

FORMULATION AND *IN VITRO* EVALUATION OF CEFIXIME LIOSPHERE

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ABSTRACT

The aim of the present study was to investigate the formulation parameters for Cefixime loaded liposphere by using lipid as a colloidal carrier. Lipid delivery system is a rapidly expanding technology for the production of controlled release dosage form. Cefixime is frequently prescribed for various bacterial infections. An attempt was made to increase the bioavailability of this drug by formulating as a controlled dosage form. Melt dispersion method was selected as a formulation method in which two phases mixed together and emulsified with the help of mechanical stirrer. Cetyl alcohol, Cetostearyl alcohol and Stearyl alcohol were used as excipients, Poly vinyl alcohol (PVA) was used as stabilizer. The liposphere dispersions were characterized with

respect to entrapment efficiency, loading efficiency, particle size, surface morphology and *in-vitro* release. Evaluation studies of lipospheres showed that shape and size of the liposphere complies with the specification of liposphere. *In vitro* release studies revealed the formulations all the formulations were showed controlled release form. The kinetic study revealed that the drug release followed non-Fickian release pattern. Finally the bio activity of the drug remains intact after micro encapsulation.

KEYWORDS: Liposphere, Cefixime, *In vitro* drug release, controlled dosage form.

INTRODUCTION

In the recent years, it has been realized that complete drug therapy for an ailment does not rely on the development of new drug alone, it needs a promising approach for a suitable delivery system. Infectious disease is one of the major causes for the death in developing countries due to lack of adequate prophylactic or therapeutic medicines for infection. Many

existing drug candidates have poor solubility in biological fluid, which results in low and highly variable and highly variable bioavailability. The therapeutic window needs a novel, safe, stable and effective drug delivery system. Numerous colloidal carrier have widely been studied as drug carrier in the field of drug delivery system.^[1,2] The use of synthetic polymer as a colloidal carrier often goes along with the detrimental effects on incorporated drug during manufacturing of the formulations or during the erosion of the polymers after application.^[3] More over the degradation of polymer might possibly cause systemic toxic effects through the impairment of Reticulo Endothelial system (RES) or after phagocytosis of particles by human macrophages and granulocytes.^[4] Therefore, alternative carrier substance have been investigated; among them lipidic material have garnered growing attention. Liposphere carrier system has several advantages over other delivery system including emulsions, liposomes and lipid micro cylinders such as better physical stability, low cost of ingredients, ease of preparation and scale- up, high dispersibility in aqueous medium, high entrapment of hydrophobic drugs, controlled particle size and extended release of entrapped drug.^[5] Liposphere have been developed as a new type of lipid based encapsulation system for drug delivery for lipophilic and hydrophilic drug.^[6] Liposphere consists of solid particles with a mean diameter usually between 0.2 – 500 μm , composed of a solid lipid matrix in which the drugs are dissolved or dispersed.

Cefixime is a third generation Cephalosporin antibiotic used in the treatment of lower respiratory tract infections, otitis media, sinusitis, urinary tract infection and gonorrhea etc. The drug has a half life of 3 to 4 h, activity against most common pathogens involved in the infections for which it is indicated. The serum and interstitial concentrations are greater than the minimum inhibitory concentration of the most of the common pathogen of the most of the common pathogens of this infection for upto 24 h.^[7] It has very low solubility in biological fluids. So to enhance the bioavailability and stability of drug it is formulated as a liposphere.

MATERIALS AND METHODS

Materials

Cefixime purchased from Yarrow Chem Products, Cetyl alcohol, Cetostearyl alcohol purchased from Hi media, Stearyl alcohol from Halewood Chemicals Ltd, London, Poly vinyl alcohol (PVA) Merck ltd, Mumbai. All other chemicals used were of analytical grade.

