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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 (API)-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 H9/HIVIIIB cells was also passed through the same set-up. Greater than 99% reduction of cell-associated HIV infectivity was achieved in the first 10 ml of milk. This proof of concept study demonstrates efficient delivery to breastfeeding infants is achievable using the NSDS.

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 can be administered by the mother during breastfeeding (Gerrard, 2011; Sokal et al., 2009). Nipple shields, typically a single molding of silicone, are available at low cost and are non-invasive, making them a suitable option for use in developing countries where breastfeeding is prevalent. However, many of the current medications associated with breastfeeding are not suitable for infants, necessitating the need for formulations that are appropriate, safe, and effective for children.

One clear example of the need for appropriate medicines for 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 yet exist in Sub-Saharan Africa, and ARVs can lead to adverse effects and resistant strains of the virus. Infection still occurs (Zahn et al., 2011).

One clear example of the need for appropriate medications in developing countries is HIV. When HIV transmission through breastfeeding (WHO, 2010b) is reduced, the infection rate is also reduced, and breastfeeding becomes more acceptable. For example, in 2000, 1.6 million children were infected with HIV through breastfeeding (WHO, 2004). However, widespread distribution of ARVs does not yet exist in Sub-Saharan Africa, and ARVs can lead to adverse effects and resistant strains of the virus. Infection still occurs (Zahn et al., 2011).

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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 insert 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 for 72 hours. To make an assay solution we measured the absorbance at 438 nm, using known SDS concentrations as standards.

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. 110 2 a-c), or weighed amounts of SDS powder were placed directly into the holder (0.1 g). Sample fluids were delivered through the SDS loaded device. Around 50 x 1 ml fractions per test were collected from the flow-through using a SuperFrac™ fraction collector (GE Healthcare Sciences, UK) to reflect typical amounts of milk consumed in a feed (Kent et al., 2006). The milk reservoir was continuously stirred to prevent fat accumulating at the top inlet. Samples were assayed in triplicate for SDS concentration using a colorimetric assay described below.

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. However, a highly reproducible further spectral shift was seen when SDS was added, thus allowing milk samples to be directly assayed for SDS concentration. The absorbance signal in milk alone, due to the presence of surfactant-like components, was measured by diluting milk samples to a fixed ratio in water prior to testing, keeping the absorbance signal caused by milk alone constant. The absorbance at 438 nm was still directly proportional to SDS concentration, allowing rapid and simple measurement in milk. Triplicate standards were assayed using an in-house SDS standard stock solution.

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, followed by thorough mixing for 30 seconds. Known SDS concentrations in milk were measured using an in-house SDS standard stock solution. 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).
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 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).

The concentrations of SDS released into early milk fractions, and the human milk itself, were both 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 by SDS 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.

3. RESULTS

3.1 Release of the edible microbicidal SDS from NSDS inserts

The release of the edible microbicidal SDS from NSDS inserts in human milk was studied using the apparatus outlined in section 2.2 and Fig. 2d. This was performed in order to provide evidence of the influence of the physiological variables within breastfeeding that could influence drug release from a NSDS. Preliminary experiments determined a suitable apparatus to mimic drug release from a drug-loaded NSDS insert. Conditions of milk flow through an NSDS insert resembling breastfeeding were achieved by maintaining the milk at 37 °C, and using a peristaltic pump to produce pulsed flow to simulate the suction produced by sucking a baby. During breastfeeding, pulse rate and volume vary greatly, so a pulse rate of 60/min with a volume of 0.07 ml per pulse was chosen that lies within the typical range of a feeding infant (Zoppou et al., 1997); this corresponds to a flow rate of 4.2 ml/min. Total feeds have been reported to have a mean of 76 g (std. dev. 12.6 g) and a range of 0-240 g per feed, i.e. mean 74 ml and range 0-240 ml of milk.
The influence of release behavior due to milk composition was studied using milk from different animal sources and varying pasteurization and homogenization. Analysis of initial release behavior provided a suitable marker for the effect of fluid type. Approximately 70% of the insert load was released after 50 ml. A higher order fit with a significantly different fluid type was observed. SDS was released into non-homogenized unpasteurized goat's milk within 10 ml, 70-90% for homogenized pasteurized cow's milk and 80% for the same milk source (Fig. 4c). SDS concentrations of above 0.1% were detected into non-homogenized cow's milk at 16 °C and 37 °C. 4°C is a commonly used temperature for infant feeding practices. The SDS concentration of milk (expressed as weight percent) exceeded by a factor of ten the concentration of SDS powder as determined by comparison to SDS powder. In all conditions tested, the majority (>70%) of SDS was released from non-woven inserts within 50 ml. A common release pattern was identified: the highest amounts of SDS releasing into early fractions, followed by decreasing concentration over time, indicating first order release kinetics. A model was fitted to the cumulative release data for all tests in the non-woven fiber insert experiments to quantify this observation (see section 3.4).

