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Rapid communication

In vitro evaluation of drug release from self micro-emulsifying drug delivery systems using a biodegradable homolipid from Capra hircus

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Abstract

Self micro-emulsifying drug delivery systems (SMEDDS) are specialized form of delivery systems in which drugs are encapsulated in a lipid base with or without a pharmaceutically acceptable surfactant. In this work, SMEDDS were formulated with a biodegradable homolipid from Capra hircus and Tween 65, and contained lipophilic drug-piroxicam, hydrophilic drug-chlorpheniramine maleate and hydrolipophilic drug-metronidazole. The SMEDDS formulated were evaluated for their drug release and drug content. The drug release studies were conducted in simulated gastric fluid (SGF), simulated intestinal fluid (SIF), and distilled water, representing different pH values. Particle size of the SMEDDS was determined by light microscopy. The results of this study indicated that drug release was affected by the particle size of the SMEDDS. It was found that piroxicam release from the SMEDDS formulated with homolipids from Capra hircus was highest in SIF compared to the other drugs. This method of drug delivery could prove to be a versatile and reliable alternative to conventional drug delivery approaches.

Keywords: Homolipids; Biodegradable; Self micro-emulsifying drug delivery systems (SMEDDS); Drug release; SIF; SGF

1. Introduction

With an increasing number of lipophilic drugs under development, homolipids and heterolipids have gained renewed interests as excipients for different drug delivery systems (Stuchlík and Žák, 2001). Homolipids are esters of fatty acids with various alcohols. Widening availability of lipidic excipients with specific characteristics offer flexibility of application with respect to improving the bioavailability of poorly water-soluble drugs and manipulating their release profiles (Neuwal and Ackard, 1966). Lipids may have considerable clinical impact. Ingested food containing lipids can significantly alter postprandial drug absorption and its bioavailability (Charman et al., 1997; Fleischer et al., 21999). Lipid based formulations have been shown to enhance the bioavailability of drugs administered orally (Hou et al., 2003; Sarkar, 2002; Gao et al., 2004;

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You et al., 2005). The oral delivery of lipophilic drugs presents a major challenge because of the low aqueous solubility. Gursoy and Benita (2004) and Kang et al. (2004) formulated self emulsifying drug delivery systems for lipophilic drugs, and showed that isoptropic mixtures of oil, surfactants, solvents, and cosolvents/surfactants can be used for the design of formulations in order to improve the oral absorption and bioavailability of highly lipophilic compounds. In his work, Pouton (2000) stated that the primary mechanism of action which leads to improved bioavailability is usually avoidance or partial avoidance of the slow dissolution process which limits the bioavailability of hydrophobic drugs from conventional solid dosage forms. Ideally, these novel formulations allow the drug to remain in dissolved state throughout the transit through the gastrointestinal tract. There are different categories of vehicles, which can be selected in order to prepare a lipidic carrier. Such formulations can be used as oral liquids or can be encapsulated into various types of capsules. The finished product is then administered to the patient as a solid dosage form (Yamahira et al., 1979). The method of drug delivery where there is spontaneous emulsification is known as self emulsifying drug delivery system. In this study, the SMEDDS containing admixtures of the homolipid and Tween 65 are expected to exhibit spontaneous emulsion formation. The formulation could be administered as parenteral formulations (as reconstitutable injectables) or orally in different forms.

The study was carried out to evaluate in vitro, drug release from SMEDDS formulated with homolipid from Capra hircus, and to determine the effect of drug physiochemistry on the characteristics of the formulated SMEDDS. The biodegradable homolipid (goat fat) used in this study has been evaluated as a basis for drug delivery (Attama et al., 2003, 2000). Tween 65 a non-ionic surfactant was chosen for this work because of its ability to form spontaneous emulsion with the homolipid, its considerably less toxicity compared with ionic surfactants and absence of charge which will greatly reduce its drug interaction potential.

