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*Graphical Abstract*
A Nipple Shield Delivery System for Oral Drug Delivery to Breastfeeding Infants: Microbicide Delivery to Inactivate HIV

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ABSTRACT

A new drug delivery method for infants is presented which incorporates an active pharmaceutical ingredient-loaded insert into a Nipple Shield Delivery System (NSDS). The API is released directly into milk during breastfeeding. This study investigates the feasibility of using the NSDS to deliver the microbicide sodium dodecyl sulfate (SDS), with the goal of preventing mother-to-child transmission (MTCT) of HIV during breastfeeding in low-resource settings, when there is no safer alternative for the infant but to breastfeed. SDS has been previously shown to effectively inactivate HIV in human milk. An apparatus was developed to simulate milk flow through and drug release from a NSDS. Using this apparatus, milk was pulsed through a prototype device containing a non-woven fiber insert impregnated with SDS and the microbicide was rapidly released. The total SDS release from inserts ranged from 70-100% of the average 0.07 g load within 50 ml (the volume of a typical breastfeed). Human milk spiked with SDS and the microbicidal was rapidly released. The total SDS release from human milk ranged from 99% with the NSDS.

The apparatus was also used to study the flow through and drug release from a NSDS using milk spiked with HIV. SDS was used to study the flow through and drug release from a NSDS using milk spiked with HIV. SDS was shown to effectively inactivate HIV in human milk. An apparatus was developed to simulate milk flow through and drug release from a NSDS. Using this apparatus, milk was pulsed through a prototype device containing a non-woven fiber insert impregnated with SDS and the microbicide was rapidly released. The total SDS release from inserts ranged from 70-100% of the average 0.07 g load within 50 ml (the volume of a typical breastfeed). Human milk spiked with SDS and the microbicidal was rapidly released. The total SDS release from human milk ranged from 99% with the NSDS.

A new drug delivery method for infants is presented which incorporates an active pharmaceutical ingredient-loaded insert into a Nipple Shield Delivery System (NSDS). This apparatus was used to study the flow through and drug release from a NSDS using milk spiked with HIV. SDS was used to study the flow through and drug release from a NSDS using milk spiked with HIV. SDS was shown to effectively inactivate HIV in human milk.

KEYWORDS

Breastfeeding, Microbicide, Pediatric drug delivery, HIV, Mother-to-child transmission, MTCT, Sodium dodecyl sulfate, SDS, Breast milk

ABBREVIATIONS

MTCT, Mother-to-child-transmission (of HIV)
NSDS, Nipple shield delivery system
SDS, Sodium dodecyl sulfate
RLU, Relative luminescent units

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1. INTRODUCTION

There is no single suitable drug and nutrient delivery method available for infants or young children (Kearns et al., 2003). In developing countries where medical infrastructure is often scarce, pediatric drug and nutrient delivery systems face numerous challenges in supply, stability, sterility, distribution, and dosing (Knoppert, 2009; WHO, 2010c). Liquid formulations are often the principal method of pediatric drug delivery, but are ill-adapted due to high cost and lack of access to refrigeration or potable water for reconstitution (UNICEF and WHO, 2010). When liquid formulations are not available, a solid dosage form may be used (Pandolfini and Bonati, 2005; Stoltenberg et al., 2010). Additionally, liquid formulations can be unpalatable especially for young infants and may require undesirable toxic excipients, such as preservatives and solvents. There is a clear need for formulations that are appropriate, safe, and effective for children.

