Formulation and Evaluation of Ciprofloxacin Mixed-Micelle Micro particles.

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Abstract

Ciprofloxacin is a fluoroquinolone antibiotics that is available in different dosage forms for the treatment of various forms of bacterial infections. In this study we developed mixed micelles microcapsule formulation consisting of ciprofloxacin (CIPRO) targeted at enhanced antimicrobial, pharmacokinetic and pharmacodynamic properties for improved patient compliance. Graded-concentrations of ciprofloxacin (0.0, 0.25, 0.5, 0.75 and 1.0 g) dissolved in Transcutol (11 mL) and Phospholipon 90 G (P90 G, 10 g) nanostructured lipid carrier to which the aqueous-phase enriched mixed micelles (Solutol and Poloxamer 118, P118) were added at same temperature (85°C) and subjected to high shear hot homogenization (24 × 1000 rpm for 10 min), before cooling to room temperature were prepared. The resultant microcapsules were characterized by % yield, encapsulation efficiency, particle size, pH storage stability, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and antimicrobial drug release study. The morphology showed spherical shapes of the particles and particle size analysis showed that particles are in micrometer range, with decreased enthalpy values, indicating less crystallinity, good CIPRO incorporation (high EE) and retention over time. There was no interaction between CIPRO and constituents of formulations. Antimicrobial drug release showed better antimicrobial activity of microcapsules than conventional CIPRO tablet. In conclusion, mixed micelle CIPRO microcapsules therefore could possibly modify the adverse effects associated with ciprofloxacin high doses in conventional oral tablets, reduce dosing frequency and improve patient compliance.

Keywords: Ciprofloxacin; Mixed micelles; Microcapsules; Solutol; Transcutol;Phospholipon 90 G.

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Introduction

The Internet The continued decline in the development of newer antibiotics has made the need for the preservation/ improvement of the efficacy of existing ones inevitable. This decline may be attributed to decrease in drug research and development [1] and difficulties posed by government regulation [2]. Also more worrisome is the ease at which these microorganisms develop resistance. The preservation/ improvement of the efficacy of these conventional antibiotics can be achieved through novel drug delivery system (NDDS). The NDDS is basically concerned with the delivery of optimum concentration of the active pharmaceutical ingredient at the required site with attendant improved therapeutic outcomes [3,4]. Microencapsulation is one of such NDDS where drug containing core is surrounded by a polymer shell. The coating material can be selected from a wide variety of natural and synthetic polymers depending on the core material to be encapsulated and the desired characteristics. This is because the physical and chemical properties of the microcapsule depend greatly on the appropriateness of the coating material selected. The polymeric materials should be non-reactive and chemically compatible with the microencapsulated drug. The formulation of drugs as microcapsule may lead to an improvement on therapeutic outcome, through protection of drug(s) from environmental factors (pH and enzymes), to prevent incompatibilities etc [5]. Ciprofloxacin (CIPRO) is a fluoroquinolone antibiotic that is effective against gram-negative and gram-positive bacteria. It can be used in the treatment of urinary tract infections[6], acute uncomplicated cystitis [7], chronic bacterial prostatitis [8], lower respiratory tract infections [9], acute sinusitis [10], skin and skin structure infections [11], bone and joint infections [12], complicated intra-abdominal infections (used in combination with metronidazole) [13], infectious diarrhea [14], typhoid fever (enteric fever) [15], uncomplicated cervical and urethral gonorrhea [16], and inhalational anthrax (post-exposure) [17] caused by susceptible organisms. The fact that ciprofloxacin has multiple applications due to its broad

spectrum of activity makes it a good candidate for novel drug delivery.

