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Formulation And Evaluation Of Sustained Release Ciprofloxacin Hydrocloride Microspheres Using Synthetic Polymers

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Abstract: Microspheres were prepared by solvent evaporation method Polymeric microspheres and microcapsules have received much attention as drug delivery systems in recent years as it improves the treatment by providing the localization of active substance at the site of action and bysustainong release of drugs . Ciprofloxacin hydrochloride is a first generation Fluroquinolone having a plasma half-life of three to five Thus, development of controlled release dosage forms would clearly be advantageous. The aim of this study was to formulate and evaluate microspheres as controlled release preparations of Ciprofloxacin HCL using Meth acrylic acid esters (Eudragit RS 100 L 100) as the retardant materials. The prepared microspheres were evaluated for drug content and entrapment efficiency and characterized by Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM). The in vitro release studies was performed in pH 7.4, phosphate buffer. The infrared spectra showed stable character of Indomethacin in the drug-loaded microspheres and revealed the absence of drug-polymer interactions. Scanning electron microscopy study revealed that the microspheres were spherical and porous in nature. The results shows that, the yield percent, the actual drug content and the incorporation efficiencies of the prepared microspheres increased with increasing the drug: polymer ratio. The release rate of drug was found to be greater from the polymer Eudragit RS 100 as compared to Eudragit L 100, and the release rates were prolonged from both types of microspheres.
Key words: Ciprofloxacin HCL, Eudragit RS 100, Eudragit L 100 ,Solvent Evaporation Technique.

INTRODUCTION

The sustained release of drugs is still one of the main objectives of drug delivery systems , which are designed to achieve a prolonged therapeutic effect by continuously releasing the drug over an prolonged period of time after administration of a single dose.[1] Microspheres are defined as homogeneous, monolithic particles in the size range of about 0.1- 1000µm and are widely used as drug carriers for controlled release. Polymeric microspheres and microcapsules have received much attention as drug delivery systems in recent years to modify and retard drug release.[2] Microspheres preparation involves coating of individual drug particles by inert polymeric material, through which the drug diffuse at a controlled and predictable rate in the surrounding medium and thus improves the treatment by providing the localization of active substance at the site of action and bysustainong release of drugs.[3,4]

Eudragits (Methacrylic copolymers) avialable in a variety of types with different water solubility and permeability properties are widwely used for drug release modification in everal oral solid dosage forms.[5-7] Eudragit L100 has pH-dependent solubility in water, whereas Eudragit RS 100 are insoluble but water

permeable. Both Eudragit RS 100 and L100 are therefore used for the production of sustained release formulations.[8]

Ciprofloxacin hydrochloride is a first generation Fluroquinolone having a plasma half-life of three to five hours and penetrates many body fluids and tissues in therapeutic concentrations. [9] Thus, development of controlled release dosage forms would clearly be advantageous. So, here an attempt is made to prepare sustained release microspheres by using Eudragit RS 100 and L100 polymers and to study some parameters affecting the preparation and performance of the microspheres.

MATERIAL AND METHOD:

Ciprofloxacin HCl was obtained as gift sample from Cipla Pharma. Pvt. Ltd. Mumbai. Eudragit RS 100 and L100, generously donated by Röhm Pharma, GmbH, Germany All other chemicals and solvents used were of analytical grade.

Physical Interaction between drug and polymers:

FT-IR spectroscopy

The FT-IR spectra were taken from dried samples. A FT-IR was used for the analysis in the frequency range between 4000 and 600 cm^{-1}.The samples (pure drug , and drug -loaded microspheres) were selected separately and dispersed in KBr powder: the pellets were made by applying 6000 kg/cm^2 and analyzed. Spectral measurements were obtained by powder diffuse reflectance on a FT-IR spectrophotometer.

