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Study on Effect of Solvents & Nonsolvents on Microspheres of Ciprofloxacin: Coacervation Phase Separation

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ABSTRACT

The objective of the study was to determine the effect of solvents like acetone, dimethyldigol, 1, 4-dioxan and non-solvents like n-hexane and chloroform on microencapsulation of ciprofloxacin hydrochloride. The microspheres were prepared by following coacervation phase separation using various non- aqueous solvents and non-solvents like solvent evaporation and non-solvent addition method with rate retarding cellulose polymers. Microspheres were characterized for the particle size distribution, wall thickness by scanning electron microscopy (SEM), angle of repose, bulk density, percent drug content, entrapment efficiency and *in vitro* dissolution studies. Drug excipient compatibility was determined by FTIR and DSC. Accelerated stability studies were also carried out following ICH Guidelines. SEM revealed that microspheres were found spherical, free flowing and porous. The wall thickness and entrapment efficiency was found in between 86.26 µ & 118.77 µ, 62.12% & 98.29 % respectively. The drug release was extended maximum upto 12hours with cellulose acetate using 1-4 dioxan as solvent and upto 12 hours with cellulose acetate phthalate using 1,4-dioxan and dimethyldigol. FTIR and XRD spectra results showed ciprofloxacin hydrochloride was compatible with excipients. The curve fitting data revealed that the release followed first order kinetics and higuchi's and peppas plots stated non-fickian and diffusion controlled where dimethyldigol and 1,4 dioxan as solvent polymer showed effect over the size and release kinetics.

Keywords: Microspheres, ciprofloxacin, cellulose polymers, coacervation phase separation, solvents, non solvents

INTRODUCTION

Microencapsulation is defined as the application of a thin coating to individual core material that have an arbitrary particle size range from 5 to 5000 µm[1, 2]. This coating can retard the release of a drug[3], modify the availability of the core and promote sustain release, change the cores chemical properties such as solubility and reactivity and physical properties such as color, and particle size[4], also alter the heat sensitivity and photosensitivity of the core[5]. Microencapsulation can improve the absorption of a drug and reduce side effects such as irritation of the gastric intestinal mucosa[6].

Cellulose acetate phthalate (CAP) and cellulose acetate (CA) is widely used as a coating material for tablets and capsules. Several researchers have investigated the use of CAP and CA as polymer[7-9]. Non-aqueous[10-12] manufacturing vehicles in microencapsulation of a drug by a coacervation phase separation procedure were reported. The solvent evaporation method and non-solvent addition method has been reported by many others[13-15]. However no study was made on the use of solvents like dimethyldigol and 1, 4-dioxan as a solvent to prepare polymer solution. Ciprofloxacin hydrochloride is a broad spectrum antibiotic which is very much effective wide

verities of bacterial and protozoa infections. The half life of drug is 5.5-6.5 hours[16]. The shorter biological half life and frequent dosing makes an ideal candidate for sustained drug release system. Therefore the objective of the work is to provide a sustained pharmaceutical composition contained ciprofloxacin in a modified release formulations and to maintain the blood levels of the active ingredients for a prolonged period of time.

MATERIALS AND METHODS

Materials

Ciprofloxacin hydrochloride was obtained as a gift sample from Cipra Life Sciences, Hyderabad, and (A.P). Cellulose acetate phthalate and Cellulose acetate was obtained as gift sample from Natco Pharma, Hyderabad, and (AP). All the solvents are procured of Merck.

Methods

Preparation of microspheres

The microspheres are prepared by three different methods with three different solvents and two non-solvents. In each of these techniques ciprofloxacin microspheres were prepared with CAP, CA as coating agents. Acetone, dimethyldigol and 1, 4-dioxan were used as solvents and chloroform and n-Hexane were used as non-solvents. Liquid paraffin is used as the encapsulating vehicle. Three batches of ciprofloxacin microspheres were prepared with each polymer and with each technique showed in Table 1.

