Bioavailability Improvement of SLMs Based Erythromycin Ethyl Succinate using Stearic Acid-Myrj-52-based SLM's

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Abstract: Solid lipid microparticles of erythromycin ethyl succinate were prepared using solvent evaporation method to improve its bioavailability and efficacy. The solvent was allowed to evaporate after which the various entrapments were determined; the best entrapment was used in the *in vivo* studies to determine the bioavailability and efficacy. This study was done with albino mice. The best entrapment obtained was 83% with a loading capacity of 2.9% (Batch D) and was used in comparison with the unformulated drug to check for the *in vivo* efficacy. The results show higher efficacy with the formulated drug than with the pure drug both *in vitro* and *in vivo*. The *in vitro* test results were better despite that some enzymes which need to act on the solid lipid microparticles were not present in the *in vitro* assay and could lead to a reduction in the release of the drugs. In conclusion, there was improvement in efficacy, and hence bioavailability.

Key words: Erythromycin ethyl succinate, solidlipid, microparticles, oral bioavailability, improved activity.

1. Introduction

Low bioavailability is a major problem of many antibiotics that leads to their over prescription. As a result of this there have been problems of adherence to medications by patients. Some of the antibiotics with such bioavailability problems as a result of instability in the stomach include gentamicin, erythromycin amongst a host of others. Thus, gentamicin is given intravenously while erythromycin is given four times daily by oral route due to bioavailability insufficiencies and its lipophilic nature. The bioavailability of lipophilic drugs can be improved using various methods which include microemulsion, nanosuspension, SLN (solid-liquid nanoparticle),

SLM (solid-lipid microparticle), SEEDS (self emulsifying drug delivery system), complexation, cyclodextrin, etc. [1]. For the improvement of gastrointestinal absorption and oral bioavailability of several drugs, lipid micro emulsions based on solid matrix have emerged as potential carriers.

Erythromycin is a broad spectrum antibiotic naturally occuring macrolide derived from *Streptomyces erythreus* (*Sacchrapolyspora erythrae*). It is used for the treatment of Gram + bacterial infections and other Gram –ve bacterial infections [2]. Erythromycin can be grouped into different classes which include erythromycin lactobionate and erythromycin ethylsuccinate. In this work, erythromycin ethylsuccinate will be greatly considered. Erthromycin ethylsuccinate is produced from a strain of *Streptomyces erythraeus* which forms

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salt with acids. The base, stearate and ester are poorly soluble in water.

Solubilizing poorly soluble drugs is a challenge frequently encountered in screening studies of new chemical entities as well as in formulation design and development [3]. Oral bioavailability is dependent on a whole lot of factors of which the most important ones are solubility in aqueous environment and drug permeability through lipophilic membrane [4]. Drugs to be absorbed must be present in the form of aqueous solution at the point of absorption [5-8]. Solubility and permeability being the deciding factor for *in vivo* absorption can be altered or even modified by enhancement techniques [9].

As a result of *in vivo* and *in vitro* characteristics and also the difficulties that are encountered in achieving predictable as well as reproducible *in vivo/in vitro* correlation, it is often difficult to develop formulation on many newly synthesized compounds due to their solubility issues [10, 11]. Traditional approaches to drug solubilization include particle size reduction, pH adjustment, and addition of co-solvents and surfactants [12].

Solubilization which involves breaking of inter-molecular or inter-ionic bonds in the solute separating molecules of the solvent [13] occurs in three steps namely: holes opens in the solvent; molecules of the solid breaking away from the bulk and the freed solid molecule integrated into the hole in the solvent [12]. Reasons for poor solubility include: poor aqueous solubility [14]; inappropriate partition coefficient; first pass metabolism; degradation as a result of low pH in the stomach; interraction with food [14, 15].

1.1 Objective of Study

When erythromycin is given orally, it is well absorbed but exhibits poor bioavailability due to its basic nature and thus destroyed by the gastric acid. Though oral formulations of erythromycin are provided with acid resistant coatings that help to facilitate the bioavailability, the coating can delay therapeutic drug levels. As a result of this, its destruction in the stomach due to acid hydrolysis led to the formulation of erythromycin using solid-lipid microparticles among other methods. It was postulated that rapid absorption might greatly reduce the acid degradation and thus bean alternative solution to the poor bioavailabilty. This method helps to carry erythromycin to the target organs or system to avoid gastric acid hydrolysis. Thus, this work is designed to enhance the oral bioavailability of erythromycin by formulating into solid-lipid microparticles.

