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# Formulation and *in vitro/in vivo* Evaluation of Zidovudine Contained in Solidified Reverse Micellar Delivery System of Immune Compromised Rats

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**ABSTRACT**

Aim of the study was to study the *in vitro* and *in vivo* evaluation and correlation of zidovudine (AZT) loaded solidified reverse micellar microparticles (SRMMs). The SRMMs composed of goat fat and Phospholipon® 90H in various ratios (1:1, 2:1, 3:1 and 2:3) were prepared by melt dispersion method. AZT (1 %w/w, 2 %w/w, 3 %w/w and 5 %w/w) were incorporated into the SRMMs and preliminary analysis of the preparations on their stability were done visually. The 1:1 formulation was evaluated for the particle size, percentage yield and *in vitro* studies which was done using SGF and SIF. The *in vivo* study was done using Wistar albino rats and the *in vitro-in vivo* correlation (IVIVC) was determined by plotting a graph of the fraction of drug absorbed *in vivo* versus the fraction of drug released *in vitro*. The yield of the goat fat extraction was 58 %. The particle size and yield of the solid lipid microparticle (SLM) containing 1 %w/w of AZT were 5.10 ± 0.10µm and 86.3 ± 4.70% respectively. The fraction of drugs absorbed *in vivo* were 0.102 µg, 0.114 µg, 0.115 µg, 0.134 µg and 0.123 µg for 1 h, 3 h, 5 h, 8 h and 12 h respectively**.** A 1:1 ratio of goat fat and Phospholipon® 90H with a high value of correlation coefficient ($r^2$ = 0.909) suggested good level-A correlation between the *in vitro-in vivo* data of the SLM obtained in the study.

## INTRODUCTION

Lipid-based delivery systems are an acceptably proven, commercially viable strategy to formulate pharmaceuticals for topical, oral, pulmonary or parenteral delivery. Whether in the form of liposomes, micelles or emulsions, formulation can be tailored to meet a wide range of product requirement dictated by disease conditions, route of administration and consideration of cost, product stability, toxicity and efficacy. There is increased use of liposome technology to control the bioavailability of small molecules and particles following localized administration e.g. to the lungs, subcutaneous tissues or brain. Solid lipid particles have been proposed as colloidal drug carrier therapeutic systems for different routes of administration such as oral, topical, ophthalmic, subcutaneous and intramuscular injection and particularly for parenteral administration.

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Solid lipid microparticles (SLM) are increasingly investigated as dosage forms for oral delivery of drugs as found in the use of methacrylic acid-based 'hydrogel microparticles for oral insulin delivery (Kumar *et al*, 2006) and SLMs of insulin prepared by solvent-in-water emulsion diffusion technique (Trotta *et al*., 2005). Biocompatible lipid micro- and nano-particles have been reported as potential drug carrier systems as alternative materials to polymers (Reithmeier *et al*., 2001; Morel *et al*., 1996; Erni *et al*., 2002). Solid lipid particles combine several advantages and avoid the disadvantages of other colloidal carriers. They offer the possibility of controlled drug release and drug targeting (Schwarz et al., 1994); provide protection of incorporated active compounds against degradation; the solid matrix is composed of physiological compatible and well tolerated lipids; allow for hydrophilic and/or hydrophobic drugs to be incorporated (Hu *et al*., 2002; Lippacher *et al*., 2002), and the possibility of large scale production at a relatively low cost (Jaspart et al., 2005).

Zidovudine is available as a 300-mg tablet, a 100 or 250 mg capsule, 50 mg/ml syrup, and a 200 mg/20ml injection solution. It is also available as a tablet in combination with lamivudine. AZT acts as a metabolic antagonist of thymidine and its antiviral effect is time dependent, an adequate zero-order delivery of AZT is desired for maintaining anti-AIDS effect and avoiding the strong side effects which are usually associated with excessive plasma level of AZT immediately after intravenous or oral administration (Kuksal et al., 2006). AZT is absorbed throughout the GIT. The drug is freely soluble at any pH and hence judicious selection of release-retarding excipients is necessary for achieving constant *in vivo* release. The most commonly used method of modulating the drug release is to include it in a matrix system (Salsa et al., 1997).

