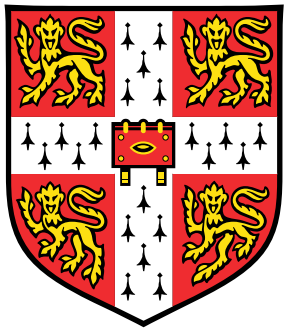
Drug delivery during breastfeeding: investigations of formulations and clinical feasibility



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This thesis is submitted for the degree of Doctor of Philosophy

St. Catharine's College February 2019

Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my thesis has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. It does not exceed the prescribed word limit for the relevant Degree Committee.

Theresa Maier
February 2019

Abstract

Drug delivery during breastfeeding: investigations of formulations and clinical feasibility - Theresa Maier

At an age when breastfeeding is the optimal nutritional support for infants, oral drug delivery can be challenging. In the past, the concept of drug delivery during breastfeeding was developed as a means to address challenges in low-income countries by facilitating administration using solid dosage forms without the need for clean water. Hereby, a silicone nipple shield, containing a formulation inside its teat, is meant to be worn by a mother during breastfeeding, enabling drug delivery to the sucking infant through the flow of human milk. Furthering past research, this doctoral work aimed to investigate novel dosage forms for this application, including a fibrous matrix and a gel formulation, as well as the clinical potential, feasibility, and acceptability of therapeutic delivery during breastfeeding. In a clinical context, a descriptive qualitative study revealed the need for alternative infant oral drug delivery technologies in high-resource settings, and parents' and nursing staff's positive response to the concept of drug delivery during breastfeeding. Findings were supported by the anecdotal evidence of difficulties in infant compliance and accurate dosing, and indicated high relevance for a use case in neonatal intensive care. Formulation investigations included zinc sulphate loaded non-woven fibre mats, and iron sulphate loaded liquid-core alginate hydrogels, using a modified and a commercially available nipple shield design. While full release during breastfeeding simulation was not achieved, both formulations enabled superior delivery of their loaded therapeutic dose compared to previously studied dosage forms. In addition, a clinical feasibility study involving the delivery of vitamin B12 from a commercially available nipple shield during breastfeeding was conducted, supported by a qualitative mixed methods approach. Results illustrated the successful delivery of vitamin B12 to breastfed infants and unanimous maternal advocacy for the availability of therapeutic delivery during breastfeeding in the future.

In memory of my grandparents.

Acknowledgements

It has been such a privilege to conduct research with a focus on improving infant health globally. I owe thanks and gratitude to many of whom have provided their generous support, guidance, and encouragement.

Foremost, I was blessed to learn from three distinguished academics, including Professor Nigel K.H. Slater, whom I am thankful for enabling me to work on such an interdisciplinary PhD project, my clinical supervisor Dr Kathryn Beardsall, who has instilled in me the love for clinical research and neonatology, and Dr Ljiljana Fruk, whose creativity was such a pleasure.

It was a joy to work with Paula Peirce, whose passion for breastfeeding and maternal support was remarkable, Lynn Thompson, whose nursing and research experience was so essential in empowering me in my day-to-day work, Andrea Edwards, who has given me the unique opportunity to support and promote neonatal research amongst both clinical staff and the public, and Laura Baird, whose help in my work on the Neonatal Intensive Care Unit I greatly appreciate.

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I am indebted to my family and friends for so much. To my parents, for their endless love and support. Cassi, for being my person and a joy from day one. Sophie, my wonderful soulmate, and Jules, for being such an inspiration. Anna, Oliver, and Robbie, for their sincere friendship. To my sisters, relatives, and friends, near and far.

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Contents

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Preface

Work in this thesis was supported by several individuals, whose contributions are summarised below as well as indicated in the main body of text.

Chapter 3. Dr Kathryn Beardsall (Department of Paediatrics, University of Cambridge) supervised the qualitative descriptive study, while also supporting me in the recruitment of parents and staff. Dr Kathryn Beardsall and Paula Peirce (Cambridge University Hospitals NHS Foundation Trust) assisted with data analysis. Oliver Bonner (Department of Engineering, University of Cambridge) acted as an adviser for study protocol preparation and interview performance. Oliver Bonner, Paula Peirce, Professor Nigel K.H. Slater (Department of Chemical Engineering and Biotechnology, University of Cambridge), and Dr Kathryn Beardsall contributed to writing the resulting manuscript, which will be submitted for publication in the coming month.

Chapter 4. Dr Rebekah L. Scheuerle (formerly Department of Chemical Engineering and Biotechnology, University of Cambridge) provided training for use of the breastfeeding simulation apparatus, guided the characterisation of human milk, and calculated the milk's protein content. She also performed the analysis of elemental zinc in human milk fractions via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), according to a protocol she had previously published, quantified the zinc content of loaded non-woven fibre mats, and guided data analysis. She was supported by Dr Sylvaine Bruggraber (formerly Medical Research Council, Cambridge). Sample preparation of Scanning Electron Microscope (SEM) analysis was performed by Dr Jeremy Skepper (Cambridge Advanced Imaging Centre), who also assisted in subsequent imaging. Sample preparation for Energy Dispersive X-Ray (EDX) analysis was conducted by Dr Karin Muller (Cambridge Advanced Imaging Centre), and analysis performed in collaboration. X-ray

Micro Computed Tomography (XμCT) measurements and data reconstruction was performed together with Dr Daniel Markl (formerly Department of Chemical Engineering and Biotechnology, University of Cambridge), supervised by Professor Axel Zeitler (Department of Chemical Engineering and Biotechnology, University of Cambridge), both of whom also guided the data analysis process. Dr Ljiljana Fruk (Department of Chemical Engineering and Biotechnology, University of Cambridge) and Professor Nigel K.H. Slater supervised the research study. Dr Rebekah L. Scheuerle, Dr Daniel Markl, Dr Sylvaine Bruggraber, Professor Axel Zeitler, Dr Ljiljana Fruk, and Professor Nigel K.H. Slater contributed to writing the resulting manuscript, published in the *International Journal of Pharmaceutics*. Dr Rebekah L. Scheuerle, a former doctoral candidate at the Department of Chemical Engineering and Biotechnology, University of Cambridge, had presented release data of zinc sulphate from non-woven fibres in the Appendix section of her doctoral thesis, referring to the support she had provided.

Chapter 5. Dr Antonina Kerbs (formerly Department of Chemical Engineering and Biotechnology, University of Cambridge) provided guidance for iron detection in human milk. Compression testing and optical analysis of resulting liquid-core hydrogels was performed together with Andrew Rayment (Department of Material Science and Metallurgy, University of Cambridge). Dr Ljiljana Fruk and Professor Nigel K.H. Slater supervised the design and realization of the research study. Dr Antonina Kerbs, Dr Ljiljana Fruk, and Professor Nigel K.H. Slater contributed to writing the resulting manuscript, published in the *European Journal of Pharmaceutical Sciences*.

Chapter 6. Dr Kathryn Beardsall was the Chief Investigator of the clinical feasibility study (18/LO/0551), providing support in its design, advice for Research Ethics Committee (REC), Health Research Authority (HRA), as well as the University of Cambridge Addenbrooke's Hospital Trust's R&D and insurance applications, and in analysis of study findings. She also provided input for the design of recruitment material together with Paula Peirce, both of whom assisted me in participant recruitment. Paula Peirce and Laura Baird (Cambridge University Hospitals NHS Foundation Trust) were present during several study feeds, and mentioned my study to mothers, whom they regarded to be a suitable fit for participation. Sophie L. Whitehouse (College of Social Sciences, Arts and Humanities, University

of Leicester) provided advice with regard to the study's mixed methods approach. Professor Nigel K.H. Slater provided financial support. Dr Kathryn Beardsall, Paula Peirce, Sophie L. Whitehouse, and Professor Nigel K.H. Slater contributed to writing the resulting manuscript, intended to be submitted for publication in the coming month. Work also highlighted a need to discuss the importance of nipple shields in clinical practice for certain health conditions in preterm and term infants. A manuscript jointly drafted with Paula Peirce, Laura Baird, and Dr Kathryn Beardsall is currently in preparation.

List of Publications

Peer-reviewed publications. Parts of the work presented in Chapter 3, Chapter 4, Chapter 5, and Chapter 6 previously appeared in:

T. Maier, O. Bonner, P. Peirce, N.K.H. Slater, and K. Beardsall. Drug and nutrient administration on the NICU – is delivery during breastfeeding an alternative to oral syringes? *Journal of Neonatal Nursing, in press*, 2019. doi: 10.1016/j.jnn.2019.09.009

T. Maier, P. Peirce, L. Baird, S.L. Whitehouse, K. Beardsall. O18 Therapeutic delivery during breastfeeding: a feasibility study. *Archives of Disease in Childhood,* **104**:e8, 2019. doi: 10.1136/archdischild-2019-esdppp.18

T. Maier, P. Peirce, L. Baird, K. Beardsall. "I didn't feel any less." - What role do nipple shields have in clinical practice? *Journal of Neonatal Nursing,* **25**(5):234-235, 2019. doi: 10.1016/j.jnn.2019.04.005

T. Maier, A. Kerbs, L. Fruk, and N.K.H. Slater. Iron delivery from liquid-core hydrogels within a therapeutic nipple shield. *European Journal of Pharmaceutical Sciences*, **131**:119-126, 2019. doi: 10.1016/j.ejps.2019.01.032

T. Maier, R.L. Scheuerle, D. Markl, S. Bruggraber, A. Zeitler, L. Fruk, and N.K.H. Slater. Zinc delivery from non-woven fibres within a therapeutic nipple shield. *International Journal of Pharmaceutics*, **537**(1-2):290–299, 2018.
doi: 10.1016/j.ijpharm.2017.12.042.

Figures and Tables originally published in the above manuscripts are indicated by * in the List of Figures and List of Tables respectively. As the first author of these

articles, use of their full content, or of individual figures and tables in this dissertation for non-commercial purpose did not require permission.

Manuscripts submitted for publication. The following manuscript, related to work presented in Chapter 6, is currently under review:

T. Maier, P. Peirce, L. Baird, S.L. Whitehouse, N.K.H. Slater, and K. Beardsall. Therapeutic delivery during breastfeeding: a feasibility study. *Under review*, 2019.

International conference presentations. Research findings were presented at the following international conferences:

T. Maier, P. Peirce, L. Baird, S.L. Whitehouse, N.K.H. Slater, K. Beardsall. Therapeutic delivery during breastfeeding: a feasibility study. Oral presentation at: *17th Congress of the European Society for Developmental Perinatal and Paediatric Pharmacology*; May 28-30, 2019; Basel/Switzerland.

T. Maier. Development of a Paediatric Therapeutic Nipple Shield. Poster presented at: C*urious2018 Future Insight Conference*; July 16-18, 2018; Darmstadt/ Germany.

T. Maier, R.L. Scheuerle, D. Markl, S. Bruggraber, A. Zeitler, L. Fruk, N.K.H. Slater. Zinc delivery from non-woven fibres within a therapeutic nipple shield. Poster presented at: *Global Young Scientists Summit*; January 21-26, 2018; Singapore City/Singapore.

Nomenclature

Acronyms / Abbreviations

BFI	Baby Friendly Initiative
CBAL	Core Biochemical Assay Laboratory
CBS	Concentric Backscattered
DDI	Distilled Deionized
DI	Deionized
EDX	Energy Dispersive X-Ray
FSOD	Flexible Solid Oral Dosage
GC/MS	Gas Chromatography/Mass Spectrometry
HC	Haptocorrin
HIV	Human Immunodeficiency Virus
HPMC	Hydroxypropyl Methylcellulose
HRA	Health Research Authority
i.d.	Inner diameter
ID	Iron Deficiency
IDA	Iron Deficient Anaemia
IF	Intrinsic Factor
IFR	Intrinsic Factor Receptor

IRAS	Integrated Research Application System
LBW	Low Birth Weight
LC/MS	Liquid Chromatography/Mass Spectrometry
LHB	Liquid-core alginate Hydrogel Beads
Mo	Molybdenum
NHS	National Health Service
NICU	Neonatal Intensive Care Unit
NIH	National Institutes of Health
NSDS	Nipple Shield Delivery System
ODF	Orodispersible Film
PIS	Patient Information Sheet
REC	Research Ethics Committee
RN	Registered Nurse
SB	Sulforhodamine B
SDS	Sodium Dodecyl Sulphate
SEM	Scanning Electron Microscope
SGA	Small for Gestational Age
TTR	Tracer to Tracee Ratio
UNICEF	United Nations International Children's Emergency Fund
VLBW	Very Low Birth Weight
WHO	World Health Organization
XμCT	X-ray Micro Computed Tomography
Zn	Zinc

Chapter 1

Motivation

"Children are one third of our population and all of our future."
- Select Panel for the Promotion of Child Health, 1981

Depending on the socio-economic setting, parents face a variety of challenges in infant enteral drug delivery. In a high-resource setting, parental challenges can comprise measuring inconvenience of liquid formulations, as well as difficulties in administering the full dose required, mostly due to a lack of infant compliance using existing devices, such oral syringes. Particularly the latter, if evoking defensive behaviour and association of therapeutic delivery as a negative experience, can make subsequent administration increasingly difficult. In a low-resource setting, additional challenges arise due to the lack of refrigerated storage, often required for liquid formulations, access limitations to clean water for the disintegration of solid dosage forms, or simply the unavailability of hygienic delivery technologies. According to the World Health Organization (WHO), more than thirty percent of the 5.4 million child deaths worldwide before the age of five are caused by conditions such as pneumonia, diarrhoea, malaria, HIV, and measles, and could be prevented with access to simple and affordable interventions [1]. With reference to the conditions in developing countries, the United Nations Secretary-General Progress Report on the Every Woman Every Child Global Strategy for Women's, Children's and Adolescent's Health emphasized that "innovation is essential to achieving the ultimate goal of ending preventable deaths among women and children and ensuring they thrive." ([2], page 60).

The following doctoral research aims to contribute to this objective. It investigates design preferences, suitable therapeutic dosage forms, as well as the clinical potential, feasibility, and acceptability of therapeutic delivery during breastfeeding to contribute towards better treatment options for breastfed infants. Hereby, a silicone nipple shield, containing a (semi-)solid dosage form, is placed on the mother's breast during breastfeeding, enabling therapeutic delivery through the flow of human milk. This approach is believed to be a promising alternative to currently existing delivery devices, because it i) overcomes the need for clean water for the disintegration of solid dosage forms in developing countries, ii) supports the call for flexible solid dosage forms by the WHO as a means to support treatment of infants and children in both high- and low-resource environments, and iii) could encourage the prevalence of breastfeeding, particularly in developed countries [3].

The objectives of this doctoral work are two-fold:

1. To investigate novel therapeutic dosage forms for delivery from a nipple shield into human milk, including
   (a) a fibrous matrix for the delivery of zinc sulphate.
   (b) a hydrogel for the delivery of iron sulphate.
2. To explore the clinical implications of therapeutic delivery during breastfeeding by means of
   (a) a qualitative study evaluating its potential and suitability for a high-resource setting.
   (b) a clinical study assessing its *in-vivo* feasibility and acceptability.

The proposed research is highly interdisciplinary, based on expertise in engineering, medicine, and natural sciences, and jointly supervised by the Department of Chemical Engineering and Biotechnology (School of Technology), as well as the Department of Paediatrics (School of Clinical Medicine). Through interaction with patients, parents, and healthcare staff, this doctoral work aims to foster an interdisciplinary development approach. Ultimately, patient-centred research facilitates the translation of scientific findings into clinical practice, and thus increases the

likelihood of reaching those in need. Beyond the scientific scope of this thesis, it also intends to encourage similarly cross-disciplinary research endeavours for the application of engineering in medicine.

The presented doctoral thesis consists of seven chapters: Following an introduction of the basic principles underlying the research of therapeutic delivery during breastfeeding (Chapter 2), the need for alternative oral therapeutic delivery technologies for infants is introduced (Chapter 3), and results of therapeutic delivery into human milk from a fibrous matrix, and a liquid-core hydrogel during *in-vitro* breastfeeding simulation presented (Chapter 4 and Chapter 5). Moreover, clinical feasibility and acceptability are discussed (Chapter 6), as well as opportunities for future research indicated (Chapter 7). A detailed outline is provided below.

Chapter 2 introduces the background underlying this doctoral thesis, amongst others the importance, prevalence, and science of breastfeeding, as well as an overview of current oral infant drug delivery technologies. Subsequently, the concept of therapeutic delivery from a silicone nipple shield during breastfeeding is introduced, and an overview of both previous research for its development and key research methodologies of this thesis provided.

Chapter 3 illustrates findings of a qualitative descriptive study conducted from May to July 2016, aimed at evaluating the perspective of parents and nursing staff on therapeutic delivery during breastfeeding in a high-resource setting.

Chapter 4 investigates the release of zinc sulphate from non-woven fibres within a lip-containing nipple shield into human milk, comparing results to previous studies conducted by Scheuerle *et al.* [4]. The chapter also provides a detailed characterisation of the non-woven fibre mats used for zinc sulphate delivery.

Chapter 5 presents the development and characterisation of a hydrogel formulation for delivery into human milk, subsequently used to evaluate the release of iron sulphate into DDI water and human milk.

Chapter 6 explores maternal expectation, experience, and acceptability of therapeutic delivery during breastfeeding by means of a single-centre feasibility study, conducted from July to November 2018. The study involved the administration of vitamin B12 from a commercially available silicone nipple shield, quantified successful delivery by means of an increase in the infant's vitamin B12 blood serum level, and made use of a mixed methods approach.

Chapter 7 provides a summary of research findings and its synthesis with previous literature. It concludes this doctoral thesis with an overview of suggestions for further work.

Chapter 2

Background

2.1 Introduction

Oral infant therapeutic delivery poses significant challenges and limitations with regard to the range of delivery technologies and therapeutic formulations available. These limitations affect patient acceptability [5, 6], defined as "the overall ability and willingness of the patient and their caregiver to administer the medicines as intended" ([5], p. 1243), and also decrease the effectiveness of treatments [5]. Liquid formulations of medicines and nutrients are preferred for infants below six years of age [7], but possess distinct disadvantages, such as dosing errors and the need for refrigerated storage. In 2006, the WHO indicated their preference for Flexible Solid Oral Dosage forms (FSOD), including dispersible, chewable or orodispersible tablets [7], to address the aforementioned shortcomings for infants globally, but with particular intend to lessen the challenges in developing countries. Flexibility in this context relates to the use of a single formulation for different age groups, and the possibility to have parts of the dosage form either disintegrated or swallowed as a whole [7]. This chapter introduces a novel approach to therapeutic delivery, namely the administration from a silicone nipple shield during breastfeeding. Since this approach of infant therapeutic administration enables the use of solid formulations, it also has the potential to overcome limitations in stability and excipient efficacy of liquid medicines [8].

2.2 Science and prevalence of breastfeeding

2.2.1 Overview

The Supplemental Nursing System, often used for formula feeding by mothers with no or only limited human milk supply, is the only infant oral delivery device that can be used while the infant is latched onto the mother's breast, and was reported to be well accepted by the infant [9]. It inspired the concept of infant drug administration during breastfeeding, which would not only provide an alternative delivery method for all infants globally, but also a solution acceptable to the 36 % of exclusively breastfed infants aged $0 - 6$ months worldwide [10]. A means of therapeutic delivery during breastfeeding would also have the additional advantage of advocating and facilitating the beneficial practice of breastfeeding in both high-income and low-resource settings. Human milk is also often referred to as "a personalised medicine for infants" ([11], p. 476) based on its nutritional and immunological characteristics [11]. Past research has shown that children breastfed for longer periods of time have a decreased infectious morbidity and mortality, fewer dental malocclusions, higher intelligence, as well as a lower risk of obesity and non-communicable diseases, such as asthma and type 2 diabetes, in later life [11–13]. According to Victora *et al.*, the scaling up of breastfeeding alone, i.e. without addition of any medicine/nutrient supplement, could prevent 823,000 annual deaths in children under the age of five [11].

2.2.2 Prevalence of breastfeeding

Despite the beneficial impact of breastfeeding on infant health, only about 40 % of infants in high-income countries at 6 months, and about 25 % at 12 months are breastfed (mixed or exclusively), while rates in low- and lower-middle income countries remain close to 90 % or above [11]. At this age, most breastfed infants originate from South Asia, sub-Saharan Africa, and certain countries in Latin America [11]. Victora *et al.* pointed out that on average breastfeeding prevalence decreases with increasing material wealth, with a longer breastfeeding duration of infants in poorer environments compared to infants in more prosperous families (see Figure 2.1) [11]. Yet, not only wealth, but also education impacts on the initiation and duration of breastfeeding: women with a higher degree of formal education in high-resource environments are more likely to breastfeed than less educated women in low-resource

environments [11]. Rollins *et al.* emphasized that women in high-resource settings need to change their belief that breastfeeding is only beneficial to prevent diseases affecting the poor [14]. Global breastfeeding prevalence of infants aged 12 months is illustrated in Figure 2.2. In the UK, 81 % of babies are breastfed at the time of birth, merely 17 % three months later [15]. Only 1 % of infants are exclusively breastfed at the age of six months [15]. Nevertheless, the 2010 Infant Feeding Survey reported a positive development in breastfeeding initiation and continuation, with any type of breastfeeding increased compared to the 2005 Infant Feeding Survey [15]. In this context, "The Baby Friendly Initiative (BFI)" is perceived as a major driver, being particularly effective in the promotion of breastfeeding [15, 16]. BFI is a worldwide programme focusing on breastfeeding promotion as part of a partnership between the WHO and UNICEF [17].

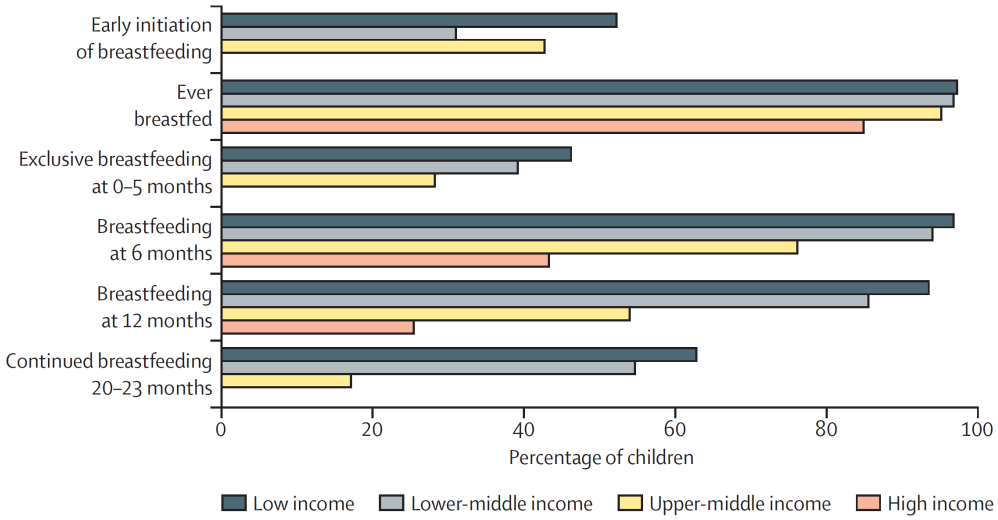


Figure 2.1: Prevalence of breastfeeding in 2010 based on the country income group. Data was obtained from 153 countries [11]. Reprinted from The Lancet, Vol. 387, Victora, C.G., Bahl, R., Barros, A.J.D., França, G.V.A., Horton, S., Krasevec, J., Murch, S., Sankar, M.J., Walker, N., Rollins, N.C., Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect, Pages 475 – 490, copyright (2016), with permission from Elsevier.

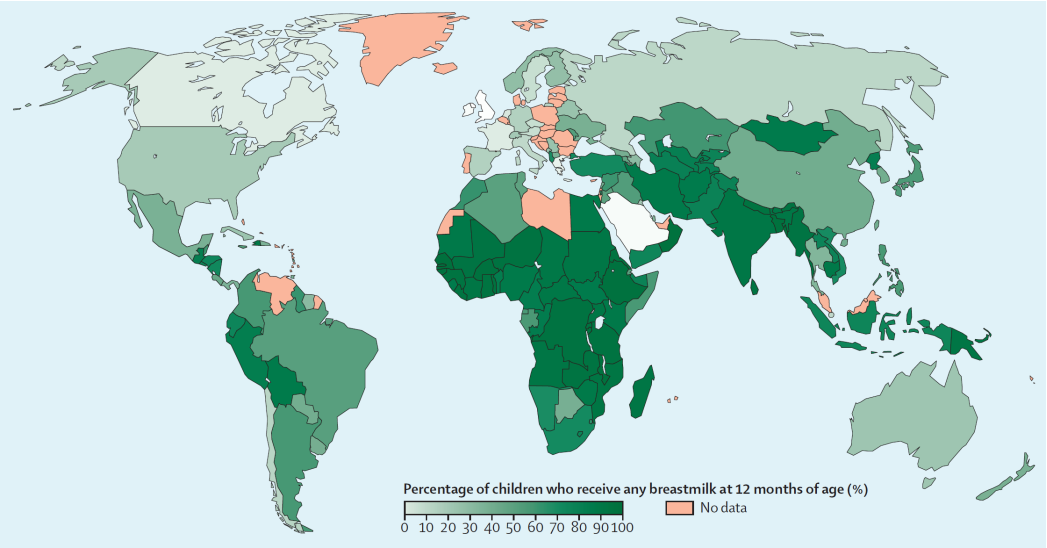


Figure 2.2: Percentage of global breastfeeding rates between 1995 and 2013 [11]. Reprinted from The Lancet, Vol. 387, Victora, C.G., Bahl, R., Barros, A.J.D., França, G.V.A., Horton, S., Krasevec, J., Murch, S., Sankar, M.J., Walker, N., Rollins, N.C., Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect, Pages 475 – 490, copyright (2016), with permission from Elsevier.

2.2.3 Anatomy and physiology of breastfeeding

The suck cycle of an infant during breastfeeding is illustrated in Figure 2.3. During breastfeeding the infant's cavity is filled with the maternal nipple as well as a significant part of areolar tissue, while the infant's tongue remains in contact with the nipple throughout the entire breastfeeding process [18, 19]. Research showed that the negative pressure created inside the infant's mouths through the intra-oral vacuum is the main driving force for human milk to be drawn out from the maternal ducts [18, 20, 21]. Throughout a suck cycle, the intra-oral vacuum increases with the lowering of the infant's tongue from the baseline pressure, also referred to as mean maximum pressure (-56.4 ± 31.4 mmHg [22], -64 ± 45 mmHg [21]), to its peak vacuum or so-called mean minimum pressure (-163.2 ± 62.0 mmHg [22], -145 ± 58 mmHg [21]), while the maternal nipple elongates and retracts rapidly with an elongation in length by up to two fold [18]. Sucks per minute vary depend-

ing on the age and type of feeding ('exclusive breastfeeding' or 'mixed feeding'), with estimated values of 46.7 ± 12.9 sucks min^{-1} for exclusively breastfed infants and 41.1 ± 10.6 sucks min^{-1} for mixed fed infants aged $21 - 28$ days, as well as 50.6 ± 14.2 sucks min^{-1} for mixed fed infants of $3 - 5$ months of age [23].

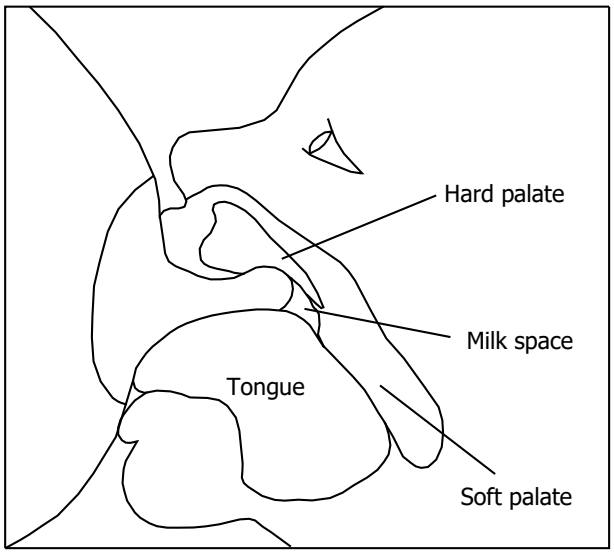


Figure 2.3: Illustration of the infant's latch during breastfeeding. Milk flow into the infant's oral cavity occurs when the infant's tongue is at its lowest point, and the intra-oral vacuum has reached its peak. Image adapted from [24].

2.3 Infant oral therapeutic delivery

2.3.1 Common oral infant therapeutics

Most commonly used therapeutics for infants up to the age of six months include nutritional supplements as well as drug formulations (Table 2.1). While some therapeutics are used for infants globally, such as antibiotics, applicability of most therapeutics is dependent on geographical and social factors, as well as on the infants' gestational age. For instance, vitamin supplements are mostly used to account for the additional needs of premature infants, as well as for infants in low-income coun-

tries with risk of deficiency. In these locations, additional drug formulations are also needed due to the prevalence of HIV, malaria, and tuberculosis. Dosage forms comprise oral liquids, tablets, capsules, and powders for the preparation of oral solutions. In accordance with the WHO Model List of Essential Medicines for Children, the term 'solid oral dosage form' is used whenever equal clinical efficacy and safety of oral formulations is applicable [25]. Tablets and capsules have to be portioned and disintegrated in water, formula or previously expressed human milk prior to delivery. Liquid or disintegrated medicines and nutrients have to be measured and administered to the infant using a delivery technology (see Table 2.2).

Therapeutic	Indication	Dosage form
Vitamin/mineral supplements		
Vitamin K [26]	Prevention of haemorrhagic disease of the newborn	Oral liquid
Vitamin A	To satisfy nutritional needs of preterm infants, and Small for Gestational Age (SGA) infants [25, 27–29]	Oral solution
Vitamin B1		
Vitamin B2		
Vitamin B3		
Vitamin B6		
Vitamin C		
Vitamin D [25, 30]	Supplement for bone metabolism, recommended for breastfed infants	Oral liquid, solid oral dosage form
Ferrous salt [25]	Supplement or antianaemia medicine	Oral liquid, tablet

Table 2.1: Overview of therapeutics commonly administered to infants up to six months of age.

Therapeutic	Indication	Dosage form
Medicines for pain		
Analgesics [31, 32]	Pain relief	Oral liquid, tablet
Medicines for epilepsy		
Antiepileptics [25]	Treatment of epilepsy	Oral liquid, tablet, solid oral dosage form, capsule
Anti-infective medicines		
Antibiotics [31, 32]	Bacterial infection	Powder, oral liquid, tablet, capsule
Antituberculosis medicines [25]	Treatment of tuberculosis	Oral liquid, tablet, solid oral dosage form
Antifungal medicines [25]	Treatment of Candida diaper dermatitis	Oral liquid, capsule, tablet
Antiretrovirals [25]	Treatment of HIV	Oral liquid, tablet, capsule, powder, solid oral dosage form
Antimalarial medicines [25]	Treatment of malaria	Oral liquid, tablet, capsule
Gastrointestinal medicines		
Oral rehydration [25]	Treatment of diarrhoea	Powder for dilution
Zinc sulphate [25]	Adjunct to oral rehydration salts for treatment of acute diarrhoea	Solid oral dosage form

Table 2.1 continued: Overview of therapeutics commonly administered to infants up to six months of age.

2.3.2 Currently available delivery technologies

A review of existing infant oral therapeutic delivery technologies is presented in Table 2.2. Administration of medicines and nutrients to neonates and young infants by means of an oral syringe is most common, while bottle feeding mothers also have the option of mixing the drug formulation with expressed milk or formula within a drinking bottle. Additional devices exist for infants, but are less commonly used or might not be suitable for use, e.g. if parents have decided against the introduction of a pacifier. Some formulations require the disintegration in water, formula or human milk, but most commercially available infant formulations are prepared in liquid form. With regard to the latter, dosing errors can occur [33].

Device	Delivery method	Note
Oral syringe	Therapeutic is displaced from a reservoir through plunger movement	Most commonly used
Drinking bottle	Therapeutic is bottle fed	Bottle feeding mothers only
Dosing cups/spoons	Therapeutic is administered via a plastic cup/spoon	Less feasible in young infants
Oral dropper	Therapeutic is displaced from a reservoir by squeezing the dropper's rubber top	Some multivitamin supplements only
Medicine dummy	Liquid is sucked from a reservoir	Applicable to infants using a dummy

Table 2.2: Overview of existing oral infant therapeutic delivery technologies.

2.3.3 Therapeutic delivery during breastfeeding

(a) Overview. To enable drug administration during breastfeeding, use of common breastfeeding adjuncts already used to support normal feeding are particularly promising. A review of the literature suggested the application of an ultra-thin nipple shield, a silicone device that is placed over the maternal nipple and areola during breastfeeding [34]. Most commonly, nipple shields are recommended when infant or maternal physiology challenge the establishment and continuation

of breastfeeding [34], supporting the infant in latching onto the breast, as well as in protecting and relieving maternal pain caused by sore nipples [35]. While maternal response to the use of nipple shields during normal feeding varies between different mothers, including both positive maternal experience as well as handling difficulties [34], past literature on ultra-thin contact nipple shields has shown that there is no statistically significant change in infant human milk intake and weight gain, while even enabling an increase in milk transfer for preterm infants [36, 37]. Moreover, as nipple shields had previously been shown to prolong the duration of mothers breastfeeding [34], their use for medicine and nutrient delivery could potentially also positively impact current breastfeeding rates. The potential to expand the currently available options of infant oral therapeutic delivery technologies, while at the same time encouraging the beneficial practice of breastfeeding, motivated the initiation of a research project at the University of Cambridge's Department of Chemical Engineering and Biotechnology in 2010: the investigation of therapeutic administration from a silicone nipple shield during breastfeeding. Thereby, a therapeutic dosage form is placed into the teat of a silicone nipple shield (see Figure 2.4).

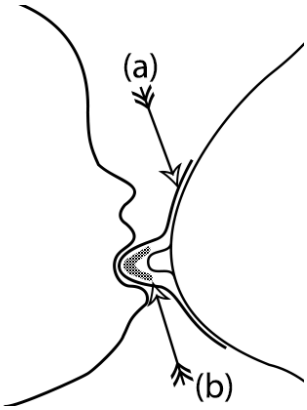


Figure 2.4: Principle of therapeutic administration from a silicone nipple shield during breastfeeding. To enable therapeutic administration during breastfeeding, a therapeutic dosage form is placed into the teat of a silicone nipple shield. Being placed on the mother's breast during breastfeeding, the therapeutic (medicine or nutrient) is delivered to the sucking infant through the flow of human milk. (a) Nipple shield, (b) Therapeutic. Image originally published in [38].

When being placed on the mother's breast during breastfeeding, it enables the therapeutic to be released and delivered to the sucking infant through the flow of human milk. A wide range of possible nipple shield designs and therapeutic dosage forms can be considered, and are discussed below.

(b) Nipple shields. Different silicone nipple shield designs could be considered for therapeutic delivery during breastfeeding, including:

- Commercially available nipple shields: Ultra-thin contact nipple shields are fabricated out of soft thin silicone in a shape that enables maximum skin contact between mother and infant.
- Modified nipple shields: Modified nipple shields could potentially facilitate therapeutic delivery during breastfeeding, e.g. through comprising a holder for drug placement.

(c) Suitable therapeutic dosage forms. To enable therapeutic delivery from a silicone nipple shield during breastfeeding, a solid-like dosage form is required. Various dosage forms exist and could be considered:

- Tablets: a therapeutic compressed into rapidly disintegrating medical tablets using standard pharmaceutical methods, such as direct compression.
- Gelatin/hpmc capsules: a therapeutic in powder form enclosed within a (pre-treated) gelatin/Hydroxypropyl Methylcellulose (HPMC) capsule to enable rapid release upon rupture.
- Membrane support materials: a therapeutic loaded onto inert fibrous mats (e.g. by means of adsorption).
- Hydrogels: a therapeutic solution used for hydrogel preparation.
- Dissolvable films: a therapeutic solution used for fabrication of thin oral matrices which dissolve in contact with mucus/the mucosal membrane.