Ciprofloxacin is a slightly soluble drug in water. Thus, it is usually formulated with non-aqueous solvents. In this study we investigated the effect of encapsulation of ciprofloxacin within nanostructured lipid carrier of Phospholipon® 90 G and Transcutol lipidic mix to which a mixed-micelle environment of two polymers (Solutol and Pluronic 5%) were added for greater solubility and antimicrobial performance.Khan GJ, Khan RA, Majeed I, Siddiqui FA, Khan S. Cipro oxacin; the frequent use in poultry and its consequences on human health. Profession

Materials and methods

Ciprofloxacin hydrochloride (Figure 1) was obtained from Hangzhou Dayang Chem. Co.,Ltd, (China), Phospholipon® 90 G (P90 G) was a gift from (Phospholipid GmbH Nattermannallee 1, Germany) while Poloxamer® 188 and Solutol® HS (BASF, Germany) were received as donations as well as Transcutol® HP by Gattefossé, France. Distilled water was obtained from Lionwater, Nigeria.

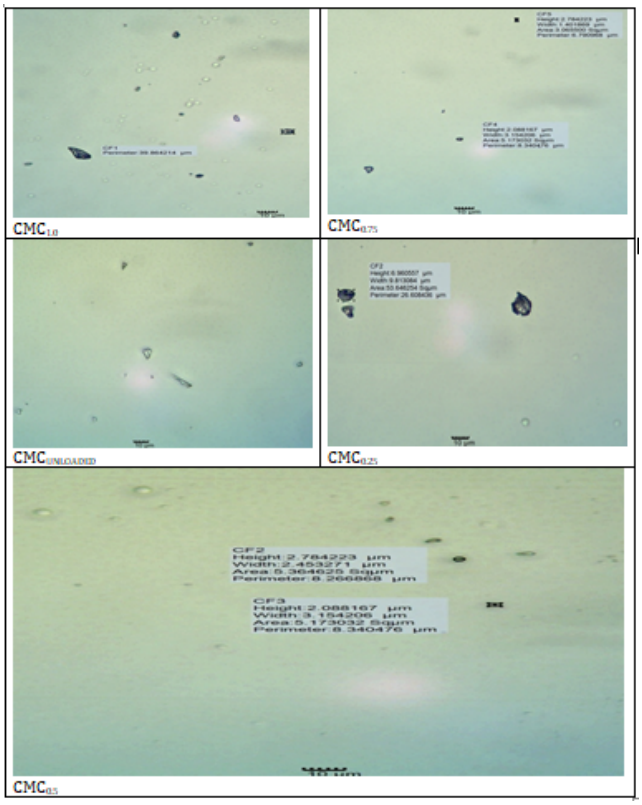

Figure 1. *Morphology of microcapsules*

2.3 Microorganisms

Strains of Bacillus subtilis, Escherichia coli, Staphilococcus aureus, Psudomonasaureginosa, Salmonella typhi (all cultured by Mr.Chijioke Clement, Department of Pharmaceutics, Faculty of Pharmaceutical Science, University of Nigeria, Nsukka).

2.4 Methods

2.4.1 Preparation of microcapsules: Hot homogenization method was adopted [18]. A 10 g weight (weighing balance, Adventurer, Ohaus China)of Transcutol(equivalent to 11.1 mL) was introduced into a beaker and separately loaded with graded concentrations of CIPRO (0.00, 0.25, 0.50, 0.75 and 1.0 g). P90 G (10%w/v) was dissolved at 85 0 C into the mixture to make the oil phase. A 5 % mixture of Solutol and Pluronic (3.75:1.25% w/v) were dissolved in a given volume of water used to make up the solution to 100 ml (aqueous phase). The two phases were mixed at 85 0 C and homogenized at 24 × 1000 rpm for 10 min using ultra Turrax (T18 basic, IKA Germany), to produce a dispersion which was stored both at room temperature and in a refrigerator.The optimized formula for the preparation of the microcapsules is shown in Table 1 below.

