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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 SDS and the microbicide was rapidly released. The total SDS release from insert ranged from 70-100% of the average 0.07 g load within 50 ml (the volume of a typical breastfeed). Human milk spiked with HIV/H9/HIVIIIB cells was also passed through the same system. 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 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 (of HIV)
NSDS, Nipple shield delivery system
RLU, Relative luminescent units
SDS, Sodium dodecyl sulfate

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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 is required, which is more challenging to administer and may require undesirable toxic excipients, such as preservatives and solvents. 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 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, '...avoidance of all breastfeeding by HIV infected mothers is recommended...' (WHO, 2000b). 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 (Gray, 2000; Lurie et al., 2001). As an alternative approach, the administration of edible microbicides into expressed infected milk which is then delivered to the baby has been previously considered (Hartmann et al., 2006a). Sodium dodecyl 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, with no detectable effects on milk composition (Hartmann et al., 2006a, 2006b). This concentration is safe for infant use, based on a maximum acceptable infant oral exposure to SDS of 1 g/kg (of infant)/day and an biochemical analysis of the effect of SDS on milk content (Hartmann et al., 2006a, 2006b). Another benefit of SDS is its broad antiviral activity by solubilizing lipid membranes; therefore unlike many anti-viral compounds SDS is strain independent and unlikely to drive HIV mutation to a resistant form (Hartmann et al., 2006b).

Given that delivery of SDS during breastfeeding may be an effective method of reducing viral load in milk, we propose a new method to deliver SDS 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 latching on (Riordan, 2005). The NSDS would have an insert containing a dose of the API inside, and a mechanism for releasing the API into the milk. The insert could be placed inside the NSDS prior to each feed or the NSDS could be preloaded with the insert prior to the mother obtaining the device, and be entirely disposable after use. This approach would be simple, easy to use, and require no refrigeration or potable water for reconstitution, and is therefore more suitable for developing countries where medical infrastructure is often scarce, and access to clean water is limited. The mother would wear the NSDS while breastfeeding, 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 pre-impregnated with the API, and would be disposed of after each use.

Although the NSDS could be washed, dried, and reimpregnated with another insert for reuse, it is possible that the NSDS could become wet, disintegrated, and released with another insert for use. If this were to happen, the NSDS would be pre-impregnated with the API, and would be disposed of after each use. If this were to happen, the NSDS would be pre-impregnated with the API, and would be disposed of after each use. If this were to happen, the NSDS would be pre-impregnated with the API, and would be disposed of after each use.
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. Their weight was measured after soaking by measurement of the difference between their wet and dry weights. The weight of each dried disc was recorded with an accuracy of ±0.01 g. Pre-conditioning of the discs was performed by ensuring that their volume was consistent with the volume of milk used in each test.

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. 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. To correct for this, SDS concentration in test samples was calculated after subtracting the absorbance signal caused by milk alone. SDS concentration was measured using an adapted stains-all colorimetric assay described below.

2.3 Summary of non-woven fiber inserts with SDS

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. This was diluted in 1 ml of PBS to a concentration of 0.1 mg/ml, followed by thorough mixing for 30 seconds. To make a assay solution to analyze 250 samples, 20 mg stains-all dye (Sigma-Aldrich, UK) was dissolved in 1 ml of PBS to a concentration of 0.1 mg/ml, followed by thorough mixing for 30 seconds. This was diluted in 1 ml of PBS to a concentration of 0.1 mg/ml, followed by thorough mixing for 30 seconds. 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. This was diluted in 1 ml of PBS to a concentration of 0.1 mg/ml, followed by thorough mixing for 30 seconds. To make a assay solution to analyze 250 samples, 20 mg stains-all dye (Sigma-Aldrich, UK) was dissolved in 1 ml of PBS to a concentration of 0.1 mg/ml, followed by thorough mixing for 30 seconds. This was diluted in 1 ml of PBS to a concentration of 0.1 mg/ml, followed by thorough mixing for 30 seconds.
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. This protocol was performed to ensure that the tested milk was representative of donor milk used in these experiments.