Preparation of Ciprofloxacin HCl microspheres :

The composition of Ciprofloxacin HCl microspheres formulations with varying proportion of Eudragit RS 100, Eudragit L 100 is presented in Table 1 and 2. Ciprofloxacin HCl microspheres were prepared by solvent evaporation method. Ciprofloxacin HCl and Polymer (Eudragit RS 100, Eudragit L 100) were dispersed in acetone and methanol at various ratios by using Tween80, as a stabilizer (0.7%) and Hexane and Dichloromethane as a hardening agent (1%). The resultant slurry was added to 100 ml of Liquid Paraffin under stirring at 2000 rpm by mechanical stirrer for 1hr until the solvent completely evaporated. The microspheres were collected by filtration and washed repeatedly with Petroleum ether 40–60^oC until free from oil. The collected microspheres were dried for 1hr at room temperature and subsequently stored in desiccators until further used.. The effect of drug-polymer ratio was studied by keeping all the variables constant.

Table 1.Composition of ciorofolxacin microspheres. showing drug –polymer ratio

Formulation Code	Ciprofloxacin HCL	Eudragit L100	Eudragit RS 100
F1	1	0.5	-
F2	1	1	-
F3	1	1.5	-
F4	1	-	0.5
F5	1	-	1
F6	1	-	1.5

Table 2.Composition of ciorofolxacin microspheres.

Variables	Drug Polymer Ratio					
	F1	F4	F2	F5	F3	F6
	1 : 0.5		1 : 1		1 : 1.5	
Stabilizer (Tween 80)	0.7 % w/v	0.7 w/v	0.7 % w/v	0.7 % w/v	0.7 % w/v	0.7 % w/v
Hardening Agent (Hexane)	1 % v/v	1 % v/ v	1 % v/v	1 % v/v	1 % v/v	1 % v/v
Stirring speed (rpm)	2000	2000	2000	2000	2000	2000
Stirring time (min)	60	60	60	60	60	60

Effect of Concentration of Solvents:

The effect of solvents like Hexane and Dichloromethane with different concentrations were studied in combination with polymer (Eudragit RS 100). Different batches with concentration 1% v/v, 2.5% v/v, 3.5% v/v and 5.5% v/v of Hexane and Dichloromethane in combination with Eudragit RS 100 were prepared in the same manner as described earlier as shown in table 2. The batches were prepared by solvent evaporation technique. The other variables were kept constant.

Table 3: Different concentration of Solvent Hexane and dichloromethane

F. Code	H1	H2	H3	H4	C1	C 2	C 3	C 4
Drug: polymer	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1
ichloromethane	1%	2.5%	4 %	5.5%	-	-	-	-
Hexane	-	-	-	-	1%	2.5%	4 %	5.5%
Stirring Time(min)	60	60	60	60				
Stirring Speed (rpm)	2000	2000	2000	2000				

Evaluatory parameters:

Entrapment Efficiency:

An accurately about 100 mg of microspheres containing drug were added to phosphate buffer (pH 7.4) solution. After diluting with sufficient quantity of phosphate buffer (pH 7.4), drug concentration was determined by UV spectrophotometry at 317 nm using pH 7.4 phosphate buffers as blank. [10] Entrapment efficiency was calculated by using following formula.

Entrapment efficiency (%)=PC/TC X 100

Where, PC- Theoretical drug content
TC- Theoretical drug content

Percentage Yield:
The yield was calculated as the weight of the microspheres recovered from each batch divided by otal weight of all non- volatile components i.e. drug and polymer used to prepare that batch multiplied by 100.[11]

In- Vitro Release Studies

In vitro drug release study was carried out in USP II Basket type dissolution test apparatus using pH 7.4 phosphate buffer as dissolution medium, volume of dissolution medium was 900 ml and bath temperature was maintained at (37±1)°C throughout the study. Paddle speed was adjusted to 100 rpm. An interval of 1 hour, five ml of sample was withdrawn with replacement of five ml fresh medium and analyzed for Ciprofloxacin HCl content by UV-Visible spectrophotometer at 278 nm. All the experimental units were analyzed in triplicate (n=3).[12]

Drug Release Kinetics:

In order to study the exact mechanism of drug release from the microsphere, drug release data was analyzed according to Zero-order, First-order, Higuchi square root, and Hixson Crowell and Peppas equation. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test. [12-15]

$R = k_0 t$ …….. (1)

$\text{Log UR} = k_1 t\ 2.303$ …….. (2)

$R = k_2 t^{1/2}$ ..…….... (3)

$(UR)^{1/3} = k_3 t$ ……(4)

$\log R = \log k_4 + n \log t$ ……(5)

Where, R and UR are the released and unreleased percentages, respectively, at time t.

