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Original Research Article

Formulation and Release Characteristics of Zidovudine-Loaded Solidified Lipid Microparticles

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Abstract

Purpose: To formulate and determine the release profile of zidovudine (AZT)-loaded solidified lipid microparticles (SLMs).

Methods: Different concentrations (0, 1, 2, 3 and 5 %w/w) of zidovudine (AZT) were formulated into microparticles in melt dispersion of Phospholipon® 90H and goat fat in the ratio 1:1, 2:1, 2:3 and 1:3 followed by lyophilization. They were characterized for particle size, yield, entrapment efficiency (EE) and loading capacity (LC). In vitro release kinetics and mechanism of release were assessed sequentially in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.2).

Results: The ratio 1: 1 formulation was the most stable in terms of physical observation. Particle size analysis indicated that the particles were irregular in shape with size ranging from 5.10 ± 0.10 to 13.40 ± 2.20 μm . Yield decreased with increase in drug concentrations in the SLMs formulations. EE data showed that the microparticles containing 1 % w/w of AZT had the highest entrapment efficiency of 74.0 ± 0.03 %. LC also decreased with increase in concentration of AZT. AZT tablet released most of its content within 5 min with a sharp decrease in the concentration but the SLMs maintained its release for 8 to 12 h in different batches

Conclusion: The results show that drug content has influence on drug release from the SLMs, but not on the mechanism of release. Furthermore, dose dumping was avoided and drug release mechanism was mostly non-Fickian while for the reference (commercial) tablet, it was Fickian.

Keywords: Phospholipon® 90H, Solidified lipid microparticles, Solidified reverse micellar microparticle, Zidovudine.

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INTRODUCTION

Zidovudine (AZT) is the first anti-retroviral compound approved for clinical use but the main limitation to its therapeutic effectiveness is its dose-dependent hematological toxicity, low therapeutic index, short biological half-life, and poor bioavailability [1, 2]. There is growing interest in lipid-based systems in drug discovery and product development to effectively overcome

physical and biological barriers related to poor aqueous solubility and stability, membrane permeability, drug efflux and availability [3, 4]. The rapid growth in the use of lipid-based drug delivery systems is primarily due to the diversity and versatility of pharmaceutical grade lipid excipients and drug formulations and their compatibility with liquid, semi-solid and solid dosage forms [5, 6].

Solid lipid microparticles (SLMs) were developed recently and have so far been considered a promising drug carrier system, especially with a view to giving the incorporated active substance a sustained-release profile [7]. Compared with other carriers such as liposomes and microparticles, SLMs combine several of those carriers' advantages (possibility of controlled drug release and drug targeting), while avoiding some of their disadvantages such as the use of organic solvent, biotoxicity of the carrier system, chemical and physical storage instability for both the carrier and the drug, high cost of ingredients, difficulty in preparation and low scale-up potential [8-11]. Uronnachi *et al* developed and characterized AZT-based SLM for control delivery [12] and demonstrated their safety and efficacy of lipid-based carriers that make them potential alternative drug carrier materials to polymers. The most important limitation of SLMs is that drugs to be incorporated into SLMs must be lipophilic enough to ensure high entrapment efficiency [13].

Thus the aim of study was to formulate and determine the kinetics and mechanism of release profile of AZT-loaded in the formulated SLMs and compare them with commercial AZT tablet.

EXPERIMENTAL

Materials

The materials used were Phospholipon® 90H, a hydrogenated lecithin (Phospholipid 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 polyoxyethylene (BASF, 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, University of Nigeria, Nsukka, Nigeria). All other reagents and solvents were of analytical grade and used as supplied.

Extraction of goat fat

This was carried out according to the procedure outlined by Attama and Nkemnele [6]. 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 °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.

Formulation of SLMs

The reverse micellar microparticles (solid lipid microparticles) were prepared to contain: lipid matrix (7.5 %w/w), zidovudine (0, 1, 2, 3, 5 %w/w), Poloxamer 188 (1 %w/w), thiomersal (0.001 %w/w), sorbitol (4 %w/w) and water (to 100 %w/w). The lipid matrix consisted of goat fat and Phospholipon® 90H (1:1, 2:1, 3:1 and 2:3).

For each batch, the lipid matrix was placed in a stainless steel bowl and heated at 70 °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 at 70 °C 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 bottle and allowed to recrystallize at room temperature for 24 h. The same procedure was repeated for the various lipid matrices.

