A nipple shield delivery system for oral drug delivery to breastfeeding infants: Microbicide delivery to inactivate HIV

Article

Accepted Version


It is advisable to refer to the publisher’s version if you intend to cite from the work.
Published version at: http://www.sciencedirect.com/science/article/pii/S0378517312005200
To link to this article DOI: http://dx.doi.org/10.1016/j.ijpharm.2012.05.035

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the End User Agreement.
www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online
*Graphical Abstract*
A Nipple Shield Delivery System for Oral Drug Delivery to Breastfeeding Infants: Microbicide Delivery to Inactivate HIV

Stephen E. Gerrard, a,b,c*, Mary L. Banieki, David C. Sokal, d Mary K. Morris, e Sandra Urdaneta-Hartman, f Fred C. Krebs, g Brian Wigdale, h Barbara F. Abrams, i Carl V. Hanson, j Nigel K. H. Slater, k Alexander D. Edwards g*

a BioScience Engineering Research Group, Department of Chemical Engineering and Biotechnology, University of Cambridge, New Museums Site, Pembroke Street, Cambridge, United Kingdom. stephen.gerrard@cantab.net, tel: +44 (0) 1223 763969, fax: +44 (0) 1223 334796
b Viral and Rickettsial Disease Laboratory, California Department of Public Health, Richmond, CA, USA.
c Division of Epidemiology, School of Public Health, University of California, Berkeley, CA, USA.
d FHI 360, Durham, NC, USA.
e Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, USA.
f Department of Obstetrics and Gynecology, Drexel University College of Medicine, Philadelphia, PA, USA.
g Reading School of Pharmacy, Whiteknights, Reading, United Kingdom.

*Corresponding authors

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 efficiently inactivate HIV in human milk. A proof of concept study demonstrates efficient drug 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

NSDS, Nipple shield delivery system

SDS, Sodium dodecyl sulfate

RLU, Relative luminescent units

MTCT, Mother-to-child transmission

NSDS, Nipple shield delivery system

SDS, Sodium dodecyl sulfate

*Manuscript - Resubmission

Click here to view linked References
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 is often the only available method for administration of medicine. 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. Infections still occur (Zahn et al., 2011).

As an alternative approach, the administration of edible microbicides into expressed infected milk which is then delivered to the baby has been previously considered (Kearns et al., 2003). 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 free 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 a 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, we propose a new method to deliver SDS 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 in latching on (Riordan, 2005). The NSDS would have an insert containing a dose of the API in dried form. In the studies reported in this publication, NSDS inserts were 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. The NSDS would be placed inside the milk prior to each feed to ensure the API is absorbed into the milk and reaches the infant. For each feeding, the mother would wear the NSDS in the nipple shield, which is then discontinued after each use. The NSDS could also be washed, disinfected, and reloaded with another insert for reuse.
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 for 72 hours to ensure complete evaporation of the SDS solution. SDS concentration was quantified using a standard SDS solution in milk (as described below). To verify the success of this process, SDS concentration was measured in each sample and found to be constant across all tests.

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), or weighed amounts of SDS powder were placed directly into the holder (0.1 g). Sample fluids were passed through the SDS loaded device using 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 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. 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, a highly reproducible further spectral shift was seen when SDS was added. By mixing milk samples with SDS at different concentrations and measuring the absorbance at 438 nm, a calibration curve was generated to determine SDS concentration in milk samples. The calibration curve was constructed 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 of SDS concentrations in milk (as described below).

2.3 Determination of non-surfactant SDS in milk

A number of non-surfactant SDS solutions were assayed in triplicate to determine the presence of SDS in milk. Samples were taken from a range of milk sources, including pasteurized and homogenized cow’s milk, unpasteurized goat milk, and other milk products. SDS concentration was determined using the stains-all colorimetric assay as described above. The results showed that SDS was present in all milk samples tested, indicating that SDS is a common contaminant in milk products.
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 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) to remove HIV particles from the milk. 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. 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. After washing, 25 μL of culture medium was added to 25 μL washed sample and 50 μL TZM-bl cells at 2 x 10^5 cells/ml were added to flat bottomed 96-well plates. After 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. 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 that resemble breastfeeding were achieved by maintaining the milk at 37 °C and using a peristaltic pump to produce pulsing flow to simulate the suction encountered by the milk at the exit of the NSDS insert. The pulsing flow was produced by the peristaltic pump and was found to closely resemble the pulsing flow (pulse rate of 60/min with a volume of 0.07 ml per pulse) 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, which results in a milk flow rate of 0.07 ml per pulse for the reported range of a feeding infant.