From biopharmaceutical standpoint, correlation could be referred to as the relationship between appropriate *in vitro* release characteristics and *in vivo* bioavailability parameters. United States Pharmacopoeia (USP) defined it as the establishment of a rational relationship between a biological property or a parameter derived from a biological property produced by a dosage form, and a physicochemical property or characteristic of the same dosage form (USP. 2004). Food and Drug Administration (FDA) defined *in vitro–in vivo* correlation (IV-IVC) as a predictive mathematical model describing the relationship between an i*n vitro* property of a dosage form and a relevant *in vivo* response. Generally, the *in vitro* property is the rate or extent of drug dissolution or release while the *in vivo* response is the plasma drug concentration or amount of drug absorbed (FDA, 1997). An IV-IVC is expected only in the case of Class II drugs though IVIVC could be expected for Class I drugs if the dissolution rate is slower than the gastric emptying the rate; with a sufficiently rapidly dissolving Class I drug, little or no IV-IVC is expected because gastric emptying (not dissolution) would be the rate limiting step (Larry and Mark, 2002). In the present work, correlation between the dissolution profiles and bioavailability of SLM containing 1 % w/w of AZT was observed; hence an *in vitro* dissolution curve can serve as a surrogate for *in vivo* performance for level-A correlation studies.

## MATERIALS AND METHODS

### Materials

The materials used were Phospholipon® 90H, a hydrogenated lecithin (Phospholipon GmbH, Köln, Germany), thiomersal, poloxamer 188 (a non-ionic tri-block copolymers composed of a central hydrophobic chain of polyoxypropylene flanked by two hydrophilic chains of polyoxythylene (Synochem City, Germany), zidovudine (a gift from Fidson Healthcare, Nigeria), hydrochloric acid, sodium chloride, sodium hydroxide, monobasic sodium phosphate (Merck, Darmstadt, Germany), cyclophosphamide (Oncomide®, Khandelwal, India) and distilled water(Lion water, Universiy of Nigeria, Nsukka, Enugu State). All other reagents and solvents were of analytical grade and were used as supplied.

### Methods

#### *Extraction of goat fat*

This was done according to the procedure outlined by Attama and Nkemnele (Attama and Nkemnele, 2005). Briefly, the adipose tissue of *Capra hircus* was grated and subjected to moist heat by boiling with about half its weight of water in a water bath for 45 min. The molten fat was filtered through a muslin cloth and later separated from the aqueous phase after cooling. The extracted fat was further subjected to purification by passing it through a column of activated charcoal and bentonite (2:1) at 100 $^{o}$C at a ratio of 10 g of the fat to 1 g of the column material. The fat was stored in a refrigerator until used.

$$\text{Yield of goat fat} = \frac{\text{weight of goat fat}}{\text{Weight of starting material}} \; x\; 100 \quad \text{Equation 1}$$

#### Formulation of the solidified reverse micellar solution (SRMS)

This was done according to the procedure outlined by Müller-Goyman and Friedrich (Müller-Goymann and Friedrich, 2003). Briefly, SRMS was prepared with different ratios of goat fat and Phospholipid as follows: 1:1, 2:1, 3:1 and 2:3 respectively. The different quantities of goat fat and Phospholipid were weighed in an analytical balance (Ohaus, Germany) and prepared by fusion using a hot plate.