2. Materials and methods

The following materials were used as procured from their suppliers without further purification: hydrochloric acid, sodium hydroxide, monobasic potassium phosphate, and Tween 65 (Merck, Germany), metronidazole (Nemel Pharmaceuticals, Enugu Nigeria), piroxicam (Pfizer, Nigeria), chlorphemiramine maleate (Juhel Pharmaceuticals, Enugu Nigeria). The homolipid was obtained from a batch processed in our laboratory. All other reagents and solvents were analytical grade and were used as supplied. Distilled water was obtained from a glass still.

2.1. Extraction and purification of homolipid from Capra hircus

The homolipid was extracted from the adipose tissue of Capra hircus by wet rendering (Attama et al., 2003). Briefly, the adipose tissue 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 separated from the aqueous phase after filtering with a muslin cloth. 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 and 1 g of the column material. The fat was stored in a refrigerator until used.

2.2. Preparation of simulated intestinal fluid (SIF) (pH 7.5) and simulated gastric fluid (SGF) (pH 1.2)

These two solutions were prepared according to pharmacopoeia standard (USP, 1995).

Table 1
Quantities of material used for SMEDDS formulation

Batch	Tween 65 (g)	Homolipid (g)	Drug	Quantity of drug (g)
A	6	24	Chlopheniramine maleate	1
B	6	24	Piroxicam	2
C	2	28	Metronidazole	5

2.3. Preparation of SMEDDS

In each case the appropriate quantities of homolipid and Tween 65 as presented in Table 1, were melted together in a crucible at 60 °C. The drug was added (as shown in Table 1) and stirred thoroughly. The mix was injected drop wise into a stirred non-solvent at 4 °C (propylene glycol for metronidazole and chlorpheniramine maleate, distilled water for piroxicam) using a 5 ml syringe fitted with an 18G needle (BDH, Germany) at a stirring speed of 1000 rpm. The SMEDDS were thereafter filtered out from the non-solvent with the aid of a filter paper (Whatman No. 1) and then dried for 72 h in a desiccator. This procedure was similar to a reported study (Schubert and Müller-Goymann, 2003). The choice of the different quantities of Tween 65 and the homolipid was informed by the result of the earlier study of self-emulsifying systems (Attama et al., 2003) and preliminary test carried out because of the presence of different drugs.

2.3.1. Evaluation of the SMEDDS

2.3.1.1. Yield of the SMEDDS. The SMEDDS formed were filtered from the solvent, dried in the desiccator and weighed to get the yield of the SMEDDS formulated per batch. Eq. (1) was used to calculate the percentage yield:

$$\%\,\text{recovery} = \frac{W_1}{W_2 + W_3} \times 100 \quad (1)$$

where W_1 is the weight of SMEDDS formulated (g), W_2 the weight of drug added (g) and W_3 is the weight of homolipid and Tween 65 (g) used as the starting material.

2.3.1.2. Particle size. A 10 mg quantity of the SMEDDS was placed inside the ring of the internally calibrated microscopic slide (Objective micrometer, KS Japan) and a drop of each non-solvent used above was added for a clearer view. The slide was covered with a cover slip and viewed under a binocular microscope at a magnification of ×100. Different particles of the SMEDDS from a particular batch were counted manually since they were sizeable enough to be distinguished ($n = 100$) and the mean value taken.

2.3.2. Drug content of the formulated SMEDDS

Beer's plots were obtained at the concentration range of (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg%), respectively for metronidazole, chlorpheniramine maleate and piroxicam using different dilutions of these drugs in distilled water for metronidazole and chlorpheniramine maleate, and ethanol for piroxicam. A 0.1 g quantity of each batch of the SMEDDS (containing 3.23, 6.25 and 14.29% of chlorpheniramine maleate, piroxicam and metronidazole, respectively) was placed in a 100 ml volumetric flask. The flask was made up to volume with the appropriate solvent in each case, and allowed to equilibrate for 24 h at 40 °C with intermittent shaking in a thermo stated water bath (Memmert, England). The solution was thereafter cooled to 0 °C in a refrigerator (Thermocool, T200), filtered through a filter paper (Whatman No. 1) and analyzed spectrophotometrically at the appropriate predetermined wavelength of 274, 225 and 325 nm for metronidazole, chlorpheniramine maleate, and piroxicam, respectively, using a spectrophotometer (Model SP6-450 UV/Vis Pye Unicam). This was repeated five times for all the batches. The drug concentrations were calculated with reference to Beer's plot for each drug prepared using the appropriate solvent and at the proper wavelength.