Table 1. *Formulation composition of microcapsules*

FORMULATION CODE	DRUG (g)	P90 G (g)	TRANSCUTOL (g)	SOLUTOL (g)	P118 (g)	DISTILLED WATER (ml)
CMC0.25	0.25	10	10	3.75	1.25	74.75
CMC0.5	0.5	10	10	3.75	1.25	74.5
CMC0.75	0.75	10	10	3.75	1.25	74.25
CMC1.0	1	10	10	3.75	1.25	74
CMCUNLOADED	0	10	10	3.75	1.25	75

2.4.2 Preparation of calibration curve for ciprofloxacin (Beer Lambert's): A 1 mg/mL stock solution was made by dissolving 100 mg of CIPRO in 100 mL of Transcutol. A 1 mL volume of the stock solution was diluted to 100 mL using the solvent to make a 10 µg/mL solution. Increasing volumes (1,2,3,4,5,6,7,8,9 mL) were collected and each diluted to 10 mL to make 1,2,3,4,5,6,7,8,9 µg/mL concentrations. The absorbance of the 10 µg/mL concentration was measured at different wavelengths. The highest absorbance was got at 277.6 nm (λmax). The absorbances of the diluted solutions were measured at 277.6 nm using a UV spectrophotometer UV/VIS spectrophotometer (6405 Jenway, UK) and the values plotted against concentrations. The slope of the graph was got which represented the constant k of the Beer Lambert's plot.

2.4.3 Determination of encapsulation efficiency: The encapsulation efficiency was determined by calculating the content of CIPRO in the microcapsules using the spectrophotometric method. The formulation poured into eppendorf tubes were each spun at 4000 revolutions per minute for 100 min using a centrifuge. 1 mL of each supernatant liquid was diluted 1000-fold and put into a cuvette. Their absorbances were measured using a UV/VIS spectrophotometer (6405 Jenway, UK) at 277.6 nm and the encapsulation efficiencies (EE) of each formulation was calculated using the following formula:

EE (%) = (Real drug loading)/ (Theoretical drug loading) ×100…….. (1)

EE (%) = (W total-W free)/ (W total) ×100……. (2)

2.4.4 Determination of morphology and particle size: A thin smear of each formulation was made on a microscope slide and covered with cover slips. The slides were then placed under a light microscope at a magnification of 100. The images were captured using Motic®images software (Motic, Xiamen China). Size measurement was diametrically determined.

2.4.5 Determination of pH (stability studies): The drug formulations were subjected to time-dependent pH analysis to determine the short-term stability. The pH of each of the dispersions was determined using a validated pH meter (SevenCompact™ pH/Ion S220, Mettler-Toledo AG 8603 Schwerzenbach, Switzerland). After calibration with pH standards (p^{H4} and p^{H7}), the electrode was rinsed, paper dried and immersed into the dispersions. The pH was read 2, 8 and 12 weeks after formulation.

2.4.6 Differential Scanning Calorimetry (thermal analysis): The thermal properties of the formulations were determined using a DSC machine (Mettler Toledo Switzerland) with an empty aluminum pan as reference after baseline correction. Each sample was measured into an aluminum crucible, covered and put into a DSC machine chamber alongside another empty aluminum crucible which was used as a reference. To investigate the transition of drug molecule from crystalline to amorphous form during the formulation process, at least 3-5 mg of each sample was weighed in an aluminum pan, heated from 60 -300 0 C at 10 C/min under constant flushing with nitrogen (10 ml/min). The DSC parameters, such as temperature onset, maximum peak and enthalpy were generated.

2.4.7 Fourier Transform Infrared spectroscopy: The compatibility of the formulations, pure drug, polymers and other ingredients were studied using a Shidmazu FTIR 8300 spectrophotometer (Shidmazu, Tokyo, Japan). For the solids, 0.4 g of potassium bromide (KBr) was weighed and ground to powder. About 0.001 g of the sample was weighed into the ground KBr and both were thoroughly mixed and molded into a disc. The disc was inserted into the sample compartment of the instrument. The scan was started and the IR spectrum generated.

Preparation of media

Nutrient Agar: A 28 g of nutrient agar powder was dissolved in 1000 mL of distilled water, and was allowed soaking for 10 mins. Similarly, some 47 g Potato Dextrose Agar (PDA) powder was dissolved in 1000 mL of distilled water, and subsequently allowed soaking for 10 mins. The two media suspensions were brought to melt by boiling in water baths. A 20 mL aliquot of each molten media was dispensed into bijou bottles, cocked, and sterilised in an autoclave at 121 0 C for 15 mins. The sterile molten media was stored at 42 0 C until used.