#### Formulation of reverse micellar microparticles (solid lipid microparticles)

The reverse micellar microparticles (solid lipid microparticles) were prepared to contain: lipid matrix (7.5 % w/w), zidovudine (1 %), poloxamer 188 (1 %), thiomersal (0.001 %), sorbitol (4 %) and water (to 100 %). The lipid matrix consisted of goat fat and phospholipon® 90H. For 1:1 batch, the lipid matrix was placed in a stainless steel bowl and heated at 70 $^{o}$C until it had completely melted. The drug was poured into the melted matrix and mixed. The remaining excipients were weighed out appropriately and mixed with the corresponding quantity of water. The excipients mixture with water was poured into the lipid matrix-drug mixture and homogenized at 5000 rpm for 10 min, with Ultra-turrax homogenizer (IKA® 25, Bonn-Bad Godesberg, Germany), a creamy emulsion was formed. The hot emulsion was then poured into an already calibrated amber-coloured bottle. The preparation was then allowed to recrystallize at room temperature for 24 h. The suspensions were left on the shelf at room temperature and monitored for one week to determine their stability. The stable suspension was then lyophilized to get the microparticles

$$\text{Percentage yield} = \frac{\text{weight of SLM obtained}}{\text{Weight of drug + excipients}} \; x\; 100$$

#### Particle size and morphological analysis

The morphology and particle size analysis of the freeze dried samples were carried out 48 h after production according to the procedure by Uronnachi *et al* (Uronnachi *et al*., 2012). Approximately 5 mg of the samples from each batch was dispersed

in distilled water and smeared on a microscopic slide using a glass rod. The mixture was covered with a cover slip and viewed with a photomicroscope (Hund®, Weltzlar, Germany) attached with a digital camera at a magnification of 1000. Triplicate readings were taken.

**Preparation of calibration plot**

The wavelengths of maximum absorption were determined by scanning some samples in the various media used (SIF, SGF, ethanol and plasma). A calibration curve was obtained at five concentration levels of a zidovudine standard solution (0.01- 0.06 mg/ml). From this, the calibration plots of zidovudine in these media were obtained and used to calculate the corresponding concentrations of drug released in each medium. Linearity was analyzed using the least square regression method in triplicate at each concentration level.

**Preparation of simulated intestinal fluid (SIF) without pancreatin**

This was done by dissolving 6.8 g of monobasic potassium sulphate in 250 ml of distilled water. The resulting solution was made up to 1000 ml with distilled water. The pH of the solution was checked and adjusted to 7.2 using 0.2 N NaOH.

**Preparation of simulated gastric fluid (SGF) without pepsin**

A 1.0 g quantity of NaCl was dissolved in 500 ml of distilled water and 7 ml of conc. HCl was added to it. The resulting solution was made up to 1000 ml with distilled water. The pH of the medium was checked and adjusted to 1.2 with a 2.0 N HCl.

***In vitro* release studies**

A sequential drug release in different release media was carried out. In each case a 0.5 g of each of the solidified reverse micellar microparticles (SRMMs) was placed in a dialysis membrane (MWCO 6000-8000 Spectrum Labs, The Netherland) tied at both ends and suspended in 250 ml of SGF, placed in a dissolution apparatus set to rotate at 100 rpm at a temperature of 37 $^{o}$C. At intervals of 5 min, 10 min, 20 min, 30 min, 1 h and 2 h respectively 5 ml aliquots of the dissolution medium (SGF or SIF) were collected and immediately replaced with 5 ml of fresh SGF. After 2 h, the dissolution medium was changed and replaced with freshly prepared SIF. The drug release in this medium was then assessed at hourly intervals for 10 h. Sequel to this the withdrawn samples were collected and analyzed using a UV-VIS spectrophotometer (UNICO-3102, England) at the appropriate predetermined wavelengths.