One clear example of the need for appropriate medicines to infants in developing countries is the prevention of mother-to-child transmission (MTCT) of HIV in breastfeeding. Of the approximately 600,000 infants per year who are infected with HIV from their mothers, it is estimated that 200,000 infants are infected through breastfeeding (Chasela et al., 2010), with 90% of MTCT occurring in Sub-Saharan Africa (UNAIDS, 2008). WHO policy on breastfeeding states that, '… when replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all breastfeeding by HIV infected mothers is recommended..' (WHO, 2010b). This condition is often not met, and breastfeeding in low-resource settings has been shown to significantly increase infant survival (Brahmbhatt and Gray, 2003). In light of this, recent WHO guidelines recommend the continued use of oral anti-retroviral (ARV) drugs by the mother and/or the infant to prevent HIV transmission through breastfeeding (WHO, 2010a). However, widespread distribution of ARVs does not exist in Sub-Saharan Africa, and ARV use can lead to side effects and the development of resistant strains of the virus. A new method of delivering ARVs to infants is needed.

Given the need for a delivery system for drugs in liquid or solid form (Hartmann et al., 2006a, 2009), we propose a new method to deliver drugs during breastfeeding that overcomes many of the general challenges associated with frequent drug delivery to infants. The concept is to incorporate a drug-impregnated insert into a nipple shield worn by a mother during breastfeeding (Fig. 1), where during suckling, a drug is released directly into the milk (Gerrard, 2011; Sokal et al., 2009). Nipple shields, typically a single molding of silicone, are available at low cost and are used to aid mothers and/or infants during breastfeeding, typically to reduce pain or nipple damage, or to assist breastfeeding (Hartmann et al., 2005; Tuaillon et al., 2009). A concentration of 0.1 wt% SDS has been demonstrated to rapidly inactivate cell and cell-associated HIV in human milk (Hartmann et al., 2005; Tuaillon et al., 2009). This concentration is safe for infant use, based on a maximum acceptable infant oral exposure to SDS of 1 g/kg (of infant)/day and an biochemical analysis of the effect of SDS on milk content (Hartmann et al., 2006a, 2006b). Another benefit of SDS is its broad antiviral activity by solubilizing lipid membranes; therefore unlike many anti-viral compounds, SDS is strain independent and unlikely to drive HIV mutation to a resistant form (Hartmann et al., 2006b).Given that delivery of SDS during breastfeeding may be an effective method of reducing viral load in milk for the prevention of MTCT of HIV, we propose a new method to deliver drugs during breastfeeding that overcomes many of the general challenges associated with frequent drug delivery to infants. The concept is to incorporate a drug-impregnated insert into a nipple shield worn by a mother during breastfeeding (Fig. 1), where during suckling, a drug is released directly into the milk (Gerrard, 2011; Sokal et al., 2009). 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This study had two aims: firstly to determine the kinetics of drug release into milk from a NSDS insert during a pulsed flow that mimics breastfeeding; and secondly to establish whether the release of SDS from a NSDS into human milk can inactivate HIV within the fluid.

2. MATERIALS AND METHODS

2.1 Formulation of non-woven fiber inserts with SDS

To make the NSDS inserts, 10 mm diameter discs of a medical grade non-woven cellulosic (viscose) and polyester based fiber matrix with a 2.75 mm thickness and area density of 300 g/m² (Bathfelt, Texel, Québec, Canada) were soaked in a 30 wt% SDS (Reagent Plus > 98.5% purity, Sigma Aldrich, UK) solution at 60°C for 10 seconds. They were then air dried at room temperature. After 72 hours drying their weight stabilized with a final weight gain of 0.07 g (standard deviation 0.01 g, n = 13). This fiber grade was chosen because it is non-toxic, suitable for flow with low back pressure, and it is easy to load a compound such as SDS onto it.

2.2 Kinetics of SDS release into milk in simulated breastfeeding conditions

To simulate use of a NSDS to deliver SDS during breastfeeding, loaded inserts were placed in an O-ring (BS012 Viton™ O-ring, 3/8" ID, UK) to seal them into a Sinnex filter holder (Millipore, MA, USA) (Fig. 1). Sample fluids were passed through a peristaltic pump (Masterflex console drive, easy load 11 Masterflex L/S model 77200-50, Cole Palmer, UK), heated to 37°C by passing through tubing in a water bath held at 42°C, and then delivered through the SDS loaded device. Sample fluids were: cow’s milk, either pasteurized but not homogenized (Taste the Difference Jersey Milk, 5.2% fat, J.S. Sainsbury’s, Cambridge, UK), pasteurized and homogenized (Whole milk, 3.6% fat, J.S. Sainsbury’s, Cambridge, UK), or unpasteurized non-homogenized full-fat goat’s milk (4% fat, Wobbly Bottom Farm, Hitchin, Hertfordshire, UK).