Ingredient	**Method-I**			**Method-II**			**Method-III**		
Formulation	CAC MA1	CAC MA2	CAC MA3	CAC MM1	CAC MM2	CACM M3	CAC MD1	CAC MD2	CAC MD3
Ciprofloxacin	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm
CA	1 gm	2 gm	3 gm	1 gm	2 gm	3 gm	1 gm	2 gm	3 gm
Acetone	30ml	30ml	30ml	--	--	--	--	--	--
Dimethyldigol	--	--	--	30ml	30ml	30ml	--	--	--
Chloroform	--	--	--	50ml	50ml	50ml	--	--	--
1,4-dioxan	--	--	--	--	--	--	30ml	30ml	30ml
n-hexane	--	--	--	--	--	--	50ml	50ml	50ml
Formulation	CAPC MA1	CAPC MA2	CAPC MA3	CAPC MM1	CAPC MM2	CAP CMM3	CAPC MD1	CAPC MD2	CAPC MD3
Ciprofloxacin	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm
CAP	1 gm	2 gm	3 gm	1 gm	2 gm	3 gm	1 gm	2 gm	3 gm
Acetone	30ml	30ml	30ml	--	--	--	--	--	--
Dimethyldigol	--	--	--	30ml	30ml	30ml	--	--	--
Chloroform	--	--	--	50ml	50ml	50ml	--	--	--
1,4-dioxan	--	--	--	--	--	--	30ml	30ml	30ml
n-hexane	--	--	--	--	--	--	50ml	50ml	50ml

Table 1. Formulation composition

METHOD-I : In this method the polymers were dissolved in acetone by stirring the mixture at 800 rpm and ciprofloxacin is dispersed as particles in liquid paraffin that contained 1% w/w polysorbate. The polymer solution was added slowly to the drug dispersion by means of a burette. The mixture was agitated at room temp (25°c) until the acetone (polymer solvent) was evaporated. The rate of stirring was kept constant for all the batches and for all the methods and the ratio of drug to polymer was varied as (D: P as 1:1, 1:2, 1:3) labeled as CACMA1, 2, 3 & CAPCMA1, 2, 3. The liquid paraffin was decanted and the microspheres were collected,

washed with petroleum ether to remove any remaining oil phase and dried under, reduced pressure for at least 12 hours.

METHOD-II: This procedure is similar to the method-I except that the solvent was replaced by dimethyldigol instead of acetone. After the addition of polymer solution a non-solvent (chloroform) of 50ml was added from a burette for a period 30 minutes. Agitation of liquid paraffin containing ciprofloxacin, polymer solution and chloroform then was performed for 20min. Microspheres collection procedure was the same as described for Method I. the microspheres are labeled as (CACMM1, 2, 3 & CAPCMM1, 2, 3).

METHOD-III: The procedure was similar to Method-I except that 1,4-dioxan was used as a solvent for dissolving the polymer and a non-solvent n-Hexane was added to the liquid paraffin (50ml) that contained dispersed ciprofloxacin particles for a period of 30 min. the microspheres collection procedure was the same as described Method-I. The formulations were coded as CACMD1, 2, 3 for cellulose polymer and CAPCMD1, 2, 3 for cellulose acetate phthalate polymer.

CHARACTERIZATION OF MICROSPHERES

Scanning electron microscopy (SEM)

Morphological characterization of the microspheres was carried using scanning electron microscopy (SEM-S-3700N, SHIMADZU). For SEM the double–sided sticking tape, and coated with gold film (thickness 200 nm) under the reduced pressure (0.001torr).

Particle Size analysis

All the batches prepared were analyzed for particle size; microspheres were placed on the set of standard sieves ranging from sieve # No. 16–60. The sieves were arranged in such a way that in descending order of the mesh size 16# on the top and 60# mesh in the bottom. The microsphere passed through the set of sieves and the amount retained on each sieve was weighed and the average mean diameter was determined. The data is given in (Figure 1 & 2).

Angle of repose

A funnel was fixed in a stand in such a way the top of the funnel was at a height of 6cms from the surface. The microspheres were passed from the funnel so that they form a pile. The height and the radius of the heap were measured and the angle of repose was calculated using the equation.

$$\Theta = h/r$$

Assay of ciprofloxacin

To determine the total drug content of the microspheres 100mg of microspheres was ground as fine powder and dissolved in 5ml of acetone and diluted with phosphate buffer pH 7.4 to 100ml. The drug content was determined spectrophotometrically at 276nm. Three determination of the microspheres content from the same batch for each ratio and method was performed. The data is represented in Table 2.

Encapsulation efficiency (EE)

Drug loaded microspheres were weighed and dissolved in phosphate buffer pH 7.4 and mixture was filtered. The percent entrapment was calculated using the Eq (1). The data is represented in (Figure 3 & 4).