Methods

a. Preparation of liposphere^[8,9]

In this study liposphere (LS) was formulated by using melt dispersion method. The chosen lipid mixture was melted at 70°C and then emulsified in to a hot external aqueous phase maintained at 70° C containing suitable surfactants. The emulsion was mechanically stirred by mechanical stirrer (Remi Stirrer 5000 rpm) and maintained the temperature at 70°C. Then the hot emulsion was rapidly cooled to about 20°C by immersing the formulation into ice bath with agitation to yield the uniform dispersion of liposphere. The obtained liposphere was washed with water and isolated by filtration through a Whatmann filter paper No 41 and dried or lyophilized if necessary. In a similar manner LS formulation was also prepared without incorporating drug into lipid matrix.

b. Evaluation of liposphere

1. Particle size analysis and photo microscopic Evaluation

Particle size of different batches of microspheres was determined by photo microscopic studies (DMWB1-123 MOTIC MICROSCOPE). Analysis was carried out by observing randomly selected **100** particles under a microscope. Mean particle size of all formulations were determined.

2. Yield of liposphere^[10]

The liposphere formed were filtered from the medium, dried and weighed to get the yield of the lipospheres formulated per batch. Eq. (1) was used to calculate the percentage yield:

$$\% \text{ Recovery} = \frac{W_1(\text{g})}{W_2(\text{g}) + W_3(\text{g})} \quad \text{----- (1)}$$

3. Scanning Electron Microscopical Evaluation (SEM)

Surface morphology of the specimen was determined by using a scanning electron microscope (SEM), (Model JSM 84 0A, JEOL, Japan). The samples were dried thoroughly in a vacuum desiccator before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120°A Knees was coated on the sample sputter coating unit (Model E5 100 Polaron U.K) in Argon at ambient of 8-10 with plasma voltage 20Ma. The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 1515 KV with the load current about 80mA. The condenser lens position was maintained between 4.4 -5.1. The

objective lens aperture has a diameter of 240 microns and the working distance was above 39mm.

4. Determination of Encapsulation efficiency (EE)^[11]

The entrapment efficiency of the system was determined by measuring the concentration of free drug in the aqueous phase. The resulting supernatant of each batch obtained after filtration was analysed spectrophotometrically at 288 nm for the drug. The absorbencies obtained were converted to concentration by using a calibration curve prepared earlier. These concentrations were used to calculate the respective entrapment efficiencies. The drug concentration in the aqueous phase and in the whole liposphere was compared to calculate the entrapment efficiency. Entrapment efficiency (EE) was achieved by the following

$$EE (\%) = \frac{[\text{Actual drug content}]}{[\text{Theoretical drug content}]} \times 100 \text{ ----- (2).}$$

5. Determination of drug content

Exactly weighed amount of the loaded liposphere (10mg) was dissolved in 10 ml of phosphate buffer pH 7.4 and sonicated for 15 minutes. The obtained sample was diluted to volume (100ml) with the buffer (pH 7.4). Then the solution was filtered and assayed by UV spectroscopy at a wave length of 288nm. (Unloaded lipospheres produced insignificant absorbance values at the same wave length). Each sample were analysed in triplicate.

6. *In vitro* release studies

It was a well known fact that the release of a drug from lipospheres influenced by the composition of the lipospheres. Hence, the *in vitro* releases of Cefixime from the formulation were studied. *In vitro* drug releases were evaluated by using a Dialysis bag diffusion technique.^[12,13] The dialysis bags were hydrated with glycerin overnight before the experiment. Lipospheres equivalent to 100 mg were placed in the dialysis bags. The dialysis bags were tied at both ends and were placed in the basket of USP Type II dissolution Apparatus (LabIndia disso2000, Mumbai, India). The baskets were immersed in 900 ml phosphate buffer (pH7.4) and maintained the temperature at (37.0 ± 0.5) °C. The baskets were rotated at 100 rpm. At regular intervals, 5.0 ml of dissolution medium was withdrawn and replaced with the fresh buffer to maintain sink condition. The withdrawn samples were filtered through a whatmann filter paper (No 41) and made appropriate dilution and analyzed

by using spectrophotometer at 288 nm to determine percentage release of Cefixime loaded liposphere (Schimadzu, UV-1800). The drug released was calculated^[14] and depicted in the Table 5, 7 and 9.