2. Materials and Methods

2.1 Materials

Erythromycin powder was a donation from Juhel Nigeria Limited. Stearic acid (BHD, England) was a gift from the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. Myjr 52 (polyethylene glycol (40) stearate) (Sigma-Aldrich, Germany). All other reagents were of analytical standard and were used without further processing.

2.2 Methods

2.2.1 Preparation of Erythromycin Solid-Lipid Microparticles

Erythromycin SLMs (solid-lipid microparticles) were prepared using solvent emulsification/evaporation method. Erythromycins (100 mg), Stearic acid (0.5 mg) were dissolved in chloroform (10 mls). The organic phase was then prepared by dissolving lecithin (0.5 g) in chloroform (5 mL) and the resulting solution was poured to the beaker containing the erythromycin. To make the aqueous phase, Myrj 52 (1.5 g) was dissolved in distilled water 30 mls and the subsequent solution obtained was heated to 75 ± 2 °C on a hot plate with a magnetic stirrer. The organic phase was then added to the aqueous phase while stirring at 1,000 rpm for 2 hours to allow the organic solvent to

completely evaporate. Ice cold water (5 mL) was added to the mixture and stirred again at 1,000 rpm for another 1 hour. The resulting suspension was then centrifuged in a super refrigerated centrifuge at 5,000 rpm for 4 hour to isolate and subsequently remove the supernatant. The procedure was done for other batches with varying quantity of stearic acid (mg) and lecithin (g) as shown in Table 1.

The calibration curve of the pure drug was obtained using a UV-Visible spectrophotometer and the various entrapments of the different formulations calculated. The best formulation was gotten based on the entrapment and this was used to carry out the rest of the study.

2.2.2 Characterization of Solid Lipid Microparticles

2.2.2.1 Aesthetic Examination

The prepared formulations were kept for about 2 days. Changes in homogeneity and colour were observed.

2.2.2.2 Particle Size

The particle size analysis of the formulation was done by light microscopy using a photomicrograph. The mean and standard deviation of three determinations were calculated.

2.2.2.3 Preparation of Calibration Curve

Beer Lambert plot of erythromycin was obtained by using 70% ethanol as solvent using concentrations of 10-100 mg% solution read off at 360 nm was adopted. Absorbance was then plotted against concentration and data were subjected to regression analysis. Regression was found to be 0.9862 as shown in Fig. 1.

2.2.2.4 Entrapment Efficiency

Entrapment efficiency was determined using an indirect method and this was done by centrifuging the various formulations at 5,000 rpm for 4 hours at 4 °C using an Eppendoff 5804 R Refrigerated Centrifuge. The supernatant was collected from each of them and the amount of free erythromycin was determined by diluting with 70% ethanol and taking its absorbance at 360 nm using a UV-Vis spectrophotometer and using the Beer Lambert plot for erythromycin. The drug entrapment efficiency within the lipid was calculated using the equation below

$$E.E = \frac{\text{Amount of drug entrapped in SLM}}{\text{Theoretical total amount of drug added to SLM}} \times 100$$

The amount of drug that was entrapped was thus calculated by subtracting amount of free drug from the theoretical amount of drug added to the SLM.

2.2.2.5 Loading Capacity

The loading capacity of the best formulation was calculated from its entrapment efficiency thus the ratio of entrapped amount of drug to total formulation mass.

Table 1   Quantities of the ingredients used in the preparation of the solid lipid micro particles.

Batch	Erythromycin (mg)	Steric acid (mg)	Myrj 59 (g)	Lecithin (g)
A	100	0.5	1.5	0.5
B	100	0.25	1.5	0.5
C	100	0.25	1.5	0.25
D	100	0.5	1.5	0.25
E	100	0.25	1.5	0.5

Table 2   Concentrations and absorbance used to plot the calibration curve.