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \qquad \text{(Eq. 1)}$$

Wall thickness

The wall thickness of the prepared microspheres was calculated using the Eq (2):

$$h = \frac{\check{r}\,(1-P)\,d_1}{3(Pd_2 + (1-P)\,d_1} \qquad \text{(Eq. 2)}$$

Fourier Transforms infrared Spectroscopy (FT-IR)

The FT-IR spectra acquired were taken from dried samples. An (FTIR-8400S SHIMADZU, IR Prestige-21) spectrometer was used for the analysis in the frequency range between 4000 and 400 cms^{-1}.

In vitro Drug Release Studies

In vitro dissolution studies were performed using (USP type II dissolution apparatus). The rotating basket method specified in USP-XXI at 75 rpm. The microspheres were weighed and tied in the muslin bag and placed in the basket. The dissolution medium (900ml) consisted of 0.1M hydrochloric acid for the first 2 hours and then changed to phosphate buffer pH 7.4 from the 3rd hour. The temperature was maintained at 37°C. An aliquot of (5ml) sample was withdrawn at specified time interval and replaced with an equivalent volume of dissolution fluid. Drug content was determined by UV-Visible spectrophotometer (Schimazdu UV 1700 E 23) at 276nm. The release studies were conducted in triplicate and the results are showed in (Figure 5).

RESULTS

Prepared microspheres were found to be discrete, spherical and free flowing and have nearly uniform size. SEM (Figure 6). Among the various formulations the formulation CAPTMD1 and CAPTMA3 showed maximum percentage yield and CATMD1 formulation showed highest drug entrapment. The average mean diameter of the microspheres was found to be ranging between 452.08 µm to 621.9 µm.

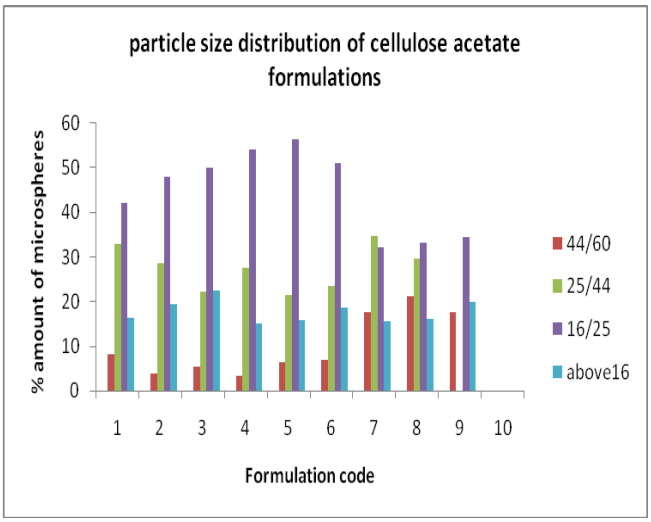

Figure 1: Sieve analysis graph of cellulose acetate microspheres of drug

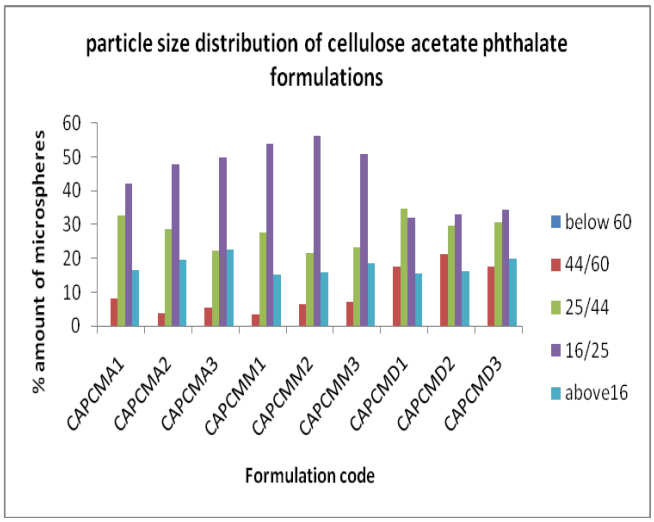

Figure 2: Sieve analysis graph of cellulose acetate phthalate microspheres of drug.