(d) Conceptual considerations. Complete delivery of a full therapeutic dose within the duration of one breastfeed is crucial to guarantee accurate dosing and

treatment compliance. Varying milk composition, flow rate, and feeding pattern represent a challenge to the development of formulations with standardized release [20]. Among others, design specifications have to account for the up to three-fold change in absolute fat content depending on the stage of lactation, dietary requirements and health status of the breastfeeding mother [39], as well as the varying human milk volume available for therapeutic disintegration based on the pattern of milk intake [40]. With regard to the latter, a cross-sectional study of 1-month- to 6-month-old infants by Kent *et al.* illustrated an average consumption of 76.0 ± 12.6 g (range $0 - 240$ g) during one breastfeed, with the average intake being unrelated to the infant's age [40]. Instead, the total amount of human milk consumed was highly dependent on whether the more or less productive breast was used, and whether breastfeeding was unpaired, paired or clustered [40]. Hereby, an additional breastfeed <30 minutes or >30 minutes after the end of the first feed was defined as "paired" or "unpaired", respectively. The notation "clustered" refers to a feed from both breasts, following which an additional feed from the first breast occurs within 30 minutes of finishing on the second breast. During clustered breastfeeding, the minimum median intake from the first breast was 42 g (IQR: $31 - 103$ g) [40]. These findings illustrate the importance of ensuring full therapeutic delivery ideally within 42 g of intake from the first breast, but in any case within a total of 76 g of human milk consumed. Likewise, it has to be ensured that therapeutic administration during breastfeeding causes no alteration in breastfeeding behaviour, i.e. no reduction in human milk consumed, as well as no change in infant compliance towards overall breastfeeding practices. Two additional factors have to be considered: the concurrent timing of feeding and therapeutic administration, as well as the dosage form's method of disintegration. The former is of importance only for medications, which need to be administered at a certain time or as subsequent doses within a certain time interval. The method of disintegration is a general design consideration, particularly focused on preventing the formation of fragments, which could pass through the nipple shield holes and might pose a risk of infant choking. Lastly, the concept of therapeutic administration from a nipple shield during breastfeeding has to take into account existing BFI guidelines, and discussions about their evaluation of therapeutic administration during breastfeeding are required whenever findings provide enough evidence that it is suitable to do so.

2.4 Research context

The concept of using a modified nipple shield, designed to differ from commercially available ones by comprising a holder (e.g. a lip or a mesh) for drug placement, was proposed by a student group during a design competition in 2008. Subsequent doctoral research by Dr Stephen Gerrard ($2010 - 2013$) and Dr Rebekah L. Scheuerle ($2013 - 2017$) at the Department of Chemical Engineering and Biotechnology, University of Cambridge, aimed to validate the scientific potential of such a modified nipple shield, referred to as the nipple shield delivery system (NSDS), to serve as an infant therapeutic delivery tool. Past lab-based investigations included the development of novel setups to mimic breastfeeding, as well as the evaluation of therapeutic release from different dosage forms using a variety of modified nipple shields. Fieldwork in Kenya and South Africa consisted of interviews exploring opinions with regard to the use of a modified nipple shield. A review of past investigations and findings is provided below.

2.4.1 Development of setups for breastfeeding simulation

Test rag [41]. Initial therapeutic recovery experiments were conducted using a test rag, enabling the investigation of therapeutic recovery from a filter holder into a flowing, temperature-controlled fluid. During experiments, fluid was transferred from a stirred reservoir by a peristaltic pump at a physiological rate through silicone tubing, heated in a water bath to 37°C, passed through the filter holder, and fractions collected via a fraction collector (Figure 2.5). Neither pressure nor suction frequency were controlled, and nipple shields were not used during experiments.

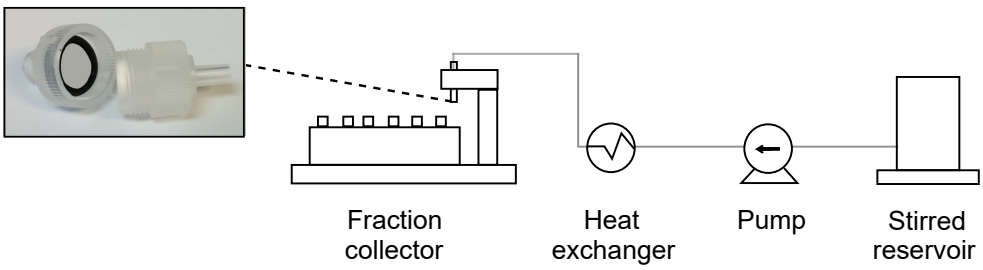


Figure 2.5: Test rag used for initial recovery experiments into human milk. Image adapted from [41].

As a result, the test rag was not capable of mimicking breastfeeding, nor of providing insight into the ability of therapeutics to be released from a nipple shield.

Breastfeeding simulation apparatus [42]. In order to improve experimental procedures, a breastfeeding simulation apparatus was developed. The apparatus enabled to mimic breastfeeding by accounting for both, fluid temperature and flow rate, as well as relevant pressure values and suction frequency, while also making it possible to investigate the release of dosage forms within a nipple shield. As a key research method used in this doctoral thesis, it will be further explained in Section 2.5.

Setup to simulate tongue movement and its applied pressure [43]. Scheuerle *et al.* investigated the impact of infant tongue peristalsis by means of a Tongue Mimic System, consisting of a fluid reservoir, a heat exchanger, a peristaltic pump, a built tongue-mimic, and a fraction collector. The tongue mimic was characterised by a shaft-like structure connected to a peristaltic pump, enabling to control the shaft's rotation rate by varying pump speed, as well as a metal plate to mimic the infants' palate (see Figure 2.6). The tablet was located in a tubing between the moving shaft and the metal plate, through which deionized (DI) water at a temperature of $33.5 - 35.5$°C was transferred at an approximate flow rate of 5 mL min^{-1}. Using two different compression settings and different rotational rates, delivery of Sulforhodamine B (SB) dye from rapidly disintegration tablets was investigated. Scheuerle *et al.* proved that not only temperature, flow rate, suction frequency, and pressure - parameters controlled by the breastfeeding simulation apparatus - but also the level of tongue compression and rotation are affecting the tablet's therapeutic release.

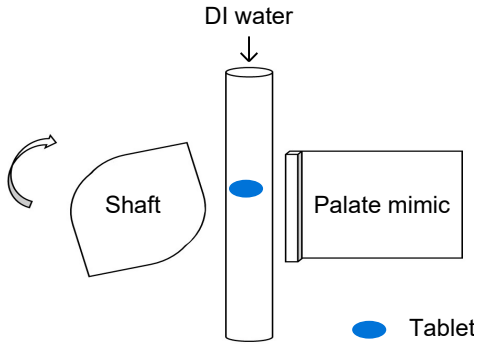


Figure 2.6: Simplified illustration of the tongue mimic. Image adapted from [43].

2.4.2 Designs of modified nipple shields

Glassfibre mesh for placement of dosage forms [42]. The initial modified nipple shield prototype consisted of a commercially available nipple shield (Maternity Silicone Nipple Shield, Boots), into which an O-ring with a fibreglass mesh was sealed at a position 9 mm away from the teat's inside tip. The commercially available nipple shield contains four 1 mm radius holes, to which Gerrard *et al.* added eight additional holes at a distance of 5 mm away from existing ones.

Circular silicone lip for placement of dosage forms [4]. Experiments by Scheuerle *et al.* made use of an advanced modified silicone nipple shield, manufactured by injection moulding, and characterised by a silicone lip for drug placement as opposed to a glassfibre mesh. The design intended for use with small dosage forms, such as tablets, was characterised by a total teat length of 23.95 mm and a silicone lip for drug placement 10.45 mm away from the teat's inside tip. Throughout this thesis, this modified nipple shield is referred to as the "lip-containing nipple shield" or "NSDS lip-containing design".

Silicone flap for placement of capsules [44]. In addition, research by Scheuerle *et al.* also investigated a modified nipple shield for use with capsules, comprising of a partially filled-in region between the teat's tip and a silicone flap for dosage form placement. It was characterised by only two holes of 2.11 mm radius, and a reduced overall teat length of 22.51 mm, whereby the silicone flap for capsule positioning was located even further away from the teat's inside tip, at a distance of 12.88 mm.

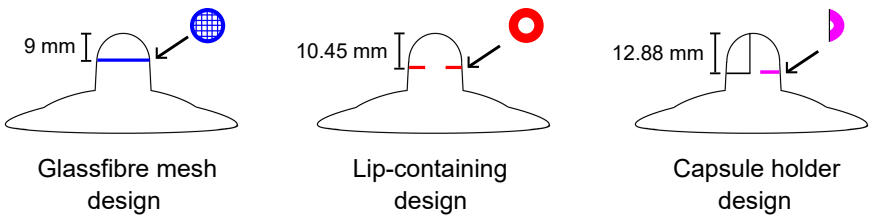


Figure 2.7: Overview of modified nipple shield designs used for *in-vitro* therapeutic delivery into human milk.

2.4.3 Dosage form investigations

Sds delivery from non-woven fibres [41]. Gerrard *et al.* investigated the release of Sodium Dodecyl Sulphate (SDS) from non-woven fibres using the breastfeeding test rag. Hereby, circular fibre mats of 10 mm diameter, consisting of non-woven viscose and polyester fibres (Bathfelt Texel, Québec, Canada), were loaded with 0.07 g SDS by soaking them for 10 sec in a 30 % wt SDS solution. Left drying for 72 hours, experiments were performed into 50 mL of human milk, whereby a recovery of $70 - 100$ % loaded SDS was obtained.

Sulforhodamine b delivery from rapidly disintegrating tablets [42]. A number of experiments investigating the release of Sulforhodamine B (SB) dye from rapidly disintegrating tablets within the mesh containing nipple shield were conducted to evaluate the impact of milk fat content, flow rate, and suction pulse rate on absolute SB recovery using the breastfeeding simulation apparatus. At a flow rate of 5 mL min^{-1}, suction rates of 30 and 60 pulses min^{-1} led to a similar SB recovery, which improved at a pulse rate of 120 pulses min^{-1}. At a physiological suction frequency of 1 suck sec^{-1}, release was neither dependent on flow rate (range: $1 - 8$ mL min^{-1}), nor on fat content (range: $2.9 - 4.2$ %). In most experiments, an absolute recovery of less than 80 % within 40 mL of milk was obtained.

Zinc sulphate delivery from rapidly disintegrating tablets [4]. Scheuerle *et al.* investigated the release of zinc sulphate from rapidly disintegrating tablets within the lip-containing nipple shield during breastfeeding simulation experiments. Four types of tablet formulations were used, characterised by different excipients and tablet hardnesses. Within approximately 100 g of human milk, an absolute recovery of $32 - 51$ % loaded zinc sulphate was achieved.

Zinc sulphate delivery from hpmc capsules [44]. Scheuerle *et al.* investigated the release of zinc sulphate from "treated rapidly disintegrating capsules" [44]. Hereby HPMC capsules were loaded with a zinc sulphate lactose mixture and dried by means of a lyophilizer until <5 % of weight change within 29.5 h was recorded. Subsequently, using the breastfeeding simulation apparatus, delivery of zinc sulphate from the modified capsule holder containing nipple shield into 100 mL of human milk was evaluated. As capsules failed to rupture, no release of zinc sulphate was achieved.

2.4.4 Qualitative research

Kenya: acceptability of the lip-containing nipple shield [45]. A qualitative study was conducted by Hart *et al.* in Kenya to evaluate the perceived acceptability of the lip-containing nipple shield to prevent mother-to-child transmission of HIV during breastfeeding, and to assess mothers' understanding of vertical HIV transmission. Eleven focus group discussions à $7 - 12$ participants were performed, including mothers, fathers, grandmothers, and mothers-in-law. In this low-resource setting, use of the lip-containing nipple shield was considered "potentially acceptable" ([45], p. 68), requiring provision to enable sustainable access and careful consideration of associated implications for barriers of use, such as religious belief or potential stigma. With regard to the prevention of mother-to-child transfer of HIV, efficacy and safety of the lip-containing nipple shield for infant antiretroviral delivery were discussed, and most participants advocated for the need of scientific validation and recommendation by healthcare staff.

South africa: acceptability of the lip-containing nipple shield [46]. Interviews (semi-structured and focus groups) were conducted with 35 infant caretakers and nine health workers, in the Vhembe District of Limpopo, South Africa, to explore their opinion towards use of the lip-containing nipple shield for therapeutic delivery. Participants responded positively, and gave preference to a disposable see-through NSDS design. Hereby, the lip-containing nipple shield was chosen over other designs without a lip, simply based on its circular base and thicker material, as mothers associated less material with an increased risk for the shield to fall off the breast. Health workers suggested a cough and de-worming medication as potential therapeutics to be delivered from the lip-containing nipple shield. Challenges with regard to community acceptance were raised and the need for education around the use of the lip-containing nipple shield for therapeutic delivery stressed.

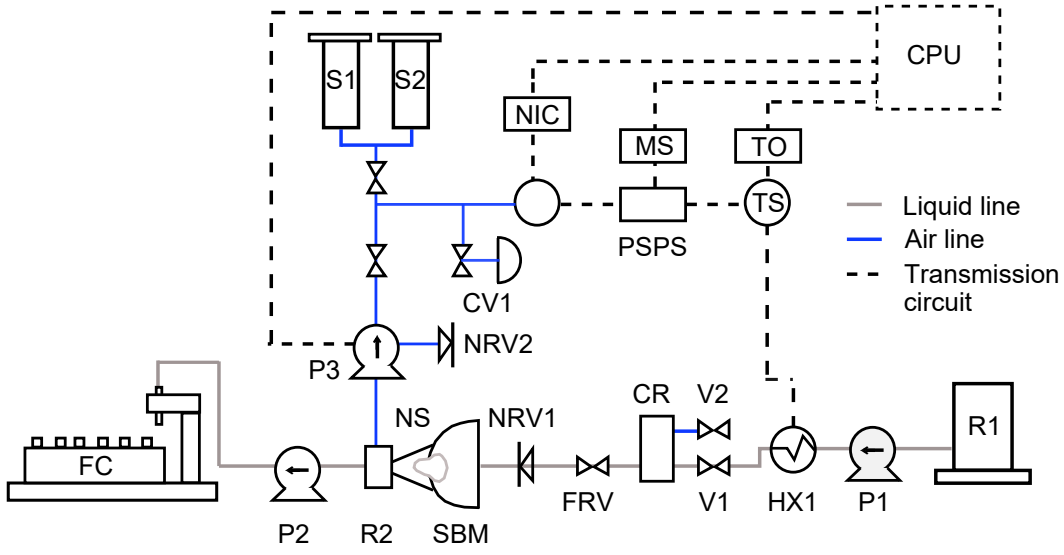
2.5 Research methods

2.5.1 Breastfeeding simulation

To ease *in-vitro* testing of different therapeutic dosage forms and nipple shield designs, a breastfeeding simulation apparatus was developed by Gerrard *et al.*, simulating both the process of lactation and infant feeding [42]. The apparatus

is capable of resembling average flow rates/patterns of milk during breastfeeding, as well as of mimicking, monitoring, and recording the sucking pressure of infants [42]. It was used for investigations in Chapter 4, Chapter 5, and Chapter 6. A process flow diagram of the apparatus is illustrated in Figure 2.8. According to Gerrard *et al.* the breastfeeding simulation apparatus has the following characteristics [42]: Using a peristaltic pump (P1, Masterflex, Cole-Parmer Instrument Co. Ltd., London/UK), milk is pumped through silicone tubing from a continuously stirred reservoir (R1) into a hot water bath (heat exchanger, HX1), controlled using a temperature sensor (TS, Digitron thermocouple, Elektron Technology, Cambridge/UK), where it is heated to a physiological temperature of $33.7 - 35.7$°C. The heated milk is subsequently fed through a reservoir valve and a non-return valve into a silicone human breast mimic (SBM), containing 25 evenly distributed holes to imitate maternal lactiferous sinuses. Using a clamp system, the nipple shield is held in place on the SBM in an airtight fashion, and positioned at a 30° angle downwards from the horizontal flow direction. A funnel (R2), having a reservoir at its lower part for fluid leaving the SBM, is connected to a vacuum pump (P3, modified Medela swing electric breast pump, Manchester/UK) through an airline. The vacuum pump (P3) withdraws milk from the SBM by means of a physiological relevant breastfeeding pressure cycle (vacuum with oscillating pattern) and is controlled by a National Instruments card (NIC, National Instruments, Austin, TX/USA), and connected to a computer system (CPU, Gigabyte Technology Co. Ltd., UK), while a second peristaltic pump (P2, Masterflex, Cole-Parmer Instrument Co. Ltd., London/UK) is delivering milk leaving the nipple shield through an exit line to a fraction collector (FC, SuperFrac, GE Healthcare Sciences, Buckinghamshire/UK). A 2011 LabVIEW software is used to accurately control P3, while the pressure within the airline system is measured using a SSOB002A pressure gauge (Sensortechnics, Munich/Germany). Two 60 mL syringes, connected to the air line, enable modification of the vacuum amplitude through adjustment of their plungers. Based on the physiology of breastfeeding outlined previously, the apparatus was designed to enable a suction frequency of 60 sucks min^{-1}, and an approximate flow rate of 5 mL min^{-1}. Breastfeeding simulation experiments are conducted as follows [42, 44]: After calibrating both P1 and P2 to have an equivalent pumping frequency, the feed pump (P1) is turned on and milk passed into the collection reservoir (CR), following which air from the airline is purged and the purge valve closed (with the feed pump P1 being on hold). Subsequently, the vacuum pump (P3) is

started by means of the LabVIEW programme and the exit pump (P2) turned on. As a last step the feed reservoir valve (FRV1) is opened to enable human milk flow through the silicone breast mimic (SBM). The breastfeeding simulation experiment is discontinued by turning off both the LabVIEW programme and the vacuum pump (P3), followed by feed (P1) and exit pump (P2).



CPU	Computer system	P2	Pump 2
CR	Collection reservoir	P3	Pump 3
CV1	Air control valve	PSPS	Current digitalized pressure sensor power supply
FC	Fraction collector	R1	Continuously stirred milk feed reservoir
FRV	Feed reservoir valve	R2	Collection reservoir and funnel connected to SBM
HX1	Heat exchanger	S1	Air syringe 1
MS	Main switch	S2	Air syringe 2
NIC	National instrument card	SBM	Silicone breast mimic
NRV1	Non-return valve 1	TO	Temperature output
NRV2	Non-return valve 2	TS	Temperature sensor
NS	Nipple shield	V1	Valve 1
P1	Pump 1	V2	Valve 2

Figure 2.8: *In-vitro* testing is performed by means of a breastfeeding simulation apparatus developed by Gerrard *et al.* [42]. Image adapted from [4]. Image originally published in [47].

2.5.2 Introduction to qualitative research

Qualitative research is often used as an initial scoping tool at the beginning of a research project, providing a direction for the establishment of a working hypothesis, which can be further investigated by means of quantitative research assessments [48]. Qualitative research is an effective measure to obtain "a deeper understanding of certain aspects of human beliefs, attitudes or behaviours" ([48], p. 235), and research interviews and focus groups are the most frequently used methods to gather data for qualitative experiments within the medical discipline [49]. Interviews can be structured, semi-structured, and unstructured [49]. Research presented in Chapter 3 and Chapter 6 has made use of semi-structured interviews, the most common interview format for qualitative medical research [49]. These interviews contain a framework created by pre-defined questions to provide guidance, while allowing the interviewer and interviewee to focus on certain aspects or responses more thoroughly as required [49]. In order to analyse the qualitative data obtained, a researcher can choose between two overall approaches: The deductive approach involves the analysis by means of a predefined theory or framework, which is either confirmed or disproved throughout the analysis process [50]. In contrast, when the inductive approach is used, little to no predefined hypothesis is applied, yet a framework developed based on themes which are derived from the data itself [50]. In both cases, the thematic content analysis is the most common approach for qualitative data analysis, and involves the identification of themes and examples for these themes within the interview transcripts [50–52]. In contrast to quantitative research, analysis in qualitative experiments already takes place during the first data collection, as preliminary results can inform the subsequent interview process, for example through modification of key questions used in semi-structured interviews [50]. Following transcription of the interview verbatim, the thematic content analysis is defined by the following analysis stages [50, 53, 54]:

1. Pre-reading: thorough reading of all transcripts.
2. Open coding: re-reading of the transcripts, adding notes capturing a summary or conclusion of the text's content.
3. Initial coding framework - generation of a short list of identified categor-

ies: Identified content categories during open coding are revised to cross out repetitions and re-group similar categories into an overarching theme. Hereby, parts of the text can be coded into different categories, while other parts of the interview may not be assigned to any category. The latter applies for segments identified as not applicable for the objectives of the conducted research.

4. Final coding framework - verification of category system: Each transcript is further analysed to identify and highlight data related to each category.

2.5.3 Introduction to mixed methods research

Studies conducted based on a mixed methods approach make use of both qualitative and quantitative components as part of their study design, data collection, and analysis [55]. Mixed methods are defined as "research in which the investigator collects and analyses data, integrates the findings, and draws inferences using both qualitative and quantitative approaches or methods in a single study or program of inquiry" ([56], p.4). Use of this approach can be advantageous if the research is of complex nature, e.g. with the intention to increase the breadth of a study [55]. Two design options exist: i) a parallel form, whereby qualitative and quantitative data are collected simultaneously and conclusions based on both analyses, ii) a sequential form, whereby data is collected successively, one type of data supporting the collection of another [55]. Research in Chapter 6 makes use of a parallel mixed methods approach.

2.5.4 Introduction to clinical studies

(a) Basic, clinical, and translational research

Basic research. Basic research focuses on lab-based investigations with the objective of contributing knowledge and of enhancing human comprehension of nature [57, 58]. According to Salter *et al.*, basic research can be differentiated as either 'curiosity-oriented' or 'strategic' [58]. While findings may address a range of practical problems, the fundamental purpose of basic research does not consist in providing actionable answers or implementable recommendations [57].

Clinical research. Clinical research comprises the following areas of inquiry: i) patient-oriented research that involves both human subjects or human tissues, the latter for which patient contact was required, ii) epidemiologic and behavioural studies, and iii) research with regard to the effectiveness of interventions and health services [57, 59, 60]. Patient-oriented research is further classified into four types of research studies: research on mechanisms of human disease, therapeutic interventions, clinical trials, and the development of new technologies [60]. Schuster *et al.* provides the following guideline [60]: "Clinical research includes any scientific investigation in which the unit of analysis is the person" (p. xvii).

Translational research. Translational research interconnects both basic and clinical research, and refers to the application of basic scientific findings for clinical use, for example to enhance patient treatment or diagnostics [57]. According to the National Institutes of Health (NIH), the pathway of "bench to bedside" consists of two translational steps, and is defined as follows [57]: "Translational research includes two areas of translation. One is the process of applying discoveries generated during research in the laboratory, and in preclinical studies, to the development of trials and studies in humans. The second area of translation concerns research aimed at enhancing the adoption of best practices in the community. Cost-effectiveness of prevention and treatment strategies is also an important part of translational science." In 2007, Westfall *et al.* proposed a third translational step, comprising of practice-based research to support the implementation of discoveries into daily clinical use [61]. The feasibility study presented in this chapter, being based on prior preclinical development at the Department of Chemical Engineering and Biotechnology, relates to translational step 1.

(b) Feasibility study. Research in Chapter 6 is classified as a "pilot" or "feasibility study", terms that are often used interchangeably. It consists of a "small study for helping to design a further confirmatory study" ([62], p. 67) of reduced duration and financial expenses [63]. The purpose of feasibility studies is to evaluate the potential of an intervention, such as general safety and effectiveness, and the benefit of allocating further resources, with the intention of subsequently investigating the intervention at a larger scale [63–65]. Consequently, feasibility or pilot studies are regarded as an essential initial step in the development process of an intervention, and believed to increase its likelihood of being successful [63]. Thereby, Bowen *et*

al. defines an intervention as "any program, service, policy or product that is intended to ultimately influence or change people's social, environmental, and organizational conditions, as well as their choices, attitudes, believes, and behaviours" ([64], p. 452). Eight focus areas of feasibility studies can be differentiated, including the acceptability, demand, practicality, adaption, integration, expansion, and efficacy of an intervention [64]. It is important to note that a pilot study does not aim to test effectiveness, efficacy, or user safety of an intervention [63].

(c) Integrated Research Application System (IRAS). Since clinical research involves the participation of human subjects, approval by major regulatory bodies is needed prior to any research being undertaken. Within the UK, applications can be submitted using the Integrated Research Application System (IRAS), a single system enabling the submission of approval requests to nine different review bodies [66].

(d) Commonly used terminology. Common terminology in clinical research, used in Chapter 6, is presented in Table 2.3.

Term	Definition
Objective	Scientific question to be answered by the clinical study [67].
Endpoint(s)	A clinical study is defined by one or more endpoints. The primary endpoint is a precise definition for reaching the study's main objective; it is evaluated by means of reproducible and accurate methods [67].
Recruitment	Process to identify and enrol suitable human subjects for participation in a clinical research study; the identification is conduced based on defined inclusion and exclusion criteria [68].
Inclusion/ exclusion criteria	Detailed description of criteria to define the study population, i.e. to determine eligibility of human subjects for participation in a clinical study [69]. A potential subject has to fulfil all inclusion criteria to be eligible for participation, while conformity with any of the defined exclusion criteria prohibits involvement in the proposed study [68].

Table 2.3: Commonly used terminology in clinical research.

Term	Definition
Informed consent process	Involves informing suitable individuals about the content and risks of a study, as well as participants' rights; individuals who match the inclusion/exclusion criteria, and who voluntarily decide to participate in the proposed study, have to sign an informed consent form to confirm their voluntary participation and acknowledge receipt of all legally required study information [70].
Research Ethics Committees	Institutional bodies, which focus on protecting those individuals involved in clinical research, either as participants or as researchers [68].

Table 2.3 continued: Commonly used terminology in clinical research.

Chapter 3

A parent and nursing perspective

3.1 Introduction

3.1.1 Overview

The potential of therapeutic delivery from a silicone nipple shield during breastfeeding in a developed setting, where dosing spoons and oral syringes are readily available, had not previously been explored. Although the concept was originally developed as a means to address challenges in developing countries, it was hypothesised that it could likewise serve as an alternative approach to common delivery technologies in high-resource environments. Potential advantages were believed to include the support of exclusively breastfeeding mothers, as well as the treatment of infants with chronic or acute conditions, requiring regular or frequent medication. More and more companies are making use of such reverse innovation by expanding their offerings in developed markets with technologies originally intended for resource-limited settings, aiming to cater towards cost-minded customers and/or to address an existing product gab [71]. Products include lower-cost developments with similar (cost innovations), market-tailored (good-enough innovations), or novel functionality (frugal innovation) [71]. Therapeutic delivery during breastfeeding was also considered as a potential means to encourage the beneficial practice of breastfeeding, with continuation rates at the infants' sixth postnatal months being over 50 % lower in high-resource environments compared to those in low-income settings [11]. In particular, the UK exhibits one of the lowest rates in breastfeeding establishment and continuation within Europe [15].

Work presented in this chapter aimed to achieve the following objectives:

1. To evaluate the potential of therapeutic delivery during breastfeeding in a high-resource environment using appropriately chosen design methodology
2. To identify design characteristics of a nipple shield intended for the use of therapeutic administration to infants

3.1.2 Study design considerations

Experimental work in this chapter was based on the following considerations.

(a) Selection of research methodology. Based on guidance for the identification of a working hypothesis at the beginning of a research project [48], use of a qualitative methodology was chosen. Hereby, semi-structured interviews were preferred over structured and unstructured interview formats, aiming at providing a certain degree of guidance while enabling the participants to direct their focus of importance. With the objective to interview mothers, fathers, and nursing staff, the study focused on gaining both primary and secondary information about potential parental challenges of infant oral therapeutic delivery, and to assess the opinion of end-users and potential groups of supporters with regard to the possible intervention of therapeutic administration during breastfeeding.

(b) Selection of study location. Research with parents of infants in a neonatal intensive care environment was chosen over assessment in outpatient groups or as part of community support, since it was believed that parents having experienced a high level of neonatal intervention, such as mechanical ventilation or total parenteral nutrition, will be more anxious and hence more critical in assessing the potential of therapeutic delivery during breastfeeding. The same interview environment was thereby also regarded as a means to avoid systematic errors arising in other study locations.

(c) Selection of nipple shields as a visual aid. Based on the concept's novelty and in order to support the participants' comprehension, it was decided to provide visual guidance in form of both a commercial contact nipple shield, potentially

already familiar to some of the parents, and a modified nipple shield, the NSDS lip-containing design, currently under investigation at the University of Cambridge's Department of Chemical Engineering and Biotechnology. Both are illustrated in Figure 3.1. The commercially available ultra-thin contact nipple shield (Medela, UK) for normal feeding has four holes of diameter 1.00 mm and is available in a variety of sizes, including small, medium, and large. Corresponding values for maximum teat diameter and teat length are illustrated in Table 3.1.

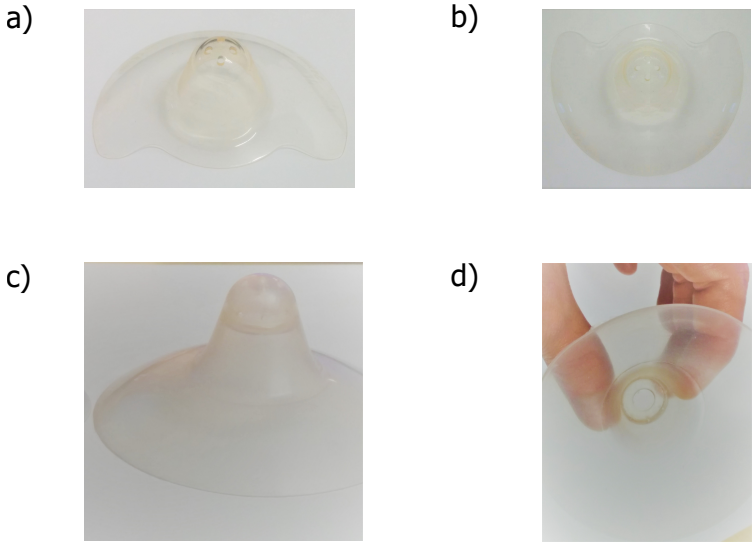


Figure 3.1: Illustration of nipple shields investigated for therapeutic delivery during breastfeeding. Commercially available ultra-thin contact nipple shield shown from a) the side, b) from below. Modified nipple shield with a retention lip for therapeutic placement from c) the side, d) from below. Image adapted from [72].

Device size	Maximum teat diameter [mm]	Teat length [mm]
Small	16	18
Medium	20	19
Large	24	20

Table 3.1: Maximum teat diameter and teat length for commercially available ultra-thin contact nipple shields (Medela, UK).

The modified nipple shield was manufactured through silicone injection moulding in one size only, containing twelve exit holes of diameter 1.23 mm evenly spread across the teat's tip, a maximum teat diameter of 17.41 mm, a teat length of 23.95 mm, and a base diameter of 75.71 mm.

3.2 Materials and methods

3.2.1 Study population and participant recruitment

The qualitative descriptive study was conducted with parents and staff on a level 3 Neonatal Intensive and a Transitional Care Unit (NICU) of the University of Cambridge Addenbrooke's Hospital NHS Trust from May to July 2016. All parents with an inpatient infant, who had previously breastfed an infant, and/or were intending to breastfeed or already doing so, and who were willing to provide written informed consent for participation were eligible for inclusion. No restrictions regarding health status, gestational age or birth weight were applied. Eligible staff comprised healthcare professionals whose role included supporting families to establish breastfeeding. Staff of varied years of experience and age were recruited by distributing information sheets on the wards, while parents were approached by the clinical nursing staff prior to being seen by a member of the research team.

3.2.2 Interview development and data collection

The interview process aligned with established procedures for semi-structured interviews [73]. Pre-defined questions were designed to (a) identify challenges of oral infant therapeutic administration, (b) assess parents' attitude towards nipple shields for normal feeding, (c) evaluate the potential of therapeutic delivery from a silicone nipple shield during breastfeeding, and (d) determine its preferred design characteristics. An excerpt of questions is shown in Table 3.2. Both a commercially available ultra-thin contact nipple shield and the NSDS lip-containing nipple shield were shown to the participants. Staff were interviewed individually in private rooms on the units, whereas interviews with parents were performed at the infant's bedside, while a nurse known to the parents was present. The interviews were conducted independently from the clinical team to provide for consistency and avoid bias, and recorded using digital voice recording software for later analysis. The interviewer did not provide any advice regarding breastfeeding or the use of nipple shields, and

parents were clearly informed that this was a research study, only investigating a novel approach of infant oral therapeutic delivery.

- In the past, have you ever given any medicine to any/your baby? Which delivery devices did you use? What was your experience? *(parents)*
- After discharge, are you/your wife/your girlfriend intending to breastfeed your baby? For what reason "no"/"yes"? *(parents)*
- What do you think about the nipple shields' design characteristics (colour, shape, size)? *(parents, staff)*
- Would you want to use/recommend a nipple shield device for therapeutic delivery during breastfeeding, and if so, which therapeutics would you like/not like to administer from a nipple shield device during breastfeeding? *(parents, staff)*

Table 3.2: Excerpt of questions used in the qualitative descriptive study.

3.2.3 Data analysis

Interviews were transcribed verbatim and potentially identifiable data of participants and their infants were anonymised during interview transcription. Analysis was conducted through code development using the inductive approach of thematic content analysis in the software package ATLAS.ti (Scientific Software Development GmbH) [53, 74]. Following pre-reading of all transcripts, notes were added line-by-line through an open coding approach, subsequently revised and re-grouped to form an initial coding framework. These initial codes were evaluated within the research team, particularly in discussion with Dr Kathryn Beardsall, together iteratively adapted and refined into a final coding framework. Advice on overall coding practices was provided by Oliver Bonner.

3.2.4 Ethics

The study was approved and registered with the Patient Office at the University of Cambridge Addenbrooke's Hospital NHS Trust, and was assessed by the Addenbrooke's Hospital Research and Development Department to not require ethics approval. All participants provided their written informed consent to be quoted anonymously in this publication.

3.3 Results

From a total of thirty invited, twenty-eight participants were interviewed (Table 3.3), including nine registered nurses (RNs), seven fathers and twelve mothers, of which six each were parents of the same infant and were interviewed together (Table 3.4 and Table 3.5). Three of the infants were born extremely preterm (<28 weeks), two very preterm (28 to <32 weeks), four moderately preterm (32 to <37 weeks), three full term (37 to <41 weeks), and one late term (41 to <42 weeks) [75, 76]. The gestational age of the participants' infants was anonymised using the WHO definitions of preterm birth [76]. Two individuals had refused participation due to language comprehension difficulties.

ID	Parents	Staff
Characteristics	n	n
Gender		
Female	12	9
Male	7	0
Average age [years]	30.2 (17 – 40)	38.8 (22 – 50)
Breastfeeding experience		N/A
Yes	13	
No	6	
Total number of children		N/A
1	9	
2-3	9	
>3	1	

Table 3.3: Characteristics of interview participants (N = 28). Table originally published in [72].

The total number of participants was determined by the point at which information saturation was reached [77].

Mothers		Fathers	
ID	Infant's gestational age	ID	Infant's gestational age
M1	Moderate to late preterm	F1	Moderate to late preterm
M2	Late term	F2	Late term
M3	Moderate to late preterm	F3	Moderate to late preterm
M4	Extremely preterm	F4	Extremely preterm
M5	Full term	F5	Full term
M6	Full term	F6	Full term
M7	Full term	F7	Moderate to late preterm
M8	Extremely preterm		
M9	Extremely preterm		
M10	Very preterm		
M11	Very preterm		
M12	Moderate to late preterm		

Table 3.4: Parent participant's ID and the gestational age of the parent's infant (n = 19).