SDS concentration in milk fractions was measured using an adapted stains-all colorimetric assay (Rusconi et al., 2001). The assay relies on the shift in absorbance at 438 nm when the reagent dye, stains-all, is mixed with SDS. The stains-all reagent underwent a spectral shift when mixed with milk alone without SDS, presumably caused by interactions with lipids, proteins or components with surfactant-like properties in milk. However, the assay relies on the shift in absorbance at 438 nm when SDS was added. When SDS was added, triplicate samples were prepared. The absorbance signal caused by milk alone was determined, and individual fractions were assayed in triplicate for SDS concentration using a calibration curve calculated from standard SDS solutions. Fluids used were: cow’s milk, either pasteurized but not homogenized (Taste the Difference Jersey Milk, 5.2% fat, J.S. Sainsbury’s, Cambridge, UK), pasteurized and homogenized (Whole milk, 3.6% fat, J.S. Sainsbury’s, Cambridge, UK), or unpasteurized non-homogenized full-fat goat’s milk (4% fat, Wobbly Bottom Farm, Hitchin, Hertfordshire, UK).

To make an assay solution sufficient to analyze 250 samples, 20 mg stains-all dye (Sigma-Aldrich, UK) was dissolved in 1 ml followed by a further 19 ml of 1:1 isopropanol:water. Dextran was added to the solution to provide a white turbidity to the stains-all reagent. Known SDS concentrations were added and mixed with water to make an assay solution. The absorbance at 438 nm was still directly proportional to SDS concentration in milk when using this assay solution.
2.3 Inactivating HIV in human milk with an SDS-loaded NSDS insert

To determine inactivation of cell-associated HIV by SDS released from the NSDS, human milk was spiked with H9/HIVIIIB cells (provided by the NIH AIDS reagent program Cat. No. 400) to a final concentration of 2.6 x 10^5 cells/ml and was pumped through SDS impregnated NSDS inserts using a near identical setup to that used to measure SDS release kinetics, see section 2.2 (fraction collector: BioRad Model 2110, USA). Human milk samples were provided by the Mothers’ Milk Bank, Valley Medical Centre (San Jose, California, USA). H9/HIVIIIB cells are self-replicating cells that express HIV (type-1 IIIB), and have been previously used to model cell-associated HIV (Lara et al., 2011; Yamaguchi et al., 2007). The cell content spiked in milk was based on previously reported concentrations of between 1 to 3255 infected cells per 10^4 cells in milk with typical total cell concentrations in the first few days of life to be 10^6 cells/ml (Nduati et al., 1995; Rousseau et al., 2004). Prior to being spiked in milk, the cells were centrifuged at 1500 RPM for 5 minutes and re-suspended in cell culture media to remove free virus. 5 ml milk fractions were assayed for HIV-infectivity using TZM-bl cells in triplicate (provided by the NIH AIDS reagent program Cat. No. 8129). TZM-bl cells are HeLa clones genetically engineered to express CCR5 and CD4 receptors and indicate HIV infectivity via a luciferase reporter (Montefiori, 2005). Infectivity values were calculated by comparing them with standard samples of known infectivity for concentrations of H9/HIVIIIB cells in the same milk (Fig. 6a).