The dispersions were left on the shelf at room temperature for one week to determine their short-term stability. At the end of the week, it was observed that only the 1:1 ratio was stable while the other ratios were unstable, thus they were discarded. The stable dispersion was then lyophilized to get the microparticles.

Evaluation of yield of SLM

The yield was determined by relating the weight of the SLMs (W_1) obtained to the total weight of the ingredients (W_2) used in producing the SLMs, as in Eq 1.

$$\text{Yield (\%)} = (W_1/W_2)100 \dots\dots\dots (1)$$

Particle size and morphology

The morphology and particle size analysis of the freeze dried samples were carried out 48 h after production. 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.

Determination of encapsulation efficiency (EE)

The entrapment efficiency was determined by solvent extraction followed by quantification. A sample (0.5 g) of the SLMs was mixed with 50 ml of ethanol. Resulting mixture was allowed to stand for 12 h and then filtered through a filter paper (Whatman no.1). The filtrate was then diluted 10-fold with ethanol and the resulting solution was analyzed spectrophotometrically (UNICO-3102, England). Determination was done in triplicates. Amount of drug encapsulated in the microparticles was calculated with reference to a standard Beer's plot for zidovudine to obtain EE as the ratio of actual drug content to theoretical drug content, expressed as percentage [14].

Assessment of loading capacity

Loading capacity (LC) was assessed as the ratio of the entrapped zidovudine to the total weight of the lipids [15], expressed as a percentage.

In vitro release studies

A sample of SLM was placed in a dialysis membrane (MWCO 6000-8000 Spectrum Labs, The Netherland) tied at both ends and suspended in 250 ml of SGF in a dissolution apparatus set to rotate at 100 rpm. The temperature was set at 37 °C. At intervals of 5, 10, 20 and 30, 60 and 120 min, respectively, 5 ml aliquots of the dissolution medium was collected and immediately replaced with 5 ml of fresh SGF. The medium was changed to SIF after 2 h, and at hourly intervals, 5 ml aliquots of the dissolution medium was collected and immediately replaced with 5 ml of fresh SIF. The drug release in this medium was then assessed for another 10 h. The withdrawn samples were analyzed spectrophotometrically (UNICO-3102, England) at a wavelength of 290 nm.

Analysis of kinetics of drug release

The dissolution data for the SLMs were also analyzed to determine the *in vitro* release kinetics. Four kinetic models viz: zero-order, first

order, Higuchi [16] and Korsmeyer-Peppas [17], as in Eqs 2 – 5, respectively were applied.

$$Q_t = K_0 t \dots\dots\dots (2)$$

$$\ln Q_t = \ln Q_0 - K_1 t \dots\dots\dots (3)$$

$$Q_t = K_H S \sqrt{t} \dots\dots\dots (4)$$

$$M_t/M_\infty = K t^n \dots\dots\dots (5)$$

where Q is the amount of drug released in time t, Q₀ is the initial amount of drug in the microparticles, K₀, K₁, and K_H are the rate constants of Zero order, First order and Higuchi rate equations, respectively. For the Peppas and Korsmeyer model, M_t is the amount of drug released at time t. M_∞ is the amount of drug released at time = ∞, n is the diffusional exponent indicative of the mechanism of drug release, K is the power law constant, M_t/M₀ is the fraction of the drug released. If n ≤ 0.43, Fickian diffusion (case I+) applies, 0.43 ≤ n < 0.89 is non-Fickian transport and n ≥ 0.89 is case II transport (zero order, drug release mechanism dominates)

Statistical analysis

All experiments were performed at least in triplicate and the results expressed as mean ± SD. ANOVA and students t-tests were performed on the data, as appropriate, using Microsoft Excel version 2007. Differences were considered significant at p < 0.05.

RESULTS

Characteristics of SLMs

The production yield, particle size, loading capacity and entrapment efficiency of the SLMs are shown in Table 1. Batch B1, containing 1 % AZT showed the highest yield.

In vitro release

The *in vitro* release profile of different formulations and the pure sample is shown in Table. 1.