Registered nurses	
ID	Profession
RN1	Nursery nurse
RN2	Nursery nurse
RN3	Junior sister
RN4	Staff nurse
RN5	Nursery nurse
RN6	Staff nurse
RN7	Staff nurse
RN8	Senior sister
RN9	Registered nurse, IBCLC® and midwife*

Table 3.5: Profession and ID of RN participants (n = 9).
*IBCLC® stands for International Board Certified Lactation Consultant®

Interviews lasted on average 15.6 min (range $9.5 - 27.5$ min), since parents were either not able to speak about previous breastfeeding or therapeutic delivery experience, or found it emotionally challenging to discuss breastfeeding intentions, given their infant's present health status. Based on the responses provided, four overall themes were identified, including current practices and challenges, perceived benefits and risks, design considerations, as well as the role of healthcare professionals. Quotations are used to illustrate key points, and if required were edited to enhance clarity and brevity (see content in square brackets). The adjunct *Mx*, *Fx*, and *RNx,* for mothers, fathers, and registered nurses respectively, refers to the participant codes from Table 3.4 and Table 3.5.

Current practice of oral drug delivery: challenges and the need for innovation

Use of commonly available infant oral therapeutic delivery devices was described by the participants as both physically and emotionally challenging, relating to either therapeutic administration to their previous infant, or to nutrient delivery to their inpatient newborn on the NICU. With regard to physical challenges, parents referred to incidents, in which the infant was actively fighting therapeutic administration, e.g. through slapping the dosing spoon or oral syringe, sudden turning of the head, or spitting out medicine after delivery. As a consequence they also expressed their worry about the reduction in the final dose received.

> "It was a nightmare, because he wouldn't take [the therapeutic] from a syringe, you put it on a spoon, he flipped it. So we were never certain, what he had." [M1]

Oral syringes were preferred over spoons for infants below the age of six months, but some mothers perceived their appropriate handling as difficult.

> "[Syringes] are not easy to put in their mouth. And they are not easy to use. So you end up shooting quite a lot of liquid in very fast." [M1]

One mother also described her observation about the limited options available for exclusively breastfeeding mothers.

> "If you are bottle feeding, you can give the medication into the milk; but when you are breastfeeding, you are kind of stuck when they are not taking the medication [with a spoon or a syringe]." [M7]

Emotionally, parents expressed their worry of hurting their struggling infant, their feeling of guilt, and their infant's anxiety.

> "[It was] quite difficult, and she is a bit scared. [...] I feel like I am torturing her." *[laughs shyly]* [M2]

Responding to a need: perceived benefits

The concept of therapeutic administration from a silicone nipple shield during breastfeeding was well perceived and positive responses were obtained from all participants, both in terms of practical and emotional benefits. It was felt that it could help to achieve the delivery of a complete dose, and thereby address a commonly held anxiety of risk with current methodologies.

> "[If] the baby was gonna get all its medication, it would be an ideal idea. Rather than trying to shove it into its cheek, and then spitting it out the other side. We have all been there with a syringe." [M4]

> "I think, mothers would probably be quite happy that their child is going to get the full dose." [RN6]

Moreover, all participants believed that therapeutic delivery during breastfeeding would lead to a reduction of infant distress.

> "It is going to be easier than trying to put a syringe with medicine into a baby's mouth, and it is not going to be distressing for the baby, if it is just going to have a breastfeed as normal." [RN5]

> "[...] they'd be calmer, I think that's the difference." [M1]

Additional statements referred to an improved relationship between mother and infant. Mothers saw the ability to deliver nutrients while breastfeeding as a means to foster mother-infant bonding.

> "It is just another way that you can be involved in your child's care. So you get so many things taken away from you [on the NICU] - that you can't do. [...] We understand that. But it would be nice to feel a bit more involved. [...] So with [therapeutic delivery from a silicone nipple shield], it is a way that you can still bond with your baby, and feel like

> you are helping them instead of being completely useless. [...] Anything that can be done to help us feel closer with our babies is absolutely excellent." [M8]

Staff expressed the belief that therapeutic delivery during breastfeeding was an opportunity of supporting and empowering mothers in challenging environments like the NICU, as therapeutic delivery during breastfeeding would allow them to regain ownership and responsibility of their infant's care.

> "In some ways we are giving ownership back to them. Giving medication is a nursing role. [...] it's lovely for them to think to take ownership of that, thinking 'It is my baby, I can do that.'" [RN9]

All participants favoured use of therapeutic delivery from a silicone nipple shield during breastfeeding, when being asked about their preference between a nipple shield device and a medicine pacifier (dummy).

> "They seem to be a bit too much in control of themselves [with a medicine pacifier]." [M8]

Noticeably, fathers appreciated the ability of enabling therapeutic delivery while encouraging the natural practice of infant feeding.

> "This is encouraging something natural, but kind of doing it [the therapeutic administration] at the same time [as breastfeeding]." [F5]

The delivery of nutrients was supported by all participants and the administration of medicines using a nipple shield during breastfeeding by the majority of the interviewees, provided potential risks can be mitigated.

Comprising challenges: perceived risks

At the beginning of the interview, questions aimed at exploring the attitudes towards using standard nipple shields in the process of establishing and maintaining breastfeeding. On the NICU, nipple shields are often used as *"a temporary measure"* [RN1], which parents associate with the positive experience of enabling them to breastfeed.

> "If the baby really struggles, [and] a nipple shield really helps [to achieve breastfeeding], then the parents are obviously very happy to

> learn that there is actually something that they can [do], that will help the baby to latch." [RN4]

Staff highlighted the importance of adequate parent education and guidance.

> "I think [it is important] to show them properly how to apply a nipple shield [...] because a lot of people don't apply it properly. You know, they just shove it on. They don't really realize that there is actually a bit of a skill to apply them." [RN9]

Subsequent questions referred to the potential use of a nipple shield for therapeutic administration. Hereby, five main areas of concern were raised. First, all participants expressed their worry that potential undesirable therapeutic taste could negatively affect normal feeding behaviour, if the infant was to associate human milk with the taste of a therapeutic.

> "So it is just ensuring that the [infant] would still latch." [RN8]

Furthermore, the need for rapidly disintegrating formulations was emphasized by all participants with the objective to avoid the risk of incomplete delivery of the required dose. RNs indicated three additional areas of concern. Firstly, they pointed out that some mothers, for example those who are in the process of establishing breastfeeding and those confidently feeding without a nipple shield, might be reluctant to use a nipple shield for therapeutic delivery, if it was to adversely impact their experience either practically or emotionally.

> "They will be worrying that maybe the baby will get used to the convenience of the silicone, and then not go back to skin-to-skin, baby's lips to mother's breast." [RN6]

> "My concern is that there are some mothers who feel already out of control, that are struggling." [RN9]

Secondly, they highlighted the potential challenge of combining therapeutic administration with the time constraints of three-hourly feeds.

> "You have to think of timings of feed with medication. [...] Then it's trying to find another method of getting the oral medication in, if it is not during a feed time." [RN2]

One RN additionally indicated that there could be a risk of maternal skin reaction when medicine in the nipple shield would be in direct contact with the mother's skin.

> "Vitamins you could do quite easily. [But] if you have the drug in there right next to the woman's nipple, you don't want to give her a rash." [RN8]

Nipple shield design considerations

When asking participants about their nipple shield design preferences, all advocated for a discrete see-through device, to be available in a range of sizes, making therapeutic delivery directly observable, personalized, and more natural.

> "I think [the nipple shield should be] see-through, because [...] I would want to know what medicine was in there, and how much was going, has been taken." [M1]

All participants preferred a contact nipple shield to a full circular one, as well as material properties with reduced thickness and enhanced softness. A preference for the ultra-thin contact nipple shield over the modified nipple shield (NSDS) was expressed, resulting from cleanliness, fit, and practicability concerns related to the NSDS retention lip. As the lip is protruding inwardly, many participants projected cleaning to be challenging, with participants preferring a reusable device for high-resource settings. RNs observed that the available space for the maternal nipple within the modified silicone teat was significantly reduced due to the retention lip, while the teat's length was similar to those of commercial shields. This was seen as particularly problematic for mothers of certain anatomy.

> "[...] I think my only concern is that depending on the mother's nipples [...] ...that [the remaining space within the nipple shield] is quite short." [RN9]

The lip was also considered to be a potential source of maternal discomfort, because of its risk to rub on, or trap the mother's nipple during breastfeeding.

> "I am worried that [the lip] could catch your nipple [...]." [F5]

> "[...] part of the nipple might then try to go through [the hole in the lip], and it might rub." [RN2]

Several parents also expressed that a commercially available device already used for normal feeding, might enable easier handling.

> "That [commercially available nipple shield] would be better. Just because if people are using these anyway [for a normal feed], it would be easier to just use this and put the medication in, I guess." [M11]

Introducing therapeutic delivery during breastfeeding: key role of healthcare professionals

Both parents and RNs felt that it would be best to introduce the nipple shield device for therapeutic delivery within a hospital environment, with the objective to provide mothers more confidence for subsequent home use.

> "If you have already used this in the hospital, you are used to it, you know what you are doing, you will be more than confident to do it [at home]." [M8]

They also indicated that the main factors driving positive perceptions of infant therapeutic delivery technologies, and consequently of a nipple shield to aid therapeutic delivery, is their customary use and formal recommendation by healthcare professionals.

> "I think if someone gave this [to us] and said 'this is the way we were giving the medicine', then I don't think you would question it. [...] Because it's... I don't know, it's almost less risk compared to something [else]." [F5]

> "I think whatever becomes normal. If the baby is able to latch on the breast with a nipple shield to be able to give medication, then that's probably a [more] normal way than having the baby away from you, and [squeezing] medicine down." [RN8]

RNs confirmed that they would recommend a nipple shield for therapeutic delivery to parents, if all stated risks could be sufficiently addressed.

3.4 Discussion

This study is the first of its kind to explore the perceived acceptability of a nipple shield for therapeutic delivery during breastfeeding in a high-resource environment. It thereby furthers work by Hart *et al.*, targeted at exploring the potential of a nipple shield for antiretroviral delivery to prevent vertical HIV transmission in a low-resource environment [45], by exploring associated benefits, risks, and design preferences. The study has highlighted practical and emotional challenges of parents in the process of infant oral therapeutic delivery, using commercially available delivery devices. It has provided evidence for the need of alternative infant therapeutic delivery technologies in high-resource settings, and suggested that a nipple shield for delivery during breastfeeding could serve as a potential solution for breastfeeding mothers.

Potential advantages

The responses provided by parents indicate that infant oral drug administration is associated with physical and emotional burden. The level of emotional anxiety is highlighted by the emotive language used, and their worry about the infant's choking and fear. The delivery of therapeutics during breastfeeding was perceived to have a positive impact on the infant's overall emotional state. The literature indicates that the release of Oxytocin during breastfeeding in both the infant's and mother's brain has the potential to decrease stress levels through counteracting certain pathways within the sympathetic nervous system [78], whilst also eliciting enhanced care-giving behaviour [78, 79]. All participants confirmed that they would use and recommend the nipple shield for nutrient delivery. This indicates the potential of a nipple shield to be used for the daily nutrient administration to infants on the NICU and its continuation up to six months after discharge [27, 28]. A nipple shield for therapeutic delivery was also perceived as a means to improve mother-infant bonding, a matter of particular relevance for parents on a Neonatal Intensive Care Unit. In such emotionally challenging environments, building of emotional closeness empowers parents to regain responsibility for their infants' care, while leading to improved infant outcomes [80–82]. The finding of this study to provide appropriate maternal support by healthcare professionals while introducing a nipple shield for therapeutic delivery aligns with past literature on the transition of responsibility in care from clinical staff to parents of preterm infants [80–83].

Potential challenges and requirements

The potential association of breastfeeding with the adverse taste of medicine was raised as a concern by all participants, due to the risk that it could negatively affect infant breastfeeding behaviour. This emphasizes the importance of therapeutic taste masking of formulations used for delivery into human milk. Yet, while appropriate taste masking of formulations is important to ensure, it is known that breastfed infants are familiar with a wide range of tastes, reflecting the maternal diet [84, 85]. Moreover, human milk is itself considered to have taste masking characteristics, as previously shown for formulations that are insoluble, characterized as irritating or bitter-tasting [86–88]. The capacity of therapeutic delivery to enable appropriate mixing of therapeutic and human milk was shown in previous lab-based studies using orally disintegrating tablets [4], whereby a linear release profile was obtained for 20 min of breastfeeding simulation at physiologically relevant conditions.

Preference for delivery using a commercial nipple shield

All participants preferred a commercially available and reusable nipple shield, providing one or more of the following reasons: inability to properly clean a lip-containing device, design limitations of a lip-containing device with regard to varying maternal physiology, perceived user convenience of a commercially available device used for normal feeding. This finding contrasts with prior efforts in developing a modified lip-containing nipple shield [4, 41, 87].

Limitations

This study has elicited parents' and staff's views revealing the potential of therapeutic delivery during breastfeeding by means of a nipple shield in a high-resource neonatal care setting. Limitations include that this is a single centre study, describing participants' opinions only based on visual assessment of nipple shields, not their practical use. Moreover, participants' preference of a nipple shield may have been biased by material appearance. It has to be noted however that most participants provided clear reasoning for their choice based on design parameters rather than the device's material composition. The study was designed to explore parents' and staff's opinions about therapeutic delivery during breastfeeding and preferred nipple shield design characteristics for further development. In order to determine

the clinical feasibility, safety, and acceptability of a nipple shield for therapeutic delivery during breastfeeding, subsequent clinical work will be required.

3.5 Conclusion

This study was the first of its kind to prove the potential of infant oral therapeutic administration in a high-resource environment, as hypothesised in previous literature [4, 42, 45]. The following two key drivers were identified: (1) the desire to overcome associated emotional and physical challenges with currently available infant oral therapeutic delivery devices, (2) the need to foster mother-infant bonding in neonatal special care environments, and to encourage parental empowerment. The findings demonstrate acceptability of a nipple shield for therapeutic delivery to address these issues, so long as concerns about potential impact on breastfeeding can be addressed and accurate dosing ensured. Raised design concerns related to a formerly modified lip-containing shield, and indicated preference for a commercially available silicone nipple shield.

Chapter 4

Fibre-based zinc delivery into human milk

4.1 Introduction

4.1.1 Overview

Based on the need to investigate novel therapeutic dosage forms for delivery from a nipple shield into human milk, presented research synthesizes previously conducted investigations by exploring the release of zinc sulphate pentahydrate from non-woven fibres during breastfeeding simulation. Neither the release properties of non-woven fibre mats, using parameters resembling the physiological process of breastfeeding, nor the delivery of zinc sulphate from dosage forms others than rapidly disintegrating tablets had yet been investigated. The following objectives where defined:

1. Characterisation of non-woven fibre mats for zinc delivery into human milk
2. Quantification of zinc release from a lip-containing nipple shield into human milk during breastfeeding simulation
3. Comparison of absolute recovery achieved with previous literature on zinc release from rapidly disintegrating tablets

4.1.2 Importance of zinc in neonates

The element zinc (Zn) is of great importance in the human body, as it is responsible for the activity of various proteins in major pathways of the human metabolism [89]. In case of deficiency, not only infant growth and development, but also the capabilities of the body's immune system are significantly affected [89–91]. Zinc is absorbed in the small intestine and uptake is independent of zinc status [89, 92]. Nonetheless, higher uptake is reported for term babies in the literature, caused by a lower zinc concentration in the blood serum [89]. Especially preterm and Small for Gestational Age (SGA) neonates have an increased risk of zinc deficiency [91], as these infants had benefited a shorter time from the maternal zinc derived via placental transfer [89]. At the same time, they are anticipated to have little intake after birth, while increasingly loosing zinc endogenously through the gastrointestinal tract [89]. Deficiency is believed to occur within the first $1-2$ months of life [90]. Although breastfed infants derive zinc through the consumption of human milk, the available concentration might not be sufficient, as it varies significantly between mothers ($0.7-1.6$ mg L^{-1}) and the stage of lactation, and constantly declines postpartum: while $8-12$ mg L^{-1} zinc are present in colostrum, the first milk the maternal breast produces after giving birth, only $3-6$ mg L^{-1} in human milk can be found seven days after birth, and only $1-3$ mg L^{-1} another three weeks later [89]. As a means to prevent deficiency, enteral zinc delivery of 0.8 mg kg^{-1} day^{-1} for term infants and 3 mg kg^{-1} day^{-1} for preterm neonates is recommended [93], while Terrin *et al.* noted that the recommended dose seemed to have increased over time [89]. Zinc deficiency in neonates is associated with conditions such as dermatitis, growth retardation, necrotizing enterocolitis, neurologic damage, bronchopulmonary dysplasia, infections, and retinopathy of prematurity [89], while its supplementation has positive impact on paediatric survival [90]. Zinc deficiency, not only in form of severe but also as moderate deficiency, is adding to "the global burden of disease" ([94], p. 1488S), as each year approximately 800,000 child deaths are documented as a result of zinc deficiency [94]. An excerpt of the literature illustrating the positive effect of zinc supplementation can be found in Table 4.1.

Author	Findings: Zinc supplementation...
Bhutta *et al.* [95] Walker *et al.* [96]	...leads to significant reduction in infantile diarrhoea and pneumonia rates in developing countries.
Sazawal *et al.* [97]	...leads to a significant decrease in mortality for small for gestational age infants.
Bhatnagar *et al.* [98]	...was suggested as an adjunct to antibiotics for the infantile treatment of bacterial infections.

Table 4.1: Excerpt of the literature illustrating positive benefits of infant zinc supplementation.

In addition to the overall health promoting effects of zinc, supplementation is also recommended by the WHO as an adjunct to oral rehydration salts for the treatment of acute diarrhoea (20 mg zinc sulphate). Guidance is included in the WHO Model List of Essential Medicines and the WHO Model List of Essential Medicines for Children [25, 99]. Based on its importance for the physical development and health of infants globally, and in order to enable comparison with previously conducted experiments [4], zinc was chosen as the therapeutic for breastfeeding simulation experiments in this chapter.

4.2 Materials and methods

4.2.1 Materials

(a) Human milk. The Cambridge Human Biology Research Ethics Committee at the University of Cambridge ethically approved all human milk sample use (HBREC.2012.01). Human milk samples from two healthy mothers, previously screened negative for syphilis, hepatitis B and C, HIV I and II, as well as HTLV I and II, were obtained from the Queen Charlotte's and Chelsea Hospital Milk Bank (Imperial College Healthcare NHS Trust). For the initial pooling and analysis, individual frozen human milk samples of $50-100$ µL volume, thawed from -80°C in repeated cycles at 4°C overnight and at room temperature for 5 h each, were pooled, thoroughly mixed, and aliquots taken for creamatocrit and

protein analysis. Protein quantification was conducted using a standard Bradford Agent assay (Sigma-Aldrich, UK). All measurements were performed in triplicate and the average taken. Cream content was evaluated in sextuplicate by means of the creamatocrit technique according to a protocol by Lucas *et al.*, whereby a Sigma Microcentrifuge (SciQuip, UK) and BRAND® Haematocrit sealing compound (Sigma-Aldrich, UK) were used [100]. Subsequently, the fat content was calculated using the following equation: fat (g L^{-1}) = (5.37 x creamatocrit %) + 5.28 [101]. A total lipid content of 43.52 g L^{-1} measured in sextuplicate was determined. Protein content measured in triplicate was 16.51 g L^{-1}. Both values correspond to previously reported literature data [40, 102, 103]. An example of human milk composition by Emmet *et al.* is illustrated in Table 4.2 [102]. Milk density measurements, covering the temperature range of $33.7 - 35.7$°C, a temperature used in previous research to simulate the milk's temperature in the infant's mouth during breastfeeding simulation [4], were conducted in triplicate using a hydrometer (VWR, Lutterworth, UK). Following linear interpolation, illustrated in Figure 4.1, a density value of 1.023 g mL^{-1} was obtained for the median temperature during breastfeeding simulation experiments. Characterised human milk was aliquoted for future experiments à 500 mL each, and stored at -80°C until further use. For the experiments conducted in this chapter, human milk was thawed for 48 h at 4°C before use. Human milk characterisation was guided by Dr Rebekah L. Scheuerle, who also calculated the milk's protein content.

NUTRIENT [g 100 mL^{-1}]	HUMAN MILK, COLOSTRUM	HUMAN MILK, TRANSITIONAL	HUMAN MILK, MATURE
Water	88.2	87.4	87.1
Protein	2.0	1.5	1.3
Fat	2.6	3.7	4.1
Carbohydrate	6.6	6.9	7.2

Table 4.2: Average water, protein, fat, and carbohydrate composition of human milk for all stages of lactation by Emmet *et al.* [102]. Colostrum is defined as human milk produced within the first few days after birth, 'mature' refers to human milk 10 days following birth, while the time period in between is classified as 'transitional' [102].

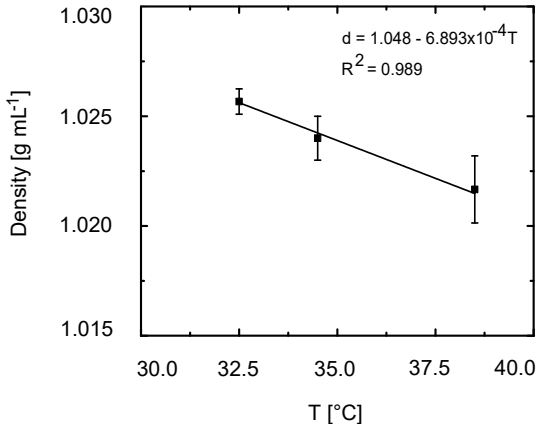


Figure 4.1: Linear interpolation used to evaluate human milk density. Milk density measurements for the temperature range of 33.7−35.7°C were conducted in triplicate using a hydrometer (VWR, Lutterworth, UK). 'T' illustrates temperature in °C, 'd' stands for density in g mL^{-1}.

(b) Nipple shield. To enable direct comparison of former experiments by Scheuerle *et al*, investigating zinc sulphate recovery from rapidly disintegrating tablets [4], the modified lip-containing nipple shield was used.

(c) Non-woven fibre preparation. Non-woven fibre mats, composed of 35 % viscose and 65 % polyester, by Bathfelt Texel (Québec, Canada) were used as the delivery matrix (see Figure 4.2). Two different types of fibre mats were evaluated: a 235 g per square metre (g m^{-2}) felt with targeted thickness of 1.8 mm (referred to as fibre mat type A), and a 335 g m^{-2} felt with targeted thickness of 2.1 mm (referred to as fibre mat type B).

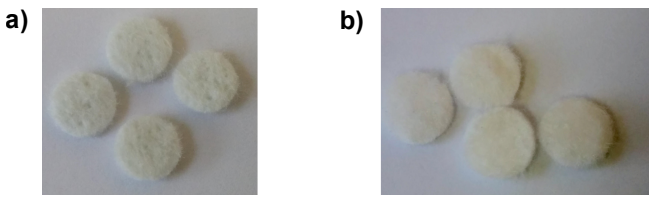


Figure 4.2: Delivery matrix. Illustration of non-woven fibre mat (a) type A and (b) type B, loaded with zinc sulphate. Image adapted from [38].

The selection of non-woven fibres was based on previous research by Gerrard *et al.* [41].

(d) Therapeutic. Zinc sulphate monohydrate was obtained from Sigma Aldrich (Dorset, UK), and preferred over elemental zinc based on its superior solubility and taste characteristics [4]. The chosen amount of zinc sulphate loaded onto the fibre mats aligned with the WHO-based dose recommendation of zinc sulphate as an adjunct to oral rehydration salts for the treatment of acute diarrhoea [25].

4.2.2 Fibre mat preparation

Fibre mat type A and type B were loaded with 6.2 mg (standard deviation 0.075 mg, n = 3) and 6.4 mg (standard deviation 0.046 mg, n = 3) of elemental zinc respectively, by pipetting a 49 % zinc sulphate monohydrate solution (wt/wt %) onto the fibre mats. The mats were air dried in a petri dish for 24 h at room temperature.

4.2.3 Fibre mat characterisation procedures

(a) Scanning Electron Microscope (SEM) analysis. Scanning Electron Microscope (SEM) analysis was applied to investigate both the fibre mats' surfaces and their cross-sections. Samples for cross-sectional visualisation were frozen in liquid nitrogen and cross-fractured with a cooled razor plate, while samples for surface visualisation did not require prior preparation. The resulting samples of zinc sulphate loaded and unloaded fibre mats were mounted on 12.5 mm Cambridge stubs with Silver Dag (Taab Ltd), and sputter coated with 15 nm of Iridium using a Quorum/Emitech K575X sputter coater. Imaging was performed by using a FEI Verios 460L at 3 kV and 25 pA with a through lens detector operated in field free mode. Sample preparation was performed by Dr Jeremy Skepper (Cambridge Advanced Imaging Centre), who also assisted in subsequent imaging.

(b) Energy Dispersive X-Ray analysis. To confirm the presence of zinc sulphate on loaded fibre mats, as well as the absence of zinc on unloaded mats, Energy Dispersive X-Ray (EDX) analysis of the mats' cross-section of loaded and unloaded fibre mats was performed. For sample preparation, the mats were frozen in liquid nitrogen and cut into appropriate size using a cooled razor blade. Following mount-

ing using carbon DAG, the samples were carbon coated using a Quorum Q150 at 50 nm. EDX spectra were recorded using an Ametec/EDAX Energy Dispersive X-ray spectrometer with a silicon drift detector. Analysed areas of the fibre mats were additionally imaged using a Scanning Electron Microscope according to the operation parameters outlined previously, as well as using a concentric backscattered electron detector (CBS). A CBS detector enables areas of higher atomic number to appear brighter in the recorded image. Sample preparation was conducted by Dr Karin Muller (Cambridge Advanced Imaging Centre), following which analysis was performed collaboratively.

(c) X-ray Micro Computed Tomography (XμCT). To enable the analysis of changes in porosity, entire fibre mats were analysed by means of X-ray Micro Computed Tomography (XμCT) using a SkyScan 1172 high-resolution XμCT scanner (Bruker, Antwerp, Belgium), combining a cone beam geometry and a 2D array detector. During the process of XμCT, shadow projections following stepwise rotations of the object at 0.25° are acquired, enabling a 3D image reconstruction of the object following a total rotation of 180°. It is characterised by high spatial resolution and negligible diffraction of investigated samples. Using an isotropic voxel size of 2.49 μm, 10 images per position were averaged, aiming to reduce noise while increasing contrast. In approximately 3 h of acquisition time, 720 images were obtained. Reconstruction of recorded data was performed using NRecon (Bruker, 159 v1.6.8.0), resulting data visualized in CTVox (Bruker, v3.3), and processed in Avizo Fire (FEI Company, 160 Hillsboro, Oregon, USA, v8.1). The protocol is based on previous literature by Markl *et al.* [104]. In order to differentiate solid material from the pore space for a selected volume of interest within the centre of the fibre mat (0.998 m^3), thresholding was applied. Subsequently, volume and surface area were calculated for the extracted solid material, while porosity values were obtained by using the relationship between pore space volume and solid material. XμCT measurements and data reconstruction was performed together with Dr Daniel Markl, who also guided the data analysis process.

4.2.4 Breastfeeding simulation procedures

(a) Breastfeeding simulation. Breastfeeding simulation conditions were chosen based on literature on breastfeeding physiology [18, 20, 21, 23]): Milk was heated to 33.7 - 35.7°C, simulating its likely temperature inside the infant's mouth dur-

ing breastfeeding, and flow rate and suction frequency adjusted to approximately 5 mL min^{-1} and 1 suck sec^{-1} respectively. For each type of fibre mat, simulation experiments were conducted in triplicate, with the human breast mimic set to an angle of 30° downwards from the vertical axis. Temperature and pressure were monitored throughout. The pressure range and amplitude represented values of physiological relevance [21], and peak vacuum values of -22.78 kPa and -22.32 kPa, as well as baseline vacuum values of -10.90 kPa and -11.38 kPa for fibre mat type A and B respectively were recorded. An average pressure profile for both types of fibre mats is illustrated in Figure 4.3. A total of 30 fractions, each for a duration of 40 sec, were collected. This duration was chosen to enable comparative analysis with previously published breastfeeding simulation experiments by Scheuerle *et al.* [4]. For each fraction, the weight was recorded using a Sartorius analytic balance (Epsom/UK), and samples subsequently stored at -80°C until further analysis.

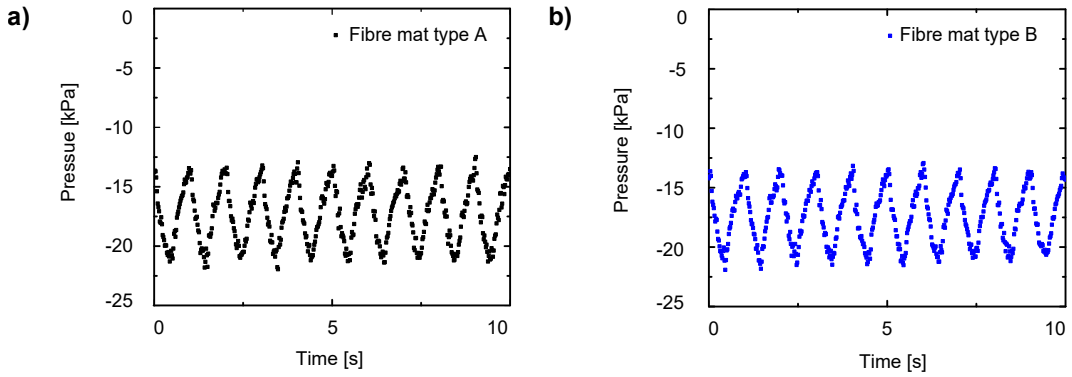


Figure 4.3: Example pressure profile during zinc delivery experiments using the breastfeeding simulation apparatus, depicted for 10 s at t = 120 − 130 s. Dosage forms used for delivery from a nipple shield were zinc-loaded fibre mats a) type A, and b) type B. Experiments were conducted using an average flow rate of approximately 5 mL min^{-1}. Image originally published in [38].

(b) Pre-analysis preparation of collected fractions and standard solutions. Preparation of collected fractions and standard solutions was performed by Dr Rebekah L. Scheuerle, and conducted based on a published protocol by Scheuerle *et al.*, illustrated in the following [4, 44]. To generate zinc calibration

curves, zinc standards (Perkin Elmer, Inc., Shelton, CT) were diluted 1:20 volumetrically in diluent A, consisting of a total concentration of 0.1 % nitric acid (SPEX CertiPrep, Metuchen, NJ/USA) in ultra-high purity water. Calibration data was retaken continuously after every few experimental samples. To evaluate the elemental zinc content in human milk samples collected during breastfeeding simulation studies, nine out of the 30 fractions obtained during each breastfeeding simulation run were chosen for experimental analysis (fraction numbers 1, 2, 3, 4, 5, 7, 10, 15, 20, 30). Hereby, samples were thawed at room temperature, following which a 1:20 volumetric dilution was performed using diluent B, a diluent of ultra-high purity water (Sartorius Stedium Biotech Arium pro UV Water polisher) with 0.002 % TritonX100 (Sigma-Aldrich, UK), as well as the internal standard 1 ppm molybdenum (SPEX CertiPrep Metuchen, NJ). To evaluate the elemental zinc content of non-woven fibre mats used for breastfeeding simulation experiments, fibre mats were added to a vial containing 1 mL rinse of ultra-high purity water (Sartorius Stedium Biotech Arium pro UV water polisher) with 0.002 % TritonX100 (Sigma-Aldrich,UK). After two ultrasonications of 10 min each, the vials were gently shaken overnight using a shaker plate, following which 1 mL of rins was added. The latter was repeated for a total of three nights/days, following which the samples were kept in a 40°C water bath overnight, and were volumetrically diluted in ultra-high purity water 1:10, and in diluent B 1:20. Prior to ICP-OES analysis being conducted, sample solutions were vortexed using a Fisherbrand Whirlimixer (Loughborough/UK).

(c) ICP-OES. Detection of elemental zinc content was performed by Dr Rebekah L. Scheuerle at the MRC Human Nutrition Research Unit (Cambridge, UK) by means of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The subsequent experimental procedure references methods by Scheuerle *et al.* [4, 44]. Experiments were performed using an ICP-OES (Jobin Yvon Horiba-ULTIMA 2C; concentric glass nebulizer with $0 - 1$ mL min^{-1} sample flow rate, 50 mL glass cyclonic spray chamber, radial torch with a 3 mm i.d. alumina injector). Samples were introduced to the concentric glass nebulizer through a sample probe of 0.75 mm i.d. sample tubing and 0.76 mm i.d. pump tubing using an auto-sampler (Jobin Yvon Hribo AS500) with a flow rate of 1 mL min^{-1}. Passing the concentric glass nebulizer, the sample was introduced to an argon plasma containing glass cyclonic spray chamber at 12 L min^{-1}, while a radio frequency power of 1300 W and

a sheath gas flow of 2 L min^{-1} was applied. Detection of zinc was performed in a photomultiplier tube at 213.856 nm and a voltage of 605 V ($\frac{kg \times m^2}{s^3 \times A}$), detection of molybdenum (Mo) at 202.030 nm and a voltage of 955 V ($\frac{kg \times m^2}{s^3 \times A}$), using a 0.5 sec integration time. Data was recorded by ICP Analyst 5.4 software (Horiba, UK).

(d) Data analysis. Analysis was conducted based on a previously published protocol by Scheuerle *et al.* [4], with guidance by Dr Rebekah L. Scheuerle, and resulting calibration curves are illustrated in Figure 4.4.

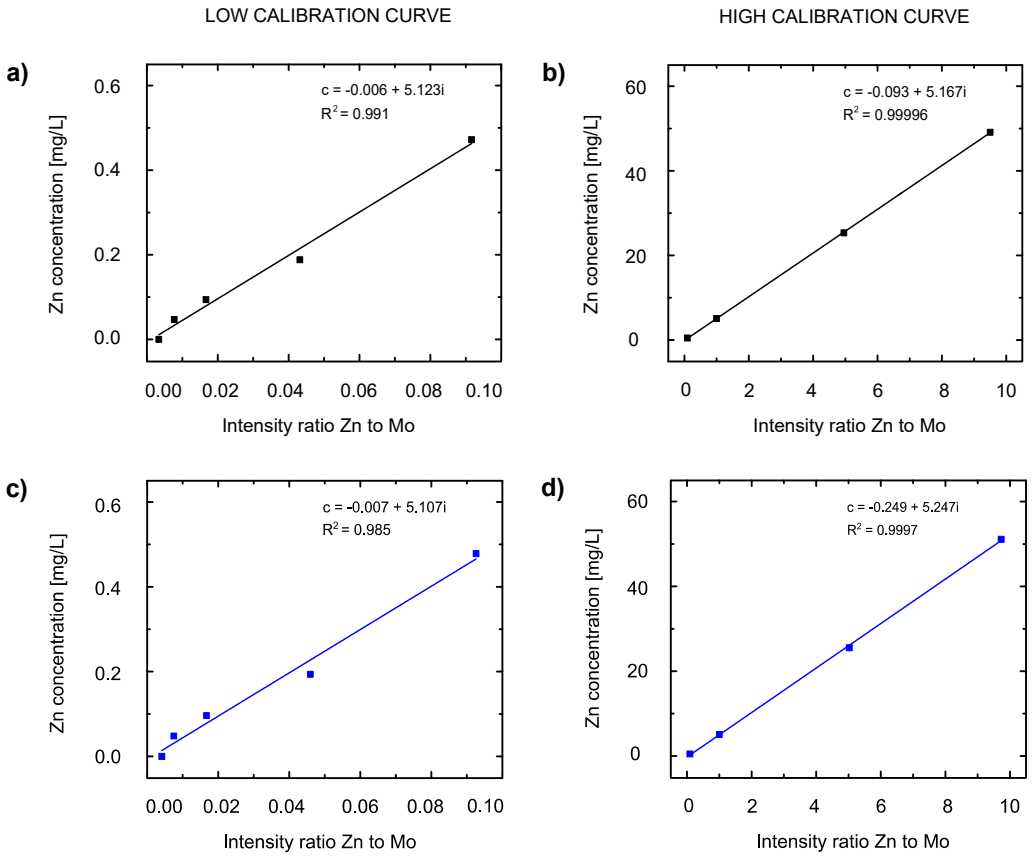


Figure 4.4: Calibration curves for a low range (0.0 – 0.5 mg L^{-1}) and a high range (0.5 – 50.0 mg L^{-1}) of elemental zinc detected vs. zinc to molybdenum intensity ratio to determine the elemental zinc content of a - b) loaded fibre mats, c - d) breastfeeding simulation samples. Calibration curves were generated through linear interpolation. 'c' represents the elemental zinc concentration in mg L^{-1}, 'i' illustrates the Zn to Mo intensity ratio.