And K_O, K_1, K_2, K_3 and K_4 are release rate constants for Zero order, First order, Higuchi, Hixson-Crowell and Peppas-Korsmeyer rate equations, respectively.

Surface Topography by Scanning Electron Microscope (SEM):

SEM photographs were taken using scanning electron microscope. The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminium stub. The stubs were then coated with gold to a thickness of ~300 A° under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with SEM.[16]

RESULTS AND DISCUSSION:

The FT-IR of pure drug (Ciprofloxacin HCl), Ciprofloxacin HCl loaded Eudragit L 100 and Eudragit RS 100 microspheres are shown in figue1.

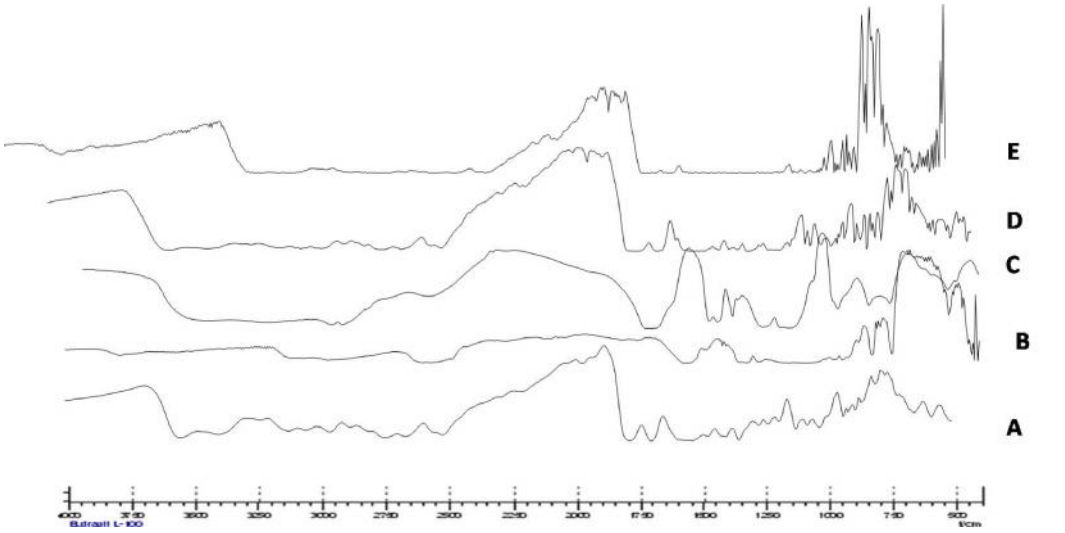

Figure 1.FTIR spectrum of Ciprofloxacin HCl (A), Edragit RS 100 (B), Eudragit L100 (C),Combination of Drug and Eudragit RS 100 (D), & Combination of Drug and Edragit L100

From the FT-IR studies, it was observed that the peaks of Ciprofloxacin HCl was detected and identified in the spectrum of Ciprofloxacin HCl loaded Eudragit L 100 and Eudragit RS 100 microspheres confirming that there was no drug- polymer interaction between Ciprofloxacin HCl and Polymer.

Effect of Drug-Polymer Ratio:

Effect of various drug-polymer ratios used for the preparation of microspheres on entrapment efficiency and mean diameter is shown in table no.4.

Table No. 4: Effect of drug-polymer ratio on evaluatory parameters.