2.3.3. Drug encapsulation efficiency

The quantities of the drugs theoretically contained in the SMEDDS were compared with the quantity actually obtained from the drug content studies i.e. the quantity loaded into the SMEDDS formulated, to get the drug encapsulation efficiency. Eq. (2) below was used for the calculation:

$$\text{EE}\,(\%) = \frac{\text{ADC}}{\text{TDC}} \times 100 \quad (2)$$

where ADC is the actual drug content and TDC is the theoretical drug content.

2.3.4. Release studies on SMEDDS

The USP paddle method was adopted in this study. The dissolution medium consisted of 500 ml of freshly prepared medium (SGF pH 1.2, SIF pH 7.5 or distilled water pH 7) maintained at 37 ± 1 °C. The membrane selected was pretreated by soaking it in the dissolution medium for 24 h prior to commencement of each release experiment. A 0.1 g quantity of the formulated SMEDDS from each batch was placed in a polycar-

Table 2
Properties of the SMEDDS

Batch	Drug	Average particle size (μm ± S.D.)[a]	Recovery (%)	Actual drug content (%)	Theoretical drug content (%)	EE (%)
A	Chlorpheniramine maleate	0.25 ± 0.12	92.26	1.03	3.23	31.88
B	Piroxicam	0.11 ± 0.07	93.13	4.63	6.25	74.08
C	Metronidazole	1.05 ± 0.11	92.86	7.69	14.29	53.80

[a] $n = 100$, S.D.: standard deviation.

bonate dialysis membrane containing 2 ml of the dissolution medium, securely tied with a thermo-resistant thread and then placed in the appropriate chamber of the release apparatus containing the dissolution medium. The paddle at 100 rpm provided agitation. At predetermined timed intervals, 5 ml portions of the dissolution medium were withdrawn, appropriately diluted, and their absorbance determined in the spectrophotometer above. The volume of the dissolution medium was kept constant by replacing it with 5 ml of fresh medium after each withdrawal to maintain sink condition. The amount of drug released at each time interval was determined with reference to the standard Beer's plot for each drug, taking note of the medium (solvent) and λ_{max} for each drug. This test was carried out in triplicate for all the batches.

3. Results and discussion

3.1. Particle size analysis

From the values in Table 2, it can be seen that metronidazole had the largest mean particle size, followed by chlorpheniramine maleate, and piroxicam had the least particle size. This may be related to the solubilities of the drugs in the homolipid. Piroxicam, which is the most lipophilic of the three, is expected to form a continuous structure with the homolipid unlike the others, which may form partly soluble mixture and partly suspended non-coherent structure. The possible effect may be that SMEDDS with larger particle sizes will possess lower rate of emulsification in vivo, than the SMEDDS with smaller particle sizes and consequently, larger emulsion droplets may be formed as a result. It has been shown that larger droplets are less neutralized by mucin solutions of different concentrations than smaller droplets (Jose and Kulkarni, 2002). Droplet sizes as well as the rate and extent of lipolysis of the emulsion products formed have also been shown to affect the bioavailability of tocotrienol administered from self emulsifying formulations (Yap and Yuen, 2004).

3.2. Drug content

The active ingredient contents varied widely from that loaded into the SMEDDS. This might be due to the solubility of the drug in the lipid. There was a direct relationship between the drug incorporated and the lipophilicity of the drugs. The solubilities of the drugs are in the order: piroxicam < metronidazole < chlorpheniramine maleate. Piroxicam with the highest lipophilicity was encapsulated more than the other drugs. This may be because the drug will dissolve in the lipid matrix until the saturation solubility is reached, whereas others would only dissolve to some degree depending on their solubility in the lipid environment. The results obtained are presented in Table 2. The low standard deviation of the drug content attests to the reproducibility and reliability of the new method of formulation. The drug loss may be as a result of differential solubility created by changing interface during SMEDDS formation.