The IR spectra of the pure drug and microspheres with polymers were compared and the characteristic peak for microspheres in spectra was found to be super imposable to that of the pure drug. There are no extra peaks, which gives evidence that there was no drug polymer interaction. The peaks at 3342.10cm^{-1}, 3308.70 cm^{-1}, 2974.33 cm^{-1}, 1606.7 cm^{-1}, 1577.8 cm^{-1}, 1288.49 cm^{-1} corresponds to NH group, OH group, CH aromatic stretch, NH bending, C=C aromatic stretch, and CN stretching respectively (Figure 7).

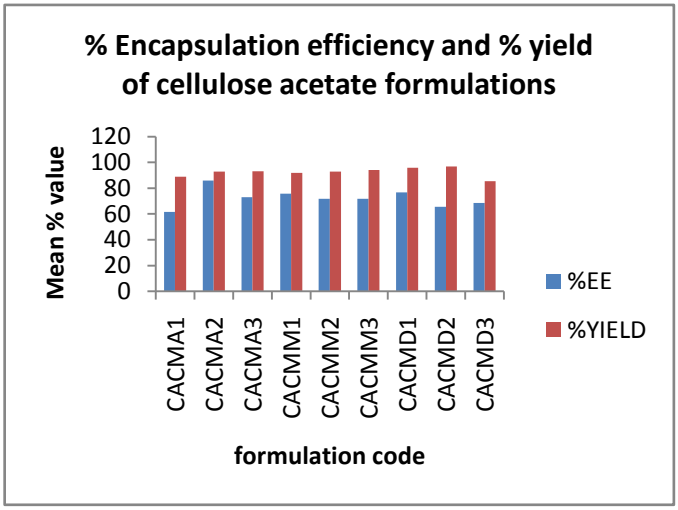

Figure 3: % yield and % entrapment efficiency of cellulose acetate microspheres.

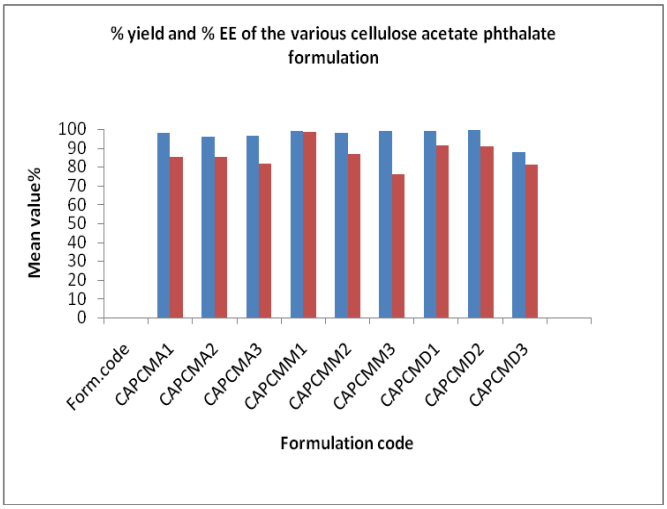

Figure 4: % encapsulation efficiency and % yield of the cellulose acetate phthalate formulation of the drug

FORMULATION	%DC ± SD	Angle of Repose	BD g/cc	AMD µ	Wall Thickness µ
CACMA1	81.51±0.833	29.67	0.585	427.88	104.04
CACMA2	86.00±1.16	27.7	0.582	429.8	112.42
CACMA3	73.12±0.50	27.02	0.560	460.57	117.43
CAPCMA1	85.43±1.02	28.53	0.572	485.21	89.56
CAPCMA2	81.06±1.10	27.98	0.534	524.26	96.00
CAPCMA3	81.82±0.47	27.73	0.528	547.60	87.46
CACMM1	75.80±0.63	26.61	0.599	480.22	109.11
CACMM2	71.84±0.60	26.19	0.592	496.66	110.76
CACMM3	76.68±1.38	25.19	0.584	569.82	112.12
CAPCMM1	98.29±0.48	26.61	0.602	508.81	100.09
CAPCMM2	86.67±0.59	26.86	0.592	516.52	103.3
CAPCMM3	76.26±1.25	26.94	0.580	523.1	118.77
CACMD1	65.53±0.66	24.56	0.580	413.84	94.26
CACMD2	68.6±1.44	24.55	0.486	461.90	94.81
CACMD3	62.12±0.57	24.89	0.462	517.72	101.52
CAPCMD1	91.60±0.68	27.09	0.585	452.23	86.26
CAPCMD2	90.61±0.82	27.28	0.492	454.90	96.22
CAPCMD3	81.19±0.09	27.52	0.468	487.08	107.9