2.4 Release of the edible microbicide SDS from NSDS inserts

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 resembling breastfeeding were achieved by maintaining the milk at 37°C and initiating pulsatile flow to produce pulses flow to simulate the suction maximizing the milk at the NSDS insert exponentially. Exponential release was observed by SDS in the apparatus, despite the pre-existing experimental conditions where milk flow was less than 100 µl/s during single pulse feeding.

The release of SDS from the NSDS inserts was studied in a mimicked breastfeeding simulation environment. The release of SDS was measured using a Chromex 96 Nicolpore Luminometer (Promega, USA). The apparatus was set up as described in section 2.2, with the fraction collector (BioRad Model 2110, USA) and a peristaltic pump to simulate the suction pressure created by a baby (Fig. 2d). During breastfeeding, pulsatile flow rate and volume vary greatly, so a pulse rate and a volume were used that were close to those found in a physiological breastfeeding condition. The milk was maintained at 37°C and was pumped through an NSDS insert exponentially. SDS release was monitored by collecting milk fractions, 100 µL, every 10 minutes at 0°C, and washing these fractions twice with PBS. SDS release was measured by the luciferase activity of TZM-bl cells exposed to the milk fractions.

The concentration of SDS released into early milk fractions was determined by comparing the luciferase activity of TZM-bl cells exposed to the milk fractions with a standard curve of known SDS concentrations. The concentration of SDS in the milk fractions was determined by comparing the luciferase activity of TZM-bl cells exposed to the milk fractions with a standard curve of known SDS concentrations. The concentration of SDS in the milk fractions was determined by comparing the luciferase activity of TZM-bl cells exposed to the milk fractions with a standard curve of known SDS concentrations.

3.2 Interaction of the edible microbicide SDS with milk

In the presence of milk, SDS forms an insoluble complex with casein, which prevents the interaction of SDS with the reporter cells. To assess the interaction of SDS with milk, a 1:10 dilution of milk was prepared in cell culture medium and assayed for luciferase activity. The results showed that the SDS-lactose complex formed in milk inhibited the interaction of SDS with the reporter cells. The interaction of SDS with milk was also assessed by measuring the inhibition of luciferase activity of TZM-bl cells exposed to milk fractions.

3.3 Interaction of the edible microbicide SDS with NSDS inserts

To determine the interaction of SDS with NSDS inserts, the luciferase activity of TZM-bl cells exposed to milk fractions was compared with a standard curve of known SDS concentrations. The results showed that the interaction of SDS with NSDS inserts was prevented by the presence of milk.

3.4 Interaction of the edible microbicide SDS with human milk

In the presence of human milk, SDS forms an insoluble complex with casein, which prevents the interaction of SDS with the reporter cells. To assess the interaction of SDS with human milk, a 1:10 dilution of human milk was prepared in cell culture medium and assayed for luciferase activity. The results showed that the SDS-lactose complex formed in human milk inhibited the interaction of SDS with the reporter cells. The interaction of SDS with human milk was also assessed by measuring the inhibition of luciferase activity of TZM-bl cells exposed to human milk fractions.
The influence of milk composition on release kinetics

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 was not a significant factor affecting the release of SDS from the non-woven fiber insert. Lower temperatures may not significantly influence SDS release rate from the non-woven fiber.

The influence of two types of flow conditions were compared between tests: the pulse rate (how quick 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 flow conditions, with SDS concentrations of above 0.1% being detected in all tests. This suggests that flow conditions did not significantly influence SDS release rate from the non-woven fiber insert. However, higher pulse rates and volumes resulted in less SDS release, indicating a possible correlation between SDS release and the speed of milk flow through the non-woven fiber insert.

The influence of the non-woven fiber on SDS release was determined by comparison to SDS powder placed in the insert holder. 0.1 g of SDS powder was used in each test. Similar release patterns were seen in all experiments, with approximately 70% of the insert load released 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 detected in the first 20 ml of milk that passed through the non-woven fiber insert.