Test microorganisms: Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Salmonella typhi were clinical isolates obtained from Pharmaceutical Microbiology Laboratory Unit of Pharmaceutics Department, University of Nigeria, Nsukka. The organisms were standardised using 0.5 macFarland turbid equivalent.

2.4.8.2 Preparation of different concentrations of the formulation: Each of the formulation was diluted with 50% DMSO a 1000-fold to make a stock solution. From the stock solutions, 100, 50, 25, 12.5 and 6.25 µg/mL was obtained using two-fold serial dilutions as the case may be.

2.4.8.3 Sensitivity test: A 0.2 mL of the standardised test microorganism suspension was seeded in 20 mL of sterile molten agar in a sterile plate under aseptic condition. The agar plates were allowed to gel. The agar plates were labelled with permanent marker, and holes of 6 mm in diameter were bored on each plate. A 0.16 mL of each of the concentrations of the formulations was dropped in the holes, using a cork borer. The plates were allowed to stand for 15 min for proper diffusion before incubation. The plates were incubated at 37 0 C for 24 h for bacteria and at 25 0 C for 48 h for fungal growth. After the due period of incubation, the plates were observed and the inhibition zone diameters were measured and recorded.

2.4.8.4 Determination of MIC: A 0.2 mL of standardised test microorganism suspension was seeded in 20 mL of sterile molten agar in a sterile plate under aseptic condition. The agar plates were allowed to gel. Each agar plate was divided into five equal segments and labelled with permanent marker in correspondence with the five different concentrations besides the stock. Holes of 6 mm in diameter were bored on each segment on the plate. A 0.16 mL of each concentration of the formulations was dropped in the corresponding hole, using a cork borer. The plates were allowed to stand for 15 min for proper diffusion before incubation. The plates were incubated at 37 0 C for 24 h for bacteria, and at 25 0 C for 48 h for fungal growth. After the due period of incubation, the plates were observed and the inhibition zone diameter was measured and recorded for the determination of MIC. The plates were further incubated for 24 h at 37 0 C for bacteria growth, and at 25 0 C for 48 h for fungal growth to determine the minimal bactericidal activity. The MIC was calculated by plotting a graph of inhibition zone diameter [IZD2] against log concentration and the anti-log of the x-intercept got [19].

2.4.9 Statistical analysis: All the data generated were expressed as mean ± standard deviation.

Results and Discussion

3.1: Encapsulation efficiency: The encapsulation efficiency was done to determine the amount of drug that was encapsulated into the polymer matrix to ascertain if the method of encapsulation was fit for the drug being encapsulated. Table 2 shows high encapsulation efficiency of Ciprofloxacin in the formulations and indicated that the method used in encapsulation (hot homogenization method) was very suitable. The CMC0.75 batch of formulation had the lowest encapsulation efficiency (46%) whereas the CMC0.5 batch had

the highest encapsulation efficiency (96%). In other words, the EE was independent of drug concentration. This good encapsulation efficiency of ciprofloxacin in this study is similar to a recent report [20].

3.2 Morphology and particle size analysis: The particle size analysis carried out on all five formulations stored at room temperature showed that the microcapsules were all within the micrometer range.

The particle sizes were within the range of 2.06 to 7.5 µm as shown in the photomicrographs (Fig.1). The highest particle size was that of the batch containing 250 mg ciprofloxacin-loaded microcapsules. The lowest particle size was that of 750 mg ciprofloxacin microcapsules. This agrees with the result of encapsulation efficiency. However, the microcapsules containing 500 mg ciprofloxacin appeared smoother, more spherical and uniform in size than any other batch (no drug crystals).

Stability studies (pH)

The results of the storage stability pH analysis carried out at room (27 °C) and refrigeration (6-8 °C) temperatures for 1 week, 2 and 3 months showed more stability of particles at room temperature (Fig. 2) than at refrigeration temperature (Fig. 3). Microcapsules of 500 mg ciprofloxacin was the most stable at room temperature as well as at refrigeration temperature compared to others. This agrees with the result of morphology and EE. This formulation was henceforth regarded as the optimized formulation.