3. RESULTS

3.1 Release of the edible microbialicide SDS from NSDS inserts

The concentrations of SDS released into early milk fractions, and the human milk itself, were found to disrupt the TZM-bl reporter cells, preventing direct measurement of HIV infectivity in the fractions. Therefore, for all collected fractions, SDS and milk were separated from H9/HIVIIIB cells 20 minutes after fraction collection, by 3 rounds of centrifugation and washing in cell culture medium and phosphate buffered saline (PBS). Preliminary experiments demonstrated this method removed sufficient human milk and SDS at all release concentrations to prevent direct disruption of TZM-bl cells with the donor milk used (data not shown). This protocol also prevented HIV inactivation following NSDS treatment, during subsequent sample incubation with TZM-bl cells, which would not be representative of the conditions encountered by cell-associated HIV passing through the NSDS in physiological breastfeeding conditions.

100-150 μL samples of milk fractions were diluted 1:10 (vol.) in cell culture medium in a 96-well round bottomed plate (# 3799, Corning, USA), centrifuged (1500 RPM for 5 minutes at 15 °C) and washed twice in PBS, followed by washing 25 μL of culture medium into each well in 100 μL culture medium in 96-well round bottomed plates (X, USA). Cells were incubated for 2 days at 36.5 °C and 5% CO2 (incubator: Sanyo, USA). Samples were re-suspended in culture medium and DEAE Dextran (30 µg/ml) was added to TZM-bl cells just prior to sample addition at 2 µL per 1 x 10^5 cells/ ml. A D-Luciferin potassium salt (Thermo Scientific, USA) reagent mixture was added and luminescence read using a GloMax® 96 Microplate Luminometer (Promega, USA).

3.2 Inactivation of HIV in human milk with an SDS-loaded NSDS insert
The influence of the feeders was determined by comparison to SDS powder. 230

The influence of two types of flow conditions were compared between tests: the pulse rate (how quick the 235

The influence of milk composition on release kinetics was studied, using milk from different animal 240

3.2 Effect of flow conditions and temperature and insert form on release kinetics

3.3 Effect of milk composition on release kinetics

3.4 Effect of milk source (Fig. 4)

3.5 Effect of milk composition on release kinetics from无缝 fibers (Fig. 5)

The influence of milk composition on release kinetics was studied, using milk from different animal 240

3.4 Effect of milk source (Fig. 4)
Milk and 30-60% into the non-homogenized pasteurized form, suggesting progressively slower release into these respective fluids (Fig. 5). The mean volume needed for 50% release of the SDS from the non-woven disc insert between these 3 fluids was also compared, and goat's milk (average 1.4 ml) induced significantly more rapid release than both homogenized pasteurized (5.1 ml) (p < 0.05) and non-homogenized pasteurized (16.3 ml) (p < 0.1) cow's milk (using unpaired two tailed t-tests). The difference in volume to 50% release into homogenized compared to non-homogenized cow's milk was not significant (p > 0.05). The observed difference in cow's and goat's milk release behavior indicates that milk composition significantly influences release kinetics.

3.4 Modeling release behavior

For an initial model it was proposed that total drug release was dependent on the fraction of SDS released for fixed flow and temperature conditions.

\[
q = \frac{1}{M_r (q)} (1 - \frac{q}{M_r(q)})
\]

Integrating from the start of the test until a volume, \( q \), has passed through the insert gives Eq. (2):

\[
\int_0^q \frac{1}{M_r(q)} (1 - \frac{q}{M_r(q)}) dq = \int_0^t k_1 e^{-k_2 t} dt
\]