Both curves were generated through linear interpolation, and intensity ratios corrected for blank values. Using the calibration equations, the amount of elemental zinc obtained in diluted samples is based on the intensity ratio of Zn to Mo [4]. The actual elemental zinc content of the simulation experiment samples was evaluated through accounting for the factor of dilution, the sample's weight, the density of human milk, and the temperature during the experiment [4]. Using the values obtained from measuring nine out of 30 samples, the concentration of zinc in the remaining 21 human milk samples was subsequently evaluated by means of linear interpolation. Data obtained was used to generate release profiles and to evaluate the cumulative zinc release during each experiment, represented as a percentage of the total amount loaded onto the non-woven fibre mats to the total amount released.

4.3 Results

4.3.1 Release of zinc from non-woven fibres

The amount of human milk passed through the nipple shield, and the absolute recovery achieved for both fibre mat types are presented in Table 4.3. Figure 4.5 illustrates the cumulative normalised proportion of zinc released and its propagated error, as well as the normalised proportion of zinc released in individual fractions over time. A similar effectiveness and duration of release were observed.

	Recovery achieved [%]	Amount of human milk passed through the shield [g]
Mat type A	64.00 ± 0.21	93.09 ± 1.08
Mat type B	61.64 ± 0.13	98.15 ± 0.97

Table 4.3: Recovery achieved and human milk passed through the nipple shield for the delivery of zinc sulphate from Texel non-woven fibre mats using the breastfeeding simulation apparatus (30 fractions à 40 sec each). Table originally published in [38].

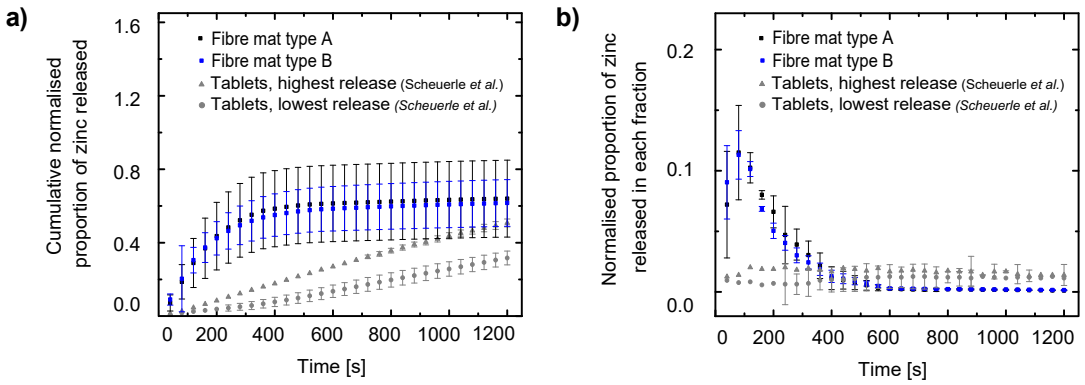


Figure 4.5: Illustration of zinc sulphate release from Texel fibre mats type A (1.8 mm targeted thickness, 235 g m^{-2}) and type B (2.1 mm targeted thickness, 335 g m^{-2}), as well as from rapidly disintegrating tablets by Scheuerle *et al.* [4] into human milk. a) Cumulative normalised proportion of zinc sulphate released, and b) Normalised proportion of zinc sulphate released in each fraction over time. Release is represented as a percentage of the total amount loaded onto the non-woven fibre mats to the total amount released. Each set of points represents the average of three experiments, using the mean of ICP-OES triplicate measurements for each fraction shown. Image originally published in [38].

4.3.2 Scanning Electron Microscope (SEM) analysis

To explore the fibre mats' morphology, SEM imaging of unloaded and zinc sulphate loaded Texel fibre mats type A and type B was conducted in triplicate (see Figure 4.6 and Figure 4.7 for fibre mats' surfaces, and Figure 4.8 and Figure 4.9 for the fibre mats' cross-sections). Hereby, an approximate fibre diameter of 20 µm was identified, and it was found that both types of mats have the same fibrous structure in spite of their difference in g m^{-2} and thickness. Adsorption of zinc sulphate was observed in localized areas scattered across the mats' network, either in form of flat patches or clumps. The mostly uniform coating on the fibre surface is characterised by a cracked pattern, because of the evaporation of water from the loaded zinc sulphate solution. Since Texel fibre mats are characterised by a porous structure, adsorption within the deeper regions of the fibre mats can be observed.

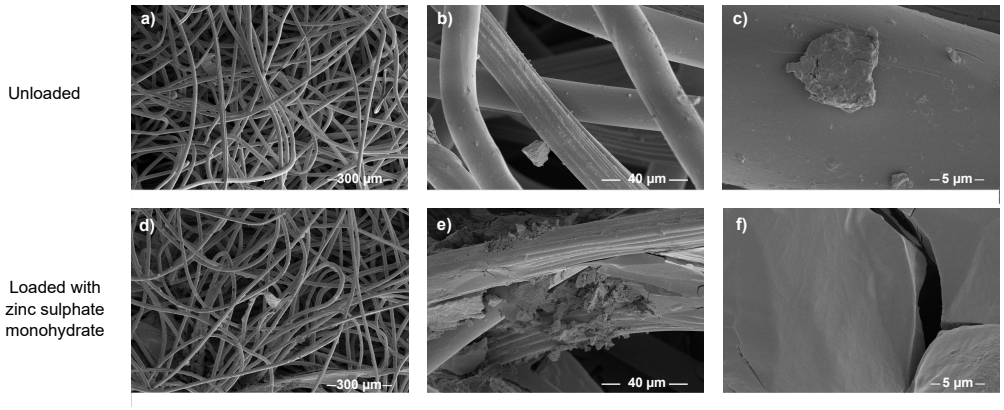


Figure 4.6: SEM results for fibre mat type A (fibre mat surface). Illustration of results obtained for SEM analysis of Texel fibre mat type A (1.8 mm targeted thickness, 235 g m^{-2}) at three different magnifications. Images a) - c) present unloaded fibre mats, images d) - f) fibre mats loaded with zinc sulphate before dissolution. Image originally published in [38].

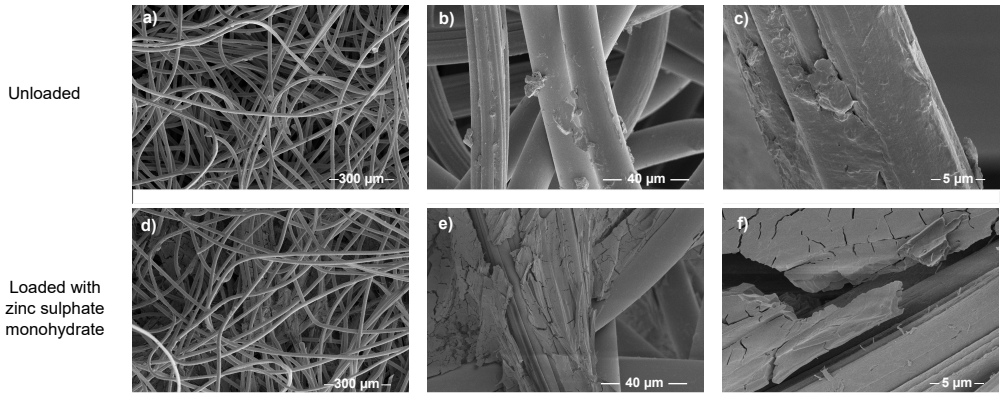


Figure 4.7: SEM results for fibre mat type B (fibre mat surface). Illustration of results obtained for SEM analysis of Texel fibre mat type B (2.1 mm targeted thickness, 335 g m^{-2}) at three different magnifications. Images a) - c) present unloaded fibre mats, images d) - f) fibre mats loaded with zinc sulphate before dissolution. Image originally published in [38].

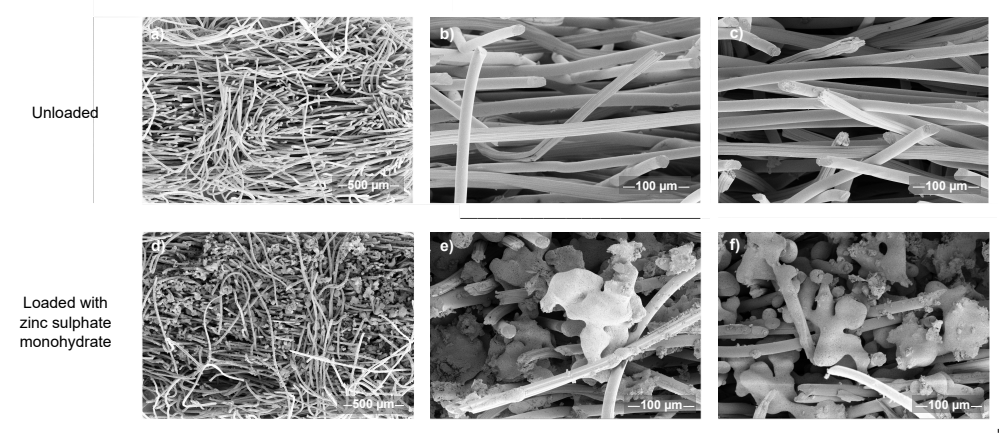


Figure 4.8: SEM results for fibre mat type A (fibre mat cross-section). SEM images are depicting the cross-section of Texel fibre mat type A (1.8 mm targeted thickness, 235 g m^{-2}) at two different magnifications. Images a) - c) present unloaded fibre mats, images d) - f) fibre mats loaded with zinc sulphate before dissolution. Image originally published in [38].

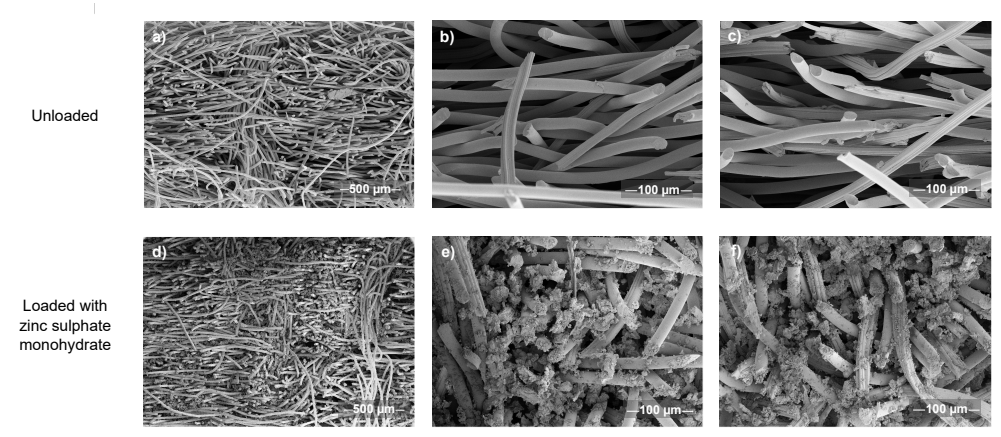


Figure 4.9: SEM results for fibre mat type B (fibre mat cross-section). SEM images are illustrating the cross-section of Texel fibre mat type B (2.1 mm targeted thickness, 335 g m^{-2}) at two different magnifications. Images a) - c) present unloaded fibre mats, images, d) - f) fibre mats loaded with zinc sulphate before dissolution. Image originally published in [38].

4.3.3 Energy Dispersive X-Ray analysis

Energy Dispersive X-Ray analysis, as well as imaging via SEM and CBS were conducted as a means to chemically characterise unloaded and loaded Texel fibre mats type A and B. Characteristic X-ray values of relevant elements can be found in Table 4.4. Results are illustrated in Figure 4.10 and Figure 4.11.

Element	Kα x-ray line [keV]	Lα x-ray line [keV]
C	0.277	-
O	0.525	-
S	2.307	-
Zn	8.630	1.012

Table 4.4: Summary of X-ray lines for the elements C, O, S, and Zn.

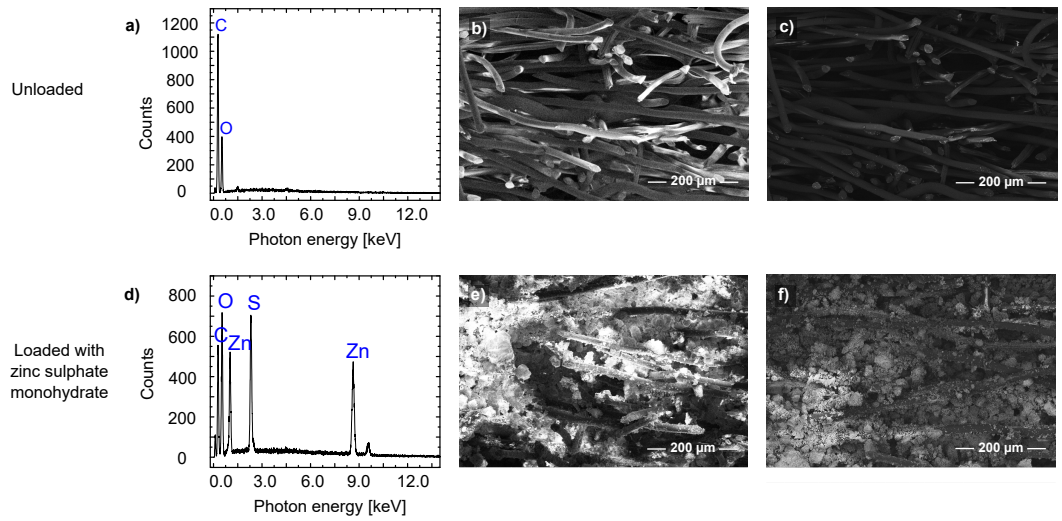


Figure 4.10: EDX spectra and corresponding imaging results for fibre mat type A (fibre mat cross-section) before dissolution. Image a) and d) illustrate the EDX spectra, images b) and e) imaging of the EDX analysed fibre mat area via SEM, images c) and f) imaging of the EDX analysed fibre mat area via a concentric backscatter detector (CBS). Image originally published in [38].

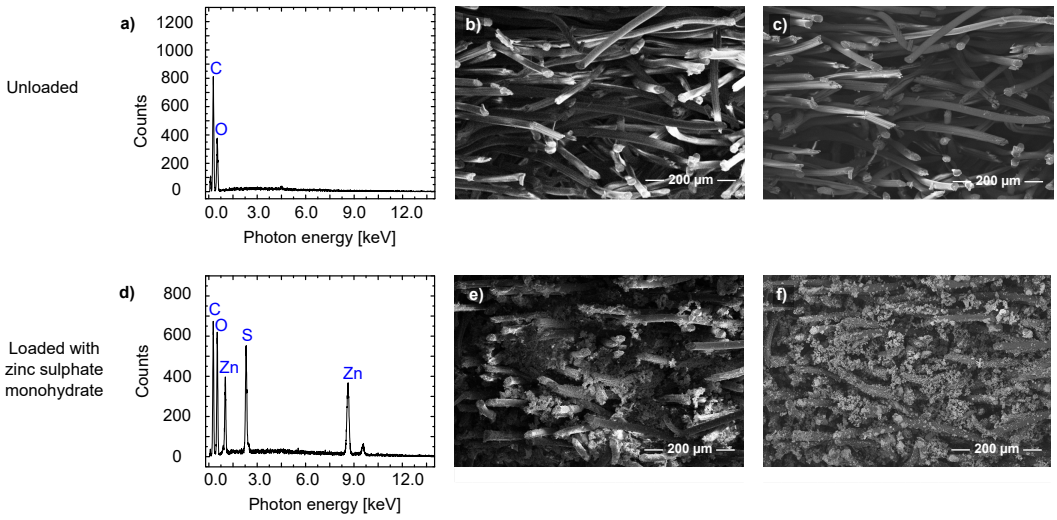


Figure 4.11: EDX spectra and corresponding imaging results for fibre mat type B (fibre mat cross-section) before dissolution. Image a) and d) illustrate the EDX spectra, images b) and e) imaging of the EDX analysed fibre mat area via SEM, images c) and f) imaging of the EDX analysed fibre mat area via a concentric backscatter detector (CBS). Image originally published in [38].

For unloaded and loaded samples, the EDX spectra showed presence of the elements C and O, the building blocks of the mats' viscose and polyester fibres, while Zn and S were only detected in zinc sulphate loaded samples. The analysis thus confirms the absence of zinc in unloaded samples.

4.3.4 X-ray Micro Computed Tomography (XµCT)

To investigate the fibre mats' porosity and 3D structure, analysis via X-ray Micro Computed Tomography (XµCT) was conducted. Loaded and unloaded Texel fibre mats type A and Type B are illustrated in Figure 4.12 and Figure 4.13, respectively. Different colours indicate material of different electron density as detected by XµCT, red indicating low, and green high-density material. Since the materials' elemental composition is not changing, the sample's electron density is equivalent to its true density values. The presented colour scale was chosen based on the density of viscose (1.52 g cm^{-3}) [105], polyester (1.38 g cm^{-3}) [105], and zinc sulphate (3.54 g cm^{-3}) [106]. As a consequence, the unloaded fibre material of comparably

lower density appeared in red, and zinc sulphate of comparably higher density in blue and green colour. 3D renderings of the XµCT data for unloaded and loaded fibre mats are illustrated in Figure 4.12 and 4.13. Through a change in the opacity (see colour maps of the individual renderings) regions of varying densities were observed. While images d) - e) depict both fibre material and zinc sulphate loaded onto the fibre mats, indicated by the mats' red and a blue to red colour, the blue and green colour of image f) solely illustrates loaded zinc sulphate. This observation is further confirmed when comparing figures of loaded with those of unloaded fibre mats in images a) – c), in which the visible fibrous material at medium density is only visible as a faint silhouette. Merely light blue spots of < 10 µm and sporadically larger spots of < 250 µm size are visible. Figure 4.14 illustrates that the bottom side of fibre mat type A, unlike those of fibre mat type B, appears similar to the fibrous structure of unloaded fibre mats.

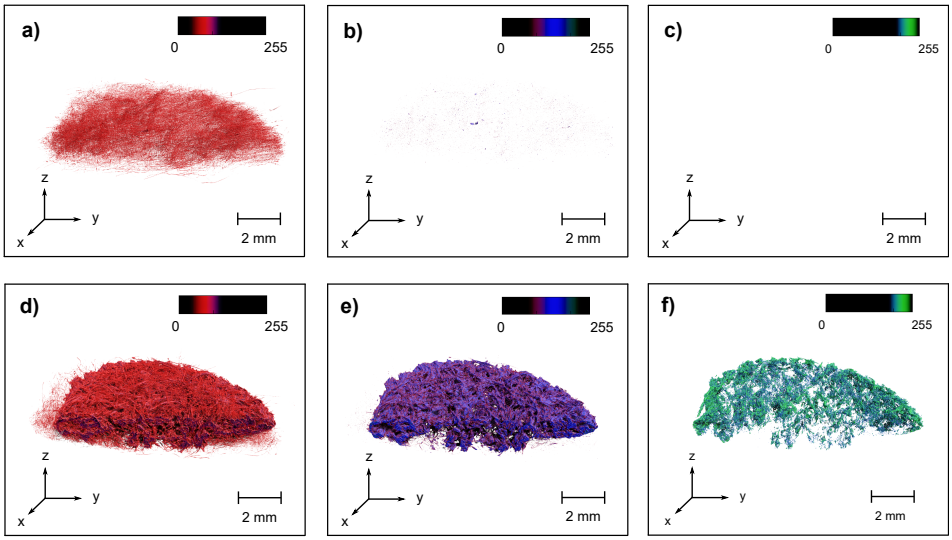


Figure 4.12: XµCT results for loaded fibre mat type A. Images generated from XµCT measurements of a) - c) unloaded, and d) - f) zinc sulphate loaded Texel fibre mats type A (1.8 mm targeted thickness, 235 g m^{-2}). The colour maps were the same for all images and only the opacity was adjusted in order to visualize components of different densities. Red colour indicates low, green colour high-density material. The images depict only half of the fibre mats in order to visualize the cross-section at the centre of each sample. Image originally published in [38].

Moreover, in alignment with SEM results, XµCT analysis confirmed an approximate fibre diameter of 20 µm for unloaded or partially loaded fibres. The diameter of loaded fibres could not be identified, as data illustrated the fusion of adjoined fibres following swelling, rendering the identification of individual fibres in areas of high zinc loading impossible. An example of representative cross-sectional cuts through a swollen fibre cluster is illustrated in Figure 4.15. To quantify changes within the fibre network following zinc sulphate loading, volume fraction, porosity, and total surface area were calculated for a cubical region of interest measuring 0.998 mm^3 from the fibre mats' centre. Results are shown in Table 4.5. A decrease in porosity can be observed as a result of an increase in solid fraction, and consequently in the volume of solid material. This is caused by both swelling of individual fibres and the deposit of zinc sulphate, in turn resulting in an increase in the surface area of the total solid material.

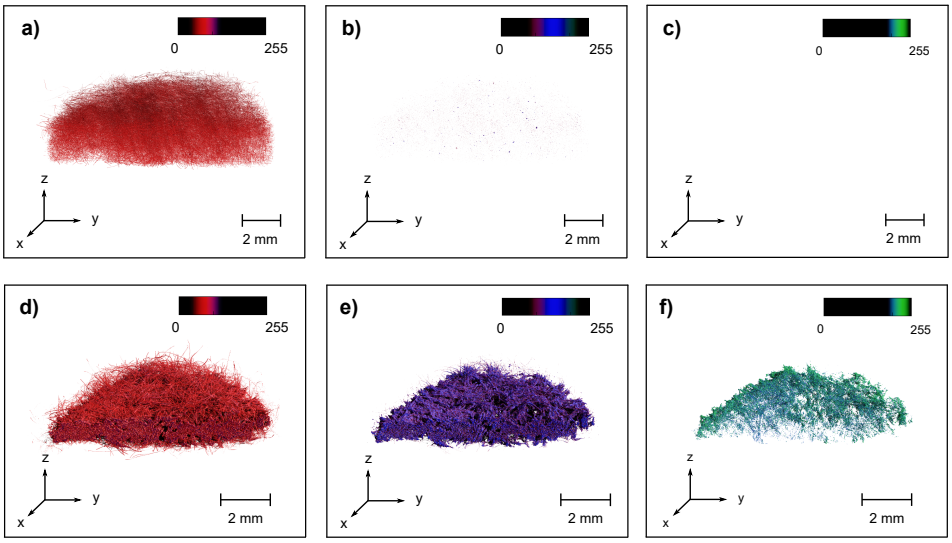


Figure 4.13: XµCT results for loaded fibre mat type B. Images generated by XµCT analysis of a) - c) unloaded, and d) - f) zinc sulphate loaded Texel fibre mats type B (2.1 mm targeted thickness, 335 g m^{-2}). Opacity was adjusted in order to visualize components of different densities using the same colour map. Red colour indicates low, green colour high-density material. In order to visualize the cross-section at the centre of each sample, only half of the fibre mats are illustrated. Image originally published in [38].

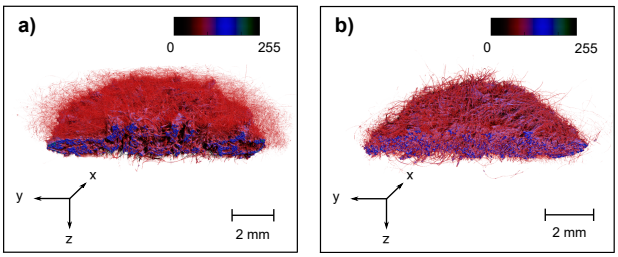


Figure 4.14: Images generated for XµCT analysis of loaded Texel fibre mats (bottom side facing up). Fibre mat a) type A, b) type B. Image originally published in [38].

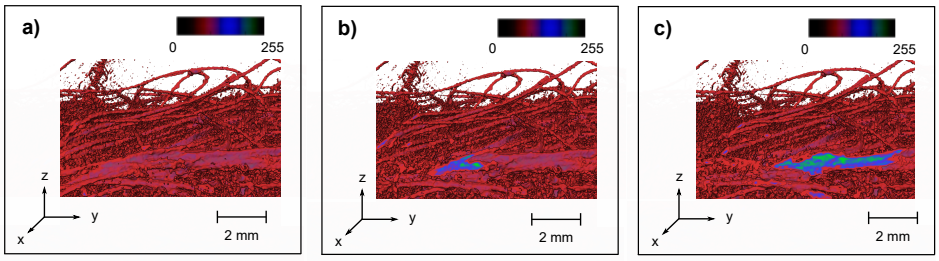


Figure 4.15: Cut through zinc-loaded fibres in the y-z plane of loaded fibre mat type B. Images a) - c) illustrate resulting cross-sectional images at different positions in the x-direction. Zinc-loaded fibres appear swollen and fused with adjoined fibres to form high-density fibre clusters. Image originally published in [38].

	Parameter	Unloaded	Loaded	Change [%]
Fibre mat type A	Porosity [-]	0.942	0.607	- 36
	Surface area [mm^2]	19.72	32.70	+ 66
	Volume [mm^3]	0.058	0.392	+ 580
Fibre mat type B	Porosity [-]	0.939	0.416	- 56
	Surface area [mm^2]	17.30	50.90	+ 194
	Volume [mm^3]	0.061	0.583	+ 856

Table 4.5: Overview of porosity, surface area, and volume of a cubical region of interest measuring 0.998 mm^3 taken from the loaded and unloaded fibre mats' centre. Table originally published in [38].

4.4 Discussion

Delivery of the full dose of loaded zinc sulphate from non-woven Texel fibres within the duration of one breastfeed, equivalent to the consumption of 76.0 ± 12.6 g of human milk for 1-month- to 6-month-old infants was not achieved [40]. Potential reasons include impact of milk properties, such as the accumulation of milk components within the porous mat structure, hampering the flow of milk and hence the delivery of zinc from the fibrous network, as well as changes in fibre conformation.

Milk properties. Although it has to be noted that the nature and adsorption properties of a therapeutic significantly affect its release rate, recent literature by Gerrard *et al.* suggests that absolute recovery is additionally dependent on both the milk's lipid content and distribution, as well as the overall milk composition, including carbohydrates, proteins, and lipids. The former was confirmed, as absolute release into cow's milk was reduced to $30 - 60$ % when non-homogenized milk (5.2 % fat) was used, as opposed to a total of $70 - 90$ % for use of a homogenized form (3.6 % fat) [41]. The latter was shown when comparing these results to the absolute recovery of 100 % into non-homogenized goat's milk (4.0 % fat) [41]. Both results pose difficulties to the development of a standardized protocol for therapeutic release via non-woven Texel fibre mats, as not only commercially available cow's and goat's milk, but in particular human milk is a complex fluid with highly variable properties. Among others, a variation of the absolute fat content of up to three fold can be observed, as a result of the stage of lactation, dietary requirements and health status of the breastfeeding mother [39]. To overcome undesired accumulation of milk components within the porous fibre network, potential surface modifications, such as plasma treatment could be applied [107, 108]. Such modifications would however increase both manufacturing efforts, as well as overall production cost of a fibre-based therapeutic insert, and potential benefits and risks would have to be carefully assessed.

Changes in fibre conformation. Moreover, XμCT results also suggest that zinc release is likely affected by the change in conformation, namely the swelling of loaded fibres, decreasing porosity and the flow of human milk through the fibrous structure, while simultaneously increasing the likelihood for milk components to accumulate. Previous literature showed a decrease in release from low surface area to volume

HPMC tablets [109], and similarly literature by Siepmann *et al.* [110] suggests that also the release from zinc-loaded fibre clusters will likely be slowed down as a result of their reduced surface to volume ratio. XµCT also revealed the presence of fibres of higher density than initially anticipated based on the true density values of viscose and polyester. The $10 - 250$ µm sized spots in rendered 3D XµCT data, observable at medium density values, indicates fibre accumulation and/or the presence of impurities. Both are believed to be a result of the fibres' manufacturing process, and/or caused by trapped dust particles. The even distribution of zinc within the fibrous network of fibre mat type B, which contrasts with the zinc adsorption pattern illustrated in Figure 4.14, can be explained by the slightly larger fibre diameter and different wetting behaviour of the two fibre mats. Liquid absorption of individual fibres within the fibre mats is driven by capillary action, while being influenced by the samples' porosity [111]. It is believed that these differences also lead to the unequal changes in porosity, surface area, and volume of fibre mat type A and B following loading, which nonetheless do not result in a noticeable difference in release from both mats.

Comparing recovery to the literature. Overall, experimental results revealed a $20 - 48$ % superior absolute recovery of zinc sulphate from Texel non-woven fibre mats, compared to rapidly disintegrating tablets manufactured by direct compression: research by Scheuerle *et al.* reported an absolute recovery of only $32 - 51$ % [4]. It has to be noted however that the total elemental zinc content of rapidly disintegrating tablets used by Scheuerle *et al.* ($17.81 - 18.21$ mg) was higher than the amount of elemental zinc loaded onto this study's Texel non-woven fibre mats ($6.17 - 6.40$ mg) [4]. Yet, when comparing the zinc delivery within the first 8 min, during which the main release from non-woven fibre mats had occurred, the estimated amount of elemental zinc released from the rapidly disintegrating tablets ranged between $2 - 4$ mg, characterised by a linear release profile [4]. It thus was significantly lower than the release from Texel non-woven fibre mats at 8 min. Not only their superior release properties, but also the enhanced loading feasibility and cost-effectiveness of Texel non-woven fibre mats, compared to the referenced rapidly disintegrating tablets, confirm their potential as a therapeutic insert for delivery from a nipple shield during breastfeeding.

Additional investigations are needed to prove the capability of Texel non-woven fibres to serve as a generalized matrix for oral drug administration, as well as to explore alternative materials with suitable characteristics for delivery from a nipple shield into human milk.

4.5 Conclusion

Texel non-woven fibre mats were investigated as a delivery matrix for zinc sulphate pentahydrate. When tested during breastfeeding simulation experiments, an absolute elemental zinc recovery of 64 % (fibre mat type A) and 62 % (fibre mat type B) respectively in approximately 93 g and 98 g of human milk passed through the fibre insert was achieved. Incomplete delivery may be attributed to i) the complex properties of human milk, ii) accumulation of milk components within the porous mat structures, as well as iii) structural changes of the fibre networks following loading, thereby hampering the delivery of zinc. Based on the non-woven fibre mats' release characteristics and cost-effectiveness, non-woven fibres can be regarded as an advantageous dosage form for oral therapeutic delivery from a nipple shield during breastfeeding simulation. Further research will be required to establish Texel non-woven fibres as a generalized matrix for oral therapeutic delivery from a nipple shield, and to further explore alternative fibrous materials with advantageous characteristics for therapeutic delivery into human milk.

Chapter 5

Hydrogel-based iron delivery into human milk

5.1 Introduction

5.1.1 Overview

Initial research on dosage forms mainly focused on rapidly disintegrating tablets and Texel non-woven fibre mats. Since neither one enabled complete delivery of a full dose during breastfeeding simulation experiments, investigation of alternative formulations was required. Hydrogels were believed to overcome some of the previously encountered limitations, such as the firmness of tablets, and the uncontrollable adsorption pattern of non-woven fibres. This chapter explores both the suitability of hydrogels for therapeutic delivery into human milk, as well as the *in-vitro* feasibility of commercially available ultra-thin contact nipple shields for oral therapeutic delivery during breastfeeding. The following objectives where defined:

1. Development and characterisation of a hydrogel formulation for iron delivery into human milk

2. Quantification of iron release from a commercially available ultra-thin contact nipple shield into human milk during breastfeeding simulation experiments

5.1.2 Importance of iron in neonates

Iron deficiency (ID), defined as "a state in which there is insufficient iron to maintain normal physiologic functions" ([112], p. 1041) and being equivalent to serum ferritin values below $10 - 12$ µg L^{-1} [113], is the most common single-nutrient deficiency worldwide, with an estimated total of two billion people affected [114]. Iron is of significant physiological importance with 80 % of the body's iron being present in the blood's haemoglobin [115]. Moreover, iron is involved in the brain's structural development due to its functional role in enzyme catalysis, among others for cell replication, the synthesis of neurotransmitters, and the cell's energy metabolism [116]. As a consequence, deficiency is reported to be associated with a decrease in mental, motor, neurophysiological, and behavioural abilities [117–120]. Three distinct ID phases can be differentiated: a first stage, during which body iron stores are reduced, followed by an increase in the amount of soluble plasma transferrin receptors during a second stage [113]. The last stage of iron deficiency is defined as iron deficient anaemia (IDA), a condition, in which the blood's haemoglobin concentration is decreased and the morphology of red blood cells altered [113]. The WHO indicates that about 500 million people suffer of IDA [121]. Iron deficiency is particularly prevalent in the developing world [112], due to nutritional limitations and the high level of parasitic diseases associated with blood loss, such as malaria [122]. Nevertheless, iron deficiency is far from being a condition solely limited to low-resource settings: Within Europe, prevalence of ID in healthy term infants and toddlers aged 1 to 3 years is reported to be as high as 26 % [113, 123]. Term infants are born with an iron storage of approximately 75 mg kg^{-1} body weight, providing a sufficient amount of iron until the infants' 4th to 6th postnatal months of age [112, 115]. Since human milk contains only small quantities of highly available iron ($0.2 - 0.4$ mg L^{-1}) [124], intake from external sources is required to guarantee adequate supply thereafter [124]. This aligns with the American Academy of Paediatrics' and the World Health Organization's recommendation for exclusive breastfeeding until the end of the infants' 4th and 6th months of age, respectively [112]. Yet, earlier supplementation is required in infants whose mothers have suffered health conditions leading to lower infant iron stores at the time of birth, such as anaemia or diabetes during pregnancy as well as for preterm infants [112]. Since eighty percent of term infants' total body iron is acquired within its 29th to 40th week of gestation [112], the amount of total body iron in preterm infants is decreased at birth based on their reduced gestational age, with subsequent

depletion during their extensive postnatal growth and rapid blood volume expansion [112, 125]. As a result, preterm infants are at particular risk of developing iron deficiency within their first six months of life. Elemental iron requirements are estimated to amount to $2-4$ mg kg^{-1} per day [112]. According to Rao *et al.* a total of 25 % to 85 % of preterm infants are shown to exhibit iron deficiency during early childhood [126]. Term infants are susceptible to developing deficiency only following their first half year of life [112]. Ultimately, supplementation recommendations are based on the infant's gestational age and the type of feeding. A summary by Baker *et al.* is provided as follows [112]:

1. HEALTHY, BREASTFED, TERM INFANTS: Exclusively and predominantly breastfed, healthy term infants are recommended 1 mg of oral elemental iron per kg body weight per day, starting at the beginning of their fifth postnatal month until iron-containing foods are introduced.

2. HEALTHY, FORMULA-FED, TERM INFANTS: For healthy formula-fed infants adequate iron supply will be guaranteed for the infants' first 12 months of life through the consumption of standard infant formula, characterised by an iron content of $10-12$ mg L^{-1} (see Table 5.1), as well as through introduction of iron-comprising complementary foods starting between their 4th and 6th months of age.

3. BREASTFED, PRETERM INFANTS: For exclusively and predominantly breastfed preterm infants, supplementation of at least 2 mg elemental iron per kg body weight per day, from the beginning of the infants' first to the end of their twelfth month of age, is required (maximum: 15 mg d^{-1}) [126]. Liquid iron supplements can be used for exclusively breastfed infants, and in combination with iron-fortified formula or iron-containing complementary foods.

4. FORMULA-FED, PRETERM INFANTS: Iron-fortified formula covers the preterm infants' iron supplementation requirements (maximum: 15 mg d^{-1}) [126], and should be fed until the infants' first year of age, with iron-containing complementary foods introduced starting between their 4th and 6th months of age (see Table 5.1).