Table 2. Various characteristics of microspheres

XRD patterns of ciprofloxacin exhibits sharp peaks at 2θscattered angle 10.57, 18.62, 21.04 and with corresponding peak intensities of 1679, 4212, 1792 linear counts respectively (Figure 8). This indicates crystalline nature of the drug. The peak intensities for formulation were also measured at the same 2θ scattered

angles of 10.7, 18.8, 21.1 and the corresponding linear counts were found to be 343, 924, 540 in case of cellulose acetate, 186, 607, 499 in case of cellulose acetate phthalate microspheres, Based on the peak intensities it shows that the degree of crystallinity of drug was reduced in presences of the polymers.

Maximum release of ciprofloxacin from the various formulations was achieved with in 12-14 hours or longer (Figure 5).The release mechanism of the ciprofloxacin formulation was determined by comparing their respective correlation coefficients. Drug release from microspheres prepared by II and III methods gave good sustained release when compared to method I. from the release profiles it can be understood that the solvent used for polymer solution influences the rate of release of the drug. The microspheres prepared with 1, 4-dioxan and dimethyldigol sustained the drug release for more than 12 hours when compared to acetone.

Formulation	*First-order* Equation		Higuchi's Equation		Peppas Equation	
	Slope	Regression Coefficient (R^2)	Slope	Regression Coefficient (R^2)	Slope	Regression Coefficient (R^2)
CACMA1	-0.005	0.919	4.705	0.966	0.396	0.982
CACMA2	-0.003	0.967	4.207	0.981	0.362	0.979
CACMA3	-0.002	0.944	4.319	0.980	0.360	0.991
CAPCMA1	-0.003	0.897	4.967	0.985	0.538	0.987
CAPCMA2	-0.002	0.955	4.744	0.979	0.556	0.983
CAPCMA3	-0.001	0.938	4.701	0.944	0.685	0.950
CACMM1	-0.005	0.925	5.674	0.983	0.465	0.985
CACMM2	-0.003	0.902	4.453	0.989	0.419	0.989
CACMM3	-0.003	0.973	4.789	0.985	0.462	0.980
CAPCMM1	0.000	0.980	3.265	0.961	0.541	0.933
CAPCMM2	0.000	0.961	2.948	0.964	0.515	0.979
CAPCMM3	0.000	0.838	2.269	0.933	0.549	0.912
CACMD1	-0.002	0.972	4.506	0.991	0.478	0.989
CACMD2	-0.000	0.929	4.605	0.990	0.503	0.990
CACMD3	-0.002	0.915	4.718	0.990	0.557	0.994
CAPCMD1	-0.001	0.948	3.392	0.944	0.635	0.965
CAPCMD2	0.000	0.974	2.208	0.951	0.555	0.946
CAPCMD3	0.000	0.958	2.165	0.940	0.520	0.941

Table 3. Kinetic values of drug release for all formulations

The formulation of CACMD3, CAPCMD3 and CAPCMM3 showed good release. In the case of microspheres prepared by cellulose acetate as the retarding membrane the solvent used has a high influence on the release rate of the drug. From the tables and graph it is clearly seen that the rapidly evaporating solvent like acetone the release rate was very fast when compared to the solvents like dimethyl digol and 1,4 dioxan. The total drug was released in less than 6hours in case of the microspheres prepared with acetone as the solvent. In the microspheres prepared by dimethyldigol as solvent the release of drug was extended upto 8hours and in case of the

microspheres prepared with 1, 4 dioxan as solvent the released was still retarded upto 10 hours. It is clearly evident that the types of solvent have much influence on the release rate of the drug. The same thing is observed in case of cellulose acetate phthalate microspheres also.