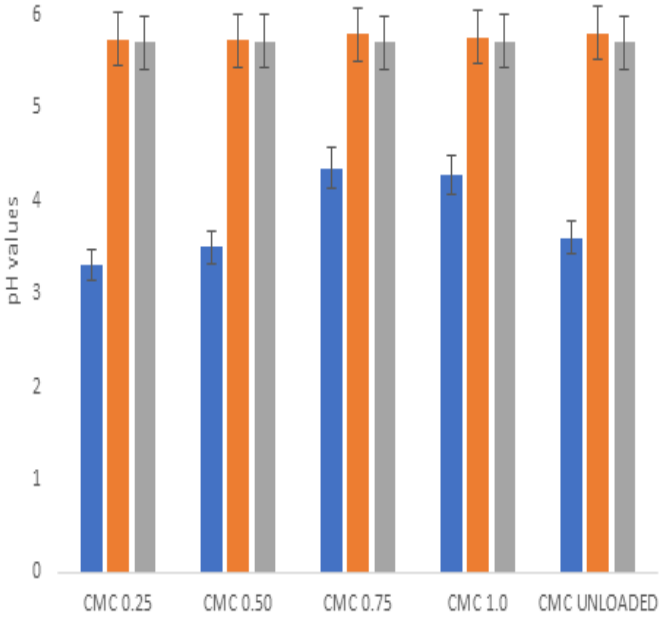

Figure 2*. pH variation of microcapsules at room temperature*

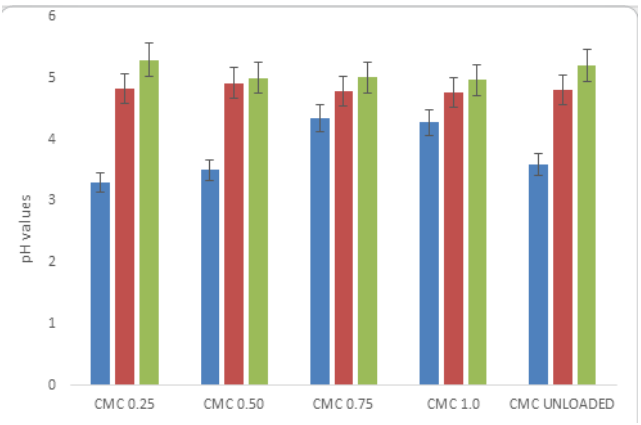

Figure 3*. pH variation of microcapsules at refrigeration temperature*

Fourier Transform Infrared Spectroscopy

The FTIR spectrophotometer was used to determine the interaction between ingredients and polymers used in producing the drug matrix as well as the drug sample. FTIR spectrum of ciprofloxacin showed major peaks at 1694.3, 2678, 2983, 1694 and 1011 /cm indicating the bonds of C=O, O-H, C=C, N-H and C-F respectively (Fig 4A). FTIR spectra of the different formulations (Fig 4B-E) still retained the major peaks indicating that formulation of the microcapsules with different compounds didn't affect the bonds characteristic to ciprofloxacin molecule. The formulation without ciprofloxacin showed absence of peaks due to ciprofloxacin molecule. (Fig. 5A). There was also no interaction due to other excipients in the formulation (Fig. 5B-E).