Products (uk)	Iron compound	Dosage form	iron [mg per 100 mL]
Term formula			
SMA® PRO First Infant Milk	Ferrous sulphate	Powder	0.7
Cow & Gate First Infant Milk		Liquid, powder	0.53 and 0.55
HiPP Organic First Infant Milk 1		Liquid, powder	0.5
Aptamil Profutura & Aptamil First Infant Milk		Powder	0.5
Aptamil Profutura & Aptamil First Infant Milk	Iron lactate	Liquid	0.52 and 0.51
Preterm formula			
SMA® PRO Gold Prem 1	Ferrous sulphate	Liquid	1.8
SMA® PRO Gold Prem 2		Liquid, powder	0.8
SMA® PRO Breast Milk Fortifier		Powder	1.8
Cow & Gate Nutriprem 2 Low Birthweight Formula		Powder	1.2
Cow & Gate Nutriprem 1 and 2 LBW Formula	Iron lactate	Liquid	1.6 and 1.2

Table 5.1: Iron compounds commonly used in preterm infant formula, term infant formula, and breast milk fortifiers [127–130].

Supplementation of higher amounts of iron apply for term infants with lower iron storage at time of birth, for preterm infants <1000 g birth weight, and for infants with special needs based on their health condition. In contrast, infants having received blood transfusions require a lower amount of external iron [112, 126]. Adjusting external iron supplementation is of importance, as the body - contrary to other nutrients - is unable to modulate iron excretion, and iron overload is reported to have negative impact on the infants' health [120]. For developing countries with high prevalence of IDA (40 % or higher), the World Health Organization's

guidelines for iron supplementation recommend a daily oral dose of $10 - 12.5$ mg of elemental iron for three consecutive months in a year [121]. A summary of most commonly used compounds in therapeutic formulations for the prevention or treatment of iron-deficiency anaemia in infants is provided in Table 5.2. The effect of providing external iron to meet the infants' nutritional needs was widely studied: among others, iron supplementation during the first six months of life in low birth weight (LBW) infants decreased iron deficiency at $6 - 12$ months of age [119, 131], as well as the prevalence of infant behavioural problems at both 3.5 years and 7 years [132, 133].

Products (UK)	Iron compound	Dosage form	Recommended in (minimum age)
Prophylaxis/treatment of iron deficiency			
Sytron®	Sodium feredetate	Syrup	Preterm and LBW infants starting 4 weeks after birth
Fersamal®	Ferrous fumarate	Syrup	Preterm and LBW infants starting $4 - 6$ weeks after birth
Galfer®		Oral solution	Preterm and term infants starting $4 - 6$ weeks after birth
Ironorm®	Ferrous sulphate	Oral drops	LBW infants starting $4 - 6$ weeks after birth
Infant vitamin supplements			
Zarbee's Naturals™ Baby Multivitamin	Ferrous gluconate	Oral solution	Infants >2 months of age
Wellbaby®	Ferric ammonium citrate	Oral drops, oral solution	Drops: from 4 months of age onward, solution: from 6 months of age onward

Table 5.2: UK products commonly used for prophylaxis and treatment of iron deficiency, as well as infant vitamin supplements [134–136].

Siddappa *et al.* proved that iron deficiency in infants at one year of age is correlated with slower motor development [116], and Angulo-Barroso *et al.* that in turn supplementation of 1 mg elemental iron per kg body weight starting at six weeks of age improved development of motor function [137]. The most commonly used compound for iron supplementation is ferrous sulphate, also referred to as iron(II)sulphate, due to its availability and low cost [126]. Ferrous sulphate has the additional advantage that it may be administered once per day based on its high bioavailability, whereas other iron compounds require more frequent consumption [126, 138]. It is used in both fortified food, prophylactic and therapeutic formulations for iron supplementation, and included in the WHO Model List of Essential Medicines and the WHO Model List of Essential Medicines for Children [25, 99].

Iron was chosen for delivery during breastfeeding simulation experiments presented in this chapter, due to i) its importance for infant development and health, ii) the prevalence of iron deficiency in both developing and developed countries, with particular need for supplementation in breastfed infants.

5.1.3 Hydrogel fundamentals

Use of hydrogels was first suggested for biological applications in the 1960s by Wichterle and Lim [139]. Such gels are defined as three-dimensional, cross-linked networks of water-soluble polymers [140], and their characteristic structure are a result of physical and chemical cross-linking: covalent and hydrogen bonds, van der Waals bindings, or "physical entanglements" ([139], p. 1639). The ability of a polymeric network to absorb water is caused by its hydrophilic groups, for example -OH, -CONH-, -$CONH_2$-, and -SO_3H [139, 141]. Since the degree of hydration is a result of the network's physiochemical characteristics, it varies based on the network's composition and environment [139, 142]. Hydrogels are classified based on a set of characteristics, such as side groups, mechanical, structural and physical properties, as well as environmental responsiveness (e.g. pH, temperature, ionic strength) [139]. For applications in biology and pharmaceutics, natural, synthetic, and combinational polymers are used. Natural polymers have particular advantages based on their biocompatibility and non-toxicity, but are prone to cause undesired immune responses [139]. On the other hand, synthetic polymers with their defined structure lead to the formation of uniform and fine-tunable hydrogels [139]. The porous structure of hydrogels enables their use as drug delivery matrices,

either through drug loading or through the conjugation of drugs to the hydrogel network [143]. Use in drug delivery requires the hydrogel to respond to either pH or temperature [144]. Three different mechanisms of release from hydrogel matrices can be differentiated: release controlled by i) diffusion, ii) swelling, and iii) chemical influences [139].

5.1.4 Formulation considerations

Prior to the development of a protocol for hydrogel fabrication, as outlined in the chapter's objectives, a suitable polymer system had to be chosen. An overview of polymers considered for hydrogel formulation and selection criteria are provided in the following.

(a) Polymer selection. Both the FDA's GRAS ("Generally Recognized As Safe") list and the database of "Indirect Additives Used in Food Contact Substances" were used as the basis for the identification of polymers considered as safe for hydrogel fabrication with intended clinical use [145]. Amongst others, six polymer systems were shortlisted: gelatin, methylcellulose, agar-agar, carbopol, hypromellose, and sodium alginate cross-linked hydrogels. Each polymer system is discussed in more detail below. Particular focus is given to its use in paediatric formulations and research, as well as to gelation temperature. As the gelation temperature of polymer solutions in water is based on the polymer's molecular weight and the polymer's concentration in solution, only a gelation temperature range is provided.

Gelatin: The natural polymer gelatin is derived through partial hydrolysis of collagen from bovine or porcine bone or skin [146, 147]. The "multi-talent" gelatin ([148], p. XI) is water-soluble, and often used in medical and pharmaceutical applications due to its biocompatibility and biodegradable characteristics [148, 149]. Hydrogels from aqueous gelatin solutions are formed when being cooled down to a temperature under 30°C following heating, but re-melt again if the temperature is raised to *in-vivo* conditions (approximately 40°C) [146, 150]. While chemical cross-linking is required to increase the chemical and thermal stability of gelatin hydrogels for a wide range of applications [146], the dissolving characteristics of gelatin at *in-vivo* temperatures seem particularly suitable for delivery into human milk. As gelatin is derived from animals, however, it might not be applicable to individuals of certain belief or dietary restrictions. Gelatin was reported to be used in both paediatric

formulations [151] and clinical research in infants aged three months and younger, including its intravenous use as a gelatin-based plasma substitute during clinical studies, and as gelatin tannate for the treatment of acute gastroenteritis [152, 153].

Methylcellulose: Methylcellulose is defined as a non-ionic polymer, which is water-soluble and stable over a wide pH range [154]. It enables gelation of solutions without precipitation, while exhibiting a long storage stability [155]. Due to these favourable characteristics, it is commonly used in food, cosmetics, and pharmaceutical products [155]. Among others, methylcellulose was the gelation agent of choice for large-scale manufacture of the first medical capsules not containing gelatin [148]. In paediatric drug formulations, methylcellulose often serves as a very thin tablet coating [156, 157]. The sol to gel transition of methylcellulose-water solutions ranges between approximately $30 - 55$°C [158, 159].

Agar-agar: Agar-agar, characterised as one of the oldest agents used for gelation [148], is derived from algae, a dried smell-, taste-, and often also colour-less gelatin-like compound [160]. The characteristic gelation of agar-agar is resulting from its high molecular weight. Standard types of agar-agar dissolve at temperatures over 85°C, "fast-dissolving" types of agar-agar regain its liquid state in a temperature range between $60 - 80$°C [148]. Agar-agar was reported to be used in paediatric gel formulations [161], as well as for the treatment of hyperbilirubinemia of jaundiced full-term newborns [162].

Carbomer polymers (carbopol): Carbomers are acrylic acid polymers widely used in the cosmetic and pharmaceutical industry [159, 163]. Advantages of carbomer gels are numerous, including a wide viscosity range (with high viscosity at low carbomer concentrations), thermal stability, and good acceptance by patients treated with carbomer-containing products [163]. Aqueous carbopol solutions gelate above its pK_a value at pH > 5.5. [159]. Use of carbopol in paediatric research was previously reported in the literature [164, 165].

Hypromellose: Water-soluble hypromellose, also called Hydroxypropyl Methylcellulose (HMPC), is a non-ionic cellulose ether [166], possessing many favourable characteristics for use in pharmaceutical products, such as low cost, stability over a wide pH range (pH 3.0 - 11.0), resistance against enzymes, and controlled release

properties [166]. Its sol to gel transition occurs at a temperature between $75-90°C$, and is decreased to approximately 40°C through lowering the hydroxypropyl molar substitution [167]. Within the class of hydrophilic matrices, hypromellose is the most often utilized cellulose ether [166]. Due to its non-reactive and pH-neutral properties, it is considered to be safe for the use in neonates [168]. It is commonly used in paediatric antibiotic eye drops/ointments, aiming at keeping the corneas moist and at preventing corneal abrasions [169], as well as in commercially available formulations, such as Bonjela Teething Gel [170].

Sodium alginate: Sodium alginate is a non-toxic, low-cost, and biocompatible anionic polymer, made by brown algae and bacteria [171, 172], and commonly used in pharmaceutical and food-grade production [171, 173]. It comprises α-L-guluronic acid and β-D-mannuronic acid residues, also referred to as G-blocks and M-blocks respectively, linearly linked by 1,4-glycosidic linkages [171]. Gels are formed by cross-linking the alginate polymer chains chemically and/or physically, whereby ionic cross-linking, i.e. the exchange of sodium ions from the guluronic acid (G) blocks with multivalent cations, is the most commonly applied method [171]. A variety of cations are suitable for cross-linking, but calcium ions are most commonly used [171, 172, 174, 175]. Elasticity and porosity of the resulting gel are a result of the number of available G- and M-blocks [171]. Spherification represents one possible approach of gelation [171, 176]. Three different techniques can be differentiated: i) basic spherification: whereby an alginate solution is slowly added into a cationic bath; ii) reverse spherification: whereby a cationic solution is added into an alginate bath; iii) frozen reverse spherification: whereby a frozen cationic solution is added into an alginate bath [171, 177]. Reverse and frozen reverse spherification both enable the formation of hydrogels with a liquid core and a solid shell [176–178]. Sodium alginate is used in commercially available formulations, and serves for example as the main ingredient in 'Gaviscon Infant', a medication used to treat infants aged $1-2$ years with gastric regurgitation, gastroesophageal reflux, and reflux associated with hiatus hernia [179–181].

The final selection was conducted sequentially by i) identifying polymer systems used in paediatrics, and specifically for infants aged three months and younger, ii) considering potential limitations of polymer systems (e.g. whether they might interfere with any religious belief or dietary requirements), iii) taking into account

the polymer's characteristics, such as gelation temperature and viscosity. The latter included considerations such as "Is the polymer system re-melting in human milk with a temperature of $33.7 - 35.7$°C, or will it release the *in-vivo* tracer through diffusion or mechanically induced disintegration?" and "Is the polymer system viscous enough to remain within the nipple shield, or would it likely drip out of the nipple shield before the breastfeeding process?" Table 5.3 illustrates an overview of important polymer characteristics, influencing polymer selection, including viscosity range, gelation temperature, and the polymer's use in paediatrics diagnostics and therapy.

Polymer	Viscosity range [MPa ×s]	Gelation at [°C]	Commonly used in infants	Used in infants ≤ 3 months
Gelatin	<50 - 125 [182] [20 % solution, 60°C]	~ 40 [146, 150]	x [151]	x [152, 153]
Methyl-cellulose	15 - 4,000 [183] [2 % solution, 20°C]	30 - 55 [158, 159]	x [156, 157]	
Agar-Agar	10 - 100 [184] [1.5 % solution, 20°C]	60 - 85 [148]	x [161]	x [162]
Carbopol	4,000 - 77,000 [185] [0.5 % solution, 20°C]	pH > 5.5 [159]	x [164, 165]	
Hypro-mellose	10 - 19,000 [186] [2 % solution, 20°C]	40 - 90 [167]	x [170]	x [169]
Sodium alginate	5.0 - 40.0 [187] [1 % solution, 25°C]	~ 32 [188]	x [181]	x [179, 180, 189]

Table 5.3: Overview of polymer characteristics and factors considered for hydrogel selection. Selection was based on the polymer's properties required for delivery during breastfeeding, as well as the polymer's use in paediatrics, and particularly neonatology. Thereby, "x" is indicating the applicability of a characteristic for the relevant polymer system.

From Table 5.3, the following ranking of polymer suitability for use within an ultra-thin contact nipple shield was created:

1. Sodium alginate (cross-linked with calcium ions): The potential to create hydrogels with a liquid core through reverse spherification, enabling burst release upon mechanically induced rupture, was regarded as the most desired option to further investigate.

2. Hypromellose: Due to its wide viscosity range and common use in paediatric formulations and neonatal treatments, hypromellose was seen as a polymer system of great interest for delivery into human milk. However, potential difficulties were projected to arise with regard to the duration of delivery, since the overall properties of hypromellose support controlled as opposed to rapid release.

3. Gelatin: The natural polymer gelatin was regarded as a suitable polymer system based on its frequent use in medical and pharmaceutical applications. Its ability to re-melt at *in-vivo* conditions following gelation was regarded as particularly ideal for use with human milk at body temperature. Yet, since gelatin is derived from bovine or porcine bone or skin, potentially interfering with religious belief and diet preferences, its suitability was believed to be limited.

(b) Crosslinker calcium and its physiological relevance. Due to sodium alginate being ranked as the most favourable polymeric system for hydrogel fabrication by means of spherification, the following paragraph provides further insights into the physiological importance of its crosslinker calcium. Since excipients used in commercially available paediatric formulations do not commonly possess additional health benefits, the positive contribution of calcium on infant health was seen as a particular advantage.

Calcium constitutes 1.9 % to the human body weight, whereby 99 % can be found as calcium hydroxyapaptide in bones and teeth, contributing to their structure and function [190]. The remaining 1 %, of which 0.9 % are present in cells and 0.1 % in extracellular fluid, fulfils vital metabolic processes, including muscle function, secretion of hormones, vascular contraction and vasodilation [190]. Calcium is also referred to as a "threshold nutrient", because an increase in bone mass with increasing calcium consumption levels off after reaching its threshold requirements for bone mechanics, and subsequently any excess calcium is excreted [191]. Human

milk is considered to be the ideal source of calcium for term infants, exhibiting a higher calcium bioavailability than bovine milk or formula [191, 192]. The average daily amount of calcium consumed by an exclusively breastfed infant amounts to $200 - 300$ mg [190], fulfilling the adequate intake recommendations for infants of $0 - 12$ months of age [192, 193]. Infants born prematurely, however, exhibit higher calcium requirements, which cannot be met through human milk consumption alone [191, 192]. In particular infants with less than 1500 g body weight at the time of birth, also referred to as very low birth weight (VLBW) infants [194], are in need for both calcium and phosphorus supplementation to ensure sufficient supply for bone mineralization [191]. As fetal calcium consumption increases significantly during the third trimester of gestation, reaching its peak of $100 - 120$ mg kg^{-1} d^{-1} at $30 - 36$ weeks of gestation, an infant born at $24 - 26$ weeks will only possess 20 % of a full-term infant's whole-body calcium [191]. Literature indicates that fortification, for example by mixing powdered fortifiers or liquid formulas with high-mineral content and human milk, has beneficial effects for infants of less than 1500 g birth weight [191, 192]. For bottle-fed infants, a variety of calcium-enriched formulas exist [190–192]. The use of such formulas even following discharge is suggested in the literature, but guidance about the optimal concentration and duration does not exist [192]. WHO recommendations exclusively relate to the first month of life, comprising a supplementation of $120 - 140$ mg kg^{-1} per day [194]. The World Health Organization recognises that supplementation is of particular importance in low- and middle-income countries, where calcium deficiency is prevalent across all age groups [195], and includes calcium gluconate in the complementary listing of the WHO Model List of Essential Medicines and the WHO Model List of Essential Medicines for Children [25, 99]. Using alginate gels fabricated by means of reverse spherification and frozen reverse spherification, might therefore also have the potential to contribute to supplementation efforts in breastfed infants, enabling to encapsulate calcium concentrations higher than those needed for spherification.

5.2 Materials and methods

5.2.1 Materials

(a) Human milk. Experiments were conducted using human milk characterised in Chapter 4, containing a protein content of 16.51 g L^{-1} and a lipid content of

43.52 g L^{-1}. The Cambridge Human Biology Research Ethics Committee at the University of Cambridge ethically approved all human milk sample use (HBREC.2012.01). Prior to breastfeeding simulation, human milk was thawed for 48 h at 4°C.

(b) Nipple shield. Based on findings in Chapter 3, demonstrating maternal preference for the use of commercially available ultra-thin contact nipple shields for therapeutic delivery during breastfeeding, as well as limitations of modified lip-containing devices, research in this chapter was conducted using a medium-sized (20 mm) commercially available ultra-thin contact nipple shield (Medela, UK).

(c) Hydrogel fabrication. For hydrogel preparation, low viscosity alginic acid sodium salt in FCC grade (G:M ratio in the range of $61-65:39-35$) was obtained from Alfa Aesar (Lancashire, UK), calcium lactate pentahydrate from Sigma Aldrich (Dorset, UK), salicylic acid and iron(III)sulphate pentahydrate from Fisher Scientific (Leicestershire, UK). A preference was given to i) iron(III)sulphate over iron(II)sulphate to prevent potential competition with Ca^{2+} during cross-linking and to facilitate subsequent iron analysis, ii) calcium lactate pentahydrate over other calcium salts, such as calcium chloride or calcium lactate gluconate, based on its neutral taste and low cost [196].

5.2.2 Hydrogel preparation

The following solutions were prepared: i) A 0.5 % sodium alginate solution of low viscosity alginic acid sodium salt in double deionized water (DDI). The solution was kept at 4°C over night to remove air bubbles formed during mixing. ii) A 1 % calcium lactate pentahydrate solution, additionally containing 60 mg mL^{-1} ferric sulphate pentahydrate in DDI water. 500 µL solution was pipetted into each hemispherical impression of a food-grade silicone mould, measuring approximately 2.5 cm in diameter and 1 cm in depth. The filled mould was kept at -20°C for at least 5 h. Gelation occurred when a frozen calcium lactate ferric sulphate hemisphere was introduced into a 4°C sodium alginate bath for 2.75 min, and access sodium alginate rinsed off through transferring formed gels into a DDI water bath. All liquid-core hydrogels were prepared immediately before use in characterisation and breastfeeding simulation experiments.

5.2.3 Hydrogel characterisation procedures

(a) Compression testing. Mechanical characterisation of manufactured liquid-core hydrogels was performed by means of uniaxial compression testing. Experiments were conducted in octuplicate using a H5Ks electric screw machine (Tinius Olsen, Redhill/UK) fitted with a 5N load cell. Hereby, the manufactured hydrogel was positioned on a flat surface, following which a flat plate applied vertical compression until the gel's plastic region of formation was reached. The setup is illustrated in Figure 5.4 a). A crosshead speed of 2 mm min^{-1} was chosen, and force displacement data recorded. Because of the complex nature of liquid-core hydrogels, no other mechanical properties were evaluated. Measurements were performed together with Andrew Rayment, Department of Material Science and Metallurgy, University of Cambridge.

(b) Visual characterisation. A bespoke optical system, consisting of a high-speed camera (Basler, Ahrensburg/Germany) and a National Instruments card (NIC, National Instruments, Austin, TX/USA) controlled via a LabVIEW software programme, was used to enable visual analysis of the gels' rupture mechanism and morphology. Calibration was performed by determining the number of pixels and the distance in mm between the top and bottom compression plate. The resulting relationship was used to calculate the hydrogels' average wall thickness, diameter, and height in quadruplicate based on images taken before compression testing. Hereby, wall thickness measurements made use of the difference in colour strength of the gel's core and shell when visualised via the LabVIEW programme. This enabled recognition of the solid core's boundary. Measurements were performed together with Andrew Rayment, Department of Material Science and Metallurgy, University of Cambridge.

5.2.4 Breastfeeding simulation procedures

(a) Breastfeeding simulation. Delivery of iron(III)sulphate pentahydrate into DDI water and human milk was conducted in triplicate using the breastfeeding simulation apparatus by Gerrard *et al.* [42]. The apparatus was operated as previously described in Chapter 4, whereby fractions were collected for a duration of 10 sec each, and recovery into approximately 10 g of human milk investigated. To explore the potential of hydrogel delivery for preterm infants, who were shown to poten-

tially benefit from the use of nipple shields to establish breastfeeding and increase milk transfer [37], the pressure values for breastfeeding simulation were adjusted in alignment with literature by Geddes *et al.* [197, 198]. A comparison of pressure values for term and preterm infants is illustrated in Table 5.4. Figure 5.1 illustrates exemplary average pressure profiles during breastfeeding simulation.

Pressure	Term infant	Preterm infant (min, max)
Baseline [mmHg]	-56.4 ± 31.4 [22], -64 ± 45 [21]	-31.1 $(-60.0, -12.7)$ [198]
Peak [mmHg]	-163.2 ± 62 [22], -145 ± 58 [21]	-106.2 $(-153.0, -65.5)$ [198]
Mean vacuum [mmHg]	-114 ± 58 [21]	-53.6 $(-89.3, -31.5)$ [198], -40.6 ± 27.8 $(-126.4$, $-0.4)$ [197]

Table 5.4: Pressure values during breastfeeding for term and preterm infants. Pressure values for preterm infants are based on two studies by Geddes *et al.* with infants born at $23.6 - 33.3$ weeks of gestation [197, 198]. While one study included 38 infants at 5.9 ± 3.8 weeks postnatal age ($1.0 - 16.3$ weeks), who were able to successfully latch and suck at the breast [197], the other study consisted of 17 infants at 4.4 ± 2.84 weeks postnatal age ($1.0 - 10.6$ weeks) when full oral feeds were introduced [198].

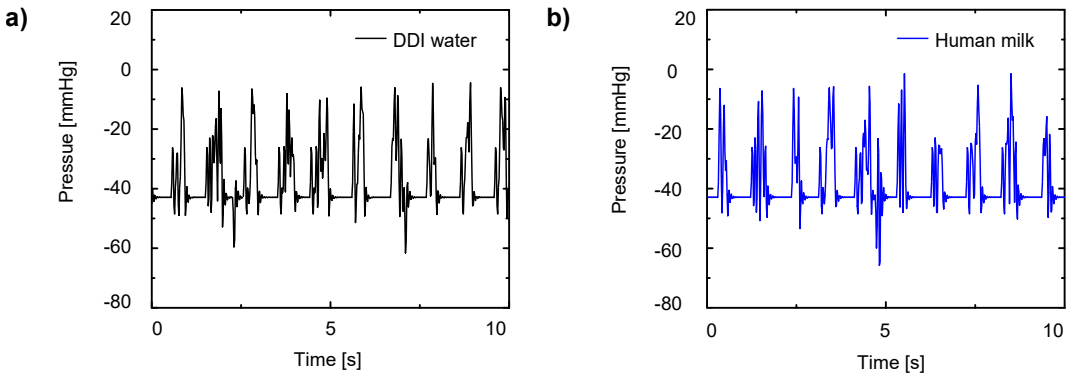


Figure 5.1: Exemplary pressure profiles during iron(III)sulphate pentahydrate delivery into a) DDI water and b) human milk, using the breastfeeding simulation apparatus. Pressure profiles are depicted for 10 s at $t = 90 - 100$ s.

Profiles obtained align with clinically obtained data for preterm infants ([197], Figure 5). During breastfeeding simulation, mean peak vacuum values of -64.62 ± 5.37 mmHg and -62.47 ± 1.76 mmHg, as well as mean baseline vacuum values of -1.01 ± 0.34 mmHg and -0.92 ± 0.37 mmHg for delivery into DDI water and human milk respectively were recorded. The mean average vacuum was -42.91 ± 9.97 mmHg and -38.30 ± 9.71 mmHg for delivery into DDI water and human milk.

(b) Iron detection and data analysis. Iron(III)sulphate pentahydrate in DDI water and human milk, including both samples collected during breastfeeding simulation experiments as well as prepared standards with known concentrations for data processing, were quantitatively analysed in triplicate as follows: 1 M hydrochloric acid was added in a ratio of 1 : 8 to the sample to achieve i) precipitation of the human milk's casein (isoelectric point: pH 4.7), ii) release of iron bound to transferrin, a protein naturally present in human milk [199, 200]. Following mixing by vortexing, all samples were centrifuged for 7 min at 16,112 x g. The resulting samples contained precipitated casein and clear supernatant, enabling detection of ferric sulphate by means of spectrophotometric analysis. From the resulting supernatant, 20 µL were transferred to 180 µL of DDI water and 1700 µL of 0.1 % salicylic acid solution added. Subsequently, absorbance of ferric salicylate in each sample was measured at room temperature at $\lambda_{max} = 524$ nm. The wavelength of maximum absorbance was evaluated by means of absorbance scans of all calibration samples over the wavelength range of $\lambda = 328 - 1000$ nm. An illustration of corresponding absorbance scans and calibration curves at ferric salicylate's maximum absorbance in DDI water and human milk can be found in Figure 5.2. Each point represents the average conductivity of triplicate measurements for a given concentration. The calibration curve in DDI water was also used to assess the total amount of ferric sulphate pentahydrate loaded into the liquid-core hydrogels. For this purpose, frozen hemispheres were melted, weighted, diluted 1:1 in DDI water, and their iron sulphate pentahydrate concentration evaluated by means of absorbance measurements at $\lambda_{max} = 524$ nm. Analysis was performed in triplicate, yielding a total of 29.59 ± 0.43 mg iron(III)sulphate pentahydrate contained within manufactured liquid-core hydrogels, equivalent to 6.75 ± 0.10 mg of elemental iron. Based on measured absorbance values of fractions collected during breastfeeding simulations, release profiles were generated. Release profiles include the cumulative iron(III)-

sulphate pentahydrate recovery of each experiment, represented as a percentage of the total amount encapsulated within the liquid-core gel, as well as the normalised proportion of iron(III)sulphate pentahydrate released in each fraction over time. To do so, the amount of iron (III)sulphate pentahydrate in each fraction was assessed by means of absorbance measurements at $\lambda_{max} = 524$ nm using the calibration curves in Figure 5.2, and normalised to the total amount of ferric sulphate pentahydrate used for liquid-core hydrogel manufacture.

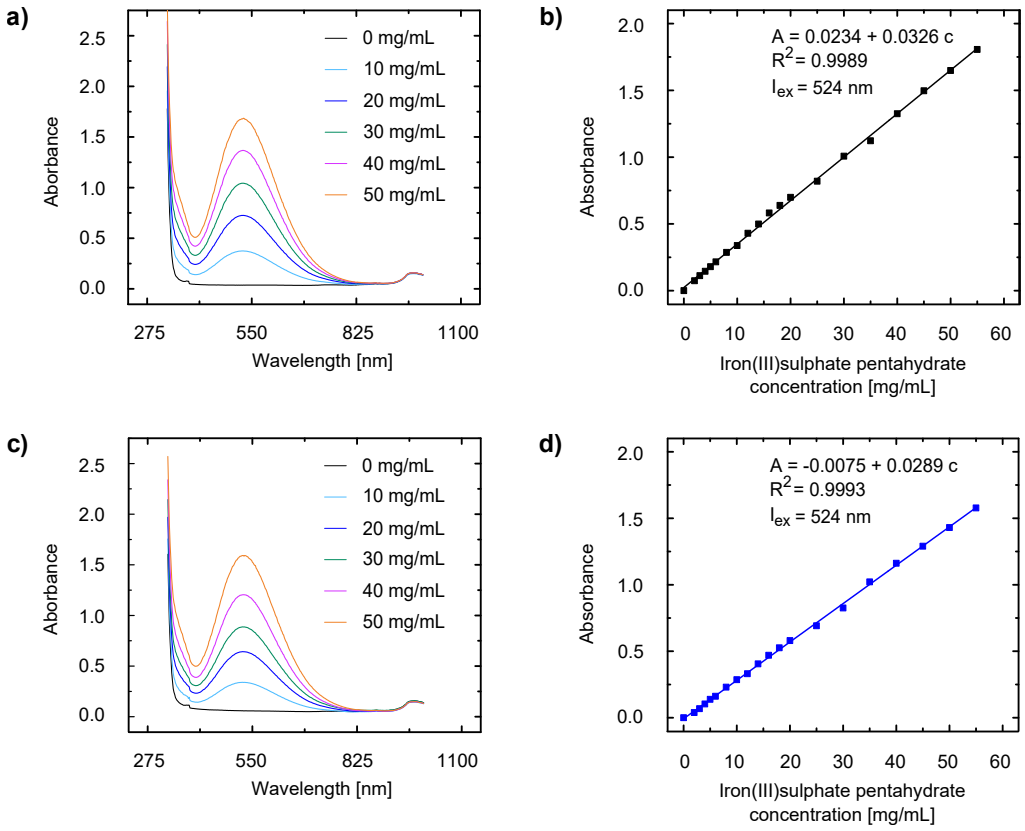


Figure 5.2: Absorbance scan for iron(III)sulphate pentahydrate samples of known concentration at $\lambda = 328 - 1000$ nm in a) DDI water and c) human milk. The ferric salicylate's maximum absorbance occurred at $\lambda_{max} = 524$ nm. Calibration curve of iron(III)sulphate pentahydrate in b) DDI water and d) human milk at $\lambda_{max} = 524$ nm. All samples were pre-processed by adding hydrochloric acid to the sample (1:8) to enable absorbance measurements following casein precipitation. Each data point represents the average of triplicate measurements. Modified image originally published in [47].

To obtain i) a graph illustrating the normalized release in each fraction over time, values were plotted for each fraction without further processing, ii) a cumulative normalised release graph, values were summed up for each new fraction with those of previous fractions.

5.3 Results

5.3.1 Hydrogel preparation

Fabrication of liquid-core hydrogels containing ferric sulphate was achieved by means of frozen reverse spherification. The fabrication protocol was iteratively adapted, including the following variables: i) type of spherification technique used to obtain reproducibly uniform spheres, ii) ratio of iron(III)sulphate pentahydrate to calcium lactate pentahydrate, in order to minimize precipitation of either compound, iii) duration of gelation to control wall thickness (increasing with increasing gelation time) and liquid-core content available for delivery, iv) size of the hydrogel in order to maximize its liquid load, but to also enable sufficient milk flow through the nipple shield's silicone teat. Spherification and compound ratio were assessed visually, while the variables gel size and gelation time were evaluated by means of breastfeeding simulation experiments. Optimising the fabrication process aimed at providing the required stability for gel handling, while at the same time maintaining the gel's mechanical sensitivity to enable rapid rupture. A range of gelation times between 1 min to 5 min were assessed, and gels stable enough for handling tested by means of breastfeeding simulation experiments. Shorter gelation duration was required for smaller amounts of liquid to be encapsulated than for larger amounts in order to achieve the same hydrogel stability. Each impression within the food-grade silicone mould was filled with 500 µL of the prepared calcium lactate pentahydrate and iron(III)sulphate pentahydrate solution, and subsequently frozen at -20°C. Gel formation occurred when the frozen hemisphere was exposed to a 4°C sodium alginate bath for 2.75 min. An illustration of the gel's manufacturing process is provided in Figure 5.3. Gelation occurs through an exchange of sodium ions from the guluronic acid blocks of alginate with calcium cations from the frozen hemisphere, and the stacking of these guluronic acid blocks to form a characteristic "egg box" structure [171].

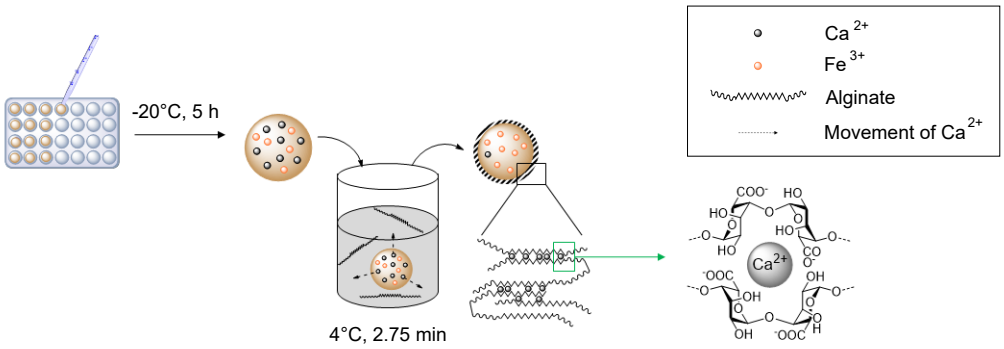


Figure 5.3: Schematic of the frozen reverse spherification process for the manufacture of iron(III)sulphate pentahydrate liquid-core hydrogels. One alginate chain can be linked with several other chains through calcium cations and form a three-dimensional gel network [171]. Image originally published in [47].

5.3.2 Compression testing and visual characterisation

Figure 5.4 b) illustrates a typical force-displacement graph, Figure 5.5 displays the gel's rupture during compression testing. Release of the hydrogel's liquid core occurs following the formation of a pinhole in the hydrogel's solid shell.

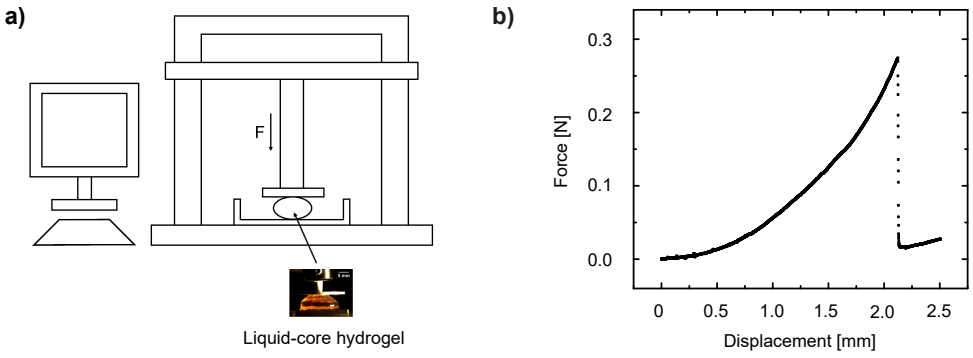


Figure 5.4: a) Simplified schematic of the experimental setup used for uniaxial compression testing of manufactured liquid-core hydrogels. b) Representative force-displacement graph for liquid-core sodium alginate hydrogels, fabricated via frozen reverse spherification. Image originally published in [47].

Average values for wall thickness, diameter, and height of manufactured liquid-core hydrogels, as well as compression force required for gel rupture are summarised in Table 5.5. Obtained values are compared to previously published data of both i) rapidly disintegrating tablets for zinc delivery into human milk by Scheuerle *et al.* [4], ii) Liquid-core alginate Hydrogel Beads by Tsai *et al.* [176, 178, 201].

		Comparison with literature data	
	Liquid-core hydrogels	Liquid-core beats (LHBs)	Rapidly disintegrating tablets [c]
Wall thickness [mm]	0.44 ± 0.10 [a]	Not provided	N/A
Diameter [mm]	14.28 ± 0.85 [a]	Range: $4.17 - 5.84$ [178]	8.093 ± 0.003
Height [mm]	4.91 ± 0.28 [a]		4.65 ± 0.03
Compression force [N]	0.245 ± 0.103 [b]	1.26 [d] [176]	22,000

Table 5.5: Overview of average wall thickness, diameter, height, and compression force of liquid-core hydrogels used for iron sulphate delivery into DDI water and human milk. Data obtained was compared to literature data by both Scheuerle *et al.* and Tsai *et al.*, using rapidly disintegrating tablets for zinc delivery into human milk [4], and Liquid-core alginate Hydrogel Beads (LHBs) for chlorogenic acid delivery into simulated intestinal fluid [176, 178], respectively. Table originally published in [47].