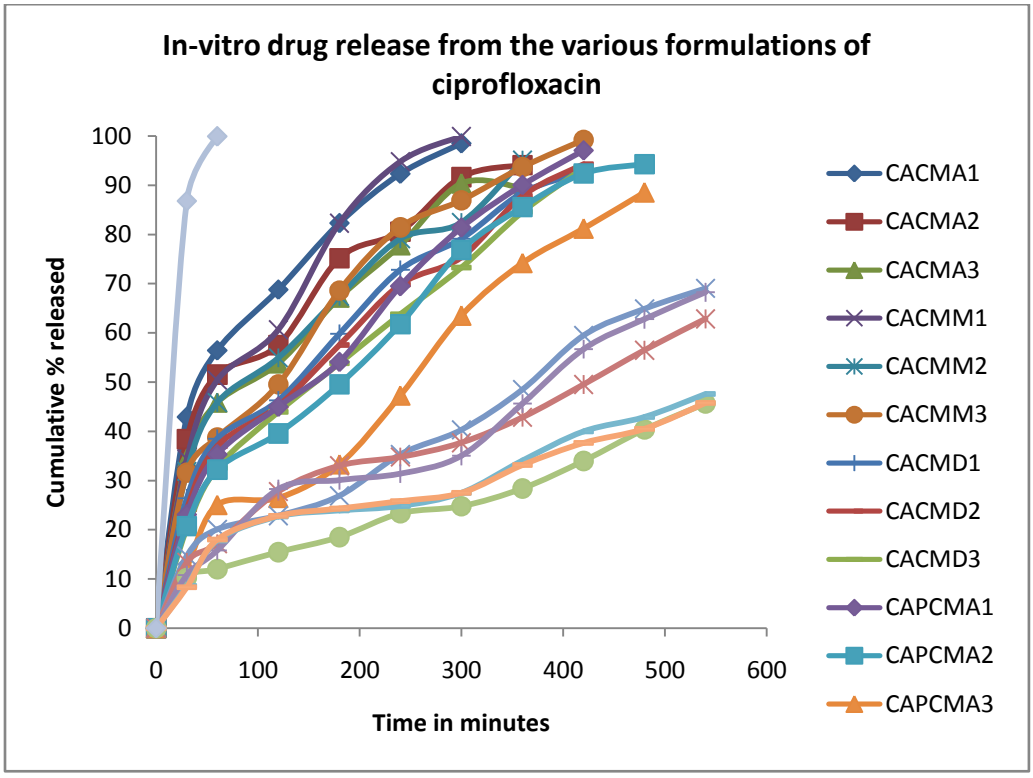

Figure 5: *In vitro* drug release profile of various formulations of ciprofloxacin microspheres