Human milk samples were provided by the Mothers' Milk Bank, Valley Medical Centre (San Jose, California, USA). Human milk was received and used to measure HIV infectivity in milk samples collected from the NSDS. 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), and the milk was pumped through the NSDS for a period of 10 minutes. SDS was then released from the NSDS and the milk was sampled at 1, 2, 6 and 10 hours after SDS release.
The influence of milk composition on the release kinetics of SDS from the non-woven fiber inserts. The release of SDS from the non-woven fiber inserts was performed with homogenized pasteurized and non-homogenized cow's milk, with and without milk content as a control. The release of SDS from the non-woven fiber inserts was determined by a spectrophotometric assay (see section 2). The release of SDS from the non-woven fiber inserts was compared to SDS powder placed into the same holder. SDS powder was used in a test to confirm the release patterns were seen.

The influence of milk composition on the release kinetics of SDS from the non-woven fiber inserts. The release of SDS from the non-woven fiber inserts was performed with homogenized pasteurized and non-homogenized cow's milk, with and without milk content as a control. The release of SDS from the non-woven fiber inserts was determined by a spectrophotometric assay (see section 2). The release of SDS from the non-woven fiber inserts was compared to SDS powder placed into the same holder. SDS powder was used in a test to confirm the release patterns were seen.

The influence of milk composition on the release kinetics of SDS from the non-woven fiber inserts. The release of SDS from the non-woven fiber inserts was performed with homogenized pasteurized and non-homogenized cow's milk, with and without milk content as a control. The release of SDS from the non-woven fiber inserts was determined by a spectrophotometric assay (see section 2). The release of SDS from the non-woven fiber inserts was compared to SDS powder placed into the same holder. SDS powder was used in a test to confirm the release patterns were seen.

The influence of milk composition on the release kinetics of SDS from the non-woven fiber inserts. The release of SDS from the non-woven fiber inserts was performed with homogenized pasteurized and non-homogenized cow's milk, with and without milk content as a control. The release of SDS from the non-woven fiber inserts was determined by a spectrophotometric assay (see section 2). The release of SDS from the non-woven fiber inserts was compared to SDS powder placed into the same holder. SDS powder was used in a test to confirm the release patterns were seen.

The influence of milk composition on the release kinetics of SDS from the non-woven fiber inserts. The release of SDS from the non-woven fiber inserts was performed with homogenized pasteurized and non-homogenized cow's milk, with and without milk content as a control. The release of SDS from the non-woven fiber inserts was determined by a spectrophotometric assay (see section 2). The release of SDS from the non-woven fiber inserts was compared to SDS powder placed into the same holder. SDS powder was used in a test to confirm the release patterns were seen.

The influence of milk composition on the release kinetics of SDS from the non-woven fiber inserts. The release of SDS from the non-woven fiber inserts was performed with homogenized pasteurized and non-homogenized cow's milk, with and without milk content as a control. The release of SDS from the non-woven fiber inserts was determined by a spectrophotometric assay (see section 2). The release of SDS from the non-woven fiber inserts was compared to SDS powder placed into the same holder. SDS powder was used in a test to confirm the release patterns were seen.

The influence of milk composition on the release kinetics of SDS from the non-woven fiber inserts. The release of SDS from the non-woven fiber inserts was performed with homogenized pasteurized and non-homogenized cow's milk, with and without milk content as a control. The release of SDS from the non-woven fiber inserts was determined by a spectrophotometric assay (see section 2). The release of SDS from the non-woven fiber inserts was compared to SDS powder placed into the same holder. SDS powder was used in a test to confirm the release patterns were seen.
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.

\[
\frac{q}{M} = \frac{k_1}{k_2} g^{1-6} (1)
\]

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

\[
\int_0^q \frac{q}{M} \, dq = \int_0^1 k_1 \exp(1 - k_2 (1 - \frac{q}{M})), (2)
\]

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 reached up to 1, significant differences between all fluids would be expected in human milk. While the NDS showed similarly release behavior for the goat’s milk, where SDS release was most rapid, k_1 reflects the total volume of fluid passed through the insert, which influences the fraction of SDS released.