[a] Values were measured in quadruplicate.

[b] Values were measured in octuplicate.

[c] Values taken from [4], referring to tablet type 2.

[d] Reverse spherification using a single spherification step.

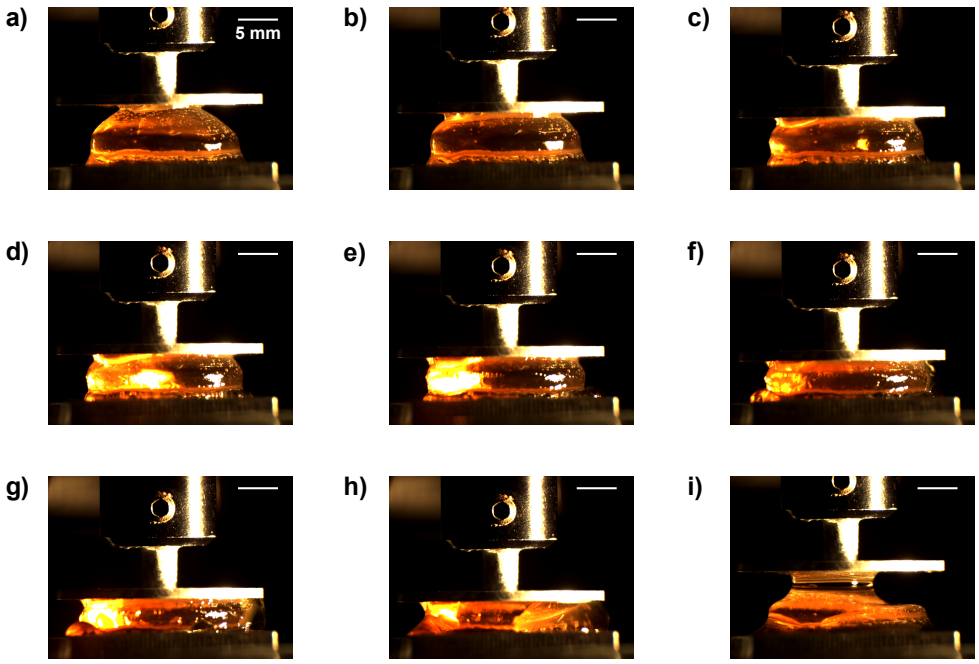


Figure 5.5: Illustration of the liquid-core hydrogel's deformation during compression testing. Rupture and release of the hydrogel's liquid core occurs following the formation of a pinhole in image f). Image originally published in [47].

5.3.3 Release of iron from liquid-core hydrogels

Breastfeeding simulation experiments evaluated both the delivery into DDI water and human milk. The amount of DDI water and human milk passed through the nipple shield, and absolute recovery data, are summarised in Table 5.6.

	Recovery achieved [%]	Amount of fluid passed through the device [g]
Ddi water	54.43 ± 4.16	10.45 ± 0.14
Human milk	44.35 ± 5.43	10.58 ± 0.09

Table 5.6: Recovery achieved and fluid passed through the nipple shield for the delivery of ferric sulphate pentahydrate from liquid-core sodium alginate calcium lactate gel formulations.

An approximately 10 % increased recovery of ferric sulphate pentahydrate was achieved into DDI water compared to delivery into human milk. Figure 5.6 illustrates the cumulative normalised proportion of ferric sulphate pentahydrate released and the normalised proportion released in individual fractions over time.

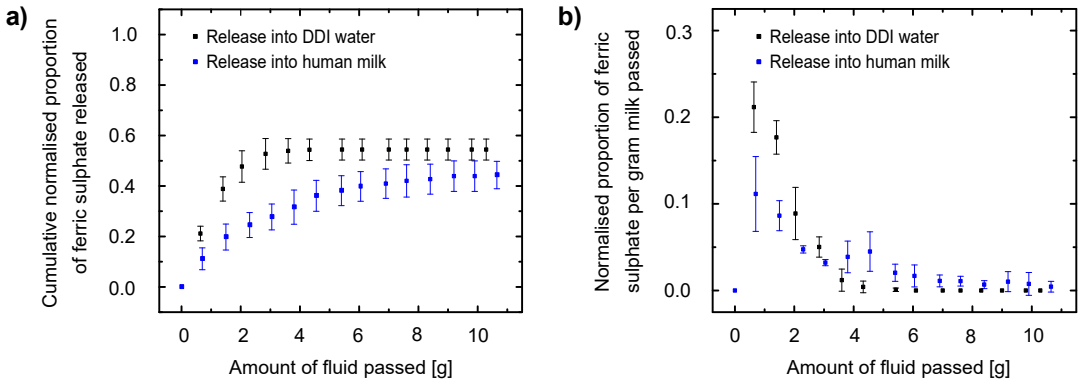


Figure 5.6: Release of ferric sulphate from sodium alginate hydrogels into DDI water and human milk. a) Cumulative normalised proportion of ferric sulphate released. b) Normalized proportion of ferric sulphate released in each fraction over time. Release is represented as a percentage of the total amount loaded within the sodium alginate hydrogels to the total amount released. Each set of points represents the average of three experiments, using the mean of absorbance triplicate measurements for each fraction shown.

5.4 Discussion

Despite the hydrogel's rapid release properties, full release during breastfeeding simulation experiments within 2 min was not achieved. Potential reasons for iron retention include a combination of both iron precipitation and iron inclusion within the shell's solid core. Liquid-core hydrogels possess a range of advantages, but also require further considerations for improvement.

Recovery and iron retention. In spite of the incomplete delivery from liquid-core hydrogels, recovery of ferric sulphate pentahydrate achieved within 2 min of breastfeeding simulation was superior to previously studied dosage forms. Release of zinc sulphate pentahydrate from rapidly disintegrating tablets and non-woven fibres in approximately 10 g of human milk (equivalent to 2 min of breastfeeding simulation) resulted in only $3 - 5$ % and $29 - 31$ % recovery, respectively [4, 38]. Gradual release as opposed to burst release can be attributed to the hydrogel's release mechanism, visualised during uniaxial compression testing, which merely entails the formation of a pinhole in the hydrogel's solid shell. As small amounts of ferric sulphate pentahydrate were still released after 10 g of human milk had passed through the nipple shield, it can be hypothesised that marginal liquid residues were still contained within the gel's solid shell. At the same time, the difference between the amount loaded into the hemispherical silicone mould as part of the hydrogels' fabrication process, and the amount recovered in collected fractions during breastfeeding simulation, results from a combination of both iron retention within the hydrogel's core shell, as well as within the complex apparatus' network. The former is a result of ferric sulphate being enclosed within the cross-linked alginate polymer chains, with the gel's solid shell being retained in the nipple shield during experimental procedures. Iron-casein precipitates occur when ferric sulphate comes in contact with human milk, as Fe^{3+} causes the neutralization of the casein's negative charge by binding to its Pser clusters [202]. Since resuspension of precipitated casein can only be achieved in collected fractions by adjusting the pH during pre-analysis processing, precipitated ferric sulphate trapped within the apparatus cannot be recovered. Comparing both the absolute recovery achieved during breastfeeding simulation experiments into human milk and into DDI water respectively, about 10 % of undelivered ferric sulphate pentahydrate can be attributed to such precipitation effects. Although *in-vitro* experiments yielded only insufficient delivery of the loaded dose, it has to be noted that a breastfed infant would be directly latched onto the nipple shield, which contains the hydrogel, preventing loss of therapeutic precipitates, and would feed for a seven times extended period of time, as shown by Kent *et al.* [40].

Advantages. In addition to their release properties, liquid-core hydrogels are characterised by distinct advantages, indicating their preferred use as a dosage form for therapeutic delivery from a nipple shield during breastfeeding. Firstly, liquid-core hydrogels are the first dosage form studied during breastfeeding simulation that enable therapeutic release by means of a mechanically induced release mechanism, i.e. the rupture of the hydrogels' solid shells as a result of flow rate, suction frequency, and vacuum applied. Comparison of manufactured gels to LHBs by Tsai *et al.* [176, 178] indicated a five-fold lower compression force needed to initiate rupture, presumably caused by the comparatively large amount of liquid enclosed within the manufactured hydrogels, leading to a marginal sphericity. The larger diameter of liquid-core hydrogels enables their central positioning within the nipple shield teat, and also increases the dosage form's surface area for human milk contact and hence subsequent rupture. Liquid-core hydrogels consequently address previous limitations of rapidly disintegrating tablets, non-woven fibres, and HPMC capsules for which the milk's fat content, homogeneity, and macromolecule composition significantly altered the dosage form's release properties. As milk composition is known to vary among others based on the maternal diet and the stage of lactation [39], the dependence of absolute recovery on the milk's properties was perceived as particularly problematic for the exploration of suitable formulations and the definition of widely applicable findings. In addition, sodium alginate hydrogels manufactured via frozen reverse spherification enable the production of low-cost dosage forms, using both a biocompatible, non-toxic polymer and the essential element calcium in form of calcium lactate, particularly advantageous for infant therapeutic formulations due to its neutral taste [196]. Since manufacture of liquid-core hydrogels does not require use of harmful or problematic excipients often present in other infant formulations, they might serve as a possible response to the consistently emphasized need for more appropriate paediatric formulations and their inclusion into pharmaceutical product portfolios [87, 157]. When considering the chosen therapeutic as part of this study, ferric sulphate delivery from a nipple shield during breastfeeding seems particularly suitable, as prolonged exposure of iron to milk products over time, e.g. through milk or yoghurt supplementation, results in both undesired change of odour and flavour, as well as the milk's rancidity due to iron-induced lipid oxidation [202].

Considerations: Three additional areas of discussion arise with regard to the hydrogels' potential *in-vivo* use: taste masking requirements, implications of the hydrogel's soft texture, as well as improvements of stability and calcium/therapeutic solubility. Although fast release of therapeutics is preferred to enable the delivery of a full dose even during short feeds, it also increases the overall ratio of human milk to therapeutic, reinforcing the concern that bitter or irritating tasting medication could negatively affect the infant's breastfeeding compliance. While liquid-core hydrogels enable 38.16 ± 0.06 % absolute ferric sulphate pentahydrate recovery already within the first minute of breastfeeding simulation, it is important to note that breastfed infants are acquainted with a wide range of tastes as a result of the maternal diet [84, 85], and that human milk itself comprises taste masking properties [86]. Likewise, encapsulation of commercially available paediatric liquid formulations, characterised by already optimised taste masking properties to enable their delivery by means of oral syringes or dosing spoons for neonates and toddlers respectively, provides a feasible means to address taste masking concerns and serves as a potential mitigation strategy. The hydrogel's soft texture also enables to mitigate potential infant or maternal discomfort due to large solid tablets or capsules within the nipple shield's teat. The potential to use commercially available nipple shields for therapeutic delivery during breastfeeding is believed to enhance user acceptability over a modified nipple shield device. Final areas of consideration comprise both the liquid-core hydrogel's stability and shelf life characteristics, as well as calcium/therapeutic solubility. According to Tsai *et al.*, the former can be improved via a secondary gelation step [176, 178, 201], whereby hydrogels are immersed into a Ca^{2+} solution following cross-linking to enable the permeation of Ca^{2+} into the previously unoccupied blocks within the hydrogels' core shell structure [201]. This approach was shown to increase hardness and storage stability of LHBs produced by means of reverse spherification, but likewise also decreased the rate of encapsulated therapeutic release over time [176, 178, 201]. It has to be noted however, that experiments by Tsai *et al.* were conducted without agitation, and that the environment during breastfeeding simulation, exposing hydrogels to repetitive mechanical stress, is of a significantly different nature [176, 178, 201]. Consequently, it can be hypothesised that secondary gelation has the potential to significantly improve the hydrogel's mechanical stability for handling and storage purposes, while at the same time causing a less pronounced alteration in therapeutic release. In fact, findings by Scheuerle *et al.* give reason to expect an even improved rate of

therapeutic release during *in-vivo* breastfeeding, as infant tongue peristalsis, shown to be an important driver for dosage form disintegration [43], cannot be mimicked using the breastfeeding simulation apparatus. Solubility considerations refer to the overall availability of calcium lactate pentahydrate for hydrogel gelation, which can be affected by both the temperature decrease during the spherification's freezing step, as well as the solution's overall concentration of calcium lactate pentahydrate and therapeutic. Since calcium ions are required to enable gelation, therapeutic- and concentration-dependent effects make it necessary to consistently optimise both the cross-linking protocol and the chosen concentration of calcium lactate pentahydrate with respect to the type and concentration of therapeutic used.

5.5 Conclusion

Liquid-core hydrogels for the delivery of iron sulphate pentahydrate were fabricated by means of frozen reverse spherification. When tested during breastfeeding simulation experiments, a total recovery of 44.35 ± 5.43 % and 54.43 ± 4.16 % was achieved after 10.58 ± 0.09 g human milk and 10.45 ± 0.14 g DDI water respectively had passed through the hydrogel-containing nipple shield. These results highlight their superior release properties to previously studied dosage forms. In addition, sodium alginate calcium lactate hydrogels benefit from their low cost, ease of fabrication, and high safety profile. Based on the hydrogel's soft texture and its compatibility with a commercially available nipple shield, an enhanced acceptability for *in-vivo* use may be anticipated. Additional research is needed to increase the hydrogel's stability characteristics to facilitate both hydrogel handling and storage.

Chapter 6

Clinical assessment of therapeutic delivery during breastfeeding

6.1 Introduction

6.1.1 Overview

Work presented in this chapter was encouraged by the positive feedback of parents and nursing staff as part of the qualitative descriptive study, presented in Chapter 3, and furthers previous *in-vitro* research conducted at the Department of Chemical Engineering and Biotechnology. While *in-vitro* breastfeeding simulation experiments are an important component for research and development, breastfeeding is a complex process with significant differences between mother-infant dyads, ultimately limiting lab-based simulation validity. The single-centre study presented in this chapter therefore aimed to investigate therapeutic delivery during breastfeeding in a clinical context, focusing on both its feasibility as well as maternal expectation, experience, and acceptability. The following objectives where defined:

1. Primary objective: demonstration of therapeutic delivery during breastfeeding. (Endpoint: change in systemic concentration post study feed)
2. Secondary objective: evaluation of mothers' perspective on therapeutic delivery during breastfeeding. (Endpoint: qualitative/quantitative assessment of maternal views pre and post study feed)

6.1.2 Tracer molecule considerations

Prior to experimental considerations for therapeutic delivery to infants during breastfeeding, as outlined in the chapter's objectives, a suitable tracer molecule had to be chosen. An overview of compounds considered for delivery from an ultra-thin contact nipple shield, and applied selection criteria are provided in the following.

(a) *In-vivo* tracer selection. To identify a suitable therapeutic for delivery and subsequent *in-vivo* detection, tracer molecules reported in published literature and those commonly used in clinical diagnostics were considered. Amongst others four different *in-vivo* tracers were shortlisted: D-xylose, [1]-^{13}C-glucose and [6,6]-$^{2}H_2$-glucose, as well as vitamin B12. Each tracer molecule is discussed in more detail below. Particular focus was given to their use in paediatric practice, as well as their associated risk profile.

D-xylose: In a clinical setting, D-xylose is commonly used to detect diseases of the upper intestine, which result in a decreased uptake of sugar molecules ("malabsorption test"). Xylose is a 5-carbon monosaccharide that is absorbed in the duodenum and jejunum, nearly not metabolized, and eliminated renally [203, 204]. Generally, a concentration of 0.4 g D-xylose kg^{-1} body weight (with a maximum dose of 5 g D-xylose) is used [203]. Although side effects like diarrhoea, vomiting, nausea, and flatulence are possible, previous research has reported no to minimal side effects when using a dose of 5 g D-xylose [203]. In neonates and infants, D-xylose has been used to detect pathological conditions of gut absorption, as well as to investigate gut permeability, during paediatric surgery, or with focus on its development in preterm neonates [204, 205]. *In-vivo* samples are usually analysed following a 5-hour long urine collection based on an adapted protocol by Roe and Rice, whereby D-xylose forms a pink colour, which can be read at 520 nm on heating with para-bromoaniline [206, 207]. Approximately 25 % can be expected to be recovered in healthy subjects [206, 207].

^{13}C- and ^{2}H-labelled glucose: Metabolic isotope tracers are defined as molecules identical in chemistry and function to their naturally occurring *in-vivo* counterpart, which only differ in their stable or radioactive isotope label [208]. Generally, non-radioactive isotope tracers are preferred, due to fewer side effects and their wide applicability for a variety of kinetic investigations within the human metabolism.

Most commonly, hydrogen, carbon, and nitrogen isotopes are used for *in-vivo* kinetic studies, with tracers exhibiting a heavier weight than their corresponding tracees, the naturally occurring unlabelled molecules [208]. Based on the weight difference of tracer and tracee, the tracer to tracee ratio (TTR, also referred to as "enrichment") can be evaluated by means of Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/Mass Spectrometry (LC/MS) [208, 209]. For research in this chapter particularly [1]-^{13}C- and [6,6]-$^{2}H_2$-glucose were considered as tracer molecules, both of which were used in neonatal research or diagnostics before. Use of ^{13}C labelled compounds (here: ^{13}C-octanoic or ^{13}C-acetic acid) is most commonly known as part of the ^{13}C-breath test to evaluate gastric emptying [210, 211], while literature also reports the use of ^{13}C-labelled glucose for ^{13}C magnetic resonance scans (MRS) in neonates and children [212–214], and to investigate pulmonary surfactant kinetics of the newborn infant [215]. [6,6]-$^{2}H_2$-glucose is a common tracer molecule in clinical research of glucose and amino acid metabolism in infants and neonates [208]. Past literature reported its application as a tracer to study intravenous amino acid intake in very low birth weight infants [216], as well as infantile hypoglycaemia in infants born small for gestational age [217], and in preterm infants (gestational age between 26 to 36 weeks) [218]. Only milligram amounts of both [1]-^{13}C- and [6,6]-$^{2}H_2$-glucose are needed for *in-vivo* applications, differing in price: 3 g of [1]-^{13}C-glucose amount to £509 (Sigma-Aldrich, Dorset, UK), 5 g of [6,6]-$^{2}H_2$-glucose to £360 (CK Isotopes Ltd, Ibstock/UK).

Vitamin B12: Vitamin B12, transformed into cobalamin, is an important cofactor for the two enzymes methionine synthase and L-methylmalonyl-CoA mutase: Methionine synthase enables the methylation of homocysteine to methionine, requiring B12 in the form of methylcobalamin, while L-methylmalonyl-CoA mutase is responsible for the "reversible rearrangement" ([219], p. 312) of methylmalonyl-CoA to succinyl-CoA, requiring B12 in the form of 5'-deoxyadenosylcobalamin [219, 220]. Since the human body cannot synthesize vitamin B12, supply has to be met through the consumption of animal products, such as fish and meat, as well as egg and dairy products [219, 221]. Deficiency can be inherited, occur as a result of vegan or vegetarian diet, or due to an abnormal absorption in the small intestine [219, 221], causing an accumulation of the precursors of both previously mentioned processes. This accumulation subsequently leads to a disruption of both DNA synthesis, the production of red blood cells, as well as the central nervous

system's development [219]. A particular risk for vitamin B12 deficiency exists for breastfed infants of a strictly vegetarian or vegan mother, as their B12 store is generally low at birth and cannot be increased due to low amounts of vitamin B12 in their mother's human milk [220]. Vitamin B12 has no reported toxicity, even being safe when administered at $300 - 3000$ times the recommended dietary allowance through intramuscular injection [220].

Table 6.1 provides an overview of important tracer characteristics, including the method of detection, costs, its use in neonates, and its suitability for delivery from a nipple shield during breastfeeding. The final selection was conducted sequentially by i) identifying tracers suitable to be delivered from a nipple shield, ii) taking into account parents' preferences (Chapter 3), and iii) considering projected tracer costs per infant feed.

Tracer	D-xylose	[1]-^{13}C-glucose	[6,6]-$^{2}H_2$-glucose	Vitamin B12
analysis	Urine	Blood, breath	Blood	Blood
costs [£/g]	0.74 (AppliChem Inc., USA)	170 (Sigma-Aldrich, UK)	72 (CK Isotopes Ltd, UK)	0.12×10^6 (JustVitamins Ltd, UK)
amount needed	5 g	0.65-0.73 g kg^{-1} BW [212, 214]	3.6 mg kg^{-1} BW [216]	1000 µg (1 tablet)
costs [£/dose*]	3.71	$442 - 496$	1.04	0.12
NS use		x	x	x

Table 6.1: Overview of tracers considered for the proposed feasibility study and key characteristics with relevance for selection. Thereby, "x" is indicating the applicability of a characteristic for the relevant tracer; BW stands for "body weight", NS for "nipple shield".
*Note: Cost per dose is shown exemplary for an infant of 4 kg BW.

With exception of the CE-manufactured sublingual vitamin B12 tablets listed in Table 6.1, expenses refer only to the purchasing costs of the respective tracers in pharmaceutical grade powder form, and additional costs need to be considered for dosage form manufacture. From Table 6.1, the following ranking of tracer molecules suitable for delivery from a nipple shield during breastfeeding was created:

1. VITAMIN B12: Due to its importance for infant health and development, its non-toxicity, commercial availability as a solid dosage form, and alignment with parents' and nurses' preference for the delivery of vitamins from a nipple shield during breastfeeding (see Chapter 3), vitamin B12 was considered as the favourable *in-vivo* tracer to be administered during the feasibility study. Its commercial availability in form of tablets was perceived as particularly advantageous, as it reduces potential risks and challenges associated with self-preparing formulations for clinical use.

2. [1]-^{13}C-GLUCOSE and [6,6]-$^{2}H_2$-GLUCOSE: Both tracer molecules were considered to be feasible for delivery during breastfeeding. While [1]-^{13}C-glucose was perceived as advantageous with regard to its less invasive mode of detection (possibility of detection through both breath test and blood test as opposed to exclusive blood sampling for [6,6]-$^{2}H_2$-glucose detection), [6,6]-$^{2}H_2$-glucose was more cost-effective. Although both labelled glucose molecules could serve as an *in-vivo* tracer for the proposed feasibility study, it was anticipated that their use might make parents more reluctant to provide their consent for participation. Projected reasons included amongst others worries about the use of an "unknown" molecule which might be perceived as "harmful" by parents, and which does not provide any benefits to their infants' health. At the same time, interaction with parents in the University of Cambridge Addenbrooke's Hospital had shown that use of the words "isotope" or "tracer" were discouraging parents from being interested in the study. In addition, intended use in the feasibility study would require costly CE-manufacture of [1]-^{13}C-glucose or [6,6]-$^{2}H_2$-glucose containing solid dosage forms.

3. D-XYLOSE: Delivery within a nipple shield for potential *in-vivo* application was identified as problematic, as the required amount of 5 g cannot be fitted within the silicone teat of a medium-sized commercially available nipple shield. Since only smaller amounts of D-xylose could be delivered from a nipple shield during breastfeeding, recovery of D-xylose in the infants' urine might subsequently be below the

detection minimum. As a consequence, suitability of D-xylose as a tracer molecule was believed to be limited.

6.1.3 Blood sampling and assay considerations

In order to identify the blood sampling time at which maximum absorption of administered vitamin B12 delivery is achieved, literature on the mechanism of vitamin B12 absorption in both adults and neonates was reviewed.

Absorption in adults: The process of absorption in adults is mediated by a vitamin B12-binding protein called intrinsic factor (IF), and requires the presence of digestive enzymes and gastric acid [222]. IF is excreted by the stomach's parietal cells, while the intrinsic factor receptors (IFRs) are located in the ileum [222]. Based on the limited capacity of IFRs, it was reported that only $1.5 - 2.9$ µg of vitamin B12 per meal can be absorbed following binding to the intrinsic factor protein, with an additional $1 - 3$ % of any oral dose being uptaken by means of passive diffusion [223]. Doscherholmen *et al.* suggested that with increasing dose, passive diffusion becomes the more dominant factor of vitamin B12 control [224]. Carkeet *et al.* reported appearance of B12 in the human blood plasma only $2 - 3$ h after administration, aligning with gastric emptying and subsequent ileum-based absorption [225]. On average, a peak of 3 % the administered dose (1.5 µg of labelled vitamin B12) was detected at 7 h after oral administration [225].

Absorption in neonates: The process of vitamin B12 absorption is believed to differ in neonates. Adkins *et al.* presents findings that suggest two mechanisms for vitamin B12 absorption in the neonate: IF-mediated and haptocorrin (HC)-mediated vitamin B12 absorption [222]. The latter mechanism was previously reported in suckling piglets and rat pups; yet the glycoprotein HC ($68 - 100$ kDa) can also be found in pico-molar amounts in human milk [222]. Due to literature highlighting low secretion levels of gastric acid and IF (both secreted by the stomach's parietal cells), and pepsin in the neonatal GI tract required for IF-mediated absorption, an IF-independent mechanism was believed to occur [222, 226, 227]. Adkins *et al.* proved i) that an IF-mediated vitamin B12 absorption mechanism seems to exist in breastfed infants, with IF receptors present in the infant's ileum, but indicated that IF levels may be too low in early life to participate in vitamin B12 absorption [222], ii) that until the IF-mediated absorption mechanism is completely

matured, HC may mediate vitamin B12 uptake [222]. IF excretion in breastfed infants was shown to gradually increase throughout the first four months of life [222]. Although the exact mechanisms of vitamin B12 absorption in neonates is unknown, studies with preterm infants by Haiden *et al.* and Wothington *et al.* provide a potential indication of the expected increase in serum vitamin B12 levels following supplementation: Hereby, supplementing vitamin B12 over $8 - 12$ weeks led to an approximate three-fold rise in the infants' serum B12 levels [228, 229].

Implications for using vitamin B12 as a tracer in breastfed infants: No evidence seems to exist about i) the rate of HC-mediated vitamin B12 uptake, and ii) how both vitamin B12 absorption mechanisms of adults (IF-mediated and diffusion-based) and term neonates correlate with the absorption mechanisms in infants born prematurely. Based on the limited information available, it was decided to apply the findings of IF-mediated and diffusion based absorption in adult populations, as well as its relevant peaks obtained as a guideline for blood sampling of both term and preterm neonates.

6.1.4 Study design considerations

Experimental work in this chapter was based on the following considerations.

(a) Selection of research methodology. In addition to the quantitative assessment of vitamin B12 delivery, the feasibility study aimed to also assess maternal expectation, experience, and acceptability. Due to the complex nature of therapeutic delivery during breastfeeding, and its evaluation with inpatient mother-infant dyads in a clinical setting, a parallel mixed method approach was chosen over purely qualitative interviews. Hereby, qualitative and quantitative data were collected simultaneously, aiming to enhance the breadth and validity of study findings.

(b) Ethical considerations and timing. Research was meant to occur at a time that is characterised by emotional and physical challenges, including maternal recovery, adjustment to parental responsibilities, as well as to experiences of birth and unexpected infant health challenges, e.g. preterm birth. As a consequence, an engaging combination of tablet-based interactions and in-person conversations was chosen: to collect quantitative data, tablet-based questionnaires (Likert scale) were

used as an interactive tool, following which semi-structured open-ended interviews were performed to more broadly discuss the questionnaires' content.

(c) Selection of a nipple shield design. The clinical study was conducted using commercially available ultra-thin contact nipple shields (Medela, UK) of either 16 mm, 20 mm, or 24 mm size.

(d) Selection of vitamin B12 tablets. Sublingual vitamin B12 tablets from JustVitamins Ltd, UK (1000 µg Methylcobalamin) were identified as a commercially available formulation, based on their suitability for vegetarians and vegans, and the absence of known allergenic components.

6.1.5 Proprietary studies

To investigate the JustVitamin tablets' disintegration properties and to evaluate whether they can serve as a suitable dosage form/formulation for the feasibility study, the following proprietary studies aimed at investigating i) the capability of JustVitamins' sublingual vitamin B12 tablets to dissolve in human milk at physiological parameters representative of *in-vivo* breastfeeding, ii) the duration for disintegration to be achieved.

Methods. *In-vitro* breastfeeding simulation experiments were performed using pasteurised standardised homogenised whole cow's milk (Coop British Whole Milk, UK), characterised by a lipid content of 36 g L^{-1}, and a protein content of 32 g L^{-1}. The vitamin B12 tablet was placed in the silicone teat of a medium-sized (20 mm) ultra-thin contact nipple shield (Medela, UK), and breastfeeding simulation experiments conducted (according to Chapter 4) in triplicate for a duration of 5 min, 10 min, and 20 min. Pressure values averaged between those of term and preterm infants were applied. Tablets and tablet leftovers after breastfeeding simulation were weighted in a petri dish at room temperature before, and for 7 days after experimental procedures until $< 5\%$ change in weight was observed.

Results. The percentage of tablets released within 5 min, 10 min, and 20 min are summarised in Table 6.2. No tablet break-off during disintegration or dislocation of

the remaining tablet through the silicone teat's three holes following the experiment was observed.

Duration of experiment [min]	Milk passed through shield [g]	Average of tablet released [%]
5	25.49 ± 0.18	41 ± 6
10	48.94 ± 0.40	62 ± 2
20	91.90 ± 0.08	74 ± 9

Table 6.2: Percentage of tablet released and milk passed through the nipple shield for breastfeeding simulation experiments of 5 min, 10 min, and 20 min duration.

Discussion. Results obtained revealed the suitability of JustVitamins B12 tablets for delivery during breastfeeding. Reasoning included the following: i) Substantial tablet disintegration even after a short duration of breastfeeding, indicating that *in-vivo* detection of vitamin B12 will also be possible for short feeds. ii) Steady tablet disintegration ensuring adequate mixing of vitamin B12 with human milk during the feed, and thus continuous delivery over time. Since the breastfeeding simulation apparatus can neither simulate infant tongue movement [43], nor the elongation of the maternal nipple during breastfeeding, enhanced disintegration properties can be anticipated during *in-vivo* delivery.

Conclusion. The experimental procedures revealed that JustVitamins' sublingual vitamin B12 tablets are capable of dissolving in human milk at physiological parameters resembling the breastfeeding process, and thus, that sufficient delivery during *in-vivo* breastfeeding can be expected. Due to their high safety profile and established efficacy for delivery from a nipple shield, JustVitamins' sublingual vitamin B12 tablets are suitable for delivery during the feasibility study.

6.2 Materials and methods

6.2.1 Study design

As part of the presented single centre feasibility study, mothers administered vitamin B12 in form of a sublingual tablet (Just Vitamins Ltd, 1000 µg vitamin B12 as

Methylcobalamin), placed within the silicone teat of a commercially available ultra-thin contact nipple shield, to their infant during breastfeeding. A qualified nurse or lactation consultant known to the mother was present, provided breastfeeding support, and advised on the appropriate use and application of nipple shields to the breast. The study involved both the evaluation of quantitative changes in vitamin B12 through blood serum tests, and assessment of maternal expectation, experience, and acceptability via a mixed methods approach. An overview of the study's design is provided in Figure 6.1. Nipple shield and vitamin tablet used in this study are illustrated in Figure 6.2.

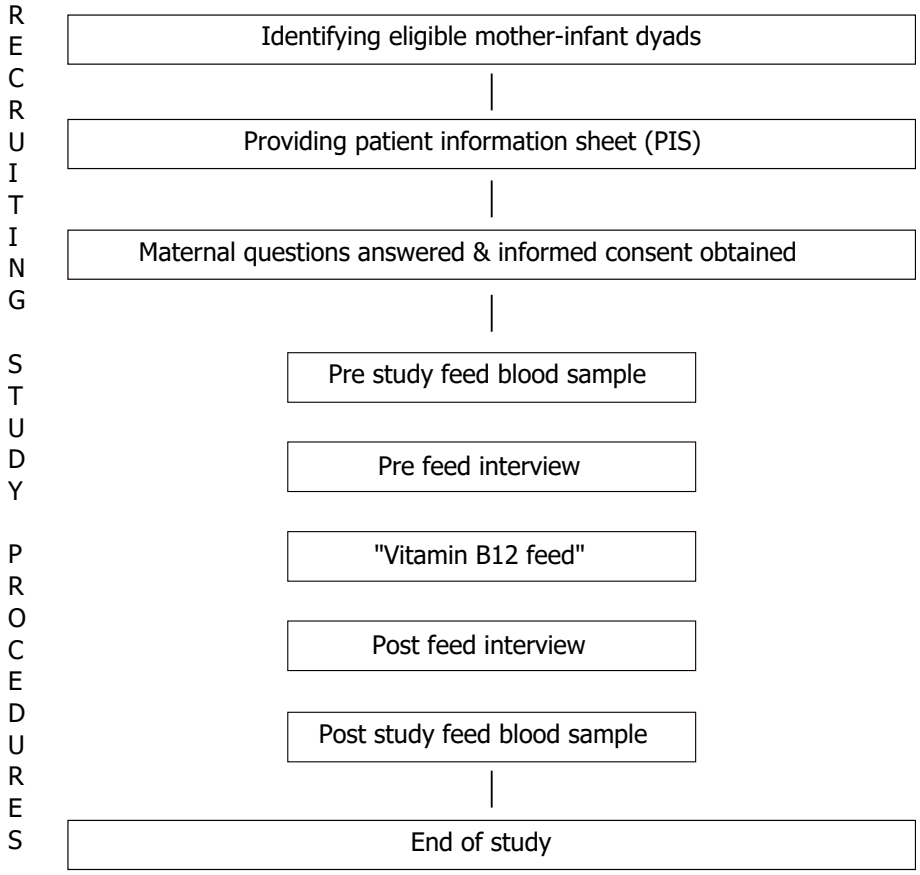


Figure 6.1: FEDD study flow chart.

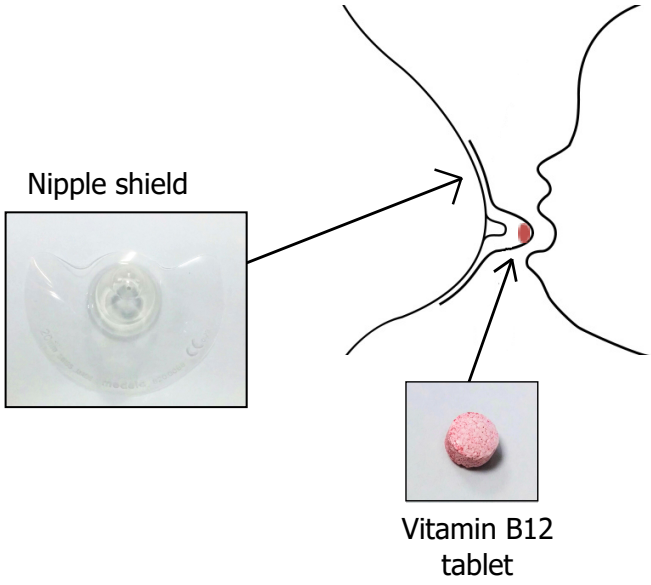


Figure 6.2: Illustration of the commercially available ultra-thin contact nipple shield design (Medela, UK) and the vitamin B12 tablet (JustVitamins Ltd, 1000 µg Methylcobalamin) used during the clinical feasibility study.

6.2.2 Study population and participant recruitment

The presented feasibility study was conducted from July to November 2018 on a UK level 3 Neonatal Intensive and a Transitional Care Unit of the University of Cambridge Addenbrooke's Hospital Trust using purposive sampling. Inpatient breastfed infants below 12 months of age, without medical conditions preventing them from participation, were eligible for inclusion. No restrictions with regard to gestational age or birth weight were made. The research team identified eligible infants in consultation with both the nursing and medical staff, as well as the units' Speech and Language Therapists and feeding lead, following which mothers were approached and study information provided. In addition, parents were informed about the study through posters located in the unit's communal area. Detailed inclusion and exclusion criteria are provided in Table 6.3.

Inclusion criteria
• Breastfeeding is established (exclusively or non-exclusively) • Infant is aged below 12 months • No known allergy or hypersensitivity of mother or infant against any ingredient of the commercially available vitamin B12 tablets (JustVitamins Ltd, UK) used in the study
Exclusion criteria
• Breastfeeding not established • Infant not feeding properly • Allergy or hypersensitivity of mother or infant against any ingredient of the commercially available vitamin B12 tablets (JustVitamins Ltd, UK) used in the study • Medical conditions that could negatively influence swallowing, and thus breastfeeding • Infant suffers from short bowel syndrome or malabsorption

Table 6.3: Inclusion and exclusion criteria for study recruiting.

