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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. 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 microbicide impregnated insert. 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 microbicide was rapidly released. The full 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 microbicide was rapidly released. The full SDS release from inserts ranged from 70-100% of the average 0.07 g load within 50 ml (the volume of a typical breastfeed).

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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
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 used to deliver drugs and nutrients. Many current medicines are only available in adult strength, so safe and accurate dosing for an infant is complicated (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 yet exist in Sub-Saharan Africa, and ARV use can lead to side effects and resistant strains of the virus. Prevention of MTCT of HIV during breastfeeding may be an effective method of reducing viral load in milk when delivered to the baby has been previously considered (Hartmann et al., 2006a). Sodium dodecyl (or lauryl) sulfate (SDS), an anionic surfactant, is a candidate for use as an edible microbicide with anti-HIV activity in human milk. It has been demonstrated that 0.1–1 wt% SDS rapidly kills sexually transmitted pathogens, including HIV in media (Howett et al., 2000, 1999; Krebs et al., 2000, 1999). 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). At this concentration, SDS is safe for infant use, based on a maximum acceptable oral exposure to SDS of 1 g/kg (of infant)/day and an independent 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 the delivery of SDS during breastfeeding may be an effective method of reducing viral load in milk, we propose a new method of delivering MTCT of HIV to infants during breastfeeding that also 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 (e.g., when mother is sick). A single molding of silicone is required for each use of the NSDS. The NSDS insert could be made from non-woven fiber, representing a flexible, high surface area support for drug incorporation. The mother would wear the NSDS as her child breastfeeds, and as milk passes through the insert the API would be released directly into the milk and pass to the infant, thus avoiding the need for administration of the drug orally in each feed. This method would be expected to be effective and superior to drug delivery, yet also avoided due to high cost and lack of access to refrigeration or potable water for reconstitution (Knoppert, 2009; WHO, 2010c). Liquid formulations are also the preferred mode of administration and distribution, and in some cases delivery of drugs to remote areas is often seen as difficult (Kearns et al., 2003).
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, Quebec, 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 until their weight remained constant, which took 12 to 24 hours. The inserts were then stored at room temperature. To make an assay solution sufficient to analyze 250 samples, 20 μl of stains-all dye (Sigma-Aldrich, UK) was dissolved in 1 ml of a 30 wt% SDS solution in water. To this stock solution, 19 ml of 1:1 isopropanol:water was added and thoroughly mixed for 30 seconds. This mixture was stored at room temperature.

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. 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. Individual fractions 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, by diluting milk sample aliquots to 1:2.5, 1:10 or 1:100 by volume with ultrapure MilliQ water and measuring the absorbance at 438 nm, the absorbance signal caused by milk alone could be maintained at a constant level. The absorbance at 438 nm was then directly proportional to SDS concentration. A range of dilution factors were used to accurately detect concentrations of SDS in milk above 0.03 wt%. SDS concentration in test samples was calculated by comparison to a calibration curve measured at the same sample dilution, using standard SDS solutions made in identical milk from a continuously stirred 5% wt/vol. (milk) SDS 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 cells/ml 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 microbicide SDS from NSDS inserts

The release of SDS from a NSDS insert in a mimicked breastfeeding simulation environment was studied using the apparatus outlined in section 2.2 and Fig. 2d. This was performed 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 were achieved by maintaining the milk at 37 °C and using a peristaltic pump to produce pulses that flow to simulate the suction minimizing the milk at 27 °C and using a peristaltic pump to produce pulses that flow to simulate the suction encountered by microporous membranes that encase the drug-loaded NSDS insert. This setup produced milk flow conditions similar to those encountered during natural breastfeeding.

In preliminary experiments, the release of SDS from the NSDS insert was found to be dependent on the milk flow rate, with a peak release occurring at a flow rate of 4.2 ml/min, which is within the typical range of a feeding infant (Zoppou et al., 1997). 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. The pH of milk was adjusted to 6.9, which corresponds to a pH of +2°C milk, and the final pH of +2°C milk. The final pH was measured to be within the physiological range of a recording of 6.0, which is within the optimal pH range for SDS to maintain its activity. A pH of +2°C milk was chosen, since it was previously found that SDS activity in milk decreased by 50% at +2°C milk and +2°C milk was assessed to be within the optimal range for SDS activity.