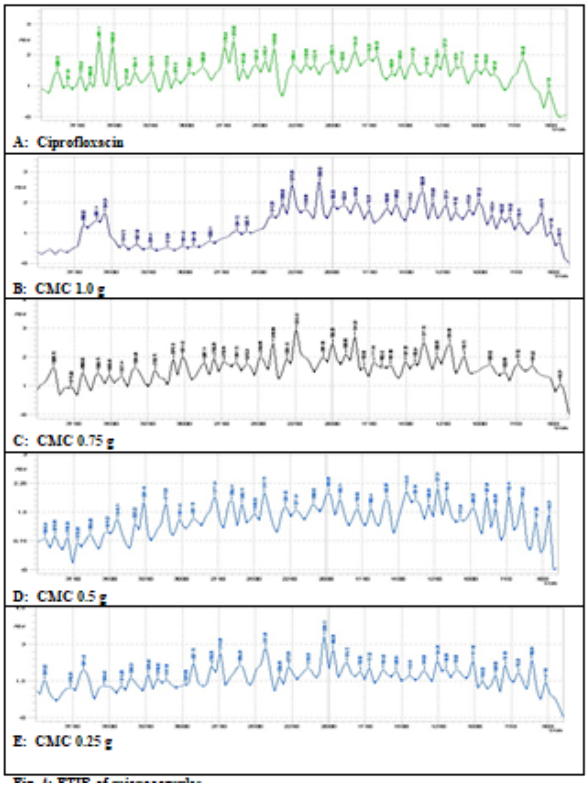

Figure 4*. FTIR of microcapsules*

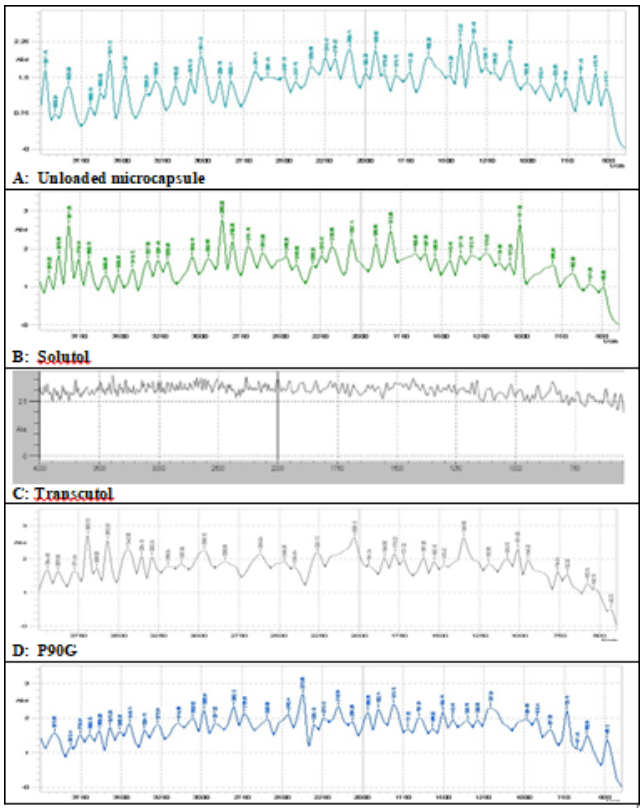

Figure 5. *FTIR of drug-free microcapsule and ingredients*

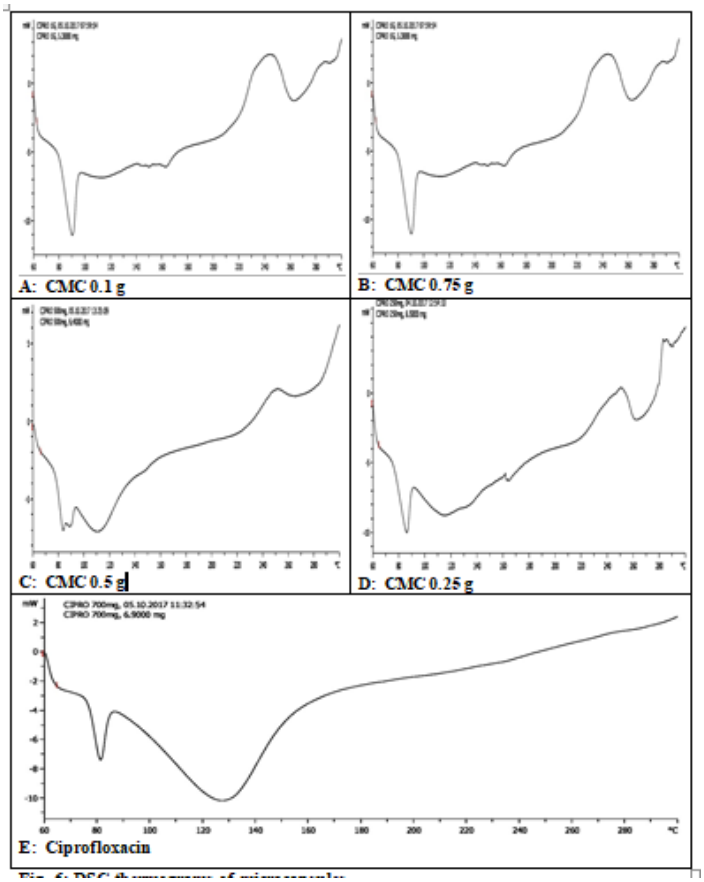

Figure 6. *DSC thermograms of microcapsules*

Differential Scanning Calorimetry analysis

DSC is an analytical tool used to detect and study thermal behaviour of materials. The summary of the DSC result is shown in Table 2. However, their respective thermograms are hereby displayed in Fig. 6 (A-E).Only microcapsule of 0.5 g ciprofloxacin showed the least enthalpy (-7.13 mW/mg) compared to other batches. Higher enthalpy values suggest more crystalline matrix and consequently lower encapsulation efficiency while a lower enthalpy is indicative of more disordered matrix (amorphous) which perhaps enhances drug loading capacity and stability. This is true of microcapsules containing 0.5 g ciprofloxacin which exhibited higher EE, stability and more uniform spherical shape without drug