***In vivo* release studies**

The animal experimental protocols were in accordance with the guidelines for conducting animal experiments stipulated by our Institution's committee Animal Ethics Committee and in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive (European Community Council Directive, 1986). Fifteen albino Wistar rats weighing 180-250 g were used for the *in vivo* test. The rats were divided into five groups of three rats per group. They were fed orally for one week for acclimatization. Afterwards, a single dose of 30 mg/kg of cyclophosphamide (Oncomide®) was administered to the rats intraperitoneally (IP) to induce immune suppression. Cytotoxic immunosuppressive drugs, particularly cyclophosphamide, can induce more long-lasting reductions in T cell Subpopulations (Bird, 1996). The immune suppressed rats were allowed to starve for 24 h. At the end of this period, blood was withdrawn from each of the rats (t=0). Subsequently, 1 mg of zidovudine was administered to the rats in one group while the equivalent weight of reverse micellar preparations that would give 1 mg of zidovudine was administered to each of the remaining four groups. After administration, blood samples were withdrawn from the retro-orbital plexus of the rats at intervals of 1 h, 3 h, 5 h, 8 h, 12 h and 15 h with the aid of heparinised capillary tubes. After collection, these samples were placed in EDTA bottles and refrigerated. Thereafter, the collected blood samples were centrifuged (Abishkar centrifuge, India) at 500 rpm for 10 min. This plasma was then diluted 50-fold with a plasma solution and their absorbance checked using a UV-VIS spectrophotometer at a predetermined wavelength of 255 nm.

***In vitro-in vivo* correlation**

The *in vitro* and *in vivo* percentage released of the drug from the Batch 1 containing 1 %w/w of AZT were determined each in triplicate for a period of 12 h. The IV-IVC graph (fraction of drug absorbed versus fraction of drug release) of the SLM containing 1 %w/w of AZT was used for comparison of the *in vitro* dissolution studies and the *in vivo* bioavailability studies according to Kuksal *et al* (Kuksal et al., 2006).

**Statistical analysis**

The quantitative data were expressed as mean ± standard deviation (SD) and statistical analysis of the data was performed using one-way ANOVA (Graph Pad Prism 5). Differences between formulations were considered to be significant at $p \leq 0.05$.

**RESULTS**

**Extraction of goat**

The extraction yielded 58 % of the goat fat**.**

**Stability**

At the end of the week, it was observed visually that only the 1:1 ratio was stable while the other ratios were unstable, thus they were discarded.

**Particle size and morphology**

The average particle size was 5.10 ± 0.10μm and the microparticles were irregular in shape. The irregular shapes of the microparticles are as shown in Figure 1.

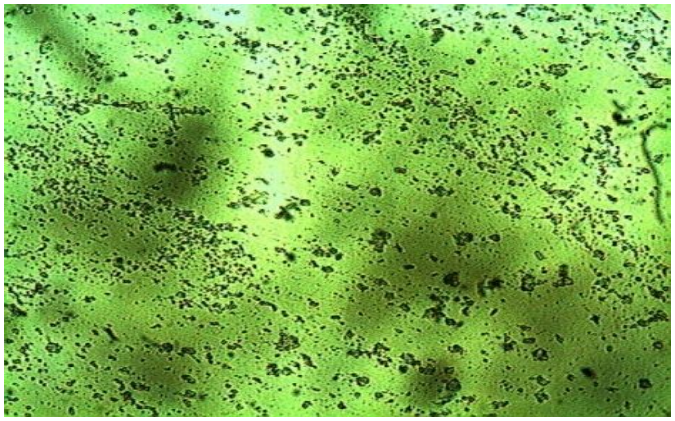

**Fig. 1:** Photomicrograph of SLM containing 1 %w/w AZT Calibration plot.

According to Beer-Lambert's law, as earlier stated;

A=klC

Since l=1, the equation can be modified to A=kC. Calibration plots indicated linear relationships between absorbance and concentration of zidovudine with all the solvents used.

### *In vitro* release studies

The *in vitro* release profile of the SLM loaded with 1 % AZT is shown in Table 1 and Figure 2.