The release of SDS from the NSDS insert in a mimicked breastfeeding simulation environment was studied by adding a known volume of milk fractions to a 96-well plate and assaying for HIV-infectivity using TZM-bl cells. In preliminary experiments, the release of SDS was found to be dependent on the milk flow rate, with a peak release occurring at a flow rate of 4.2 ml/min, which is within the typical range of a feeding infant (Zoppou et al., 1997). 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. The pH of milk was adjusted to 6.9, which corresponds to a pH of +2°C milk, and the final pH of +2°C milk. The final pH was measured to be within the physiological range of a recording of 6.0, which is within the optimal pH range for SDS to maintain its activity. A pH of +2°C milk was chosen, since it was previously found that SDS activity in milk decreased by 50% at +2°C milk and +2°C milk was assessed to be within the optimal range for SDS activity.
The influence of release behavior due to milk composition was studied using milk from different animal sources with varying pasteurization and homogenization. Analysis of initial release behavior provided a suitable marker for the effect of different fluid types. In all conditions tested, the majority (>70%) of SDS was released from non-woven inserts within 50 ml. A common release pattern presented itself: the highest amounts of SDS releasing into early fractions, followed by decreasing concentration over time, indicating approximately first order release kinetics. A model was fitted to the cumulative release data for each experiment to qualify this observation (see section 3.5).

3.2 Effect of flow conditions and temperature and insert form 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 after 30 ml in all tests. This suggests that milk temperature is not a significant factor affecting SDS release rate from the non-woven fiber.

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 at similar rates for all these flow rate conditions, with >50% of release of the disc's load after 20 ml for all tests (Fig. 4c and d). SDS concentrations of above 0.1 wt% SDS (previously reported to be highly anti-viral—see 1. Introduction) were seen for the tests in the first 20 ml of milk that passed through the non-woven fiber insert.

The influence of the non-woven fiber on SDS release was determined by comparison to SDS powder placed into the insert holder. 0.1 g of SDS powder was used for each test. Similar release patterns were seen compared to SDS powder when SDS was released into homogenized, pasteurized cow's milk at 37°C. Release from the flow chamber ranged from 40% to 70% after 50 ml for 16°C pasteurized and homogenized cow's milk and 80% for the same milk source (Fig. 4e). The influence of milk composition on release kinetics was studied using milk from different animal sources with varying pasteurization and homogenization. Analysis of initial release behavior provided a suitable marker for the effect of different fluid types. In all conditions tested, the majority (>70%) of SDS was released from non-woven inserts within 50 ml. A common release pattern presented itself: the highest amounts of SDS releasing into early fractions, followed by decreasing concentration over time, indicating approximately first order release kinetics. A model was fitted to the cumulative release data for each experiment to qualify this observation (see section 3.5).

3.3 Effect of milk composition on release kinetics

The influence on release behavior due to milk composition was studied, using milk from different animal sources and with varying pasteurization and homogenization. Analysis of initial release behavior provided a suitable marker for the effect of different fluid types. In all conditions tested, the majority (>70%) of SDS was released from non-woven inserts within 50 ml. A common release pattern presented itself: the highest amounts of SDS releasing into early fractions, followed by decreasing concentration over time, indicating approximately first order release kinetics. A model was fitted to the cumulative release data for each experiment to qualify this observation (see section 3.5).

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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 (Eq. (1)) for fixed flow and temperature conditions.

$$qM = k_1 (qM)^{k_2}$$

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

$$q = k_1 (qM)^{k_2}$$

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 R² > 0.969 for all tests apart from one with the highest flow rates, with R² 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, the amount of HIV inactivation at least in the first position of milk passed through the insert predicted that similar release would be expected in human milk, and thus the NSDS showed significantly more rapid release into human milk than the non-woven disc insert used in these studies. HIV inactivation (inactivation to a level of at least 99.99%) was studied using the same apparatus and test conditions as the release studies, but using human milk. For this second element of this proof of concept study the reduction of cell-associated HIV by SDS was

3.5 HIV inactivation by a SDS loaded NSDS insert

HIV inactivation was developed into an algorithm to measure the inactivation of HIV in milk and was anticipated to reduce the apparent and incorporate HIV-1 with removal of the HIV protease and reverse transcriptase. TZM-bl cells, which express HIV-1, were spiked into human milk at various dilutions and used as the control for each test. The cells were treated with human milk with minimal SDS present (20% of total volume) to prevent HIV-1 infection as the control for each test.

$$\text{Volume of fluid passed through insert (ml)} = \frac{b}{p} \cdot \left( \frac{b}{p} \right)^{k_2}$$

For an initial model it was proposed that total drug release was dependent on the fraction of SDS released.
4.2 Viral inactivation in human milk