Concentration (mg/mL)	Absorbance (360 nm)
0	0
0.1	0.004
0.2	0.010
0.3	0.013
0.4	0.016
0.5	0.020
0.6	0.026
0.7	0.029
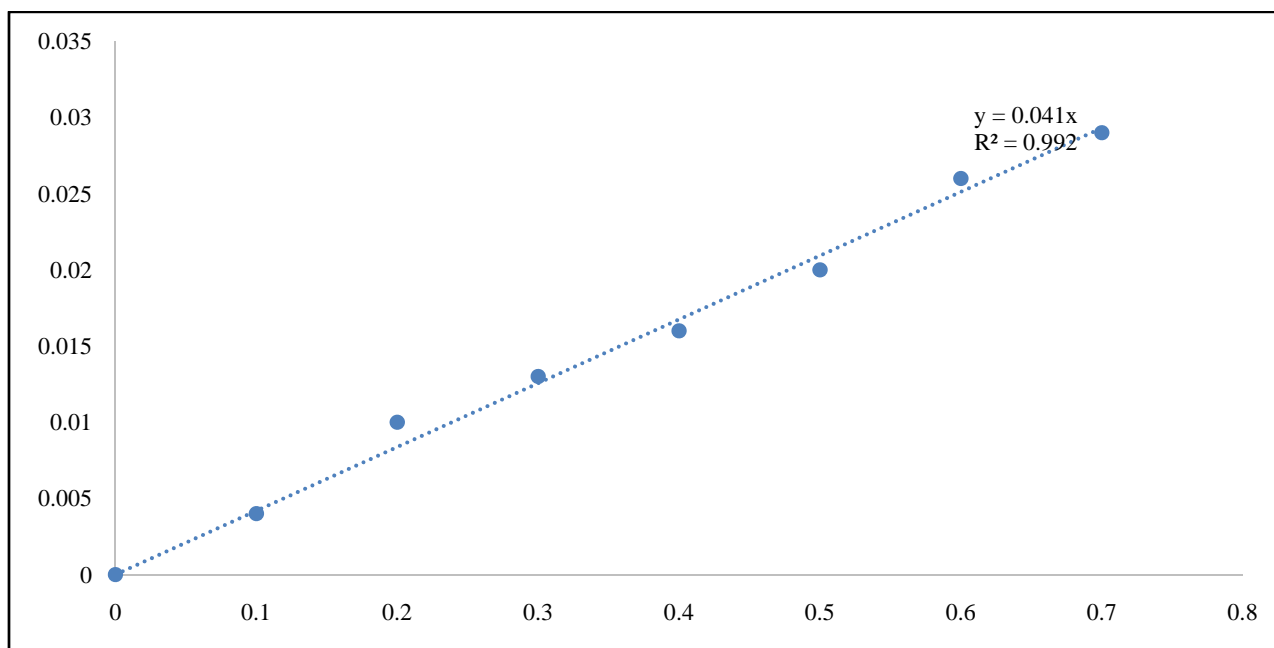
Fig. 1 Calibration plot of erythromycin.

Loading Capacity =

$$\frac{Amount\,of\,drug\,entrapped}{Amt.of\,lipid\,used+amt.of\,drug\,entrapped+amt.of\,surfactant}$$

2.2.3 In Vitro Antimicrobial Screening

The *in vitro* antimicrobial screening was carried out using agar well diffusion method (Okore, 2009), sterile molten nutrient agar was prepared following standard method and also, the sterile petri-dishes were assembled under aseptic condition. 0.1 mL of *E. coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Bacillus subtilis* were respectively added into the separate petri-dishes and 20 mL sterile molten nutrient agar was added as well. The petri-dishes were carefully swirled clock wisely and anti clock wisely to attain a homogenous mixture and the resultant mixture was allowed to solidify. A sterile cork borer of about 8mm diameter was used to bore two cups on the solidified agar medium. Using 1 mL sterile syringe, 0.01 volume of each sample was added to the wells and allowed for 20 to 30 pre-diffuse into the media. The preparations were incubated at 30 °C for 24 hours and the IZD (in hibitionzone diameter) were measured and calculated. The process was done for the pure drug, the SLM and excipients without drug.

2.2.4 In Vivo Studies

Twelve (12) albino mice were divided into four groups A, B, C and D. Broth culture of test *E. coli* was used to obtain 0.5 McFarland standard with dilution to 10^4 CFU/mL using sterile normal saline, while the broth was prepared according to the manufacturer's specification. Groups A, B and C were infected with *E. coli* by IP (intra-peritoneal) inoculation of the test organism, while group D was left uninfected. After infecting, 0.5 mL blood samples were taken by tail milking from the albino mice and cultured in nutrient broth. The nutrient broth was incubated for two days. The nutrient agar was also prepared according to manufacturing procedures. The blood was added to a vial containing 2 mL sterile nutrient broth for bacterial resuscitation from blood and the vials were incubated for 48 hours, after which, they were mixed with sterile molten nutrient agar and poured into a petri-dish by pour plate method and allowed to solidify. The resultant preparation was incubated for 24 hours and the number of colonies were counted and calculated as

Colony forming unit =

$$\frac{No\,of\,colonies}{volume\,per\,drop}\,X\,Dilution\,factor\,(CFU/ml)$$

Table 3   Groups of albino rats and treatment given.