6.2.3 Data collection and analysis

(a) Vitamin B12

Data collection. In order to assess the increase in vitamin B12 in the infants' blood following delivery, two infant blood samples were taken: a pre study feed sample within one week before the study feed serving as the infants' baseline vitamin B12 level, and a sample $6-8$ hours after the study feed to evaluate the infants' vitamin B12 level increase and percentage uptake. Hereby, sampling time was based on literature referencing vitamin B12 absorption in adult patients [225].

Vitamin B12 assay. Blood serum was separated from whole blood following clotting by centrifugation at 6100 rpm for 10 min, transferred into a separate vial, and frozen at -80°C until further analysis. Serum vitamin B12 levels were analysed using a LOCI Vitamin B12 assay (Siemens Healthcare, Munich/Germany) by the Core Biochemical Assay Laboratory (CBAL) at the NHS Addenbrooke's Hospital Cambridge.

Analysis of assay data. The percentage increase of blood serum vitamin B12 was calculated based on the difference in vitamin B12 levels pre and post study feed. The percentage uptake of the administered dose was obtained via the following steps: i) The infant's haematocrit (Hct) data was used to calculate the infant's corresponding percentage of serum. ii) The infant's blood volume was subsequently calculated by means of published correlations [230], taking into account the infant's current weight. iii) The infant's blood volume and the increase in vitamin B12 serum level concentration was used to calculate the percentage uptake of the administered dose. Data of the infants' haematocrit and weight were taken from the medical notes, ideally recorded/evaluated for clinical reasons on the study day itself or - if unavailable - from a day as close to the study day as possible.

(b) Mixed methods approach

Data collection. A mixed methods approach was used to evaluate maternal expectation, experience and acceptability, including both tablet-based questionnaires (offline survey app: Feed2go) and recorded semi-structured interviews, developed based on established guidelines [73]. Mothers were asked to compare their expectations and experience of vitamin B12 delivery during breastfeeding to the previous use of an oral syringe via the Likert scale from 0 to 10. The tablet was also used to collect demographic data, e.g. asking mothers about their infants' gestational age or their previous experience of breastfeeding using a nipple shield. Excerpts of the semi-structured questionnaire script and structured tablet-based questions are illustrated in Figure 6.3 and Figure 6.4, as well as in Table 6.4 and Table 6.5, respectively.

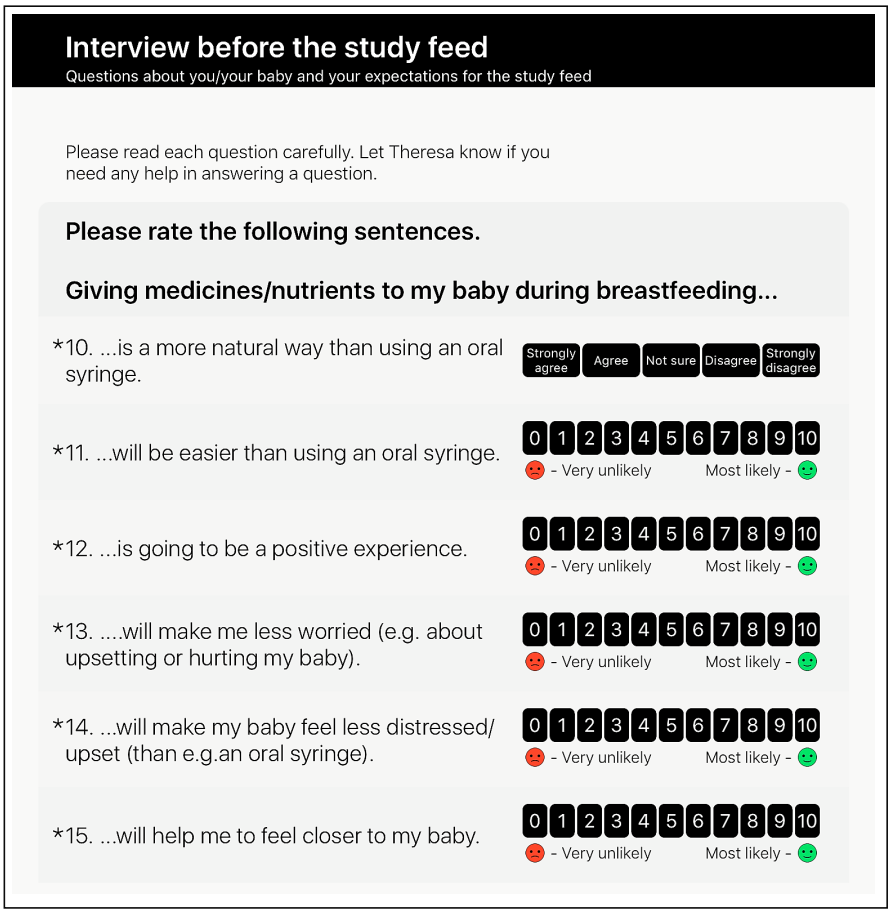


Figure 6.3: Excerpt of structured interview questions used before the study feed, illustrated as presented to mothers on a tablet via the Feed2go survey app.

Semi-structured pre-delivery interview questions
• In the past, has your baby been given medicines/nutrients using commercial devices, e.g. oral syringes, and how did you feel about it? • What is your biggest worry for giving medicines or nutrients to your baby during breastfeeding, and why? • Why are you/are you not worried about using a nipple shield to give medicines or nutrients to your baby during breastfeeding?

Table 6.4: Excerpt of semi-structured interview questions used (pre study feed).

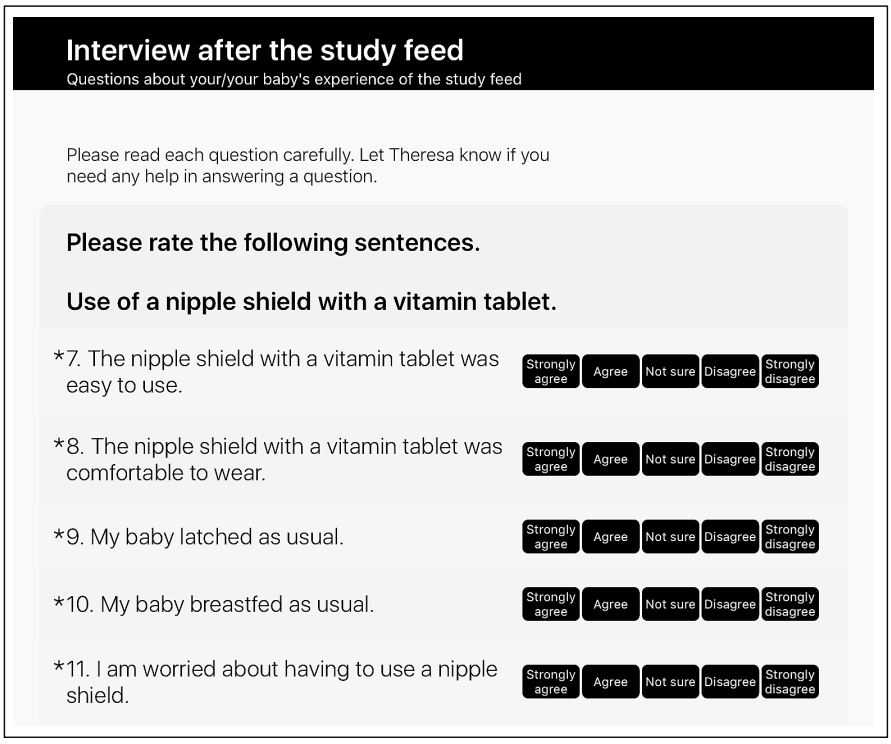


Figure 6.4: Excerpt of structured interview questions used after the study feed, illustrated as presented to mothers on a tablet via the Feed2go survey app.

Semi-structured post-delivery interview questions
• What did surprise you the most when you compare your expectations to your experience of giving the vitamin to your baby during breastfeeding?
• Which of your expectations was least met, i.e. which experience was not at all similar to your expectation?
• Why do you/do you not prefer to give medicines or nutrients during breastfeeding over oral syringes? What would have to be changed, so that you would prefer it?

Table 6.5: Excerpt of semi-structured interview questions used (post study feed).

Data analysis. Semi-structured interviews were transcribed verbatim and potentially identifiable data anonymized; tablet-based questionnaires were evaluated quantitatively. Interview analysis was facilitated by ATLAS.ti (Scientific Software Development GmbH) using an inductive approach of thematic content analysis [53, 74]. Hereby, an initial coding framework emerged following both pre-reading, a line-by-line open-coding approach, and re-grouping steps. The final coding framework was developed by means of iterative revisions within the research team.

6.2.4 Ethics approval

The study was approved by the London - Brighton & Sussex Research Ethics Committee (18/LO/0551), with subsequent approval by the Health Research Authority (HRA), as well as the Cambridge University Hospitals R&D and Insurance Department. Applications were submitted via the IRAS system, comprising almost 100 pages of documentation. All participants provided their written informed consent to be quoted anonymously in this publication.

6.3 Results

Out of a total of 60 infants screened, including four twin pairs, 43 were eligible for participation. Reasons for ineligibility included: Health problems or feeding difficulties (9), change to bottle feeding (4), discharge (4). 26 mother-infant dyads provided their consent, of which 20 dyads completed the full study protocol (Table 6.6). Reasoning to decline participation included: Mother felt overwhelmed with the establishment of breastfeeding (3), mother refused non-clinical blood samples (3), mother refused vitamin administration (1), mother had stopped using a nipple shield (2), no reason provided (8). Non-completion and exclusion from analysis was a result of one of the following: change to bottle feeding (1), discharge before study feed (2), blood sampling time not kept (2), parent-led withdrawal (1). The total number of participants was determined according to guidelines for qualitative research and feasibility studies [231–233], and based on reaching information saturation [77, 234].

Characteristics	Mean (range) or N (%)
Mother's age, mean (range) [years]	32.4 (23 - 39)
Total number of children, N (%)	
1	9 (45)
2-3	10 (50)
>3	1 (5)
Infants' gestational age at birth, N (%)	
Extremely preterm (<28 weeks)	1 (5)
Very preterm (28 to <32 weeks)	2 (10)
Moderately preterm (32 to <37 weeks)	4 (20)
Full term (37 to <41 weeks)	8 (40)
Late term (41 to <42 weeks)	5 (25)
Infants' birth weight, mean (range) [gram]	2769 (890 - 4145)
Stay of infant on NICU, N (%)	
Yes, up to 1 week	5 (25)
Yes, 1 week or longer	7 (35)
No	8 (40)
Infants' age at time of study, mean (range) [days]	16.2 (2 - 70)
Infants' corrected gestational age at time of study, mean (range) [days]	-3.7 (-30 - 15)
Duration infant has been breastfeeding, mean (range) [days]	6.7 (2 - 17)
Exclusive breastfeeding at time of study, N (%)	
Yes	7 (35)
No, also NG	9 (45)
No, also bottle	4 (20)
Use of nipple shield, N (%)	
For current infant	9 (45)
Only for a previous infant	1 (5)
Never	10 (50)

Table 6.6: Characteristics of study participants (mother-infant dyads, N = 20).

6.3.1 Vitamin B12 delivery

In all study feeds, complete tablet disintegration and delivery of the full vitamin B12 dose was achieved. No residual tablet was left after the study feeds, and the tablet's presence in the shield did not appear to affect feeding. Percentage uptake of the administered dose and the percentage increase in blood serum vitamin B12 are illustrated in Table 6.7. A pharmacokinetic-dependent increase to 3856 pg/mL $(1506 - 8413)$ from a baseline of 498 pg/mL $(236 - 681)$ in infants aged 19-70 days, and 1136 pg/mL $(610 - 1743)$ from a baseline of 549 pg/mL $(303 - 925)$ (infants <7 days of age) was observed. Detailed blood serum vitamin B12 levels pre and post study feed for each infant are illustrated in the Appendix section.

	Increase of blood serum vitamin B12 [%]	Uptake of the administered dose [%]
Infants aged <7 days	126 ± 79	0.0072 ± 0.0029
Infants aged 19-70 days	656 ± 619	0.0329 ± 0.0173

Table 6.7: Vitamin B12 serum increase and uptake of the administered dose (n = 16). Four pairs of blood samples (pre and post study feed) were excluded from analysis due to haemolysis, affecting accuracy of the vitamin B12 assay used.

6.3.2 Mixed methods approach

Semi-structured interviews before and after the study feed, assessing maternal expectations and experiences, lasted 7.7 min (range $4.4 - 16.6$ min) and 7.0 min (range $3.5 - 12.2$ min), respectively. Results and relevant quotes of expectation are illustrated in Table 6.8. A summary of the maternal experience and perceived acceptability of therapeutic delivery from a nipple shield during breastfeeding, including quotes of experience, is provided in Table 6.9 and Table 6.10. For all quotes used, content in square brackets was edited to improve clarity and brevity. The abbreviation "NS" (nipple ´shield) and "no NS" (no nipple shield) provides an indication about the current nipple shield use of the participating mother-infant dyad.

Reported expectations

Maternal expectations about therapeutic delivery during breastfeeding can be classified into two dyads of themes: worry and curiosity, as well as perceived emotional and practical benefits. Concerns arose with regard to the nipple shield itself, as well as to the vitamin inside its nipple teat. Mothers without previous experience of using a nipple shield, worried predominantly about the infants' reaction and behaviour during the study feed, including both the infants' latching and overall feeding.

> "It might take some [time] getting used to. I don't know how she is going to do with a nipple shield. And I don't know whether it is going to affect her, I mean the way that she latches." [M12, no NS]

> "I guess it feels like this barrier in between the breast and them feeding. [...] And it might feel a bit alien to the baby [...]." [M4, no NS]

For mothers who had previously used nipple shields, worries focused exclusively on the tablet's disintegration properties, its potential taste, and implication on breastfeeding practice.

> "[...] will it dissolve, and will she taste, be able to taste it?" [M2, NS]

> "I don't have any particular worries. Oki, I suppose if I was going to have a worry it would be that it would give them a negative experience of breastfeeding and then would put them of breastfeeding." [M6, NS]

In spite of their worries, mothers expressed their positivity and curiosity in attempting vitamin delivery from a nipple shield during breastfeeding.

> "I think it's worth looking into. It is something I have never thought about. I think it is a good idea." [M12, no NS]

> "It is just quite exciting to see how it works."[M2, NS]

Therapeutic delivery whilst breastfeeding was associated by all participants with the expectancy of an improved emotional situation and enhanced convenience (see Table 6.8). On an emotional level, mothers assumed a reduction of stress for both mother and infant, while some mothers also referred to an enhancement in physical

intimacy (see Table 6.8). With regard to practical considerations, anticipated benefits were related to time saving, as well as fewer dosing errors using solid dosage forms and its association of being "less messy" [M2, NS]. Both emotional and practical benefits were associated by all mothers with breastfeeding as a more "natural" method of delivery compared to oral syringes.

> "It seems a more natural way of administering medication. Because he would have to nurse in this manner. So this shouldn't be too different from that natural process."[M5, no NS]

Reported experiences

Discussing their experience of therapeutic administration during breastfeeding, mothers most frequently discussed the following: the experience as being positive, their feeling of surprise about its ease and the infant's positive perception, as well as consolidated positive thoughts around the use of nipple shields for therapeutic delivery. Aligning with their previous expectations, mothers associated use of the nipple shield with the vitamin B12 tablet with an ease of use and comfort, and referred to it as positive. At the same time they shared their surprise about their infant's contentment during both feeding and vitamin delivery (Table 6.9), admitting that they had expected a difference in the infant's breastfeeding behaviour. Hereby, mothers attributed their worries prior to the study feed to the lack of experience and emotional circumstances.

> "I think those worries were probably fear of the unknown. Not ever using a nipple shield before. Sort of remembering how they were three years ago, when I saw them in the shops, and they were a little bit alien-looking. [...] I suppose not having that practice or that experience made me think 'Oh, what is this going to feel like? And is it going to be a barrier to feeding? And is he going to latch properly?' But actually, all of that was fine." [M4, no NS]

Based on the positive reaction of breastfed infants and the maternal assessment that "the nipple shield didn't hinder that natural breastfeeding sensation or process" [M5, no NS], 95 % of mothers expressed that they are not worried to use a nipple shield at times for therapeutic delivery, particularly in light of its beneficial implications for therapeutic administration.

> "I think the thing is, the benefits outway any of the potential problems. So I think that [since I am a midwife] I would definitely advocate the use of nipple shields under those circumstances to give medication, because actually, I think the benefits of this far outway the risks of any nipple confusion, which - to be honest with you - if you are gonna use a nipple shield for ten minutes and the drug dissolves, I don't really think you can cause any harm." [M6, NS]

Appropriate guidance was requested for feeds, during which only incomplete delivery of the full dose was achieved.

> "So my thought would be: What would you do - if it was an actual drug - and [you had] given only part of a dose? " [M2, NS]

Overarching themes: perceived advantages and acceptability

When asked to comment on the overall method of therapeutic delivery during breastfeeding, considering its implications for future use, mothers expressed their belief that it was "less invasive" [M7, no NS], and "not an aggressive method of delivery" [M16, no NS], which meant to them that "you are not forcing them" [M20, no NS]. It emphasised the potential of therapeutic delivery during breastfeeding to de-medicalise infant treatment by combining it with the natural process of breastfeeding. This was seen as a particular advantage for mothers with prior neonatal intensive care experience, providing a means to ease the emotional burden of the infants' past medical journey.

> "I think what's really nice, especially for [my daughter] - because she started off her life being poked and prodded, and having things stuck in her - that this is such a lovely... like for babies who've had to undergo all that, to have something so natural, is lovely." *[moved to tears]* [M12, no NS]

> "In particular, I think, for my daughter, because she has been in a hospital for three weeks, I would like something more natural for her from now on. She had - she has - a tube in her nose, and I hope that in the future, we don't have anything clinical, you know, to deal with. [...] the thought [of it] brings us back here. And not that it has been a horrible experience [on the NICU], but it has been very scary. [...] at the

> moment, for us everything that has to do with syringes and medication makes me think of NICU and, you know, this very scary part of her life" [M19, NS]

All mothers advocated for oral infant therapeutic administration during breastfeeding to become available to parents in the future, with the majority preferring this method of infant therapeutic delivery to existing options (see Table 6.10). Reasoning for availability included that it would increase the range of technologies available to them, and provide "choices to make things simpler" [M8, no NS]. A range of different technologies was seen as a possibility to respond to a mother's and infant's individual preferences, to adjust to the type of therapeutic to be administered, while also making it possible for the partner and family to help with therapeutic administration.

> "[...] at the end of the day, as parents we have to make sure to give our children medications when they need them, and in the most calm, you know, not upsetting way possible for them. So, having the choice, since every child is different... there isn't just one way to make it easier, I think. So having more ways means that there will be more children having the best way." [M19, NS]

	Likert scale evaluation			
	Before	After	Change	Quote of expectation
	study feed			
... will be/was easier than using an oral syringe.	7.0 ± 1.6	8.3 ± 1.8	+19 %	"They are upset, but you need to give the medicine first. So then you have to get that all sorted out. Then they are crying, because they are hungry, and by the time you actually start to feed, they are already really distressed. Whereas I guess, if you can do it all in one go, then that is just going to be a bit easier." **[M9, NS]** "[...] you are doing breastfeeding anyway. Hopefully it will be less messy. There is no sort of error with calculating, mismeasuring with the syringes. You have just one tablet and that's the dose." **[M2, NS]** "Because you are already breastfeeding, it makes it a bit simpler in that sense. That it's faster with what you are already naturally anyways doing." **[M8, no NS]**
... will make/ made me less worried.	7.2 ± 2.0	8.6 ± 1.5	+19 %	"[...] I think it would be positive for the mother, if it is positive for the baby. [...] So I am just making the assumption it will be nicer for [my baby] and in turn it will be nicer for me." **[M6, NS]** "Probably [I'd be] more confident in giving medicine, to be honest. Because then it's not having to stress him out, nor do I stress me out." **[M10, NS]** "I would probably feel more comfortable doing it this way, just 'cause it's something we are already doing." **[M17, NS]**

Table 6.8: Comparison of maternal experience and expectation of vitamin B12 delivery during breastfeeding (N = 20).

	Likert scale evaluation			
	Before	After	Change	Quote of expectation
	study feed			
... will help/ helped me to feel closer to my baby.	7.7 ± 1.6	8.4 ± 1.7	+9 %	"I would probably going to be closer to him and it would feel more like a natural part of the feed, rather than kind of going away, taking the medicine out of a bottle, putting it in a syringe, and then feeding it to him." **[M9, NS]** "I don't know if it really changes closeness, because it is such a short... it's such a snapshot of time." **[M4, no NS]**
... will make/ made my baby feel less upset/ distressed.	7.7 ± 1.5	8.6 ± 1.4	+12 %	"I think, if you are able to give your baby medication in a more natural way where it is just part of their routine anyway, I think that's good. I think it's less stressful." **[M12, no NS]** "I think it's a good idea, 'cause it's a way of them getting the medication that's doing something they would normally do. You are not forcing them. It's a natural process." **[M20, no NS]** "I imagine the nipple shield would be less stress for the baby. Because it's part of a regular interaction. Whereas a syringe would be something completely new, external coming in." **[M16, no NS]** "I guess, if baby is already familiar with a nipple shield, it shouldn't notice the difference of receiving something else as well. So I guess that would be the least invasive way of delivering. If a baby is not familiar with a nipple shield, then I don't know if it would be as smoothly received. Because that's another thing that the baby has to adapt to, that would change." **[M16, no NS]**

Table 6.8 continued: Comparison of maternal experience and expectation of vitamin B12 delivery during breastfeeding (N = 20).

	Agree (strongly)	Quote
The nipple shield with a vitamin tablet...		
... was a positive experience	95 % (45 %)	"I think it surpassed my expectation. Mainly because I was slightly concerned about using this silicone and having the tablet, and how it would all work. Whereas now that I have done it, I can see that it is actually quite a natural process. [...] I wish I'd be able to administer all medicines like that rather than using syringes." **[M5, no NS]** "It was pleasantly surprising, really. [...] You know, it was just an idea before, and now having done it, I feel good about it. I would say that it exceeded my expectations." **[M1, NS]** "I think after doing it, using the shield, and having him breastfeeding normally on it, I'd say it was a really positive experience. [...] I really think it is a more natural way to deliver the vitamin." **[M4, no NS]**
... was easy to use.	95 % (65 %)	"I think this method of being able to give medication is so much easier. [...] You are not having to use a syringe necessarily to give the medication, which can be quite difficult. [...] It's part of what would have been your everyday routine anyway, instead of having to include something that's not necessarily something that they would want to do." **[M20, no NS]** "It was really easy to put on." **[M7, no NS]** "[...] I didn't feel that I was giving him medicine at all." **[M6, NS]** "[...] this was just such a smooth, easy process." **[M5, no NS]**
... was comfortable to wear.	95 % (65 %)	"[Breastfeeding] is a bit different with a shield, 'cause you don't necessarily get the same contact. But I think for the benefits it's not enough to worry about. Because the benefits far outway that." **[M20, no NS]** "How comfortable the shield was for me [...] - I was quite surprised." **[M5, no NS]** "You know, I was aware that there was a layer in between. But then it was less painful for me - the whole idea of the nipple shield is to ease the pain of the nipple, so that actually made it more comfortable anyway." **[M4, no NS]** "I thought it might be... I don't know awkward or difficult. I thought the shield might slip off, or something. But it was actually very comfortable." **[M12, no NS]**

Table 6.9: Maternal experience of vitamin B12 delivery during breastfeeding using a commercially available contact nipple shield (N = 20).

	Agree (strongly)	Quote
My baby....		
...latched as usual.	95 % (65 %)	"I was surprised how easily he still latched, and that he didn't even notice that there was a nipple shield in the way." **[M7, no NS]** "I think he thought it was a bit strange at first, because he has not had to use a breast shield before. So it was obviously a different texture to what he was used to. But once he got used to it, it didn't stop him at all. [...] it [also] hasn't stopped him then going onto the other side, which didn't have the breast shield on – which is a positive." **[M20, no NS]**
...breast-fed as usual.	90 % (65 %)	"I couldn't tell any difference between feeding him with the tablet in there or without. [...] it was completely flawless, it just worked perfectly." **[M6, NS]** "I was expecting more hurdles there. But there weren't any. She did very well. She didn't have any problems with taste or anything changing. [...] I think that was my main concern initially: How would she react to it having the change? And she was absolutely comfortable with it." **[M8, no NS]** "That surprised me the most, that he just didn't seem bothered." **[M5, no NS]**

Table 6.9 continued: Maternal experience of vitamin B12 delivery during breast-feeding using a commercially available contact nipple shield (N = 20).

	Agree (strongly)	Quote
I prefer to give medicines/ nutrients using a nipple shield over using an oral syringe.	85 % (30 %)	"It's because [breastfeeding is] what she's used to. And that means there is not gonna be a traumatic experience. It's not gonna be something scary or something that she doesn't know, that she doesn't understand what's going on. Which is always helpful [...] especially when the baby is poorly you don't want to add more stress to the whole procedure." **[M19, NS]** "It would definitely be something I would consider doing over a syringe, if I had the option." **[M17, NS]** "Just because it's natural, it's the best. That's how she is been giving her food." **[M12, no NS]** "To have something simple as that... then I would always choose the nipple shield, and that option." **[M6, NS]** "Whether I would use it all the time, I don't know. [...] I might use a combination of ways. But definitely the fact that it caused him less stress having it, was a major plus. [...] I am glad I tried this way as well." **[M4, no NS]** "It has not been a bad experience at all. But I just don't know if it is the preferred method for me." **[M11, no NS]**
I think the nipple shield could be an acceptable method for nutrient delivery.	100 % (65 %)	"I would say vitamins would be more preferable, because (for) medicine it depends on how distressed the baby is. Because sometimes you have to hold them and give them the medicine. They are not interested in feeding, they are not feeling that well, and everything. And then it could become a bit hard." **[M8, no NS]** "I think anything you can save a baby from having to kind of have to have syringes and things like that." **[M6, NS]**
I think the nipple shield could be an acceptable method for medicine delivery.	95 % (60 %)	"I think, as long as it's proved that they absorb the amount they need to absorb, I wouldn't have an issue with it being medication as well at all. If anything it's less stressful, so [...] you are actually more likely to get the full amount of it [delivered] in this scenario." **[M20, no NS]** "It could be anything, as long as there is a way to know that he has definitely got everything. Which... It was really easy to tell, that the tablet had completely dissolved, and therefore he had taken the whole tablet. So it's just making sure he got the right dose." **[M7, no NS]**

Table 6.10: Perceived acceptability of vitamin B12 delivery during breastfeeding (N = 20).

	Agree (strongly)	Quote
I would like that medicine/ nutrient delivery during breastfeeding becomes possible for parents in the future.	100 % (55 %)	"I think it would be good, if it is possible. As long as there is proper training on how to give the medicine. But then you have to have training on how to give a syringe anyway. So it shouldn't make much difference, it's just a different way of doing it. Similar, but in a less invasive way." **[M7, no NS]** "It's very intimate with the baby. [...] You are not imposing anything on the baby, it's not an aggressive method of delivery. I think if someone is breastfeeding, this should definitely be presented as an option, as a way to deliver the medicine or the nutrient supplement." **[M16, no NS]** "I think it is a perfectly good option. Just 'cause I don't particularly get on with the shield, I think for other mums, [...] it would be a good idea for some. [...] I think options are always a good idea."**[M11, no NS]** "It would be much easier in the future if that's a method. Because the syringe... yes, you have the odd child that likes the syringe, but not every child does. It varies quite a lot." **[M20, no NS]** "I think it is more of a personal choice. It would always come down to having a choice there. But having a choice is good." **[M8, no NS]**

Table 6.10 continued: Perceived acceptability of vitamin B12 delivery during breastfeeding (N = 20).

6.4 Discussion

This study is the first to demonstrate the feasibility and acceptability of therapeutic delivery during breastfeeding using a commercially available silicone nipple shield.

Vitamin B12 delivery

The study showed that vitamin B12 (in tablet form) can be delivered from a commercially available silicone nipple shield during breastfeeding. Since none of the administered dose was lost, a common held anxiety with oral syringes and dosing spoons, uptake of only 0.0072 ± 0.0029 % (infants <7 days of age), and

0.0329 ± 0.0173 % (infants aged $19 - 70$ days) of the administered dose can be attributed to vitamin B12 pharmacokinetics. Observations of this study align with reported literature on a less matured mechanism of absorption in infants, e.g. the gradual increase in IF excretion throughout the first 4 months of life [222], leading to a lower uptake of vitamin B12 in infants than suggested for adult subjects ($1 - 3$ % of the administered dose). In addition, dependence of vitamin B12 absorption on the infants' actual age, independent of prematurity or birth weight, represents a previously unreported finding. Based on the infants' creatinine values, no concerns about renal function existed. In accordance with the literature [224, 225], adjustment of vitamin B12 values within $10 - 24$ hours to those detected in the infants' blood serum prior to the study feed were expected.

Themes of advocacy

The study also demonstrated maternal advocacy for the availability of therapeutic administration during breastfeeding, which was motivated by two main themes: choice and absence of worry. Throughout the study, mothers emphasised the importance of choice, a factor shown to be liberating parents from experiencing a lack of control [235, 236], while at the same time enabling them to assume responsibility of their infants' care and to further their ability in making informed decisions in favour of their infants' well-being [237, 238]. The advocacy of choice in oral delivery technologies, particularly for neonates, might also be driven by a projected relief in fear of potentially being "kind of stuck when they are not taking the medication" (see Chapter 3), enabling to elevate the emotional burden associated with therapeutic delivery to infants. Oral syringes, albeit commonly supplied with commercially available infant liquid formulations, were associated with a predominantly negative, medical sensation by mothers. Reasoning included their stimulation of worry and their role as a reminder of previously experienced medical challenges, with the latter being especially pronounced for mothers whose infants had undergone a variety of intensive care procedures. It can be assumed that previously experienced emotional burden during intensive care is again reinforced by the infants' efforts in fighting therapeutic administration [237].

Use of nipple shields

The exceedingly positive evaluation of therapeutic delivery from a nipple shield was at odds with literature-based reporting on maternal beliefs around the use of nipple shields: hereby, nipple shields were characterised to have both significant benefits in establishing and maintaining breastfeeding [10,11], as well as negative associations of discomfort, inconvenience, and the fear of nipple confusion [12]. While it was anticipated that recruitment and the interventions' overall assessment might be affected by potential negative prejudice, mothers in fact presented a very positive and curious attitude towards this novel approach of therapeutic delivery, indicated by a 60.5 % recruitment rate. Moreover, mothers, supported by nursing staff in the application of the nipple shield to the breast, described the experience as positive for both themselves and their infants, and indicated that their infant did not show problems in latching following removal of the nipple shield after vitamin delivery. Even though half of the participants were not acquainted with the use of a nipple shield, and the majority of mothers referred to breastfeeding as their infant's sole method for oral feeding at the time of study, concerns about nipple confusion were limited. On the contrary, mothers in fact reported their relief in having become familiar with the use of a nipple shield under nursing supervision, enabling them to be prepared if temporary use might be required in the future. While this observation highlights the need for maternal education on the practice of feeding with a nipple shield, it also stimulates considerations to relieve maternal fear of nipple confusion, empowering them to make use of available tools - if needed - to support them in breastfeeding. The observation of complete tablet disintegration and positive infant acceptance aligns with literature on the infant's acquaintance with a variety of tastes and the milk's taste masking properties [13–16]. Yet, based on maternal idioms of expressing surprise following vitamin delivery during breastfeeding, it seems likely that in spite of their hope for a positive experience, previous encounters of therapeutic delivery and potential prejudice for the use of nipple shields might have reinforced the connotation of a more challenging encounter.

Limitations

Limitations of this study include its design as a single centre study with a limited sample size. Moreover, participants might have been biased towards a more favour-

able evaluation of therapeutic delivery during breastfeeding, as only mothers willing to use a nipple shield for the delivery of vitamin B12 consented to participate in the study. It has to be highlighted however that the majority of participants had not used a nipple shield before for feeding their current infant, and that potentially biased expectations would have been resolved when reporting on their experience after the study feed. Further research is needed to investigate use of a variety of formulations for different medical indications and with an increased sample size in both developing and developed countries.

6.5 Conclusion

This study evaluated the feasibility and acceptability of therapeutic delivery to infants during breastfeeding by means of i) vitamin B12 delivery from a commercially available ultra-thin contact nipple shield, ii) measurement of the infants' vitamin B12 blood serum levels $6-8$ hours after the study feed, and iii) a mixed method approach. Delivery of the full dose was achieved, and results showed that therapeutic delivery during breastfeeding was perceived as a feasible approach. Mothers provided their unanimous advocacy for its availability to parents in the future, relating to the following two themes of advocacy: (1) the desire of parents to be given choices with regard to their infants' health, (2) their preference of replacing a mostly medically associated practice of delivery with one dominated by the natural process of breastfeeding. Further investigations could focus on a variety of infant therapeutic formulations and indications.

Chapter 7

Concluding remarks

7.1 Final conclusions

The exploration of therapeutic delivery from a nipple shield during breastfeeding has taken place within the Department of Chemical Engineering and Biotechnology since 2010, and progress was made in establishing a means to enable *in-vitro* breastfeeding simulation in order to analyse different formulations and modified nipple shield designs. Yet, due to incomplete therapeutic delivery, the need for further formulation investigations for delivery into human milk remained, so did the evaluation of the *in-vivo* feasibility and acceptability of therapeutic delivery from a nipple shield during breastfeeding. The presented doctoral work provided insight into both areas of need by means of lab-based and clinical investigations. In the following, the objectives outlined in Chapter 1 will be reviewed and study findings summarised. Subsequently, their contribution to previous knowledge will be presented, resulting implications synthesised, and suggestions for further research provided.

7.1.1 Overview of results

Objective 1: To investigate novel therapeutic dosage forms for delivery from a nipple shield into human milk

(a) Fibrous matrix: non-woven Texel fibre mats for zinc sulphate delivery. The ability of Texel non-woven fibre mats to deliver zinc sulphate pentahydrate from a modified lip-containing nipple shield into human milk was investigated, and quantitatively compared to previous research by Scheuerle *et al.,* using rapidly disintegrating tablets. Two types of Texel non-woven fibre mats of varying thickness and different gram per square metre values were used, and zinc detection performed via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). A total recovery of 64.00 ± 0.01 % and 61.64 ± 0.01 % was achieved after 93.09 ± 1.08 g and 98.15 ± 0.97 g of human milk had passed through the modified lip-containing nipple shield. This totals $20 - 48$ % superior release compared to previous zinc delivery studies using rapidly disintegrating tablets. Incomplete delivery is believed to be attributed to the accumulation of milk components within the porous mat structures, and structural changes of loaded fibres. Further research is required to establish Texel non-woven fibres as a generalized matrix for oral therapeutic delivery.

(b) Hydrogel: liquid-core sodium alginate calcium lactate hydrogels for iron sulphate delivery. The feasibility of liquid-core hydrogels for therapeutic delivery from a nipple shield during breastfeeding simulation was investigated by means of ferric sulphate pentahydrate. It was the first time that a semi-solid dosage form and a commercially available ultra-thin silicone nipple shield have been used during *in-vitro* breastfeeding simulation into human milk. Release of ferric sulphate pentahydrate was quantified using absorbance measurements of a salicylic assay. An absolute recovery of 44.35 ± 5.43 % loaded ferric sulphate pentahydrate was obtained after 10.58 ± 0.09 g of human milk had passed through the nipple shield. This finding is superior to the recovery of previously studied dosage forms. Consequently, based on their superior delivery properties, ease of fabrication, and cost-efficiency, liquid-core alginate hydrogels represent a promising dosage form for delivery during breastfeeding. Incomplete recovery may be attributed to a combination of both iron retention within the hydrogel's core shell, as well as formation and accumulation of iron-casein precipitates within the complex apparatus' network. Further research is required to improve the gel's handling stability and shelf life characteristics.