The influence of milk composition on release kinetics 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. Approximately 100% of the insert load had released into non-homogenized unpasteurized goat's milk within 10 ml, 70-90% for homogenized pasteurized cow's milk, and 80% for homogenized unpasteurized cow's milk within 10 ml. 70-90% for homogenized pasteurized cow's milk.
3.4 Modeling release behavior

For an initial model, it was proposed that total release was dependent on the fraction of SDS released from the insert (Eq. (1)) for fixed flow and temperature conditions.

\[
q_{M} = k_{1} \cdot \frac{1}{(1 - e^{-k_{2}q})}
\]

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

\[
q_{M} = k_{1} \cdot \frac{1}{(1 - e^{-k_{2}q})}
\]

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 in homogenized cow's milk, the total cumulative release for goat's milk was significantly higher. This means that in order to release more SDS from the insert, a higher release rate is required. The observed difference in goat's and cow's milk release behavior indicates that milk composition significantly influences release kinetics.
associated HIV by the NSDS, whereby both human milk and SDS were removed 20 minutes after collection followed by measurement of HIV infectivity (see section 2.3).

When known doses of H9/HIVIIIB cell samples were assayed with this method using TZM-bl cells, H9/HIVIIIB cell concentrations between \(0.26 \times 10^4\) – \(2.6 \times 10^4\) cells/ml were quantitatively detected, indicating a sensitive assay of HIV infectivity (Fig. 6a). Relative HIV infectivity levels in samples of NSDS-treated milk were then determined by comparing the measured luminescence from TZM-bl cells exposed to test samples with the calibration data in Fig. 6a. Using this method, it was found that treatment of HIV-spiked human milk with the NSDS SDS-loaded insert resulted in a significant reduction in the mean correlated infected cell content of 3 tests, compared to input cell content (Fig. 6b). Average infectious cell content reductions were significant at the following levels: More than 2 log reduction for 0-5 ml (p < 0.0001), 1.5 log reduction for 5-10 ml (p < 0.0001) and 0.6 log reduction for 10-15 ml (p < 0.05) (using paired single t-tests). A 0.4 log reduction for 15-20 ml, 0.4 log reduction for 20-25 ml and 0.3 log reduction for 25-30 ml in mean infected cell content was observed but these reductions were not significant (p > 0.05). The individual infectious cell content in each volume fraction is illustrated in Fig. 6b. The small variation in reduction of infectivity between repeat tests is likely due biological variations in inactivation between tests, given the small variance observed between replicate HIV infectivity assays. The time course of virus release into milk from the NSDS is shown in Fig. 6c.

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 upon application of the release medium, resulting in a more protracted pattern of SDS release. In contrast, the presence of naproxen sodium in the micronized formulation caused an immediate burst release of the API within the first 15 minutes of soaking into milk, which resulted in rapid release of the API and an immediate reduction in efficacy. These results highlight the importance of formulating the API in a manner that prevents rapid initial release into milk, while still allowing for sustained release over time.

The first-order cumulative release model presented here, observed data well, and the constants derived

4.2 Viral inactivation in human milk

There was a high inactivation of cell-associated HIV in early fractions (0-10 ml) of human milk passed through the NSDS SDS-insert (> 99%), followed by a much smaller reduction in later fractions. This high level of inactivation was observed in all samples tested, regardless of milk type.

In contrast to the high levels of virus inactivation observed in early fractions, there was a much smaller reduction in later fractions. The reported threshold of rapid HIV inactivation (> 0.1 wt%) also occurred within the first 10 ml for goat's milk, but then rapidly decreased to below reported microbicidal concentrations (therapeutic ca. 10-6) within the first 50 ml (Fig. 6a). When milk samples were assayed using TZM-bl cells, viral infectivity levels were measured post-release, indicating that the NSDS-loaded insert was effective in reducing virus levels in milk. However, the high levels of inactivation observed in early fractions were not maintained in later fractions, suggesting that other factors, such as the presence of milk components, may play a role in the observed inactivation kinetics.

When known doses of H9/HIVIIIB cell samples were assayed using TZM-bl cells, cell-associated HIV was measured by means of measurement of HIV infectivity (see section 2.3)
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 viral infectivity than seen in a simplified study. Further study will be required to better predict the effectiveness of a given NSDS microbicidal surface within the milk.