F.Code	F1	F2	F3	F4	F5	F6
Mean Diameter (µm) ± S.D.	240 ± 33	260 ± 27	310 ± 41	280 ± 34	270 ± 37	340 ± 48
Entrapment Efficiency (%) ±S.D.	58.42 ± 2.1	73.01± 2.4	77.23 ± 1.7	75.78 ± 1.2	78.55 ± 2.4	89.58 ± 1.49
Yield (%)	78.31	92.88	63.88	73.42	84.64	69.53

From the results shown in the table 4, it was revealed that entrapment efficiency value is high in case of Eudragit L 100 and Eudragit RS 100 i.e. more than 70 % upto drug-polymer ratio of 1:1, while it is increases in both the polymers, if we further increase the polymer ratio to 1:1.5. But there was drastic increased in particle size and decreased in percentage yield, which might due to, formation of agglomerates of microspheres and polymers stick to the vessels and stirrer. Thus optimum drug-polymer ratio, for the preparation of Eudragit RS 100 and L100 microspheres with high entrapment efficiency , particle size and percentage yield was found to be in 1:1

In vitro release studies were performed, in pH 7.4 phosphate buffer. Effect of drug-polymer ratio on *in vitro* release profile of Ciprofloxacin HCl with Eudragit RS 100 and L100 as a carrier is shown in figure 2.

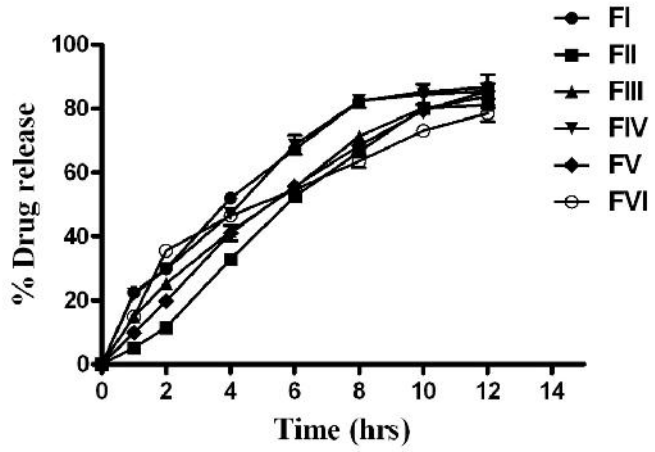

Figure2 Cumulative % drug releaseof Formaulation F1-F6: Efect of Polymer concentration

From the figure 2, it was observed that the drug: polymer ratio 1:1 shows greater release as compared to other because of finer particle size and hence increasing the surface area for dissolution. Eudragit RS 100 and L100 is lipophilic substance, and it might reduce the rate of water penetration into the microspheres, resulting in a slower rate of drug release.

Ciprofloxacin HCl is a water soluble drug and is insoluble in organic solvents, so it might have higher affinity with Eudragit RS 100 and L100 than that of dissolution medium leading to slower drug release from microspheres. From figure 1 it was observed that the release rate of Ciprofloxacin HCl was greater from the polymer Eudragit RS 100 as compared to Eudragit L 100.

Effect of Concentration of Solvents Hexane and Dicloro methane :

Effect of various concentrations of Hexane and Dicloromethane as a solvent on evaluatory parameters is shown in table 5 and 6 Respectively.

Table 5 : Effect of solvent Hexane concentration on evaluatory parameters.

Batch Code	H1	H2	H3	H4
Mean Diameter (µm) ± S.D.	230 ± 12	280 ± 24	260 ± 32	240 ± 43
Entrapment Efficiency (%) ± S.D.	51.49% ± 2.4	49.62% ± 3.4	39.62 ± 2.1	59.27% ± 2.9
Yield (%)	65.53	57.60	56.09	50.0

Table 6 : Effect of Dichloromethane concentration on evaluatory parameters.