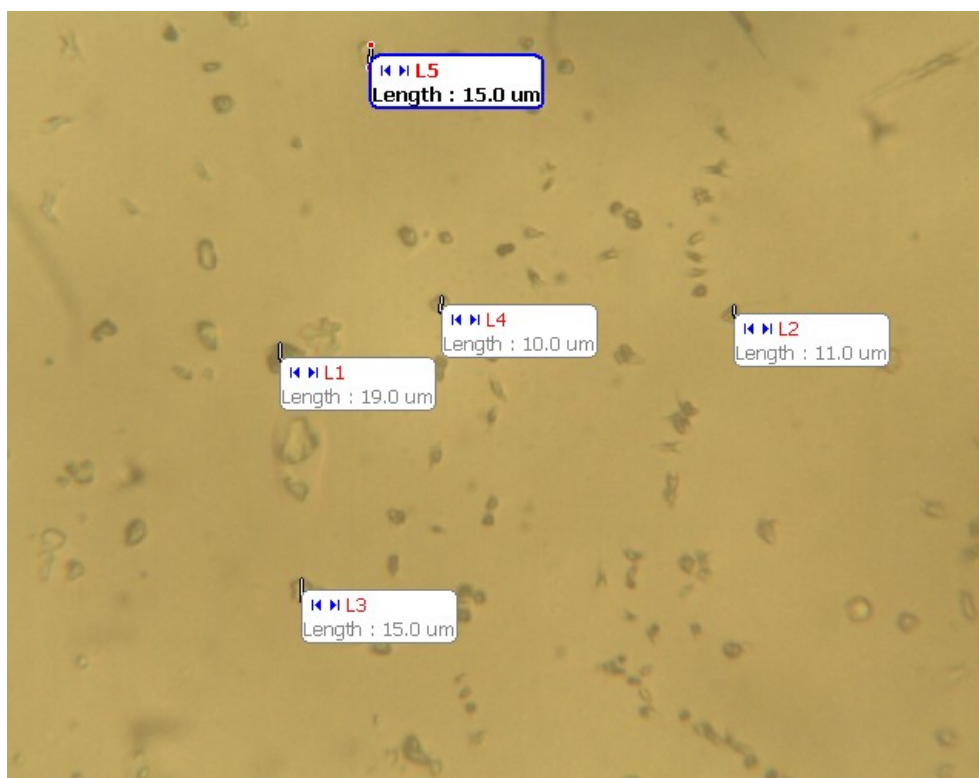| Group	Treatment
A	Formulation
B	Pure drug
C	Sterile water
D	Sterile water +without infection

Fig. 2   Photomicrograph of the formulation.

Table 3 shows the various groups and the manners they were treated. The dose of erythromycin for treatment was calculated for 7.14 mg/kg given twice a day whether as formulation or unformulated drug.

3. Results

3.1 Appearance

All the different preparations of solid-lipid micro particles appeared milky and translucent. After 3 days the system remained homogenous. About after 2weeks the formulation began to separate and developed small sediments which were seen at the bottom of the flask.

3.2 Particle Size

After the entrapment was calculated the best formulation according to the result which the entrapment gave was collected and the particle size was analysed using a photomicrograph and the result is shown in Fig. 2. The mean size was given as 14 μm $\pm$ 3.

3.3 Entrapment Efficiency

Entrapments of the various formulations are shown in Table 4.

Batches not represented did not form good emulsions.

3.4 In Vitro Antimicrobial Assay

The results of the IZDs of the pure drug, the formulated drug and the excipients are shown in Tables 5, 6 and 7 respectively.

Table 4   Entrapment efficiency and loading capacity of different batches of solid-lipid microparticles.

Batch	Concentration equivalent unentrapped (mg/mL)	Concentration entrapped (mg/mL)	Entrapment Efficiency (%)	Loading Capacity (%)
A	1.800	0.730	73.00	2.26006
B	1.625	0.756	75.62	2.51546
C	1.225	0.816	81.62	2.89823
D	1.125	0.831	83.15	2.69836

Table 5   IZD for pure erythromycin.

Test Org.	Concentrations (µg/mL)				Controls	
	30	15	7.5	3.75	Eryth. (30 µg/mL)	Sterile water (10 mL/kg)
	Inhibition zone diameter (mm)					
E. coli	4 ± 0.0	0	0	0	4 ± 0.0	0
K. pneumonia	16 ± 0.5	14 ± 0.0	12 ± 0.0	8 ± 0.5	16 ± 0.5	0
S. aureus	18 ± 0.0	16 ± 0.0	12 ± 0.5	8 ± 0.0	18 ± 0.0	0
B. subtilis	0	0	0	0	0	0

Table 6   IZD of the formulation.