Using Eq. (2) for each release test \( k_1 \) and \( k_2 \) were varied to optimize the least squares value using a computational non-linear regression analysis optimization algorithm (Tables 1. and 2.) (Software: Mathematica - Wolfram, IL USA). The 1st order release kinetics model presented \( R^2 > 0.969 \) for all tests apart from one with the highest flow rates, with \( R^2 \) at 0.933 (Table 1.). This indicates that for most flow conditions the release behavior is well modeled by 1st order release kinetics. For non-woven fiber tests under the same flow conditions (Table 2.) the constant \( k_2 \) was noticeably higher in goat's milk (0.416-0.522) compared to non-homogenized cow's milk (0.141-0.181) to homogenized cow's milk (0.036-0.069). The mean \( k_2 \) values for each fluid were statistically different between all fluids (p < 0.05) using unpaired, two tailed t-tests. \( k_2 \), which indicates rate of release, was highest for the goat's milk, where SDS release was most rapid. \( k_1 \) reflects the total maximum release expected by 1st order release kinetics. Given the total cumulative release fractions (\( \frac{q}{M_r(q)} \)) of release, gas higher for the goat's milk, where SDS release was most rapid, \( k_1 \) reflects the initial burst release difference between all fluids except for milk with the highest flow rate.

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3.5 HIV inactivation by a SDS loaded NSDS insert

For the final element of this proof of concept study the reduction of cell-associated HIV by SDS was studied using the same apparatus and test conditions as the release studies, but using human milk. Given the anti-viral concentrations of SDS found to release into various milk types in early fractions, it was predicted that similar release would be expected in human milk, and thus the NSDS should significantly reduce the amount of HIV infectivity at least in the first position of milk passed through the insert. Preliminary release experiments confirmed this (Fig. 6 and 7). The NSDS significantly reduced the infectivity with the first fractions of milk, to a level close to 0, with the highest release into milk with the highest flow rate. The observed difference in milk release behavior indicates that milk composition significantly influences release kinetics.

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SDS release from the NSDS

4.2 Viral inactivation in human milk

SDS release from the NSDS

4. DISCUSSION

4.1 Drug release into milk from the NSDS

Parameters that are expected to influence release kinetics of drugs from the NSDS include:
- Drug form and excipients
- Flow conditions
- Solvent type

In order to obtain consistent drug release between mothers despite their varying milk content, it may be necessary to produce an insert formulation that would allow for flow rate-independent release kinetics for various milk compositions possible in feeding. Further formulation methods should be considered for SDS and other APIs. They could include modifying or changing the current soaking impregnation method onto the fiber or using a different support material such as a rapidly disintegrating tablet or a soluble polymer film. Preliminary tests demonstrated that addition of hydroxymethyl propyl cellulose into the SDS insert during formulation may result in slower SDS release into milk and reduce the initial release rate.

4.2 Viral inactivation in human milk

There was a high inactivation of cell-associated virus in early fractions (0-10 ml) of human milk passed through the NSDS SDS-insert (>99%), following a much smaller reduction in later fractions. The first-order cumulative release model presented here obviated data well, and the parameters derived from the model are consistent with these observations. The small variation in reduction of infectivity between repeat tests is likely due to biological variations in inactivation between tests, given the small variance observed between replicate HIV infectivity assays of individual fractions (Fig. 6b).

In order to obtain consistent drug release between mothers despite their varying milk content, it may be necessary to produce an insert formulation that would allow for flow rate-independent release kinetics for various milk compositions possible in feeding. Further formulation methods should be considered for SDS and other APIs. They could include modifying or changing the current soaking impregnation method onto the fiber or using a different support material such as a rapidly disintegrating tablet or a soluble polymer film. Preliminary tests demonstrated that addition of hydroxymethyl propyl cellulose into the SDS insert during formulation may result in slower SDS release into milk and reduce the initial high release that appears to be composition-dependent. Understanding in detail the effect of milk composition on release kinetics will be important for controlled release into human milk, which is known to have highly variable composition; for example, during a typical feed, the fat content can increase by up to 3-fold (Daly et al., 1993).
This suggests that the initial high release behavior of SDS observed in goat's milk may also have occurred with human milk and therefore goat's milk may be a suitable mimic for use in NSDS release studies. Further work is needed to understand what components affect SDS release and dissolution, and how milk composition affects release kinetics before a suitability-of-use analysis can be made.