Table 1: Some characteristics of the SLMs (± SD, n = 3)

Batch code	Yield (%)	Mean particle size (µm)	LC (%)	EE (%)
B1	86.3 ± 4.70	5.10 ± 0.10	90.80 ± 0.20	74.00 ± 0.03
B2	85.4 ± 2.60	7.40 ± 0.70	88.17 ± 0.60	55.88 ± 0.01
B3	78.5 ± 1.50	9.73 ± 0.50	82.19 ± 0.35	34.60 ± 0.01
B4	78.2 ± 2.20	13.40 ± 2.20	74.23 ± 0.40	20.16 ± 0.05
B5	75.4 ± 1.20	3.30 ± 0.00	-	-

* B1 to B5 denote 1, 2, 3, 5 and 0 % AZT, respectively, LC = loading capacity; EE = entrapment efficiency

Table 1: Quantity of drug release in SGF and SIF after 12 h

Time (h)	B1 (mg/ml)	B2 (mg/ml)	B3 (mg/ml)	B4 (mg/ml)	B5 (mg)
0.08	3.57	7.14	10.71	20.94	146.67
0.17	5.24	8.81	14.35	17.14	13.09
0.33	5.24	10.47	15.6	17.14	14.29
0.5	5.24	8.57	15.6	17.85	8.81
1	5.47	9.28	17.88	14.04	11.19
2	6.9	10.47	19.14	17.37	10.71
3	8.112	11.23	6.66	13.94	11.67
4	9.152	12.48	5.82	16.22	13.37
5	8.32	11.86	4.99	14.98	16.05
6	9.152	12.48	4.58	15.81	15.84
7	7.904	23.3	5.41	15.6	17.7
8	13.31	32.03	5.62	14.77	17.08
9	13.52	13.94	4.99	19.34	17.08
10	14.35	12.89	6.45	17.68	17.9
1	14.35	13.73	6.24	17.68	19.55
12	15.6	14.77	5.82	11.02	9.88

Table 2: Release kinetic parameters for the formulations

Batches	Zero order		First order		Higuchi		Korsmeyer	
	r ²	K ₀	r ²	K ₁	r ²	K _n	r ²	n
B1 in SGF	0.8970	0.054	0.8370	0.007	0.9910	1.166	0.9970	0.538
B1 in SIF	0.8930	0.024	0.9390	0.002	0.9860	1.173	0.9870	0.520
B2 in SGF	0.8340	0.087	0.7860	0.008	0.9660	1.311	0.9590	0.580
B2 in SIF	0.9420	0.025	0.9330	0.002	0.9800	0.599	0.9800	0.466
B3 in SGF	0.7990	0.159	0.7850	0.020	0.9700	0.361	0.9530	0.865
B3 in SIF	0.8740	0.012	0.8710	0.001	0.9310	0.381	0.9820	0.351
B4 in SGF	0.8870	0.144	0.8050	0.010	0.9800	5.971	0.9060	0.882
B4 in SIF	0.9200	0.035	0.8330	0.002	0.9220	1.374	0.9490	0.559
AZT in SGF	0.9000	0.186	0.7350	0.008	0.9920	0.485	0.9700	0.285
AZT in SIF	0.8730	0.034	0.8230	0.002	0.9580	0.283	0.9800	0.288

Key: r² = correlation coefficient, K₀ = zero order rate constant, K₁ = first order rate constant, K_n = Higuchi rate constant, n = Korsmeyer constant

Release kinetics

Drug release from monolithic spheres has been reported variously as taking place by numerous differing mechanisms, which include surface erosion, total sphere disintegration, microsphere hydration (or swelling), drug diffusion and desorption, particulate diffusion and leaching

DISCUSSION

The LC and EE of the AZT in the SLMs showed a decrease in the quantity of AZT entrapped with increasing drug concentrations as shown in Table 1. The matrix incorporating 1 % AZT (B1) had the highest percentage entrapped while the formulation containing 5 % AZT (B4) had the least entrapment efficiency. This could be as a result of saturation of the lipid matrix [18] as adequate drug loading is the prerequisite to obtaining a high solubility of the drug in the lipid melt. Generally, solubility decreased after cooling

down the lipid melt and might even be lower in the solid lipid. The presence of mono and diglycerides in the lipid used as matrix material promotes drug solubilisation [19]. The chemical nature of the lipid is also important because lipids that form highly crystalline particles with a perfect lattice (such as monoacid triglycerides) lead to drug expulsion [20].

The particle size analysis of the microspheres revealed a variation in the sizes of the different formulations with the 1 % AZT (B1) having the least average particle size while the 5 % AZT (B4) had the greatest average particle size. Differences in sizes of individual particles may be adduced to the surface of viewing which could have been 'edge-on' or 'side on'. The particle sizes of the AZT-loaded SLMs were significantly higher than the particle size of the unloaded batch (B5).