3.2 Effect of flow conditions and temperature on release kinetics

The initial focus was to identify the principal release behavior of SDS from the non-woven fiber over a range of flow conditions. This was intended to examine the basic influence of fluid kinetics on release behavior, which may vary significantly from a feeding infant using the NSDS. The effect of milk temperature upon release behavior was studied to provide evidence of the importance of fluid temperature for future laboratory studies. The release of SDS from the non-woven fiber insert into homogenized, pasteurized cow's milk at 16 °C (laboratory temperature) was similar to that detected at 37 °C (temperature of human milk) into homogenized, pasteurized cow's milk (Fig. 4a and b). Around 70-100% release was detected into non-homogenized cow's milk at 16 °C (boiled condition), in agreement with previous reports (Fig. 4c). The release of SDS from non-woven fibers was significantly higher than that from SDS powder in a flow chamber. The influence of two types of flow conditions were compared between tests: the pulse rate (how quickly the infant sucks) and the pulse volume (how much milk is extracted from the breast per suck); these were controlled by altering the size of tubing used by the peristaltic pump and the operating speed. Two test sets were run using non-homogenized pasteurized cow's milk; (1) maintaining the pulse rate at 60 pulses/min and varying pulse volume at 0.02, 0.07 and 0.45 ml/pulse and (2) maintaining the pulse volume at 0.07 ml/pulse and varying the pulse rate to 40, 60 and 80 pulses/min. The release results demonstrated that SDS was released into non-homogenized cow's milk with similar release for all flow conditions, with >50% of release of the disc's load after 20 ml. SDS concentrations of above 0.1% were seen for all tests in the non-woven fiber insert experiments (Fig. 4c and d). SDS concentrations of above 0.1% were detected into non-homogenized cow's milk at 16 °C and 37 °C. A high quality SDS release pattern was observed into all milk types, with the majority of release occurring within the first 20 ml. SDS concentrations of above 0.1% were seen for all tests in the non-woven fiber insert experiments (Fig. 4c and d). SDS was released into non-homogenized cow's milk at 16 °C and 37 °C. A high quality SDS release pattern was observed into all milk types, with the majority of release occurring within the first 20 ml.
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.

\[ q = \frac{qM}{k1 + k2} \]

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

\[ q = \frac{qM}{k1 + k2} \]

Using Eq. (2) for each release test \( k1 \) and \( k2 \) 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 \( k2 \) 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 \( k2 \) values for each fluid were statistically different between all fluids (p < 0.05) using unpaired, two tailed t-tests. \( k2 \), which indicates rate of release, was highest for the goat's milk, where SDS release was most rapid. \( k1 \) reflects the total maximum release expected by 1st order release kinetics. Given the total cumulative release reached 70-100% within most flows (less than 10% of release occurred after the 1st hour) for the goat's milk release tests, the model of release was highly applicable in this condition. However, it was noted that the model was not as applicable to the non-homogenized cow's milk test, where more rapid release was observed in early fractions. This suggests that the model may be less applicable to other milk compositions.