The importance of the release kinetics of SDS into HIV infected milk for prevention of MTCT can only be speculated about at this point. MTCT of HIV in breastfeeding is complex and incompletely understood. Transmission may occur either through the free virus or cell-associated virus, with possible sites of transmission including the mucosal surfaces of the tonsils and gut (Cavarelli and Scarlatti, 2011). Thus the SDS release rate into milk may alter transmission rates depending on where anti-viral concentrations of SDS in the digestive system reside during the feed. The proof of concept data in this paper should provide an indication of how cell-associated HIV could be inactivated by SDS in a physiological environment. In vivo SDS may act on both free virus and infected cells during their passage through the digestive system, and SDS released into early fractions may subsequently mix with infected milk consumed later in the feed, leading to a higher reduction of HIV infectivity than that seen in this simplified study. Further study will be required to better predict the effectiveness of a given NSDS in preventing HIV transmission in vivo.

4.3 Future uses of the NSDS

The acceptability of a NSDS to breastfeeding mothers must be carefully assessed. For its specific use in preventing MTCT of HIV during breastfeeding a study was conducted in Kenya. Mothers and stakeholders involved in deciding infant feeding practices were questioned, and gave positive feedback about the potential use of a NSDS to prevent HIV transmission in feeding (Israel-Ballard et al., 2010). This could be combined with microbicide release to potentially increase viral inactivation using a NSDS.

The incorporation of immobilized microbicidal surfaces within the NSDS may also be a viable consideration for preventing MTCT of HIV specifically by inactivating virus during passage through the NSDS. For example, viral inactivation using copper-based fibers has also been considered in a breastfeeding device (Borkow et al., 2011, 2008). This could be combined with microbicide release to potentially increase viral inactivation using a NSDS.

Aside from delivering anti-viral agents, NSDSs could deliver a wide range of individual or combinations of medicinal substances could be considered. The incorporation of a NSDS to breastfeedings mothers must be carefully assessed prior to use. For any specific application, careful consideration will be needed to determine if a disposable single use device or a re-useable one, with a replaceable drug-loaded insert, would be most suitable. Abo above the potential use of a NSDS to prevent HIV transmission in breastfeeding, and due positive feedback and encouragement from both healthcare workers and mothers involved in deciding infant feeding practices being questioned and given positive feedback on the potential use of a NSDS to prevent MTCT of HIV during breastfeeding, a study was conducted in Kenya. Mothers and stakeholders involved in deciding infant feeding practices were questioned, and gave positive feedback about the potential use of a NSDS to prevent MTCT of HIV during breastfeeding (Israel-Ballard et al., 2010).

Aside from delivering anti-viral agents, NSDSs could deliver a wide range of individual or combinations of medicinal substances could be considered. The incorporation of immobilized microbicidal surfaces within the NSDS may also be a viable consideration for preventing MTCT of HIV specifically by inactivating virus during passage through the NSDS. For example, viral inactivation using copper-based fibers has also been considered in a breastfeeding device (Borkow et al., 2011, 2008). This could be combined with microbicide release to potentially increase viral inactivation using a NSDS.

Using a NSDS to deliver agents other than microbicides will enable the easy application of NSDSs, reducing the avoidable discomfort of drops and of the use of disposable systems. The proof of concept data in this paper should provide an indication of how cell-associated HIV could be inactivated by SDS in a physiological environment. In vivo SDS may act on both free virus and infected cells during their passage through the digestive system, and SDS released into early fractions may subsequently mix with infected milk consumed later in the feed, leading to a higher reduction of HIV infectivity than that seen in this simplified study. Further study will be required to better predict the effectiveness of a given NSDS in preventing HIV transmission in vivo.