Objective 2: To explore the clinical implications of therapeutic delivery during breastfeeding

(a) Descriptive qualitative study: need and design preferences in a high-resource setting. The concept of drug delivery during breastfeeding has originally been developed as a means to overcome the challenges associated with therapeutic administration to infants in low-resource settings. In this low-resource environment, therapeutic delivery during breastfeeding was considered "potentially acceptable" ([45], p. 68). Yet, its suitability in a developed country, where dosing spoons and oral syringes are readily available, had not been previously explored. The qualitative descriptive study conducted at the University of Cambridge Addenbrooke's Hospital NHS Trust provided evidence that use of a nipple shield for therapeutic delivery during breastfeeding was believed to be acceptable to parents and staff in a high-resource environment and to address previously neglected challenges faced with current delivery technologies. Particular benefits were anticipated for infants in neonatal special care, among others the potential to foster mother-infant bonding and encourage parental empowerment. Concerns raised related among others to taste masking and timing of feeding and therapeutic administration. Parents advocated for use of a commercially available ultra-thin contact nipple shield over a modified lip-containing design.

(b) Clinical feasibility study: experience, acceptability, and advocacy in a high-resource setting. Feasibility and acceptability of therapeutic delivery from a nipple shield during breastfeeding was investigated as part of a clinical study with twenty mother-infant dyads below 12 months of age. The study involved the delivery of vitamin B12 to infants during breastfeeding from a commercially available ultra-thin contact silicone nipple shield, and the assessment of maternal expectation and experience by means of a mixed methods approach. Participants reported a positive experience, noting that the nipple shield, containing a small tablet in its silicone teat, did not affect their infants' feed or comfort of breastfeeding. It also demonstrated delivery of the full dose of vitamin B12. Mothers unanimously advocated for this approach to become available to parents in the future, with 85 % expressing their preference for therapeutic administration from a nipple shield during breastfeeding over the use of oral syringes. Reasoning included the desire (1) of being provided with choices in relation to their infants' health, (2) to replace

a mostly medically annotated practice of therapeutic delivery with one dominated by the natural process of breastfeeding.

7.1.2 Synthesis

Formulation investigation. Three formulation types have been investigated, using both the modified lip-containing nipple shield and a commercially available ultra-thin contact nipple shield. While the work on formulation development for disintegration during breastfeeding is far from being exhaustive, research findings complement previous investigations using rapidly disintegrating tablets and HPMC capsules, both of which were inferior to non-woven fibres and liquid-core hydrogels [4, 44]. A summary of previously studied formulations and therapeutics via *in-vitro* breastfeeding simulation experiments is provided in Table 7.1, key information on formulation evaluations conducted as part of this doctoral thesis is illustrated in Table 7.2. Out of all formulations studied for use in human milk, liquid-core hydrogels allowed for the highest and non-woven fibres for the second highest release within 2 min of breastfeeding simulation, amounting to 44.35 ± 5.43 % of loaded ferric sulphate pentahydrate (Chapter 5, [47]), as well as 28.95 ± 9.52 % and 30.50 ± 4.33 % of zinc sulphate pentahydrate (Chapter 4, [38]), respectively. In comparison, only about $3 - 5$ % of zinc sulphate was released from rapidly disintegrating tablets within the same time and no detectable amount from HPMC capsules [4, 44]. When comparing the total release values, it has to be noted that experimental procedures for the investigation of liquid-core hydrogels were adapted to account for breastfeeding physiology of premature infants, with a decreased pressure amplitude and a less effective mean pressure to drive milk from the breast and through the nipple shield, and that Fe^{3+}-casein precipitation occurred. To date, liquid-core hydrogels are the only dosage form, studied using breastfeeding simulation, that enable therapeutic release simply by means of mechanical drivers, i.e. flow rate, suction frequency, and vacuum applied. In contrast, previous research has shown the dependence of tablet disintegration on the milk's fat content, homogeneity, and macromolecule composition, also assumed to be affecting the release from fibrous networks. Differences in release between *in-vitro* and *in-vivo* breastfeeding have been highlighted as part of the feasibility study, demonstrating limitations of breastfeeding simulation experiments. These include its lack of mimicking tongue peristalsis, shown to be an important factor for therapeutic release from dosage

forms within a nipple shield [43], as well as the apparatus' void space, making it possible for therapeutic-milk precipitates to be trapped and accumulate. Sublingual vitamin B12 tablets were also analysed by means of *in-vitro* breastfeeding simulation, before being used for *in-vivo* delivery from a nipple shield.

Parameter	Rapidly disintegrating tablets		HPMC capsules
Reference	[42]	[4]	[44]
Nipple shield used	Modified nipple shield, mesh holder	Modified lip-containing nipple shield	Modified capsule nipple shield
Dosage form related			
Diameter [mm]	8	$8.08 - 8.09$	4.8
Height [mm]	N/A	$3.65 - 4.65$	12
Physical state	Solid	Solid	Solid shell, powdered core
Therapeutic related			
Therapeutic delivered	Sulforhodamine B	Zinc sulphate	Zinc sulphate
Analysis method used	Absorbance	ICP-OES	ICP-OES
Approximate amount of human milk passed [g]	100	100	100
Time of breastfeeding simulation [min]	20	20	20
Absolute recovery achieved into human milk [%]	<80	$32 - 51$	No recovery detected

Table 7.1: Summary of *in-vitro* breastfeeding simulation experiments, reported in previous literature.

Parameter	Non-woven fibre mats	Alginate hydrogels	Sublingual tablets
Reference	Chapter 4, [38]	Chapter 5, [47]	Chapter 6
Nipple shield used	Modified lip-containing nipple shield	Commercial ultra-thin contact nipple shield	Commercial ultra-thin contact nipple shield
Dosage form related			
Diameter [mm]	10	14.28	8
Height [mm]	1.8/2.1	4.91	5
Physical state	Solid	Solid shell, liquid core	Solid
Therapeutic related			
Therapeutic delivered	Zinc sulphate	Iron(III) sulphate	Vitamin B12
Analysis method used	ICP-OES	Absorbance of trisalicylate complexes of iron(III)	Weight difference
Amount of human milk passed [g]	93.09 ± 1.08/ 98.15 ± 0.97	10.58 ± 0.09	91.90 ± 0.08
Time of breastfeeding simulation [min]	20	2	20
Absolute recovery achieved into human milk [%]	64.00 ± 0.01/ 61.64 ± 0.01	44.35 ± 5.43	74 ± 9

Table 7.2: Summary of conducted *in-vitro* breastfeeding simulation experiments conducted as part of this doctoral thesis.

Thereby, release of 74 ± 9 % was achieved within 20 min of breastfeeding simulation, contrasting to findings from the feasibility study, whereby vitamin B12 tablets dissolved completely in all study feeds of $15 - 20$ min duration. For those infants whose breastfeeding was paired (feeding on both breasts) or who were losing their latch at some time during the study feed, it could be noted that tablet disintegration was already completed after $5 - 10$ min of breastfeeding. These findings emphasize both the importance of *in-vitro* experiments in order to provide a general assessment of formulation suitability for delivery into human milk, but likewise the need for clinical investigations in order to account for current limitations in breastfeeding simulation.

Qualitative research. Presented qualitative data complements research conducted in Kenya and South Africa [45, 46], by providing views of parents in a developed setting. In addition, the descriptive qualitative study also benefited from enabling trained medical staff to contribute their thoughts on the potential of therapeutic delivery from a nipple shield during breastfeeding. It was the first time that this potential end-user group and staff, likely to act as advocates or opponents for therapeutic administration from a nipple shield, were interviewed. As the work on therapeutic delivery during breastfeeding is of interdisciplinary nature, particularly medical input is key, but unfortunately had been lacking prior to research presented in this doctoral thesis. These studies have been helpful in identifying previously unrealized limitations of the modified nipple shield designs, presented in Chapter 2. These included among others the decreased space within the modified nipple shield, which was believed to be not compatible with maternal physiology. Likewise, parents gave preference to a reusable commercially available ultra-thin contact nipple shield, which contrasts to findings by Hart *et al.* and Flynn *et al.* [45, 46]. In both studies, mothers had advocated for a disposable nipple shield with a circular shape, based on the fear that a reduced base would increase the likelihood of the shield to fall off the breast [45, 46]. No assessment on the lip contained within the shield's teat was made, indicating that maternal preference for a modified nipple shield was likely based on appearance of the shield's base. Following multiple years of work with modified nipple shields, research presented in this doctoral thesis thus sets a new direction for the development of therapeutic delivery during breastfeeding. It moved its focus away from the design of a modified nipple shield towards the identification of approaches to enable use of commercially available ultra-thin contact

nipple shields for convenient and reliable administration of therapeutics to breastfed infants.

Clinical study. Clinical work presented in Chapter 6 was unique in being the first *in-vivo* assessment of therapeutic delivery during breastfeeding. Previously, interactions with parents and staff had only been based on the visual assessment of nipple shields and dosage forms, as well as its hypothetical use during breastfeeding (Chapter 3, [45, 46]). Findings of the presented feasibility study confirmed the assumption that therapeutic delivery from a nipple shield could serve as an alternative to existing oral infant delivery technologies. Even more so, 95 % of mothers were happy using a nipple shield for therapeutic delivery and 85 % preferred this method to oral syringes. This highlights that therapeutic delivery during breastfeeding is not just a tolerated alternative, but in fact, an alternative regarded as superior by the majority of mothers who participated in the study. Irrespective of personal preference, experience in using nipple shields, or exposure to neonatal intensive care, the mothers' unanimous advocacy of therapeutic administration during breastfeeding acknowledges the need to provide parents with a choice in oral infant therapeutic delivery. "Choice" was identified as a main driver for advocacy of therapeutic delivery from a nipple shield, alongside the desire to turn the negative, medical sensation of therapeutic administration into a positive experience associated with the natural process of breastfeeding. As both themes are not constrained to a country's state of development or available resources, it can be hypothesised that therapeutic delivery from a nipple shield during breastfeeding has the potential to serve as an alternative for therapeutic administration to infants globally.

7.2 Outlook

Further progress is needed to move closer towards making therapeutic delivery from a nipple shield during breastfeeding available to parents in the future. The work of interdisciplinary nature focuses on complementing formulation investigations, furthering clinical research, as well as seeking support in the translation of research efforts into medical practice.

7.2.1 Formulation investigations

Non-dissolvable and dissolvable fibres. In order to continue presented work on Texel non-woven fibre mats (Chapter 4), research to confirm its *in-vivo* suitability could be undertaken, including the investigation of fibre cohesion to exclude the possibility of fibre break-off, palatability, biocompatibility, and long-time stability. In addition, further research using different polymeric networks could be conducted, including the use of different fibre types and materials, such as the natural polymers cellulose and xanthan with enhanced biocompatibility characteristics [239–241]. Particularly fast-disintegrating electrospun fibres could be promising for two reasons: they are capable of enhancing the solubility of even poorly water-soluble drugs through incorporation in their amorphous form or as nanocrystals [242, 243], and enable their full delivery when disintegrating, thereby actualising the end-users' preference to visually observe the delivery process (Chapter 3). During electrospinning, electrostatic forces are used to achieve fibre formation of both natural and synthetic polymers [243].

Dissolvable films. To date four out of the five types of dosage forms, believed to be suitable for delivery into human milk as outlined in Chapter 2, have been explored, leaving dissolving films as an investigation yet to pursue. Such films, designed to dissolve in the oral cavity, are referred to by the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) as orodispersible films (ODFs) and soluble films, respectively [244]. Originally developed to help address the needs of geriatric patients as well as younger children with swallowing difficulties, dissolvable films are designed to enable release of a full dose within less than one minute when being exposed to saliva [244, 245]. In addition, ODFs enable convenient alteration of doses by adapting the number of strips used for administration [87]. Most common are polymeric films fabricated using a single or a mixture of polymers, comprising celluloses, starch, semi-synthetic, synthetic, and other polymer classes [244], but also protein or protein-polymer films [246]. Particularly promising in the context of therapeutic delivery into human milk is the investigation of films or 'melts' made from casein, a protein naturally occurring in milk. Films of CaCas or NaCas blended with glycerol (Gly) are transparent and tasteless [246], characteristics preferable for administration during breastfeeding. Originally developed for intended use as edible food packaging [246], these films would also possess the stability needed to be placed within the nipple shield's teat. For each type of therapeutic and its inten-

ded concentration, iterative adaption of the original fabrication protocol is required to ensure film formation despite the presence of a therapeutic in the CaCas/Gly or NaCas/Gly mixture. Film formation is achieved by spreading the mixture on a silicone baking tray, followed by a controlled trying process in an environmental chamber [246].

Liquid-core capsules fabricated through crystallization. Another potential approach for the fabrication of liquid-core dosage forms is the encapsulation of molecule solutions by means of crystallisation. The literature documents its wide range of applications, such as the fabrication of dissolvable milk capsules for use in hot drinks or of small pastilles with liquid content for pharmaceutical applications [247, 248]. The process is also already used commercially, e.g. in the confectionery industry for the manufacture of sugar coated liquid-core sweets [248]. Formation is most commonly based on the presence of a sugar or sugar substitute, such as xylitol, in a saturated liquid formulation, poured into moulds lined with seeded particles [247, 248]. A cooling steps subsequently leads to the formation of a crystal shell [247, 248]. Crystallisation protocols have to be optimised for each therapeutic or food product solution, the type of sugar or sugar substitute used, and the size of pastille/capsule to be obtained [247, 248]. This method of dosage form fabrication seems particularly suitable for formulations containing a high amount of sugar, such as CALPOL® Infant Suspension. One dose of CALPOL® Infant Suspension (5 mL) contains 2.2 g of sucrose.

3D-printed dosage forms. Computer-assisted three-dimensional printing is a technique whereby an ink-jet printing technology enables a binder material to be printed into layers of powder, resulting in a 3D object [249]. 3D printed dosage forms provide a range of beneficial characteristics, such as personalization, precise dose control, and the production of dosage forms with complex therapeutic release profiles [250, 251]. This technique is particularly advantageous for paediatric applications, allowing for the production of smaller quantities and providing an incentive for the development of formulations optimised for use in paediatrics, particularly in its even smaller neonatal sub-population. It can also help to reduce potential adverse reactions and enhance tolerance [251]. Evidence of the potential of 3D printed dosage forms is shown in the literature, and gains increasing commercial interest. The first 3D printed orally disintegrating tablet was approved by the Food and

Drug Administration (FDA) in 2015 [252]. A range of 3D printed dosage forms are possible, for example tablets or films [87, 253].

7.2.2 Clinical investigations

The positive feedback received by mothers as part of the clinical feasibility study is promising and encourages further clinical research with the objective to increase total sample size, as well as to evaluate use of different formulations and their application for a range of therapeutic indications. Since acceptability can vary based on the cultural, social, and geographical setting, investigations should be designed to account for a range of factors believed to impact acceptability of therapeutic delivery from a nipple shield during breastfeeding.

High-resource settings. In order to further presented clinical work in a high-resource environment, future research could investigate use of additional commercially available formulations for delivery during breastfeeding, aiming at supporting therapeutic administration for a range of clinical neonatal indications. Investigations could among others include the delivery of dextrose gel for the treatment of neonatal hypoglycaemia, affecting $5 - 15$ % of newborns and putting them at risk for irreversible brain injury [254]. It was hypothesized that administration of dextrose gel from a nipple shield during breastfeeding could be beneficial in two respects. i) It could support the stimulation of maternal milk production. ii) It could enable a slower, more favourable rise of the infants' blood glucose levels, as the dextrose is swallowed as opposed to absorbed via the buccal mucosa. Yet, care has to be taken with regard to the gel's viscosity, which could hamper its delivery from a nipple shield, and with regard to early use of a nipple shield, requiring guidance based on the expertise by both trained midwives and healthcare professionals. Additional applications could include the delivery of vitamin and antioxidant supplements to premature neonates [255], probiotics [256], and adjustable human milk fortification [257]. Since commercially available formulations, both with regard to suitable solid or semi-solid dosage forms and appropriate dosing for the delivery from a nipple shield during breastfeeding, do not exist, formulation development would be required.

Low-resource settings. Due to the high prevalence of breastfeeding [11], and by drawing on previous qualitative interviews conducted in Kenya and South Africa [45, 46], investigations in low-income countries are of particular interest. Hereby, literature indicates the importance of focusing on the intervention's suitability for cultural and religious circumstances, while also considering obstacles in realising its availability and access [45]. The Cambridge University Hospital Global Network has established links with maternal and neonatal hospital units, and academic institutions in Botswana and El Salvador, which could facilitate in expanding investigations internationally. A range or research interventions could be considered. First, the study protocol could be designed to have the same objectives and endpoints as the feasibility study presented in Chapter 6, enabling direct comparison of findings in a low- and a high-income environment. In addition, use of alternative therapeutics could be considered, whereby use of vitamin and mineral supplements seem most appropriate due to their safety and commercial availability in form of sublingual tablets or capsules. Due to the high prevalence of anaemia in children below five years of age, amounting to more than 40 % in Botswana and 30 % in El Salvador [25], administration of ferrous salt would seem particularly suitable. Literature reports that oral delivery of 25 mg ferrous iron in six healthy adult subjects led to a maximum increase in serum iron (µmol L^{-1}) concentration by 10 µmol L^{-1} 4 h post delivery [258], enabling detection by means of blood serum analysis.

7.2.3 Support for translation into medical practice

As indicated previously in the context of design considerations for therapeutic delivery during breastfeeding, guidance and support by external organisations is required to make its translation into clinical practice possible. This includes both support on a policy as well as on an industrial pharmaceutical level. Initial responses to the overall concept of therapeutic administration from a nipple shield, obtained in 2016 from the Deputy Programme Director at the UNICEF UK Baby Friendly Initiative, as well as the Research Engagement Officer and VOICES Co-ordinator at NCT Excellent Practitioner (Antenatal), were generally positive, but highlighted the need for further investigations, including clinical research. Based on results presented in Chapter 6, further opportunities for conversations can now be sought. Likewise, the Royal College of Paediatrics and Child Health, focusing to increase breastfeeding rates within the UK, could assists in providing advice. To enhance

visibility of this potential alternative to existing infant therapeutic delivery technologies, publications of short articles in magazines for professional practice and research communication, such as "Infant", a journal for neonatal and paediatric healthcare professionals available on all neonatal units within the UK, the University of Cambridge Magazine "Research Horizons", as well as the IChemE magazine, are intended. Engaging in discussions with healthcare experts, policy professionals, and the pharmaceutical industry is of high importance in emphasising the need of and parents' interest in innovation of infant oral therapeutic administration. Ultimately, only in collaboration it will be possible to provide parents with a choice to facilitate drug and nutrient delivery and lessen current struggles in children - an endeavour worthwhile, because they are all of our future.

Appendix

A1. A parent nursing perspective (page 140 – 146**)**

Appendix A1 illustrates study recruitment material used as part of the qualitative descriptive study, presented in Chapter 3, including patient information sheets for parents and healthcare professionals, as well as consent forms.

A2. FEDD feasibility study (page 147 – 153**)**

Appendix A2 illustrates study recruitment material used as part of the FEDD feasibility study, presented in Chapter 6, including a poster, the patient information sheet, as well as consent forms for mother and infant.

A3. FEDD vitamin B12 blood serum levels (page 154**)**

Appendix A3 relates to the clinical investigation in Chapter 6, and illustrates raw data of the infants' vitamin B12 blood serum levels before and after the study feed.

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Drug and Nutrient Delivery System: Exploratory interviews

Participant information sheet

We would like to invite you to take part in our research study. Before deciding whether you take part you need to understand why this research is being done and what it involves. Please take time to read the following information carefully and talk to others about the study if you wish. Please ask us if anything is not clear or if you would like more information. Contact details can be found on the last page.

If you would like to take part, please speak to the nurse who gave you this sheet.

Introduction to the project
My name is Theresa Maier and I am a PhD student and WD Armstrong Scholar developing a novel device for delivering medicines to infants during breastfeeding. A first prototype of the device exists, which has been tested in the laboratory. Its design will now be optimized based on end-user feedback.

Purpose of the study
This interview study is designed to listen to the opinions and experiences of parents and healthcare professionals on their experience in oral drug administration to infants, breastfeeding habits, as well as preferred design characteristics of the novel drug delivery device. By receiving end-user feedback, I hope to be able to optimize the design of the novel infant drug delivery device in the most effective way possible, making infant drug administration safer, simpler and more convenient.

Do I have to take part?
Participation in this study is entirely voluntary. You may withdraw your participation at any point, including after the interview has started. If you do decide to withdraw, all information gathered will be immediately destroyed. You will be asked to sign a consent form prior to the interview starting.

What will happen if I do take part?
The interviews will take place in a meeting room close to the neonatal unit. The whole process will last for approximately 20 minutes. There will be a pre-interview briefing and a post-interview debriefing, with the interview itself taking around 15 minutes. The interviewer will ask questions on the topics of drug delivery, breastfeeding, and on your opinion related to the novel infant drug delivery device.

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The interview will be recorded using a digital voice recorder, which will be kept in a secure location (see below for more information about confidentiality).

Are there any risks in taking part?
The topic of your baby's healthcare is clearly a sensitive one, and it may be upsetting for you to talk about it. There is no obligation to answer any of the questions, and you may withdraw from the study at any time. Along with the researcher, a research nurse will usually be present during the interview who can answer any clinical questions you have which arise during the interview. Care has been taken when designing this study to minimise any negative impact on the participants.

What possible benefits will there be to taking part?
By taking part in this study you will be contributing to the background research about current oral drug delivery practices in infants, your attitude towards breastfeeding, as well as towards using a drug delivery device during breastfeeding. Your answer will aid the development of a device optimized for clinical use, in particular for a clinical investigation with mothers and babies in early 2017.

Will my taking part in this project be kept confidential?
Digital recordings of the interviews will be securely stored at Addenbrooke's Hospital for the duration of the study. Transcripts of the recordings will be made – where identifiable data exists in the interview, the transcript will anonymise it. For example, if a birth weight of 745g is mentioned during the interview, this will be presented in the transcript as 'extremely low birth weight' as per the Bliss Charity guidelines[1]. The recordings will be destroyed after six months, or the publication of results, whichever is sooner. Transcripts will be destroyed after 12 months.

What will happen to the results of the research project?
After writing up the transcripts from all the interviews I will identify common themes and ideas through the texts. We hope to report the results of this study in a relevant academic journal. The results will be reported as a discussion of the identified themes using direct quotes from the interview to help the reader to understand the point of view of the interviewee. All quotes will be completely anonymous with no identifiable data present.

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July 2016

Ethical approval
This project has been registered with the patient experience department at Addenbrooke's Hospital.

Contacts for further information
The first point of contact for any queries about the study is the project lead investigator, Miss Theresa Maier. Any clinical queries should be directed to Dr. Kathryn Beardsall at Addenbrooke's Hospital.

Project lead investigator: Miss Theresa Maier, University of Cambridge
email: tm520@cam.ac.uk

Project supervisor: Prof Nigel Slater, University of Cambridge
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Clinical supervisor: Dr. Kathryn Beardsall, Addenbrooke's Hospital
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Drug and Nutrient Delivery System: Exploratory interviews

Participant information sheet

We would like to invite you to take part in our research study. Before deciding whether you take part you need to understand why this research is being done and what it involves. Please take time to read the following information carefully and talk to others about the study if you wish. Please ask us if anything is not clear or if you would like more information. Contact details can be found on the last page.

Introduction to the project
My name is Theresa Maier and I am a PhD student and WD Armstrong Scholar developing a novel device for delivering medicines to infants during breastfeeding. A first prototype of the device exists, which has been tested in the laboratory. Its design will now be optimized based on end-user feedback.

Purpose of the study
This interview study is designed to listen to the opinions and experiences of parents and healthcare professionals on their experience in oral drug administration to infants, breastfeeding habits, as well as preferred design characteristics of the novel drug delivery device. By receiving end-user feedback, I hope to be able to optimize the design of the novel infant drug delivery device in the most effective way possible, making infant drug administration safer, simpler and more convenient.

Do I have to take part?
Participation in this study is entirely voluntary. You may withdraw your participation at any point, including after the interview has started. If you do decide to withdraw, all information gathered will be immediately destroyed. You will be asked to sign a consent form prior to the interview starting.

What will happen if I do take part?
The interviews will take place in a meeting room close to the neonatal unit. The whole process will last for approximately 20 minutes. There will be a pre-interview briefing and a post-interview debriefing, with the interview itself taking around 15 minutes. The interviewer will ask questions on the topics of drug delivery, breastfeeding, on your opinion related to the novel infant drug delivery system and to its first clinical investigation at Addenbrooke's Hospital in early 2017.

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The interview will be recorded using a digital voice recorder, which will be kept in a secure location (see below for more information about confidentiality).

Are there any risks in taking part?
There are no risks associated with this study. There is no obligation to answer any of the questions, and you may withdraw from the study at any time. Care has been taken when designing this study to minimise any negative impact on the participants.

What possible benefits will there be to taking part?
By taking part in this study you will be contributing to the background research about current oral drug delivery practices in infants, your evaluation of parents' attitude towards using a drug delivery device during breastfeeding, and your opinion about the current device prototype. Your answer will aid the development of a device optimized for clinical use, in particular for a clinical investigation with mothers and babies in early 2017.

Will my taking part in this project be kept confidential?
Digital recordings of the interviews will be securely stored at Addenbrooke's Hospital for the duration of the study. Transcripts of the recordings will be made – where identifiable data exists in the interview, the transcript will anonymise it. For example, if a birth weight of 745g is mentioned during the interview, this will be presented in the transcript as 'extremely low birth weight' as per the Bliss Charity guidelines[1]. The recordings will be destroyed after six months, or the publication of results, whichever is sooner. Transcripts will be destroyed after 12 months.

What will happen to the results of the research project?
After writing up the transcripts from all the interviews I will identify common themes and ideas through the texts. We hope to report the results of this study in a relevant academic journal. The results will be reported as a discussion of the identified themes using direct quotes from the interview to help the reader to understand the point of view of the interviewee. All quotes will be completely anonymous with no identifiable data present.

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Who is funding the research?
This PhD project is funded by the WD Armstrong Trust of the University of Cambridge, supporting PhD research in the application of engineering in medicine.

Ethical approval
This project has been registered with the patient experience department at Addenbrooke's Hospital.

Contacts for further information
The first point of contact for any queries about the study is the project lead investigator, Miss Theresa Maier. Any clinical queries should be directed to Dr. Kathryn Beardsall at Addenbrooke's Hospital.

Project lead investigator: Miss Theresa Maier, University of Cambridge
email: tm520@cam.ac.uk

Project supervisor: Prof Nigel Slater, University of Cambridge
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Clinical supervisor: Dr. Kathryn Beardsall, Addenbrooke's Hospital
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Theresa Maier
PhD Student and WD Armstrong Scholar

Participant Consent Form
Drug and Nutrient Delivery System: Exploratory Interviews

July 2016

Dear Participant

Your signature below indicates that you have read and understood the information presented in the attached Participant Information Sheet, and consent to your participation in the study.

You are reminded that you may withdraw from the study at any point for any reason. If you do so, all recordings and personal information will be destroyed.

If you have any further questions do not hesitate to ask.

If you would like to be informed of the publication of the results of the study, please write your email address below and tick the box indicating you are happy for us to keep a record of your email address. We will not contact you for any reason other than informing you of the results of the study, and the information will not be passed on to any other individuals.

I would like to be informed when the results of the study are available: ☐

Email address: _____

Participant signature: _____

Participant name (printed): _____

Date: _____

Researcher signature: _____

Researcher name (printed): _____

FEDD

Feasibility of drug delivery to infants during breastfeeding

Are you breastfeeding your baby?

Would you be interested in helping to develop a new way of giving vitamins and medicines to babies?

What it will involve?

Trying a new way of giving a vitamin to your baby during breastfeeding.

To find out more, please contact:

Dr. Kathryn Beardsall, Consultant in Neonatology
Email: kb274@cam.ac.uk
Office Phone: 01223 746791

Theresa Maier, PhD Student
Email: tm520@cam.ac.uk
Phone: 07778627858

Cambridge University Hospitals NHS
NHS Foundation Trust

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Version 1.0, 1 February 2018

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Cambridge University Hospitals NHS
NHS Foundation Trust

FEDD
Feasibility of drug delivery to infants during breastfeeding

Information Sheet for Parents

We would like to invite you and your baby to take part in our research study. Please take time to read the following information carefully and talk to others about the study if you wish. Please ask us if anything is unclear, or if you would like more information.

Dr. Kathryn Beardsall
Principal Investigator
Neonatal Consultant
Email: kb274@meschl.cam.ac.uk
Phone: 01223 746791

Miss Theresa Maier
Project Lead Investigator
PhD Student
Email: tm520@cam.ac.uk
Phone: 07778627858

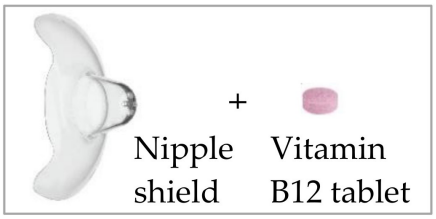
Paula Peirce (Lactation Nurse)
Email: paula.peirce@addenbrookes.nhs.uk
Phone: 01223 256950, Bleep 157-933

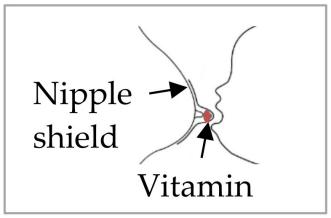
What is the purpose of the study?
This feasibility study aims to look at a new way of giving medicine/vitamins to babies during breastfeeding, without using syringes or spoons. It is conducted as part of a doctorate degree and aims to obtain information about its suitability for delivery, as well as expectations and experience of mother and infant.

Who is being invited to take part?
Mothers who are breastfeeding their babies.

Does my baby have to take part?
Taking part in this study is completely voluntary. If you agree for your baby to take part, you are free to change your mind and leave the study at any time without giving a reason. If you do wish to withdraw at any time, it will not affect your baby's care in any way, and you can choose to have your baby's blood sample/collected data disposed of, and we will document this in your baby's notes.

What will taking part in the study involve?
The study has two parts. 1) You will be asked to breastfeed your baby using a nipple shield that contains a vitamin supplement (1 feed only). Staff will be available to support you during feeding. You will also be asked some questions before and after the feed about your expectations/experiences (total length approx 30-50 min, recorded). 2) We will take a total of two small blood samples (heel prick or venous samples) from your baby – one before and one after the feed. We will try to take theses samples at the same time as your baby is having samples taken for clinical reasons.





What do I have to do?
You have received this information leaflet, because the clinical team and a lactation consultant had identified your baby as suitable for this study. If - following a conversation with the research team, and having all your questions answered - you are interested in participating in the study, please let one of the nurses know. Please take time to decide whether to join the study. If you do, you will be asked to sign a consent form. You will be given a copy of your signed consent form to take away and refer to later. The study itself will be scheduled for a day suitable for you.

What are the possible risks of my baby taking part?
The study uses a nipple shield and a vitamin supplement. Both are commercially available – it is the combined use which is novel. You will get your own nipple shield which will be sterilized before the feed. Use of nipple shields is recommended only if advised by an appropriate professional, as use for each feed over a long period may impact breastfeeding. Literature about the use of nipple shields for premature babies has indicated beneficial feeding outcomes. We are taking two small blood samples, but this is only a small amount and we will try to take them at the same time as clinical samples that your baby may be having. There is a small risk of bruising with any blood sample being taken. The study is covered by NHS indemnity insurance.

What are the possible benefits of my baby taking part?
We cannot say that your baby will directly benefit from taking part in this study. However, the information collected may inform medicine and nutrient delivery for babies in the future. Many parents feel that involvement in research studies, which aim to improve clinical care of future babies, is a positive experience.

Will my/my baby's taking part in this study be kept confidential?
We will follow ethical and legal guidance, and all study information will be handled in confidence, with exception of safeguarding issues, in which case we would have to take appropriate action. The medical team looking after your baby will be informed of their involvement. You/your baby will be given a unique study number, which will be used on all study documentation and to record study data. The study data (including interview recordings) will be stored securely in keeping with the data protection act, and it won't be used to contact you at any point in the future. Following completion of the study, your baby's blood samples will be disposed in accordance with the NHS's code of practice.

What will happen to the results of the study?
When the results of this study are available, they may be published in peer-reviewed journals, used for presentations and conferences, and shared with parent support groups/organisations. If you would like to obtain a copy of the published results, please let the study team know who will be able to arrange this for you.

Who is organising (sponsoring) and funding the study?
This study is sponsored by Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge.

Who has reviewed this study?
All research within the NHS is reviewed by an independent group of people called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by the National Research Ethics Service Committee London - Brighton & Sussex.

Thank you for taking the time to read this information sheet.

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Participant study number (for office use) | | | | | | | |

Feasibility of drug delivery to infants during breastfeeding

Maternal consent for their own involvement in the study

Name of Researchers: Dr. K Beardsall (Neonatal Consultant), Theresa Maier (PhD Student)

I _____ being the legal guardian of _____ (subsequently referred to as baby) hereby give my permission fully and freely to participate in the research study stated above: ***'Feasibility of drug delivery to infants during breastfeeding'***

Please initial each box

1. I confirm that I have read and understood the attached Information Sheet for Parents entitled '*Feasibility of drug delivery to infants during breastfeeding*' (Version: ……..) and have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I understand that my participation is voluntary and that I am free to withdraw from the study at any time, without giving any reason, and without my legal rights being affected in any way.	☐
3. I agree to the study team retaining my contact details for potential future follow-up.	☐
4. I understand that relevant sections of my notes and data collected during the study may be looked at by the research team or individuals from regulatory authorities or from the NHS Trust where it is relevant to them taking part in this research. I give permission for these individuals to have access to this information.	☐

_____	_____	_____
Name of Mother	Signature	Date
_____	_____	_____
Name of Person taking consent	Signature	Date

Please file the original in the study folder, one copy in the participant's notes, and give one copy to the participant.

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Participant study number (for office use) | | | | | | | |

Feasibility of drug delivery to infants during breastfeeding

Maternal consent for involvement of her baby

Name of Researchers: Dr. K Beardsall (Neonatal Consultant), Theresa Maier (PhD Student)

I _____ being the legal guardian of _____ (subsequently referred to as baby) hereby give my permission fully and freely for my baby to participate in the research study stated above: ***'Feasibility of drug delivery to infants during breastfeeding'***

Please initial each box

1.	I confirm that I have read and understood the attached Information Sheet for Parents entitled '*Feasibility of drug delivery to infants during breastfeeding*' (Version: ……..) and have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2.	I understand that my baby's participation is voluntary and that I am free to withdraw him/her at any time, without giving any reason, and without their legal rights being affected in any way.	☐
3.	I agree that blood samples are to be taken from my baby to check for vitamin levels.	☐
4.	I agree to the study team retaining my baby's contact details for potential future follow-up.	☐
5.	I agree to the clinical team caring for my baby being informed of my baby's participation in this study.	☐
6.	I understand that relevant sections of my baby's medical notes and data collected during the study may be looked at by the research team or individuals from regulatory authorities or from the NHS Trust where it is relevant to them taking part in this research. I give permission for these individuals to have access to my baby's records.	☐

Name of Parent/Guardian	Signature	Date
Name of Person taking consent	Signature	Date

Please file the original in the study folder, one copy in the participant's notes, and give one copy to the participant.

Infant id	Serum vitamin B12		Haemolysis
	pre study feed [pg/mL]	post study feed [pg/mL]	
1	575	3484	
2	681	2577	
3	449	1743	
4 (*)	371	705	Pre study feed sample
5 (*)	395	13516	Post study feed sample
6	858	1285	
7	303	1045	
8	236	4981	
9	593	1928	
10	596	4104	
11	430	1006	
12	925	1121	
13	565	1321	
14	325	610	
15	660	1259	
16 (*)	297	2193	Post study feed sample
17	582	1104	
18	397	866	
19 (*)	452	8413	Post study feed sample
20	352	1506	

Table A3.1: FEDD vitamin B12 blood serum levels in breastfed infants pre and post study feed. Blood samples of infants indicated by (*) were not considered for data analysis, since at least one blood sample was haemolysed. Four pairs of blood samples (pre and post study feed) were excluded from analysis due to haemolysis, affecting accuracy of the vitamin B12 assay used.

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