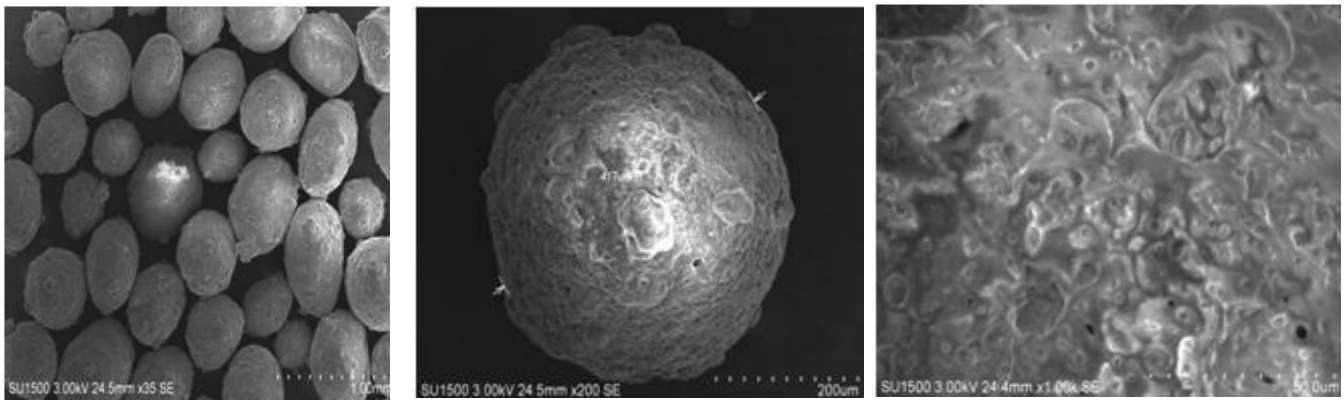

Figure 6: SEM pictures cellulose acetate microspheres of ciprofloxacin

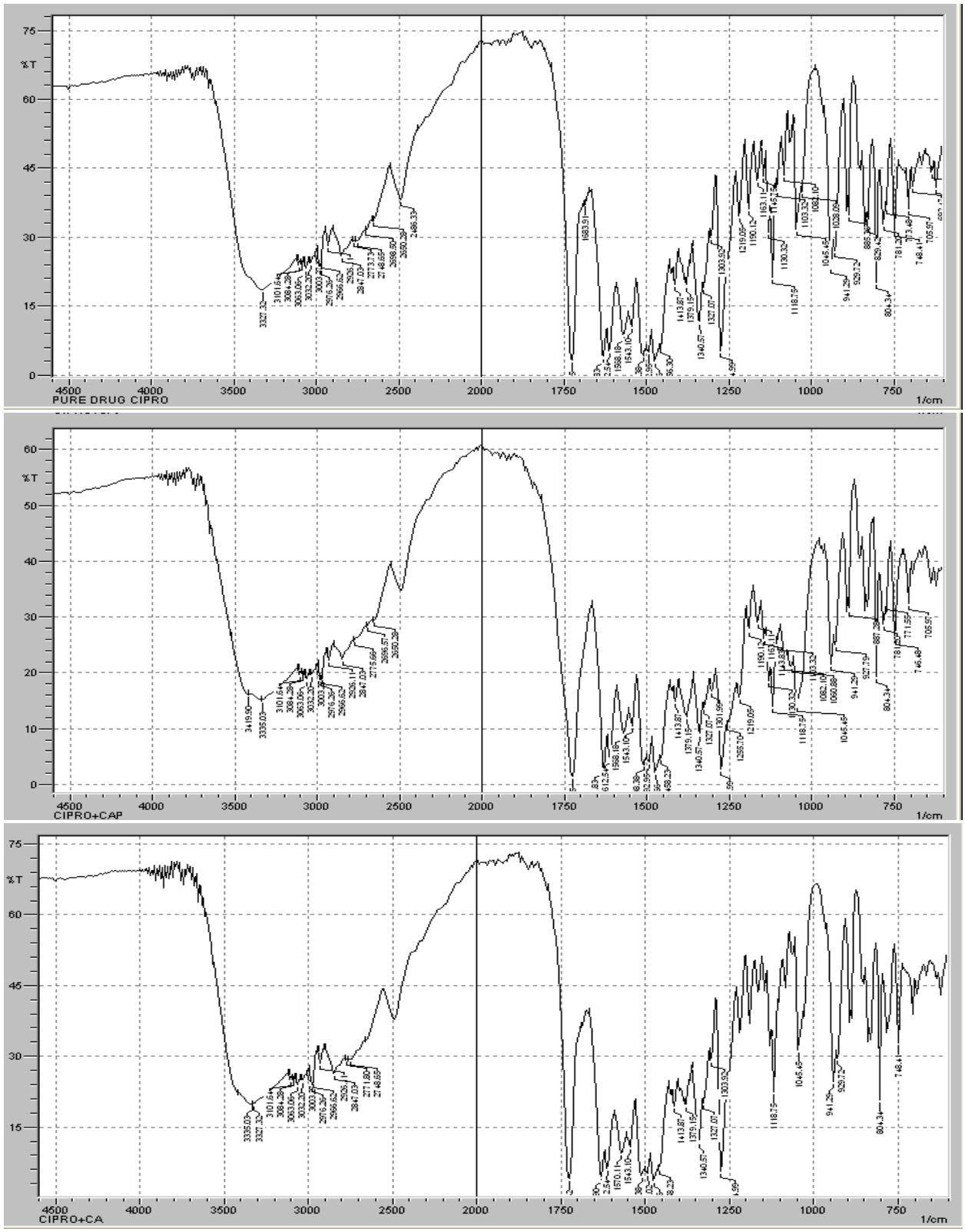

Figure 7: FTIR spectra of (a) pure drug ciprofloxacin, (b) cellulose acetate phthalate microspheres of drug, (c) cellulose acetate microspheres of drug.