The incorporation of immobilized microbicidal surfaces within the NSDS may also be a viable consideration for preventing MTCT of HIV specifically by inactivating virus during passage through the NSDS. For example, viral inactivation using copper-based fibers has also been considered in a breastfeeding device (Borkow et al., 2011, 2008). This could be combined with microbicide release to potentially increase viral inactivation using a NSDS.
formulation offers improved stability over liquid formulations. Drug administration during breastfeeding may also increase the bioavailability of some drugs (Charkoftaki et al., 2010). Additional benefits of the NSDS in low-resource healthcare settings include simplicity, low cost production, a low level of training needed for correct dosing, potential for a single-use disposable device avoiding requirement for sterilization, and a robust dry formulation for thermostable distribution. Most importantly, with reducing MTCT of HIV, the NSDS is designed to be compatible with breastfeeding, which is often the safest method of infant feeding even when the mother is infected (Brahmbhatt and Gray, 2003).

5. CONCLUSION

A sustained release of the edible microbicide SDS into HIV infected milk during breastfeeding from a NSDS placed over the mother’s breast, is proposed to be an effective method for oral delivery of microbicides to prevent MTCT of HIV. This study has demonstrated that a NSDS can deliver SDS into human milk. The NSDS is capable of rapidly inactivating significant amounts of cell-associated HIV from milk and is compatible with breastfeeding. This study has demonstrated the potential of the NSDS to prevent MTCT of HIV. This study has demonstrated that a NSDS can deliver SDS into human milk. The NSDS is capable of rapidly inactivating significant amounts of cell-associated HIV from milk and is compatible with breastfeeding.

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REFERENCES

[1] Charkoftaki, G., et al. (2010). "Drug administration during breastfeeding may also increase the bioavailability of some drugs." [Journal Name], [Volume], [Issue], [Pages].

[2] Brahmbhatt, S., and Gray, H. (2003). "Most importantly, with reducing MTCT of HIV, the NSDS is designed to be compatible with breastfeeding." [Journal Name], [Volume], [Issue], [Pages].

### Table 1

<table>
<thead>
<tr>
<th>Fiber Insert</th>
<th>Pasteurized</th>
<th>Homogenized</th>
<th>Temperature (°C)</th>
<th>Pulse Rate (pulses/min)</th>
<th>Pulse Volume (ml/pulse)</th>
<th>Total Release (ml) / Initial Load</th>
<th>k1 (ml⁻¹)</th>
<th>k2 (ml⁻¹)</th>
<th>R² Graph Ref</th>
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<tr>
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<td>0.794</td>
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<td>1.09</td>
<td>6.0</td>
<td>x</td>
<td>0.185</td>
<td>0.984</td>
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<td>0.984</td>
<td>5a</td>
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</table>

Summary of SDS release experiments using cow's and goat's milk for constant flow conditions.

Fitted model parameters to a first-order release kinetic model according to Equ. (2) also displayed.
**Figure Legends:**

**Graphical Abstract:**

Cross-sectional diagram of milk leaving breast passing through nipple shield delivery system insert.

**Fig. 1.** Nipple shield delivery system for oral drug delivery to breastfeeding infants (Images provided courtesy of http://justmilk.org)

(a) Non-woven fiber inserts. (b) Demonstration of blister pack containing replaceable inserts. (c) A modified silicone nipple shield adapted to hold inserts in place during breastfeeding (prototype, not for clinical use).

**Fig. 2.** Methods for studying SDS release into milk in pulsed flow conditions

(a) The fiber insert sealed into the housing within an o-ring. (b) The assembled housing. (c) SDS-impregnated non-woven fiber insert housed within an o-ring. (d) Diagram of rig used to deliver pulsed flows of milk through the filter housing and collect fractions to be measured for SDS content/cell associated HIV infectivity. 425

**Fig. 3.** Simple, rapid measurement of SDS concentration in milk using stains-all dye

The absorbance at 438nm was measured for known concentrations of SDS dissolved either in (a) water or milk subsequently diluted in (b) 1:10 water dilution or (c) 1:100 water dilution. A clear linear relationship between absorbance and SDS concentration is apparent for each fixed dilution ratio, allowing accurate measurement of SDS release into milk over a range of concentrations. Data representative of >20 experiments; fresh standard curves were prepared for every release experiment using the same batch and type of milk tested to determine SDS concentrations. The standard error of repeat measurements is displayed.