Batch Code	C1	C2	C3	C4
Mean Diameter (µm) ± S.D.	210 ± 19	230 ± 42	260 ± 25	290 ± 31
Entrapment Efficiency (%) ± S.D.	50.35% ± 1.4	48.20% ± 2.2	42.09 ± 3.19	55.61% ± 3.21
Yield (%)	76.92%	63.02%	58.34%	52.08%

Table 5 clearly indicates that , as the concentration of solvents increases entrapment efficiency and the practical yield decreases. Use of Hexane (1% v/v) resulted in discrete, spherical uniform microspheres with high entrapment efficiency, optimum particle size and good practical yield. Thus, Hexane in the concentration of 1% v/v was found to be optimum solvent for microsphere preparation.

With regard to mean diameter, the solvent concentration seems to an important parameter. As the concentration of solvent was increased, mean diameter of microspheres was increased. At low solvent concentrations, the droplets were greatly stabilized and tend to inhibit coalesce and became smaller particle. Another reason for forming good microspheres might be decrease in interfacial tension.

table 6 it shows that, the concentration of solvents increases entrapment efficiency is decreases and the practical yield is also decreases. Use of Dichloromethane (1% v/v) resulted in discrete, spherical uniform microspheres with high entrapment efficiency, optimum particle size and good practical yield. Thus, Dichloromethane in the concentration of 1% v/v was found to be optimum solvent for microsphere preparation.

With regard to mean diameter, the solvent concentration seems to an important parameter. As the concentration of solvent was increased, mean diameter of microspheres was increased. At low solvent concentrations, the droplets were greatly stabilized and tend to inhibit coalesce and became smaller particle. Another reason for forming good microspheres might be decrease in interfacial tension.

In vitro release studies were performed, in pH 7.4 phosphate buffer. The drug release from

the formulations was different at different solvents ;Dicloromethane (C1,C2,C3) and Hexane (H1,H2 ,H3) on *in vitro* release profile of Ciprofloxacin is shown inand figure 2.

From the results shown in figure 3, it was observed that batch H1 shows greater release profile of Ciprofloxacin HCl as compared to other three batches i.e. H2, H3 and H4. Thus, the 1% v/v concentration of hexane shows the greater release as compared to other because of finer particle size. Similarly batch C1 shows greater release profile of Ciprofloxacin HCl as compared to other three batches i.e. C2, C3 and C4. Thus, the 1% v/v concentration of hexane shows the greater release as compared to other because of finer particle size.

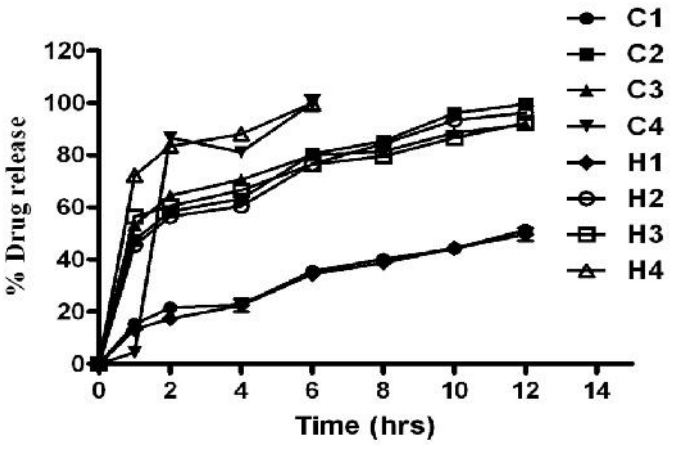

Fig. No. 3: Figure1 Cumulative % drug releaseof Formaulation C1-H4: Effect of solvent (Hexane/Dicloromethane) concentration

The mechanism of Ciprofloxacin HCl release from microspheres was studied by fitting the data obtained from *in vitro* release studies into Zero-order, First order, matrix, Hixson-Crowell's and Peppas kinetic models. Obtained values of correlation coefficient are given in table 7.

Table 7.Correlation coefficient values for release kinetics of polymeric microspheres.