3.5 HIV inactivation by a SDS loaded NDS insert

For the final element of the 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 NDS should significantly reduce the amount of HIV infectivity at least in the first position of milk passed through the insert. 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.

\[
\frac{q}{M} = \frac{k_1}{k_2} g^{1-6} (1)
\]

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

\[
\int_0^q \frac{q}{M} \, dq = \int_0^1 k_1 \exp(1 - k_2 (1 - \frac{q}{M})), (2)
\]

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 reached up to 1, significant differences between all fluids would be expected in human milk. While the NDS showed similarly release behavior for the goat’s milk, where SDS release was most rapid, k_1 reflects the total volume of fluid passed through the insert, which influences the fraction of SDS released.
4.2 Viral inactivation in human milk

SDS release from the NSDS appears to follow a first-order approximation. This order is supported by the observation that the measured release of SDS correlates with the changes observed in the milk samples. The amount of SDS released into the milk was calculated using the first-order approximation model, which was then compared to the SDS concentrations in the milk samples. The calculated SDS concentrations in the milk samples were found to be consistent with the theoretical predictions, indicating that the model can be used to predict SDS release in milk.

The first-order approximation model is valid when the concentration of SDS in the milk is low. However, at higher concentrations, the release kinetics may deviate from the first-order approximation. In such cases, more complex models may be required to accurately predict SDS release.

4.3 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. In 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 hydroxethyl propyl cellulose into the SDS insert during formulation may result in slower SDS release into milk and reduce the initial high SDS release from the NSDS.

4.4 Discussion

Indirect evidence (Y), (66), that milk production has increased in recent years has been observed between reduced HPAI H5N1 virus detection in milk and the increased milk production in the USA. The reported reduction of 0.1% HPAI H5N1 virus detection (Y) also occurred with the first 10 ml of milk. The reported reduction of 0.1% HPAI H5N1 virus detection (Y) also occurred with the first 10 ml of milk passed through the NSDS insert (>99%), followed by a much smaller reduction in later fractions. The NSDS-inserted milk was then determined to be free of reported HPAI H5N1 virus detection (Y). 0.1% HPAI H5N1 virus detection (Y) was observed in 99% of human milk passed through the NSDS-inserted milk. The use of a NSDS-inserted milk was expected to effectively reduce the risk of HPAI H5N1 virus detection in milk.

When known doses of HPAI H5N1 cell cultures were assayed with this method, using TZM-bl cells, associated HPAI H5N1 by the NSDS, whereby both human milk and SDS were removed 20 minutes after
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 accordingly, a greater possibility of an effective device delivered to infants fed with formula or expressed milk via a bottle. Effective devices delivered to infants fed with formula or expressed milk via a bottle.

Aside from SDS delivery, a wide range of individual or combinations of antimicrobial substances could be considered.

The acceptability of a NSDS 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 NSDS 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 more suitable. Further study will be required to better predict the effectiveness of a given NSDS in reducing HIV transmission in recipients. In addition, if promising MTCT of HIV drug-releasing devices are developed in Kenya, it would be important for HIV drug-releasing devices to be considered by other agencies involved in the development of the devices.

Potential advantages of the NSDS over other infant drug delivery routes and devices include ease of use,

4.3 Future uses of the 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 breastfeeding devices (Borkow et al., 2011, 2008). This could be combined with microbicide release to potentially increase viral inactivation within a NSDS.

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). 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). 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).
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 non-human milk. Future work is needed to fully understand the effects of milk composition on release kinetics. Modifying the non-woven fiber composition, the addition of cellulose based compounds onto the fiber, or the addition of microbicides and cellulose in fiber construction, may enable controlled release patterns. With better understanding of the sites of transmission in breastfeeding these methods could be adapted to provide maximum reduction of MTCT of HIV.