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 inactivation at least in the first position of milk passed through the insert. The 1st order release kinetics model that predicted similar release would be expected in human milk, and thus the NSDS should significantly reduce HIV infectivity if tested under experimental conditions. HIV was used as a model of cell-associated HIV. H9/HIVIIIB cells were used as a model of cell-associated HIV. The cells were spiked into human milk to mimic milk from HIV positive mothers, and were then passed through SDS loaded NSDS inserts at 60 pulses/min and 0.07 ml/pulse (used in release tests and typical of infant feeding conditions). HIV infectivity was then determined using the same apparatus and test conditions as the release tests, but using human milk. Given the time elapsing between the release tests and the inactivation tests, it was predicted that significant release of HIV would occur. The reduction of HIV infectivity was determined using a luciferase reporter gene assay and H9/HIVIIIB cells spiked into human milk. The percentage of HIV infectivity was determined by comparing the luciferase activity of the infected cells before and after passage through the NSDS insert. The reduction of HIV infectivity was determined by comparing the luciferase activity of the infected cells before and after passage through the NSDS insert.
4. DISCUSSION

4.1 Drug release into milk from the NSDS

Parameters that are expected to influence release kinetics of an API from a NSDS are drug form, support material/excipients, flow conditions and solvent type. For this study where flow conditions and milk type were changed the greatest variation in release behavior was seen between the differing milk types, with goat's milk producing the most rapid SDS release rate.

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).

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 that occurs. This polymer inclusion modulated the diffusivity of the neutral surfactant allowing one and other APIs. They thus included modulations in the drug content to produce a constant surfactant concentration during the release period. Formulations possible in feeding will include non-woven fabrics, polymeric and solubilization leaders. For the study, however conditions that approximate in vivo experiments should be tested in NSDS and drug perform as expected.

The first-order cumulative release model presented here was observed well, and the constants derived from a NSDS

4.2 Viral inactivation in human milk

There was a high inactivation of cell-associated virus in early fractions (0-1 ml) of human milk passed through the NSDS SDS-insert (> 99%), followed by a much smaller reduction in later fractions. The threshold concentration of rapidly microbicidal SDS was observed within the first 10 ml of milk and then rapidly decreased to below reported non-potential concentrations (< 0.1 wt%) as the flow rate was increased with the NSDS. The results are consistent with previous findings in other studies. The clinical implications of this finding are significant, as the concentration of SDS was observed to remain above the threshold for rapid inactivation (> 0.1 wt%) within the first 10 ml of milk through the NSDS. The high inactivation of cell-associated virus in early fractions (0-1 ml) of human milk was significant, as it was observed in the clinical implications of this finding are significant, as the concentration of SDS was observed to remain above the threshold for rapid inactivation (> 0.1 wt%) within the first 10 ml of milk through the NSDS. The high inactivation of cell-associated virus in early fractions (0-1 ml) of human milk was significant, as it was observed in the clinical implications of this finding are significant, as the concentration of SDS was observed to remain above the threshold for rapid inactivation (> 0.1 wt%) within the first 10 ml of milk through the NSDS.
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. This would lead to a higher reduction of HIV transmission in recipients, and could positivley feedback and support further research in developing infant feeding practices that are effective and acceptable. Effective and deliverable oral formulas may be considered with expressed milk via a bottle.

Aside from delivery via milk and infant milk feeding practices, 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 breastfeeding devices (Borkow et al., 2011, 2008). This could be combined with microbicide release to potentially increase viral inactivation within 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 breastfeeding (Israel-Ballard et al., 2010). For any specific application, careful consideration will be needed to determine if a disposable single use device or a re-usable one, with a replaceable drug-loaded insert would be most suitable. Furthermore, if speciflc composition and configuration of the NSDS is needed to deliver the drug in a desirable way, appropriate drug delivery devices need to be considered.