In vitro drug dissolution studies Fig. 1 showed that the 1 % AZT (B1) gave a gradual release of AZT. The 3 % AZT (B3) and 5 % AZT (B4) formulations had an initial high release which could be adduced to the presence of zidovudine on the surface of the microparticles as a result of saturation of the lipid matrix. According to Reddy *et al* [21] this initial *in vitro* burst release could be as a result of adsorption on the surface of the microparticle, or precipitation from the superficial lipid matrix. This could be advantageous in a formulation since it provides a quantity of drug to the body within a short time for therapeutic activity to commence before subsequent quantities are delivered gradually to maintain this activity. In contrast, the commercial AZT tablet, released almost all of its drug content within 5 min. According to Galinski and Svenson [22], prolonged release dosage forms have the additional advantage of giving a lesser fluctuation in blood levels of the drug than with rapidly absorbed dosage forms. Also, the study of White [23] revealed that the reduction in the rapidity of change of blood concentrations of some drugs could lead to an improvement in the efficacy, and a decrease in adverse effects of these drugs. There is significant difference in the release of the AZT from the SLMs (B1-B4) and the commercial tablet (B5) within the first 5 min of administration. This explained that those dumping is experienced in the commercial sample but not on the SLMs-loaded with AZT

Determining the correlation coefficient assessed the fitness of the data into various kinetic models. The rate constants for the respective models were calculated from their slopes [24] The results obtained as shown in Table 2 revealed that the best fit release kinetics was obtained with the Korsmeyer's plot while the Higuchi plot also showed a high correlation for the data. Thus drug release from the matrix of the solid lipid microparticles could be described as non-Fickian for the 1 %, 2 % and 3 % AZT-loaded solidified lipid microparticles, with a value of $0.43 < n < 0.85$, while drug release from the zidovudine tablets was Fickian with an n -value < 0.43 . This thus means that the drugs are released via a diffusion and dissolution mechanism. The difference in the release mechanisms of the formulated solid lipid microparticles and the commercial tablets may be due to the tortuosity of the polymer matrix which thus restricted the path of diffusion, whereas the simple tablet matrix of the commercial tablets made diffusion from its matrix to be easier and faster.

The difference in the release mechanisms of the formulated microparticles and the AZT tablets

may be due to the tortuosity of the polymer matrix which thus restricted the path of diffusion whereas the plain tablet matrix of the AZT tablets facilitated diffusion from its matrix. This confirms that the release of AZT-loaded SLMs followed a diffusion controlled mechanism for a non sequential analysis while none of the kinetics was followed for the sequential release. Since the discrete granular particles were non-disintegrating because of the dominance of lipid materials in the formulations, the only logical process of drug release would be by diffusion. This is accentuated by the fact that the granules did not swell, and so drug release would not be by gel-erosion.

The presence of surface-active agent in the SLMs would be expected to influence the pattern of drug release. Clearly, the presence of the Phospholipon[®] 90H caused a shift of drug release kinetics from Fickian type exhibited by the AZT tablets. This indicates that drug release mechanism is independent of drug release kinetics [25]. Drug release mechanisms generally relate to the interactions between component ingredients in a formulation, and this includes the possible interactions between active ingredients and excipients.

CONCLUSION

The results indicate that drug concentration has influence on the rate of drug release from the SLMs, but not on the mechanism of release. However, dose dumping was avoided and drug release was mostly non-Fickian while that of reference (commercial) tablet was Fickian.

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CONFLICT OF INTEREST

The authors declare no conflict of interest and have received no funding for the research or in the preparation of this manuscript.

REFERENCES

1. Kiebertz KD, Seidllin M, Lambert JS, Dollis R, Reichman R, Valentine T. Extended follow-up of neuropathy in patients with AIDS and AIDS related complex treated with dideoxyinosine. *J Acquir Immuno Defic Syndrom*. 1992; 5: 60-64.