crystals. CMC1.0 had the highest enthalpy of -11.06 mW/mg.However, increase in enthalpy seemed to be dependent on drug concentration. Enthalpy values of other ingredients were much higher than formulations. The enthalpies of Transcutol and Solutolwere -26.76 and -21.3 mW/mg respectively and shown in Fig. 7 (AB)

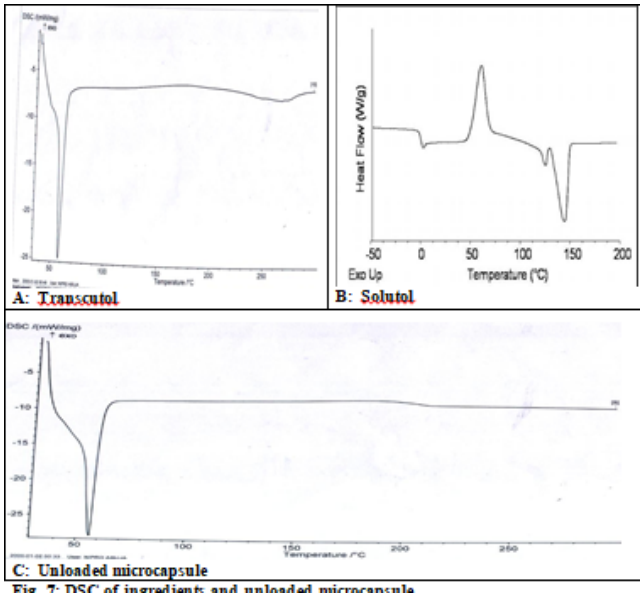

Figure 7. *DSC of ingredients and unloaded microcapsule.*

Whereas ciprofloxacin was -7.17 mW/mg at both onset temperature (80.7 °C) and peak temperature (129.6 °C). This dual peak due to ciprofloxacin was flatter or more spread in microcapsule formulations showing a pleasant interplay between the ingredients and the drug.

Table 2. *Characterization of microcapsules.*

Formulation code	Encapsulation Efficiency (%)	Particle size (µm)	Melting point(0 C)	Enthalpy (mW/mg)
CMC0.25	80±1.02	7.5±0.03	84.67	9.82
CMC0.50	96±1.22	2.78±0.02	109.74	7.13
CMC0.75	46±0.89	2.06±0.04	127.1	10.23
CMC1.0	84±1.03	5.08±0.03	89.62	11.06
CMCUNLOADED	-	-	147.2	7.3