F.Code	Zero-order	First-order	Higuchi	Korsmeyer-peppas	Hixson-crowell
F1	0.5286	0.8128	0.9774	0.9676	0.7679
F2	0.953	0.9422	0.9292	0.9690	0.9498
F3	0.924	0.9457	0.9126	0.9589	0.9779
F4	0.5236	0.7938	0.9674	0.9746	0.7259
F5	0.9140	0.9332	0.9252	0.9610	0.9508
F6	0.9374	0.9247	0.9066	0.9610	0.9579
H1	0.9318	0.7238	0.9132	0.9634	0.6432
H2	0.9641	0.8428	0.9564	0.9732	0.6310
H3	0.2816	0.8376	0.9659	0.9649	0.8260
H4	0.4731	0.7418	0.9651	0.9712	0.9101
C1	0.4381	0.6752	0.3739	0.9729	0.8460
C2	0.3954	0.8170	0.8328	0.9652	0.9401
C3	0.5298	0.8128	0.9638	0.9850	0.7590
C4	0.5976	0.8438	0.6328	0.9641	0.7141

From Table 7, it was found that all the batches showed better fitting with the Korsmeyer-Peppas model. The Korsmeyer-Peppas equation gave consistently higher values for the correlation coefficient (above 0.95) than the other release models. Thus, the drug release from all the microsphere preparations confirmed Korsmeyer-Peppas model indicating the release mechanism is not well known or when more than one type of release phenomena could be involved. This may be due to the fact that microspheres are matrix type and swelling or erosion of microspheres took place during drug release experiments. Thus, fitting of drug release data into Korsmeyer-Peppas equation indicates, the possible mechanism of drug release may be by diffusion and erosion of the polymer matrix.

Scanning electron microscopy was utilized to observe the surface and inner part of the microspheres. Figure 4. shows SEM photographs of microspheres (batch A5) at 100X magnification, 110X and 1000X magnification. SEM photographs showed discrete, spherical and uniform microspheres. SEM photographs also showed the absence of any drug crystal on the surface of microspheres revealing that the microspheres were smooth.

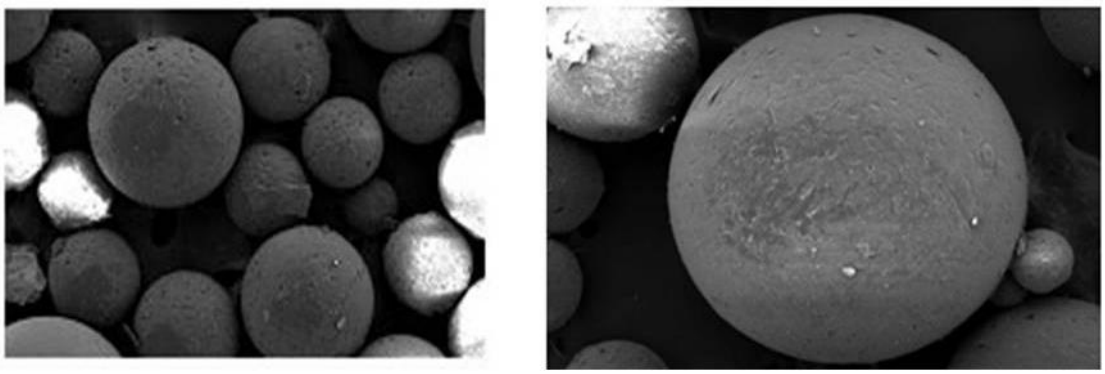

Figure 4. SEM photographs of microspheres

CONCLUSIONS

In-vitro data obtained for floating microspheres of ciprofloxacin HCL showed good entrapment efficiency and prolonged drug release The infrared spectra showed stable character of Indomethacin in the drug-loaded microspheres and revealed the absence of drug-polymer interactions. From SEM it was evident that the microspheres were spherical and porous in nature. In all the formulations, with the increase in the polymer concentration, the rate and amount of drug release was found to decrease, which can be attributed to the greater binding of the drug with the polymer.

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