2. Kuksal A, Tiwary AK, Jain NK, Jain S. Formulation and in vitro, in vivo evaluation of extended- release matrix tablet of zidovudine: Influence of combination of hydrophilic and hydrophobic matrix formers. *AAPS PharmSciTech*. 2006; 7(1): Article 1. DOI. 10.1208/pt070101.
3. Westesen K, Sielkmann B. Solid lipid nanoparticles of bioactive agent and method for the manufacture and use thereof. *United States Patent*. 1998; 5785-976.
4. Attama AA, Okafor CE, Builders PF, Okorie O. Formulation and in vitro evaluation of a PEGylated microscopic lipospheres delivery system for ceftriaxone sodium. *Drug Delivery* 2009; 16: 448-457.
5. Craig DQM. Lipid matrices for sustained release. An academic overview. *Bull Tech Gatefosse* 2004; 97: 9-19.
6. Attama AA, Nkemnele MN. In vitro evaluation of drug release from self-emulsifying drug delivery systems using a biodegradable homolipid from *Capra hircus*. *Int. J. Pharm*. 2005; 304: 4-10
7. Jaspert S, Piel G, Delatte L, Evrad B. Solid lipid microparticles: Formulation, preparation, characterization, drug release and applications. *Expert Opin Drug Deliv*. 2005; 2: 75–87.
8. Reithmier H, Hermann J, Gopferich A. Lipid microparticles as a parenteral controlled release device for peptides. *J. Control Rel*. 2001; 73: 339–350.
9. Milak S, Medicott N, Tucker IG. Solid lipid micro particles containing loratadine prepared using a micro mixer. *J. Microencapsul*. 2006; 23: 823–831.
10. Jaspert S, Bertholet P, Piel G, Dogne JM, Delattre L, Evrad B. Solid lipid microparticles as sustained release system for pulmonary drug delivery. *Eur. J. Pharm. Biopharm*. 2007; 65: 47–56.
11. Umeyor EC, Kenechukwu FC, Ogbonna JD, Chime SA, Attama A. Preparation of novel solid lipid microparticles loaded with gentamicin and its evaluation in vitro and in vivo. *J. Microencap*. 2012; DOI: 10.3109/02652048.2011.651495
12. Uronnachi EM, Ogbonna JDN, Kenechukwu FC, Attama AA, Chime SA. Properties of zidovudine loaded solidified reverse micellar microparticles prepared by melt dispersion. *J Pharmacy Res* 2012; 5(5): 2870-2874.
13. Long C, Zhang L, Qian Yu. Preparation and crystal modification of ibuprofen loaded solid lipid microparticles. *Chin J Chem. Eng*. 2006; 14(4): 518–525.
14. Morkhade DM, Joshi SB. Evaluation of gum dammar as a novel microencapsulating material for ibuprofen and diltiazem hydrochloride. *Indian J. Pharm. Sci*. 2007; 67: 263–268.
15. Doktorovova S, Souto EB. Nanostructured lipid carrier-based hydrogel formulations for drug delivery: A comprehensive review. *Expert Opin Drug Deliv*. 2009; 6: 165–176.
16. Higuchi T. Mechanism of sustained action medication. Theoretical analysis of rate of solid drugs dispersed in solid matrices. *J. Pharm. Sci*. 1963; 52: 1145–1149.
17. Korsmeyer RW, Peppas NA. Solute and penetrant diffusion in swellable polymers III. Drug release from glassy polymers (HEMA-co-NVP) copolymers. *J. Control Rel*. 1983: 1:89-98.
18. Vivek K, Harivardhan R, Ramachandra SR. Investigation of the effect of the lipid matrix on drug entrapment, In vitro release, and physical stability of olanzapine-loaded solid lipid nanoparticles, *AAPS PharmSci Tech*, 2007; 8(4). Article 83. (<http://www.aapspharmstech.org>).
19. Muller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery: a review of the state of the art. *Eur J Pharm Biopharm*. 2000; 50: 161-177.
20. Westesen K, Bunjes H, Koch MHJ. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. *J Control Release*. 1997;48:223–236. doi: 10.1016/S0168-3659(97)00046-1.
21. Reddy HL, Murthy RSR. Etoposide-loaded nanoparticles made from glyceride lipids: Formulation, characterization, in vitro drug release, and stability evaluation. *AAPS PharmSciTech*. 2005; 6(2): E158–E166. doi: 10.1208/pt060224
22. Galinsky ER, Svenson CK. Basic Pharmacokinetics. In *Remington: The Science and Practice of Pharmacy*, 19th edn. Easton P.A, Mack Publishing, 1995; vol II pp.1127-1144.
23. White PF. *Anesthesiology*, 1983; 59, 294.
24. Pachua L, Sarkar S, Mazumdar B. Formulation and evaluation of matrix mic rospheres for simultaneous delivery of salbutamol sulphate and theophylline. *Tropical J. Pharm. Res*. 2008; 7(2): 995-1002.
25. Okore VC, Ogbonna JDN, Kenechukwu FC. In vitro release mechanism of acetylsalicylic acid from melt-extrusion granules of dika wax. Abstract, 14th Annual Conference of Chemical Society of Nigeria, Enugu State Chapter, Nsukka (Nigeria), 2012; p 15.