**Table 1: *In vitro* Drug release studies**

| Time (h) | Amount released (µg/ml) |
|---|---|
| 0.08 | 3.570 |
| 0.17 | 5.240 |
| 0.33 | 5.240 |
| 0.50 | 5.240 |
| 1.00 | 5.470 |
| 2.00 | 6.900 |
| 3.00 | 8.112 |
| 4.00 | 9.152 |
| 5.00 | 8.320 |
| 6.00 | 9.152 |
| 7.00 | 7.904 |
| 8.00 | 13.310 |
| 9.00 | 13.520 |
| 10.00 | 14.350 |
| 11.00 | 14.350 |
| 12.00 | 15.600 |

**Media: SGF (0-2h); SIF (3-12h)**

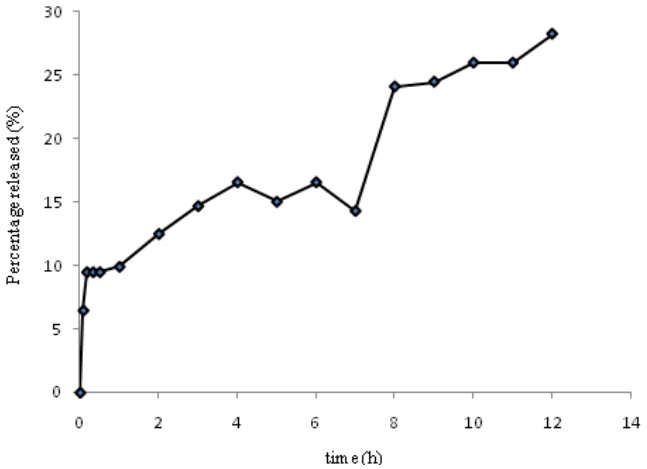


**Fig. 2:** Percentage released *in vitro* of the 1 %w/w AZT SLM formulation.

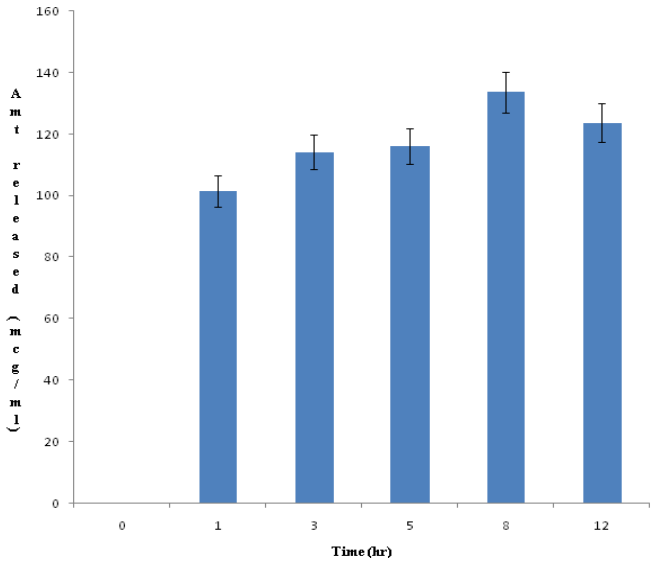


**Fig. 3:** *In vivo* drug release studies.

### IV-IVC correlation

Fraction of drug absorbed versus fraction of drug release (*in vitro-in vivo* correlation) for formulation containing 1 %w/w of AZT in SLM is shown in Figure 4**.**