3.3. Percentage recovery of the SMEDDS

The result presented in Table 2 was obtained for the SMEDDS formulated. The percentage recovery values were less than 100% due to loss accruing form transference, filtration, drying, and weighing. However, they had overall high percentage recoveries. This may be as a result of adoption of a reliable production process technology.

3.4. Drug encapsulation efficiency, EE (%)

From the values of EE (%) shown in the Table 2, it can be inferred that the lipophilic drug piroxicam had

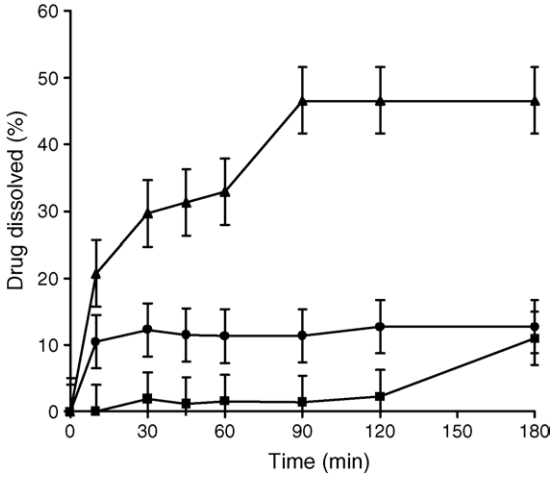

Fig. 1. Release profile of the drugs from the SMEDDS in SGF: (■) piroxicam, (▲) metronidazole, and (●) chlorpheniramine maleate.

the highest drug encapsulation efficiency followed by the hydrolipophilic drug, metronidazole and lastly by the hydrophilic drug, chlorpheniramine maleate. This shows that the EE (%) obtained varied directly with the liphophilicity of the drug.

3.5. Release studies

The results of the release studies are presented in Figs. 1–3, respectively, for SGF, distilled water and SIF. From the figures, it could be seen that metronidazole was released faster than chlorpheniramine maleate and piroxicam in SGF (pH 1.2). Metronidazole, which is a hydrolipophilic drug, might have dissolved faster in the SGF due probably to peripheral attachment of the drug in the SMEDDS, and its release increased as the duration of release increased until at 90 min when the highest quantity was released. This implies that when formulated as SMEDDS, metronidazole may be significantly absorbed when transit of the dosage form is 'delayed' in gastric environment. The release of chlorpheniramine maleate, a hydrophilic drug was higher than piroxicam, which is a lipophilic drug in SGF. This may be due to the more basic nature of chlorpheniramine maleate which may have favoured partitioning to the release medium. But the overall release was still low. Since piroxicam, which is a lipophilic drug released less until at 180 min when the peak concentration was achieved, its formulation as SMEDDS

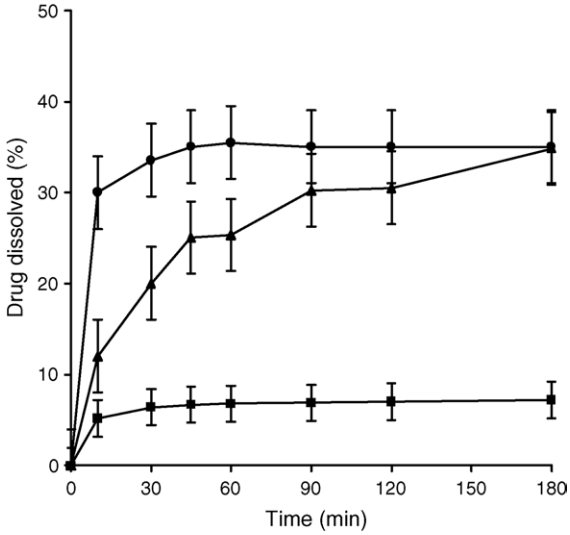

Fig. 2. Release profiles of the drugs from the SMEDDS in distilled water: (■) piroxicam, (▲) metronidazole, and (●) chlorpheniramine maleate.