Potential increases viral inactivation within a NSDS breastfeeding device (Borkow et al., 2011, 2008) could be combined with microbicidal release to further increase viral inactivation within NSDS containing infant milk during passage through the digestive system. The incorporation of immobilized microbicidal surfaces within the NSDS may also be a viable consideration.

Using a NSDS to deliver agents other than microbicides will generally require simple direct API release rather than potentially sustained release, with the primary focus to ensure full dose release within a typical feed. Taste, solubility and the effect of the formulation on the milk would be primarily considered. Effective and deliverable oral formulas could be considered with expressed milk via a bottle.

For any specific composition and configuration of the NSDS, potential increases viral inactivation within a NSDS breastfeeding device (Borkow et al., 2011, 2008) could be combined with microbicidal release to further increase viral inactivation within NSDS containing infant milk during passage through the digestive system. The incorporation of immobilized microbicidal surfaces within the NSDS may also be a viable consideration.

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are inventors of the nipple shield delivery system (patent pending US 12/536,219, PCT/US10/44589).

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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 in human milk. The NSDS is especially valuable for use in developing countries where no safer alternative to breastfeeding exists. The NSDS has also demonstrated that its presence over the mother’s breast can prevent the spread of HIV to the infant. A sustained release of the edible microbicide SDS into HIV infected milk during breastfeeding from a NSDS is a promising method for the prevention of MTCT of HIV. This study has demonstrated that a NSDS can deliver SDS into human milk and has shown that HIV can be inactivated by the NSDS.

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### Table 1

<table>
<thead>
<tr>
<th>Fluid Temperature (°C)</th>
<th>Pasteurized</th>
<th>Homogenized</th>
<th>Total Release (initial load)</th>
<th>k1</th>
<th>k2</th>
<th>R²</th>
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<td>x</td>
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</table>

### Table 2

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<th>Fluid Temperature (°C)</th>
<th>Pasteurized</th>
<th>Homogenized</th>
<th>Total Release (initial load)</th>
<th>k1</th>
<th>k2</th>
<th>R²</th>
</tr>
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<tbody>
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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 Eqn. (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

Pasturised cow's milk was flowed through SDS loaded onto non-woven fibre discs (a-d) or SDS powder (e) 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 measured. 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 as a line in each case. SDS release is significantly greater than background levels in all cases.

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

The release of SDS from loaded non-woven discs through non-homogenised cow, milk (a) or non-homogenised sheep (b) milk was measured with a flow rate of 4.3 ml/min and pulse rate of 60 pulses/min. Data are displayed as concentration of SDS in collected 1 ml fractions and cumulative SDS release relative to input disc load. In all cases, each set of symbols represents an individual release experiment, with the mean of triplicate measurements displayed as a line in each case. SDS release is significantly greater than background levels in all cases.

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 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 5ml aliquots were collected to measure infectivity. The standard error of repeat measurements is displayed.
REFERENCES


SDS concentration in milk (wt%)
Absorbance 438 nm

1 in 10 milk dilution
0.03 to 0.5 wt%

1 in 100 milk dilution
0.5 to 5.0 wt%

Figure 3.
Cumulative SDS release fraction of input SDS in each fraction (wt%)
Cumulative SDS release fraction of input SDS in each fraction (wt%)

Fig. 5.

Total volume passed through SDS insert (ml)

ai) Non-homogenized unpasteurized goat’s milk 37 ºC

bi) Homogenised pasteurised cow’s milk

cii) Non-homogenised pasteurised cow’s milk 37 ºC

cii) Homogenised pasteurised cow’s milk 37 ºC
Fig. 6.

**a**

3 repeat experiments

**b**

Original H9/HIV\textsuperscript{Illb} cellular content

\(0\) not detected

Lower limit of H9/HIV\textsuperscript{Illb} cellular content detection

Volume of human milk passed through SDS insert (ml)

Correlated infected H9/HIV\textsuperscript{Illb} cellular content (cells/ml x 10\textsuperscript{4})

\(\text{RLUs}\)