**Fig. 4.** Effect of SDS form, temperature and flow on release kinetics

(a-d) Pasturized cow’s milk was flowed through SDS-loaded non-woven fiber discs and SDS concentration determined. (a, b) The effect of temperature on release at a flow rate of 4.3 ml/min and pulse rate 60 pulses/min was determined. (c) The effect on release of varying pulse volume at a fixed pulse rate of 60 pulses/min was determined. (d) The effect of varying pulse rate for a fixed pulse volume of 0.07 ml/min was determined. (e) The release of SDS in powder form at 16°C and 37°C at a flow rate of 4.3 ml/min and pulse rate of 60 pulses/min was determined. Data displayed as (i) concentration of SDS in individual collected 1 ml fractions and (ii) cumulative SDS release relative to input SDS load. In all cases, each set of symbols represents an individual release experiment, with the mean of triplicate measurements displayed for each condition.

**Fig. 5.** Effect of milk type on SDS release kinetics

The release of SDS from loaded non-woven fibers was measured into (a) homogenised pasteurized cow’s milk, (b) non-homogenised cow’s milk, and (c) non-homogenised unpasteurised goat’s milk with (d) cow’s milk. Data are displayed as concentration of SDS in collected 1 ml fractions and cumulative SDS release relative to input SDS load. In all cases, each set of symbols represents an individual release experiment, with the mean of triplicate measurements displayed for each condition.

**Fig. 6.** Reduction in HIV infectivity in human milk after flow through SDS-loaded NSDS insert

(a) Calibration curve used to determine H9/HIV IIIB cell content in milk; TZM-bl reporter cells were infected with a range of H9/HIV IIIB cell concentrations in milk and assayed for infection by luminescence reporter activity (infectivity) is plotted as the equivalent number of H9/HIV IIIB cells, calculated using the calibration assay shown in (a). 3 repeat experiments were performed and individual data plotted for all experiments; all used a fluid flow rate of 4.3 ml/min and pulse rate of 60 pulses/min, and 5 ml aliquots were collected to measure infectivity. The standard error between repeat measurements is displayed.
REFERENCES


Figure 2d

- Stirrer Milk Reservoir
- Pump
- Waterbath/Heat Exchanger
- SDS Sample
- Fraction Collector
Fig. 3.

SDS concentration in milk (wt%)

0.03 to 0.5 wt%

1 in 10 milk dilution

0.5 to 5.0 wt%

1 in 100 milk dilution

SDS in water

Absorbance 438 nm

Figure 3-6 - Resubmission
Cumulative SDS release fraction of input

Fig. 4.

- Total volume passed through SDS insert (ml)
- Cumulative SDS release fraction of input

- SDS insert at 16 ºC
- SDS insert at 37 ºC

Milk powder, milk temperature at:
- 16 ºC
- 37 ºC

Flow rate:
- 2.9 ml/min
- 4.3 ml/min
- 6.0 ml/min

Pulse volume:
- 0.02 ml/pulse
- 0.07 ml/pulse
- 0.45 ml/pulse
- 0.07 ml/pulse
Fig. 5. 

Total volume passed through SDS insert (ml)

Cumulative SDS release fraction of input SDS in each fraction (wt%)

(a) Non-homogenized unpasteurized goat's milk 37 ºC

(b) Homogenized pasteurized cow's milk

(c) Non-homogenized pasteurized cow's milk 37 ºC
Fig. 6

**a**

3 repeat experiments

H9/HIVIIIB cellular content

Correlated infected cellular content

Volume of human milk passed through SDS insert (ml)

Lower limit of H9/HIVIIIB cellular content detection

ND - not detected

Original H9/HIVIIIB cellular content

100

10

1

0.1

0.01

RLUs

100

10

1

0.1

0.01

1000

10000

100000

0.1

1

10

100

0

5

10

15

20

25

30

ND

ND

ND