may delay the release in SGF. In distilled water (pH 7.0), metronidazole was also released faster than chlorpheniramine maleate and piroxicam, which released at almost a constant rate. This may also be attributed to the fact that metronidazole may not be uniform in the lipid matrix and may have been encapsulated peripherally in the SMEDDS. The high release of chlorpheniramine

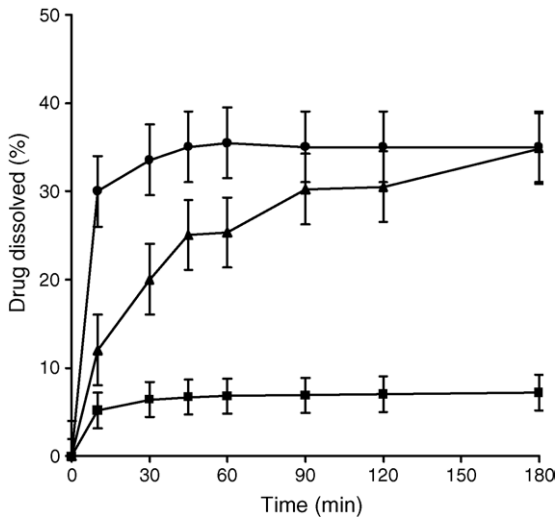

Fig. 3. Release profile of the drugs from the SMEDDS in SIF: (■) piroxicam, (▲) metronidazole, and (●) chlorpheniramine maleate.

maleate in distilled water may be as a result of its very soluble nature. In SIF (pH 7.5), piroxicam an acidic drug may be favoured at that alkaline pH and this enhanced its solubilization and subsequent release from the SMEDDS. Chlorpheniramine maleate probably due to its high aqueous solubility was released faster and much higher than piroxicam and metronidazole. The release of chlorpheniramine maleate was higher from the SMEDDS in SIF than in SGF and distilled water. This may be as a result of enhanced release possibly caused by the favourable environment contributed by the dissolved and dissolving chlorpheniramine maleate and sodium hydroxide present in SIF on lipid miscibility with aqueous fluid. Although the aqueous solution of chlorpheniramine maleate is acidic (pH 4–5), the acidic moiety (maleic acid) is a soluble organic acid that is miscible with lipids. For instance, maleic acid is used as a rancidity retardant in fats and oils (Swinyard and Harvey, 1970a,b). In SIF, the release of piroxicam reached maximum after 30 min, which became relatively constant even at 3 h. This shows that the formulation may achieve a fast onset of action with a subsequent sustained effect. The inclusion of Tween 65 in the formulation may improve in vivo bioavailability by enhancing the rate and/or the extent of drug solubilization into aqueous intestinal fluids. Hence, the drug will be present in fine droplets of the fat/surfactant mixture, which will spread readily in the gastrointestinal tract. The experiment performed without Tween 65 did not produce any useful result as it was not stable. In vivo studies using this SMEDDS was not carried out. This work is a preliminary report on the use of this novel homolipid for the formulation of SMEDDS. The result of this study will thus serve as a guide for designing in vivo experiments using this novel homolipid.

4. Conclusions

The SMEDDS showed good release profiles as drug delivery systems. Results of this study indicated that in vitro drug release varied with the release media and particle size. Piroxicam, which has the least particle size released lower than chlorpheniramine maleate and lastly metronidazole in SGF and distilled water. From the result of the study, piroxicam, a lipophilic, drug, may be formulated as SMEDDS to provide a high initial therapeutic concentration of the drug when administered orally. Chlorpheniramine maleate had the highest release in SIF while piroxicam had the fastest release in SIF. This method of drug delivery may be best suited for lipophilic drugs where resulting emulsification brought about by the presence of Tween 65 gives faster dissolution rates and absorption mainly in the gastrointestinal region. The optimized formulations could be encapsulated in hard or soft gelatin capsules and administered as solid dosage form or under appropriate control, dispersed in sterile water for injection and administered parenterally, where a more sustained effect may be achieved.

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