SDS release into milk

combination of suspension phenomena and solid and liquid particulate release from the fiber only
when the model is least well: Further work is required to refine the model. We propose that a
not encompass all the actions influencing release from the non-woven fiber, especially in higher flow rates
not shown in this work. Further, the authors provided no information about the nature of the SDS
not shown in this work. Further, the authors provided no information about the nature of the SDS
understood 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 hydroxyethyl methyl cellulose into the SDS insert during formulation may result in slower SDS release into milk and reduce the initial rapid release that was observed in the present study. The authors included in vitro data showing the current inactivation mechanism of SDS only one of three possible in vivo mechanisms that should be considered for SDS and other APIs. Further work is required to refine the model. We postulate that a combination of dissolution phenomena and solid and hydrated particulate release from the fibers govern SDS release from fibers.

4.2.1 Drug release into milk from the NSDS

individual fractions (F6, 66).

mean correlated infected cell content from NSDS-negative milk with the NSDS H9-positive milk was 0.70 ± 0.06. Using this method and then performing SDS-negative milk were then determined to be below reported microbicidal concentrations (thrombomodulin and thrombin-antithrombin III complex). The unbound association of H9 HIV IIIB cell cultures had the highest correlation between H9 HIV IIIB cell cultures and the measured infected cell content from NSDS-negative milk.
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 who consumed milk from a feed where SDS was delivered in early fractions. Further study will be required to better predict the effectiveness of a given NDSD in reducing HIV transmission in recipients who consumed milk from a feed where SDS was delivered in late fractions.

The incorporation of immobilized microbicidal surfaces within the NDSD may also be a viable consideration for preventing MTCT of HIV specifically by inactivating virus during passage through the NDSD. 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 NDSD.

4.3 Future uses of the NDSD

The acceptability of a NDSD to breastfeeding mothers must be carefully assessed prior to use. 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 NDSD to prevent HIV transmission in feeding (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.

Aside from delivering anti-viral agents, NDSDs can also be used to deliver medications or combinations of nutritional substances. For example, using a NDSD, milk could be fortified with vitamins, minerals, or probiotics to improve infant health. Alternatively, a NDSD could be used to deliver medications such as antibiotics or antimalarials.

Using a NDSD to deliver antibiotics before a milk feed could reduce the number of antibiotic-resistant bacteria in the gut. This could be particularly important in regions with high rates of antibiotic resistance. Additionally, a NDSD could be used to deliver vaccines to infants who are unable to receive them through oral administration. This could be particularly important in areas with high rates of childhood illness and mortality.

The NDSD could also be used to deliver medications to preterm infants who are at risk of developing infections. Preterm infants are particularly vulnerable to infections due to their immature immune systems. A NDSD could be used to deliver medications to prevent or treat infections in these infants.

In conclusion, the NDSD has great potential for preventing MTCT of HIV and other infections during breastfeeding. Further research is needed to determine the most effective way to deliver anti-viral agents and other medications using the NDSD.

2006a, 2006b, 2005). This suggests that the initial high release behavior of SDS observed in goat’s milk may be a suitable mimic for use in human milk and may be used to predict the release behavior of other anti-viral compounds. However, further work is needed to understand how milk composition affects release kinetics.

NSDS release studies have shown that the initial high release behavior of SDS observed in goat’s milk may be a suitable mimic for use in human milk and may be used to predict the release behavior of other anti-viral compounds. However, further work is needed to understand how milk composition affects release kinetics.
are inventors of the nipple shield delivery system (patent pending US 12/362,195, PCT/US10/44589).

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. SDS release using the NSDS is capable of rapidly inactivating significant amounts of cell-associated HIV. SDS release using the NSDS is capable of rapidly inactivating significant amounts of cell-associated HIV.

ACKNOWLEDGMENTS AND ASSOCIATIONS

We are grateful to the Bill and Melinda Gates Foundation, the Clinton Global Initiative, the UK EPSRC, Cambridge University, and King’s College. We thank Wobbly Bottom Farm, Hertfordshire for their supply of goat’s milk and Pauline Sakamoto of the Milk Bank, Santa Clara Valley Medical Centre (San Jose, California) for coordination use of human milk samples. We thank the JustMilk team including Geoff Galgon, Elizabeth King, Ryan Hubbard, Arron Rodrigues of EWH Cambridge, Krishnaa Mahbubani, Yucy Fang, Samantha Gooneratne and David McNally of the Bioscience Engineering Group, Department of Chemical Engineering and Biotechnology, University of Cambridge, UK. Peter Patiris, Leo Oceguera and Haynes Sheppard of the California Department of Public Health, Richmond and David Jenkins of FHI 360 for helpful discussions. Stephen Gerrard and David Sokal are inventors of the nipple shield delivery system (patent pending: US 12/362,195, PCT/US10/44589).
Summary of SDS release experiments using cow’s and goat’s milk for constant flow conditions.