Antimicrobial analysis

The antimicrobial activity of the microcapsule decreased with increasing concentration such that the microcapsule 0.25>0.5>0.75>1>g. The 0.250 g microcapsule had the best MIC values against B. subtilis and E. coli when compared with the other formulations including the positive control Ciprotab®. S. typhi showed similar susceptibility pattern to the 0.250 g, 0.500 g microcapsule and Ciprotab®. However, Ciprotab® showed a marked superiority against P. aeruginosa and moderately S. aureus (Table 3). MIC value is the least concentration of the antimicrobial agent that prevented visible growth of the organism(s). It is a measure of the potency of the agent and the smaller the value the higher the antimicrobial efficacy. The inhibition zone diameter (IZD) measurement as a determinant for CIPRO release followed drug concentration dependence as highest drug-containing microcapsule (1.0 g) had the highest IZD (Table 4). Overall, since IZD of microcapsules containing both 0.75 and 0.5 g of CIPRO equally performed better than the commercial CIPRO tablet, it could be deduced that the microcapsules could serve as prolonged regimen for treatment of bacterial infections rather than the tablet form. This will make for a once-daily dosing instead of twice-dosing of the conventional tablet culminating in improved patient`s compliance.

Table 3. *MIC Values of different ciprofloxacin formulations.*

Formulation code	B. subtilis	E. coli	Staph. Aureus	Pseud. aeruginosa	S. typhi
CMC0.25	2.042±0.01	2.51±0.02	3.16±0.03	6.31±0.02	3.16±0.03
CMC0.50	3.45±0.02	3.98±0.03	3.02±0.01	6.76±0.08	3.31±0.02
CMC0.75	5.01±0.01	5.01±0.02	8.32±0.03	6.30±0.01	4.79±0.03
CMC1.0	7.94±0.03	6.31±0.11	8.13±0.02	7.94±0.02	5.13±0.02
CMCUNLOADED	25.12±0.74	31.62±0.82	31.62±0.18	-	-
CIPROTAB	2.51±0.03	3.16±0.02	2.51±0.01	1.20±0.01	3.55±0.04

Table 4. *Growth inhibition zone diameters as drug release index.*

Formulation code	B. subtilis	E. coli	Staph. Aureus	Pseud. aeruginosa	S. typhi
CMC0.25	20±0.09	18±0.08	12±0.01	11±0.01	12±0.03
CMC0.50	31±0.12	22±0.11	20±0.09	19±0.06	21±0.05
CMC0.75	32±0.23	24±0.10	22±0.11	19±0.09	21±0.11
CMC1.0	34±0.14	25±0.13	30±0.14	30±0.12	23±0.12
CMCUNLOADED	0	0	0	0	0
CIPROTAB	31±0.13	21±0.11	25±0.09	18±0.09	20±0.07

The 0.5 g microcapsule had the best EE, pH stability upon storage, DSC and particle uniformity values hence the optimal concentration for further studies. This study is in agreement with other studies targeted at novel delivery of ciprofloxacin for enhanced therapeutic outcome [20-22]. In this study, ciprofloxacin was formulated as microcapsules based on matrices of P90 G and Pluronic/Solutol co-polymers in order to obtain a slow release formulation, and further enhance CIPRO dissolution by first dissolving it in Transcutol, liquid oil. The formulation presented microcapsules with good encapsulation efficiency, good particle uniformity and stability upon storage. The antimicrobial property was not compromised rather there was an enhanced antimicrobial activity with decreasing drug concentration. This means that the reduction in particle size led to enhanced antimicrobial activity; hence formulation with reduced side effect and dosing frequency yet with ultimate improvement of patient compliance.

Conclusion

This study has shown that microencapsulation of ciprofloxacin using P90 G and Pluronic/Solutol co-polymers, which in more recent terms refer to mixed-micelles has improved therapeutic outcome of Ciprofloxacin over its conventional tablet form in lower doses. The microcapsules containing 500 mg of CIPRO could be regarded as the overall best formulation in terms of physical and pH storage stabilities, encapsulation efficiency, particle uniformity, drug content and CIPRO release as an index of antimicrobial growth action. An outlook from the present investigation could explore further strategies to either produce liquisolid compacts of the 500 mg CIPRO-loaded microcapsules or capsules for more prolonged release (once daily and/or once alternate days) and user-friendliness at reducing dose and dosing-frequency of the conventional tablet regimen.

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