ACKNOWLEDGMENTS AND ASSOCIATIONS

We are grateful to the Bill and Melinda Gates Foundation, the Clinton Foundation (Clinton Global Initiative), the UK EPSRC, the Cambridge University - UC Berkeley Exchange, and the International Design Development Summit (Cambridge University - Pembroke College). The authors would like to thank the JustMilk team including Geoff Galgon, Elizabeth Kneen, Ryan Hubbard, Arron Rodrigues of EWHCambridge, Krishna Mahbubani, Yucy Fang, Samantha Gooneratne and David McNally of the Bioscience Engineering Group, Department of Chemical Engineering and Biotechnology, University of Cambridge, Elizabeth Kneen, Ryan Hubbard, Tomo Banda (inventors), Anou Koudinge of Goel, and Pauline Sakamoto of the Milk Bank, Santa Clara Valley Medical Centre (San Jose, California, USA) for their supply of goat’s milk and Paulette Renzoni of the Milk Bank, Santa Clara Valley Medical Centre (San Jose, California, USA) for coordinating use of human milk samples. We thank the JustMilk team including Geoff Galgon, Elizabeth Kneen, Ryan Hubbard, and Arron Rodrigues of EWHCambridge, Cambridge University - Pembroke College (Cambridge University - Pembroke College, Pembroke College (Cambridge University), Pembroke College (University of Cambridge), Pembroke College (University of Cambridge), and Pembroke College (University of Cambridge) for their support. We thank Wobbly Bottom Farm, Hertfordshire for supplying goat’s milk, and Pauline Sakamoto of the Milk Bank, Santa Clara Valley Medical Centre (San Jose, California, USA) for coordinating use of human milk samples.
<table>
<thead>
<tr>
<th>Fluid</th>
<th>Temp (°C)</th>
<th>Pulse Rate (pulses/min)</th>
<th>Pulse Volume (ml/pulse)</th>
<th>Total release (ml)</th>
<th>Initial load (ml)</th>
<th>Homogenized Ref</th>
<th>Pasteurized Ref</th>
<th>Flow Cell Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurized</td>
<td>37.0</td>
<td>0.70</td>
<td>0.07</td>
<td>0.80</td>
<td>0.794</td>
<td>0.185</td>
<td>0.984</td>
<td>5a</td>
</tr>
<tr>
<td>Pasteurized</td>
<td>37.0</td>
<td>1.14</td>
<td>1.180</td>
<td>0.069</td>
<td>0.994</td>
<td></td>
<td></td>
<td>4c</td>
</tr>
<tr>
<td>Pasteurized</td>
<td>37.0</td>
<td>1.04</td>
<td>1.026</td>
<td>0.183</td>
<td>0.994</td>
<td></td>
<td></td>
<td>5b</td>
</tr>
<tr>
<td>Pasteurized</td>
<td>37.0</td>
<td>1.07</td>
<td>1.057</td>
<td>0.416</td>
<td>0.978</td>
<td></td>
<td></td>
<td>5c</td>
</tr>
<tr>
<td>Pasteurized</td>
<td>37.0</td>
<td>1.02</td>
<td>1.030</td>
<td>0.452</td>
<td>0.995</td>
<td></td>
<td></td>
<td>4d</td>
</tr>
</tbody>
</table>

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

In a first-order release kinetic model according to Eqn. (2) is also displayed.
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 matrices in which a range of H9/HIV cell concentrations is measured and assessed for interaction with milk and assessed for interaction with milk. 

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. 

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 60 pulses/min was measured. Data displayed as (i) concentration of SDS in 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 individual measurements of SDS concentration in each 1 ml fraction shown.

Fig. 5. Effect of milk type on SDS release kinetics
The release of SDS from loaded non-woven fibers during pulsed flow into (a) homogenised pasturised cow's milk, (b) non-homogenised cow's milk and (c) non-homogenised unpasturised goat's 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 (i) and cumulative SDS release relative to input disc load (ii). In all cases, SDS release was measured with a flow rate of 4.3 ml/min and pulse rate of 60 pulses/min. 

Fig. 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/HIV cell concentrations in milk and assayed for interaction with milk. 

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

Fig. 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) Non-homogenized pasteurized cow's milk

ci) Homogenized pasteurized cow's milk 37 ºC
**Fig. 6.**

- **b**

Correlated infected H9/HIVIIIB cellular content

- Original H9/HIVIIIB cellular content

- Lower limit of H9/HIVIIIB cellular content detection (ND - not detected)

- 3 repeat experiments

- Volume of human milk passed through SDS insert (ml)

**Fig. 6.**

- RLU

<table>
<thead>
<tr>
<th>RLU</th>
<th>0.01</th>
<th>0.1</th>
<th>1</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1000</td>
<td>10000</td>
<td>100000</td>
<td>0 5 10 15 20 25 30</td>
<td></td>
</tr>
</tbody>
</table>