<table>
<thead>
<tr>
<th>Graph</th>
<th>Pasteurised</th>
<th>Homogenised</th>
<th>Total</th>
<th>Fluid Temp (°C)</th>
<th>Pulse Rate (pulses/min)</th>
<th>Pulse Volume (ml/pulse)</th>
<th>Initial Load (ml)</th>
<th>k1 (ml⁻¹)</th>
<th>k2 (ml⁻¹)</th>
<th>r² Graph</th>
<th>Ref</th>
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<tbody>
<tr>
<td>5a</td>
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<td>0.80</td>
<td>0.794</td>
<td>0.185</td>
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<td>5b</td>
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<tr>
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<td>1.180</td>
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<td>0.994</td>
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<tr>
<td>4e</td>
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Fitted model parameters to a first-order release kinetic model according to Equ. (2) also displayed.

Summary of SDS release experiments using cow’s milk with varying flow conditions.

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<tr>
<th>Fluid Temp (°C)</th>
<th>Pasteurised</th>
<th>Homogenised</th>
<th>Total</th>
<th>k1 (ml⁻¹)</th>
<th>k2 (ml⁻¹)</th>
<th>r² Graph</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>Cow</td>
<td>0.95</td>
<td>0.939</td>
<td>0.132</td>
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<td>0.125</td>
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<td>0.939</td>
<td>0.132</td>
<td>0.995</td>
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</table>

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

**Figure 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.

**Figure 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.

**Figure 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 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 as (i) mean concentration of SDS in individual collected 1 ml fractions and (ii) cumulative SDS release relative to input SDS load. In all cases, individual collected 1 ml fractions and cumulative SDS release relative to input SDS load were displayed as mean ± standard error.

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

The release of SDS from loaded non-woven discs was measured with milk type of (a) homogenised pasturised cow's milk, (b) non-homogenised cow's milk and (c) non-homogenised unpasturised goat's milk. Data are displayed as concentration of SDS in collected 1 ml fractions (i) and cumulative SDS release relative to input disc load (ii). In all cases, each set of symbols represents an individual release experiment, with the mean of triplicate measurements displayed as (i) mean concentration of SDS in individual collected 1 ml fractions and (ii) cumulative SDS release relative to input disc load. In all cases, individual collected 1 ml fractions and cumulative SDS release relative to input disc load were displayed as mean ± standard error.

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

**(a)** Calibration curve used to determine H9/HIVIIIB cell content in milk; TZM-bl reporter cells were infected with a range of H9/HIVIIIB cell concentrations in milk and assayed for infection by luminescence reporter activity. Reporter activity (infectivity) is plotted as the equivalent number of H9/HIVIIIB cells, calculated using the calibration assay shown. 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 between repeat measurements is displayed.

Average reduction in HIV infectivity was significant with p < 0.0001 (**) or p < 0.05 (*) based on paired t-test.


Figure 1a
Click here to download high resolution image
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

Figure 3: SDS in water

SDS concentration in milk (wt%) - Absorbance 438 nm
Cumulative SDS release fraction of input

Fig. 4.

Total volume passed through SDS insert (ml)

Cumulative SDS release fraction of input (wt%)

- SDS insert at 16 ºC
- SDS insert at 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

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

- 60 pulses/min
- 0.07 ml/pulse

- 60 pulses/min
- 0.02 ml/pulse

- 60 pulses/min
- 0.45 ml/pulse

- SDS insert at 37 ºC
- SDS insert at 16 ºC
Fig. 5. Cumulative SDS release fraction of input [SDS] in each fraction (wt%).

- Total volume passed through SDS insert (ml)

- ai) Non-homogenized unpasteurized goat's milk 37 ºC
- bi) Homogenised pasteurised cow's milk 37 ºC
- ci) Non-homogenised pasteurised cow's milk 37 ºC
Correlated infected H9/HIV<sub>IIIB</sub> cellular content (cells/ml x 10<sup>4</sup>)

Volume of human milk passed through SDS insert (ml)

**Fig. 6.**

**a**

3 repeat experiments

**b**

ND - not detected

Lower limit of H9/HIV<sub>IIIB</sub> cellular content detection

Original H9/HIV<sub>IIIB</sub> cellular content

RLUs