Test Org.	Formulation concentrations (µg/ml)					Controls	
	191.47	95.73	47.9	23.93	11.96	Positive	Negative
						Eryth. (30 µg/mL)	Sterile water (10 mL/kg)
E. coli	12 ± 0.0	10 ± 0.0	8 ± 0.0	4 ± 0.0	2 ± 0.0	4 ± 0.0	0
K. pneumonia	24 ± 0.0	21 ± 0.5	19 ± 0.0	17 ± 0.5	15 ± 0.5	16 ± 0.5	0
S. aureus	28 ± 0.0	26 ± 0.0	22 ± 0.0	20 ± 0.0	18 ± 0.0	18 ± 0.0	0
B. subtilis	0	0	0	0	0	0	0

Table 7   IZD for excipients without erythromycin.

	6.6 mg/mL	3.3 mg/mL	1.65 mg/mL	0.875 mg/mL	0.4875 mg/mL
E. coli	0	0	0	0	0
K. pneumonia	0	0	0	0	0
S. aureus	0	0	0	0	0
B. subtilis	0	0	0	0	0

The starting concentration of 191.4 µ/mL corresponded to a total formulation concentration of 6.6 mg/mL was chosen because beyond this concentration, the reaction medium became turbid, and that could interfere with results.

3.5 In vivo Studies

The results of the *in vivo* studies using *Escherichia coli* as the microorganism are shown in Table 8.

4. Discussion

Erythromycin solid-lipid microparticles were prepared and in each case it was noticed that during the preparation the different formulations appeared clear but on addition of water and continuous stirring the formulations gradually became semi-transparent. After about 2 hours the formulation had condensed to about 5mls and then cold water was added to it in drops and stirring continued. It was observed that the different formulations turned milky and also were semi-transparent (emulsion). SLM-D gave the best entrapment while SLM-A gave the least entrapment. Thus, the best formulation was selected as batch D and used for further assays.

The mean particle size of SLM D using the photomicrograph was 14 µm. From the results of the *in*

Table 8 Bacteraemia after infecting the mice with *E coli*.

Groups of albino mice	A	B	C	D
Bacterial cell count at 24 h	300 ± 8	$350 \pm 15^*$	$270 \pm 10^*$	0
Bacterial cell count at 56 h	150 ± 5	$220 \pm 13^*$	$500 \pm 12^*$	0
% Bacteraemia	50 %	62.9%	185%	-

Key: A = mice treated with the formulation; B = mice treated with the pure drug; C = mice that was not treated (control) D = uninfected
* Significantly different from A values at $p < 0.05$
Note: *E. coli* was used for infection because *in vitro* work showed some minimal effect of erythromycin.

vitro microbiological assay, it was observed that the formulation had more activity (i.e. larger IZD) as compared with that of the pure drug. This is an indication formulation enhanced the penetration into the microorganisms. There is a tendency that there are some enzymes in the body which need to act on the SLM for it to exert its optimal action which are not present in the microorganism, thus a higher activity might be expected *in vivo*.

The various concentrations that were used in comparison showed that as the concentration of the drug decreases there is a decrease in IZD and thus decrease in activity as regards the formulated drug. Therefore, an increase in the concentration of the drug signifies an increase in activity. Our result shows that erythromycin did not have activity against *Bacillus subtilis*.

The results also show that the excipients used in the formulation did not have any antimicrobial property. This serves as a control. Preliminary studies had shown that after infecting with *E coli*, mice left untreated had severe stooling and died after 48 hours. Before 24 hours, the infection had not built up sufficiently. Hence, the optimal time to start the treatment was after 24 hours of infection. Thus, the treatment of the animals commenced after 24 hours of infection and this lasted for 56 hours with continuous monitoring. It was observed that the animal group that were left untreated had continuous defecation and died. As for the treatment groups, it was observed that the group which was given the pure drug just had a little decrease in their defecation while that given the formulated drug were healthier and much less stooling

than that the group treated with the unformulated drug. It was also observed that some of the mice that were treated with the unformulated drug died while all the animals treated with the formulation survived.

Bacteraemia count after treatment showed results in the order: formulation (50%) < pure drug (62.5%) < untreated (185%). Hence, the formulation was superior to the unformulated drug in antibacterial efficacy. This shows increased bioavailability of erythromycin ethyl succinate when formulated into solid-lipid microparticles.

5. Conclusion

The solid-lipid microparticles of erythromycin ethyl succinate were more efficacious in term of bioavailability than the unformulated erythromycin.

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