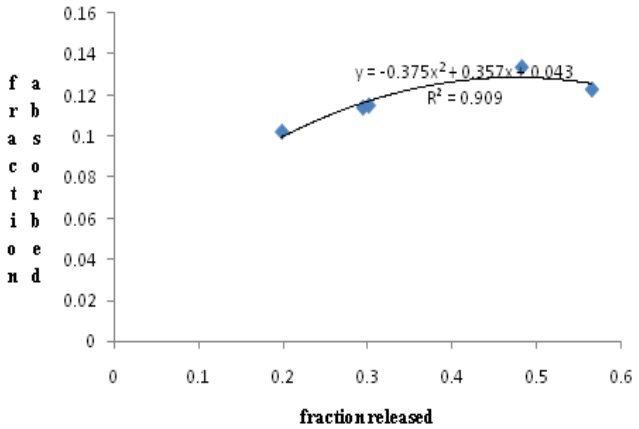


**Fig. 4:** IV-IVC plot for formulation containing 1 % of AZT SLM.

## DISCUSSION

The reduction in amount of goat fat obtained with respect to the original size of the unprocessed fat was probably due to losses during removal of the 'skin' of the fat and during the purification process of filtration, drying and weighing. The particle size analysis of the microsphere as shown in Fig. 1 revealed a variation in the sizes and such differences in sizes of the individual particles may be related to the orientation of the particles during imaging ( USP, 2004). For the SRMM containing 1 % zidovudine the fraction of drugs released *in vitro* at 1 h, 3 h, 5 h, 8 h and 12 h were 0.198 µg/ml, 0.294 µg/ml, 0.301 µg/ml, 0.482 µg/ml and 0.565 µg/ml respectively.

The dissolution studies showed that the formulation gave an increased value of the drug released till the 12$^{th}$ h as shown in Table 1. In Table 1, the sequential *in vitro* findings in the SGF

showed that the percentage of drug released at 5 min, 10 min, 20 min, 30 min, 60 min and 120 min were 6.46 %, 9.48 %, 9.48 %, 9.48 %, 9.90 %, and 12.49 % respectively, and the results in SIF showed that the percentage of drug released at 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h and 12 h for the formulation were 14.68 %, 16.56 %, 15.02 %, 16.56 %, 14.30 %, 24.09 %, 24.47 %, 25.97 %, 25.97 % and 28.24 % respectively as shown in Fig. 2. The findings showed that the SLM formulation containing 1 %w/w zidovudine can sustain the plasma concentration of the drug thereby reducing the number of dosing which would have been increased due to the fast elimination of the drug.

From Fig. 3, the quantity of drug released *in vivo* for the SLM formulation at 1 h, 3 h, 5 h, 8 h, 12 h and 15 h were 101.58 µg, 114.21 µg, 111.05 µg, 133.68 µg, 123.68 µg and 122.11 µg respectively. In Table 2, the *in vivo* studies showed that the fraction of drug absorbed for formulation containing 1 %w/w of AZT in SLM at 1 h, 3 h, 5 h, 8 h and 12 h were 0.102 µg/ml, 0.114 µg/ml, 0.115 µg/ml, 0.134 µg/ml and 0.123 µg/ml respectively.. The *in vivo* findings revealed that the concentration of the formulation containing 1 %w/w AZT has its absorption peaked at $8^{th}$ h and decreased at the $12^{th}$ h. In Fig. 4, the IV-IVC graph (fraction of drug absorbed versus fraction of drug release) of the formulation containing 1 %w/w of AZT according to Kuksal *et al* (Kuksal et al., 2006) showed a level-A correlation with a regression coefficient of 0.909. The *in vitro-in vivo* correlation determined gave credence that a good correlation between the dissolution profiles and bioavailability respectively can serve as a biomarker for clinical investigations.

This showed to a reasonable extent that the *in vitro* data of the 1 % SRMM could be used to predict the *in vivo* properties of the formulation. With the Level A correlation established, it is possible that *in vitro* testing may be utilized for establishing the effects of manufacturing modifications such as minor formulation changes, manufacturing site and equipment change, alternative excipient suppliers, and a change in dosage form strength in the same formulation (Bird GA, 1996).

## CONCLUSION

In this study, Phospholipon® 90H and natural lipid, goat fat extracted from *capra hircus* were used to formulate SLMs of zidovudine. The *in vivo-in vitro* correlation studies revealed that the formulation containing 1 %w/w of zidovudine-SLM had level-A correlation with a correlation coefficient of 0.909, thus an *in vitro* dissolution curve can serve as a surrogate for *in vivo* performance for level-A correlation studies.

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