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

4.3 Future uses of the NSDS

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-useable one, with a replaceable drug-loaded insert, would be most suitable. For example, a re-useable NSDS would be more sustainable and lower cost, in low-resource settings where sanitation equipment may be limited. However, re-useable NSDS would need to be disposed of after use, which could be a potential disadvantage.

Aside from delivering nanoscale or microscale of medicinal substances, the NSDS may also be a viable delivery vehicle for complex formulations of medicinal substances. Using a NSDS to deliver agents other than microbicides will generally require simple direct API release. The potential increases when the NSDS is combined with microbicide release to potentially increase viral inactivation and be released from SDS delivery within nanoscale or microscale of milk contents. For example, the use of copper-based fibers in a breastfeeding device (Borkow et al., 2011, 2008) could be combined with microbicide release to potentially increase viral inactivation and be released from SDS delivery within nanoscale or microscale of milk contents.
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-toxic microbicidal concentrations. However, additional tests are 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 steps of transmission in breastfeeding these methods could be adapted to more effectively reduce the risk of MTCT of HIV.

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## Table 1

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<th>Fluid Temp (°C)</th>
<th>Pasteurized Fluid</th>
<th>Homogenized Fluid</th>
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<td>Total release</td>
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### Summary of SDS release experiments using cow's milk for constant flow conditions.

Fluid temperature 7°C, 60 pulses/min, 0.07 ml/pulse and SDS-fibre insert. Fitted model parameters to a first-order release kinetic model according to Equation (2) also displayed.

### Table 2

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<tr>
<th>Graph Ref</th>
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### Summary of SDS release experiments using cow's and goat's milk for constant flow conditions.

Fluid temperature 7°C, 60 pulses/min, 0.07 ml/pulse and SDS-fibre insert. Fitted model parameters to a first-order release kinetic model according to Equation (2) also displayed.
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 masks in which integrated with a range of HIV protease inhibitors and assayed for infection by luciferaseexpressing adenovirus vectors which was used to determine HIV protease inhibitor release into milk. 420

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. 430

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 of 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 multiple measurements expressed as means ± standard deviation (n=3). In each experiment, SDS content in collected 1 ml fractions and cumulative SDS release relative to input SDS load 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 as a function of time (min) and volume of milk collected (ml). 435

Fig. 5. Effect of milk type on SDS release kinetics. The release of SDS from loaded non-woven fabric discs was measured during pulsed flow into (a) homogenised pasturised cow’s milk, (b) non-homogenised cow’s milk and (c) non-homogenised unpasturised goat’s milk with a flow rate of 4.3 ml/min and pulse rate of 60 pulses/min. Data are displayed as concentration of SDS as a function of time (min) and volume of milk collected (ml). 440

Fig. 6. Reduction in HIV infectivity in human milk after flow through SDS-loaded NSDS insert. (a) Calibration curve used to determine HIV infectivity in milk; TZM-bl reporter cells were infected with a range of HIV protease inhibitors and H9/HIVIIIB cells and assayed for infection by luciferase expression. Reporter activity 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. 445

Average reduction in HIV infectivity was significant with p < 0.0001 (**).
REFERENCES


Figure 2a

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 - Resubmission
Cumulative SDS release fraction of input [SDS] in each fraction (wt%)

Fig. 4.

Total volume passed through SDS insert (ml)

ai)

bi)

[SDS] in each fraction (wt%)

ci)

di)

ei)

SDS insert at 16 ºC

SDS insert at 37 ºC

60 pulses/min

SDS powder, milk temperature at:

16 ºC

37 ºC

Flow rate:

2.9 ml/min

4.3 ml/min

6.0 ml/min

Pulse volume:

0.02 ml/pulse

0.07 ml/pulse

0.45 ml/pulse

0.07 ml/pulse
Cumulative SDS release fraction of input [SDS] in each fraction (wt%)
Fig. 6. a, b: 3 repeat experiments. ND - not detected. Lower limit of H9/HIV cellular content detection.