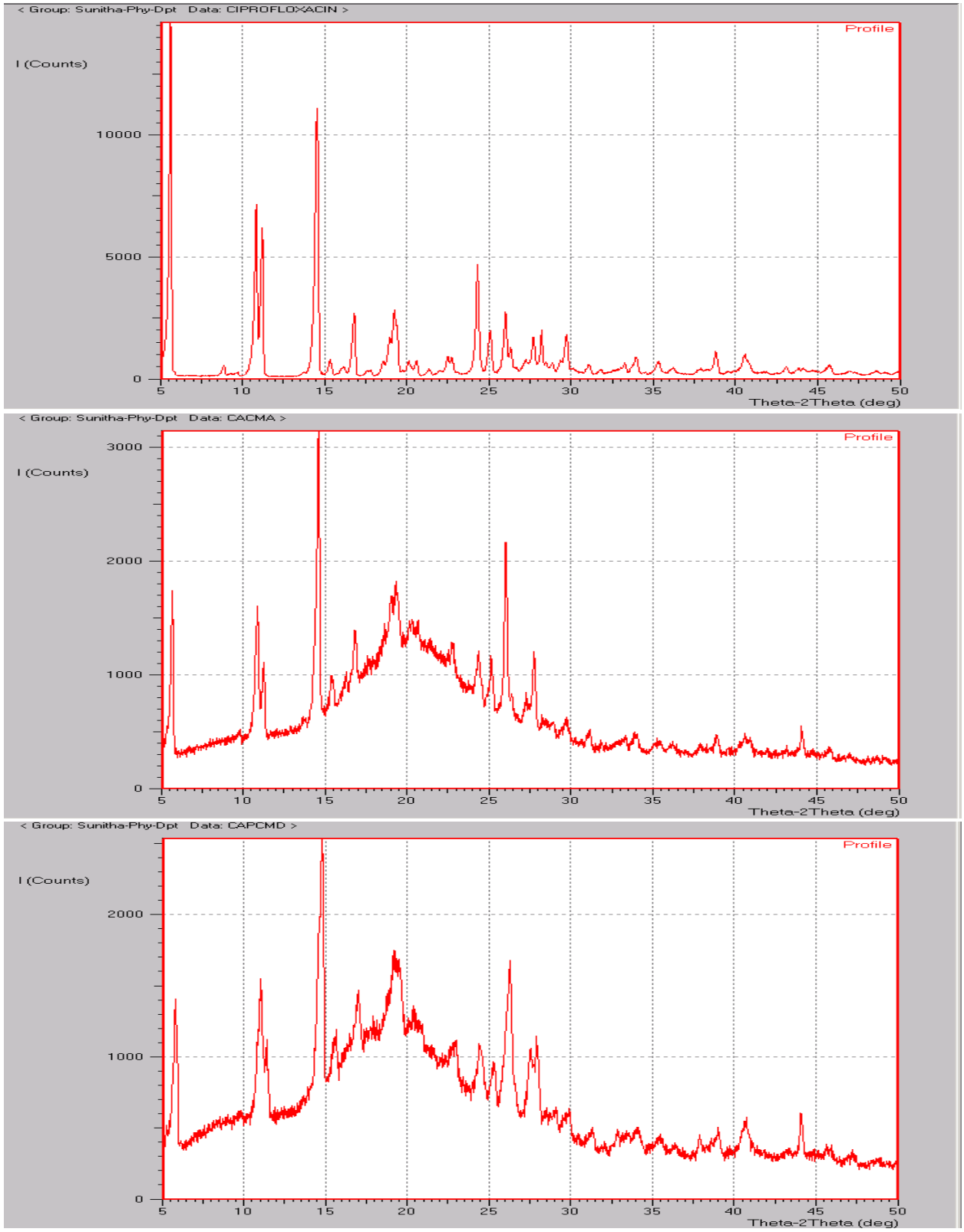

Figure 8: XRD spectra of pure drug, CA microspheres and CAP microspheres of ciprofloxacin

DISCUSSION

The formulation either followed first order release kinetics, higuchi's and peppas release plots stated non-fickian and diffusion controlled showed in Table 3. The release mainly depends on the ratio of the polymer and also the method of preparation technique used. In ciprofloxacin spectrum C-H, O-H, N-H bands were found. The same bands were also found in the spectra of the formulations indicating that there was no drug-polymer interaction. The accelerated stability studies showed the stable nature of the drug and showed a good correlation between the original and the aged samples. Good entrapment efficiency was observed. SEM demonstrated the spherical nature of the microspheres and the presence of the drug particles on the surface.

CONCLUSION

The ciprofloxacin microspheres sustained drug release for 12 hours longer. This retard release of the drug can be related to the size of the microspheres. The size of the microspheres prepared with dimethyldigol and 1, 4-dioxan have high mean diameter when compared to the acetone. It can be concluded that the technique used and the type of solvent and non-solvent has an effect on the entrapment of drug as well as on the drug release. No drug polymer interaction was found and formulations remained stable over a long period of time.

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