RESULTS AND DISCUSSION

The lipid based carriers were selected to eliminate the toxic effects associated with the use of polymers as carriers.^[15]

Compatibility study of drug and lipids has been done by using FT-IR analysis.

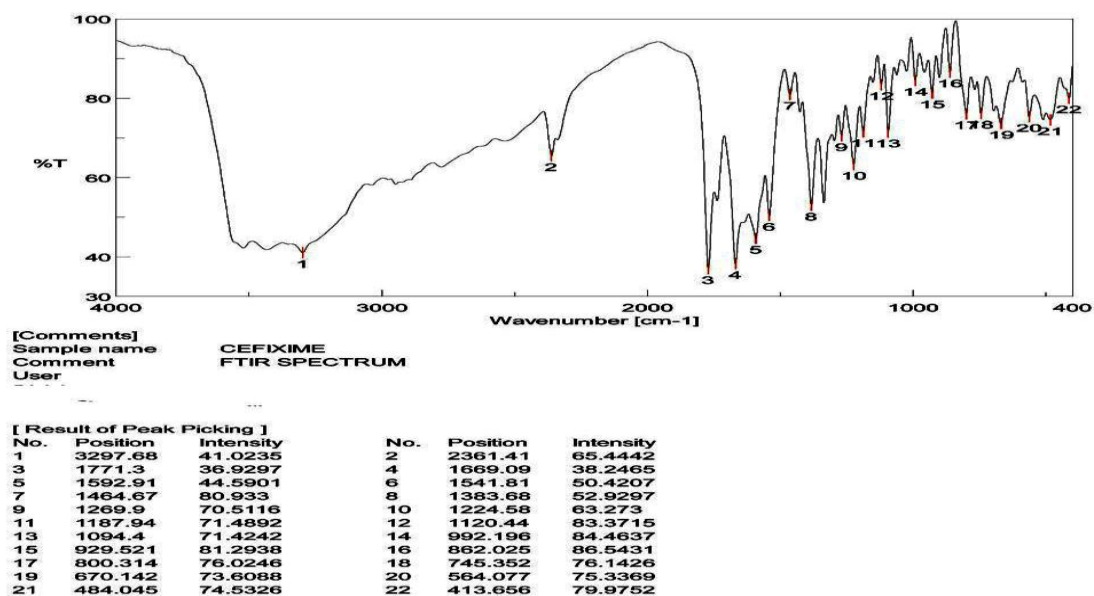


Figure.1: FT-IR spectrum of pure Cefixime

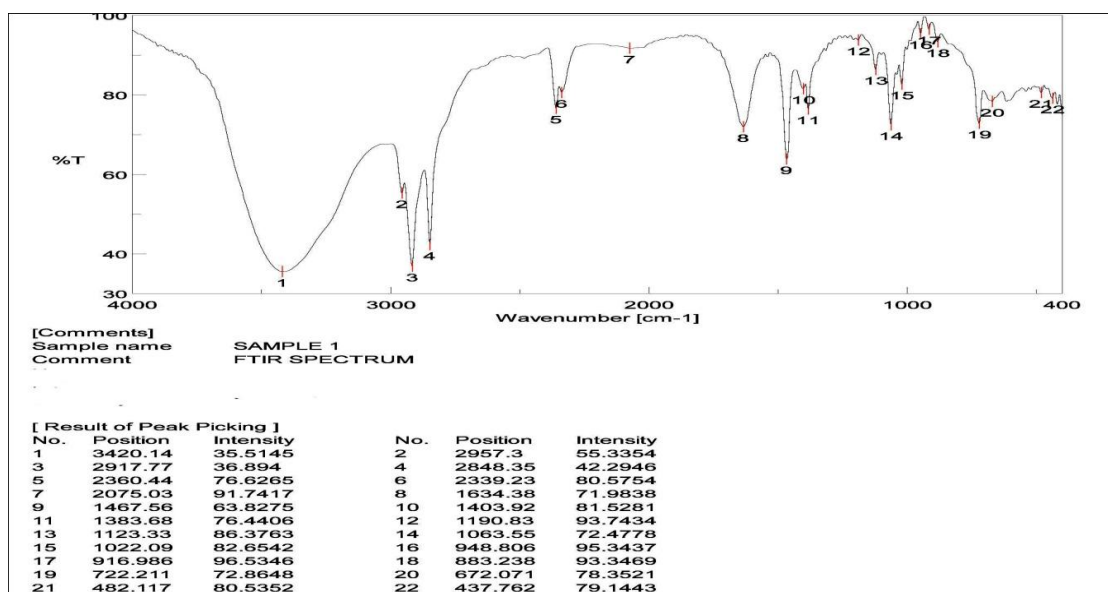


Figure.2: FT-IR spectrum of Cefixim + cetyl alcohol physical mixture

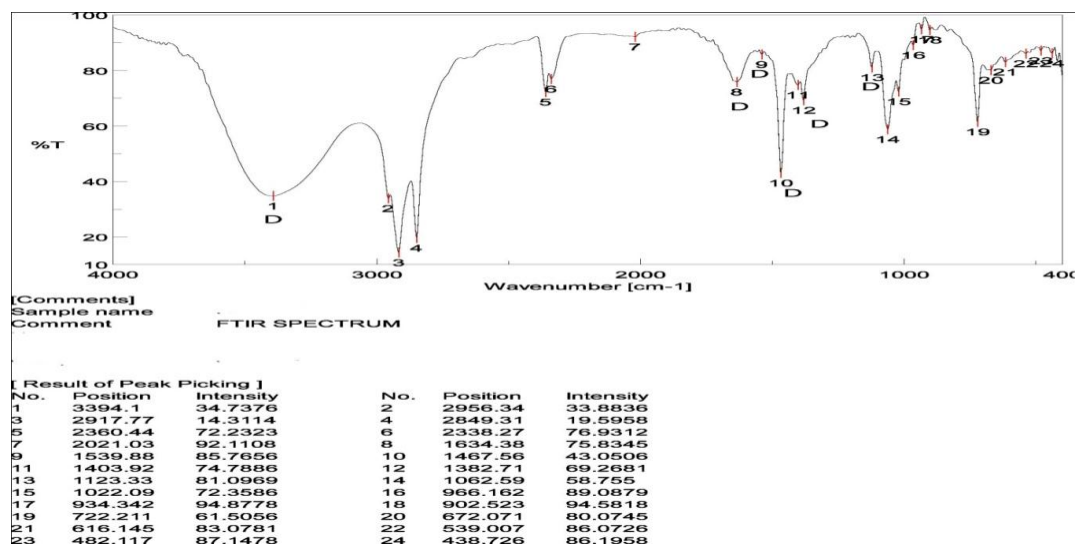


Figure.3 : FT-IR spectrum of CFX2

The FT-IR spectra of pure Cefixime (Figure 1) powder display a characteristic -NH_2 absorption peak at 3296 cm^{-1} , which is a normal range of absorption of primary amines. It exhibits a strong band for C=O stretching of the non conjugated carboxylic acid at 1770 cm^{-1} whereas the second band which is expected to shift to lower frequency (owing to conjugation) appears as an overlapping band. The carbonyl of cyclic as well as acyclic amide appears at 1670 cm^{-1} . These peaks are agreed with the reported one which indicates the purity of the drug.^[16,17]

The results indicate that there was no chemical incompatibility between drug and polymer as all the characteristic IR peaks related to pure drug were also appear in the IR spectrum of the formulations.

Preparation of liposphere

Different lipid ratios (1:2, 1:5, 1:10) were used for preparing Cefixime loaded LS with cetyl alcohol, cetostearyl alcohol and stearyl alcohol. Based on higher entrapment than other method melt dispersion method was used for the incorporation of Cefixime in the lipids and the emulsion was stirred at the rate of 1000 rpm speed. The present study was aimed to achieve maximum entrapment with reduced particles size and controlled drug release from the liposphere using a suitable processing condition (Table 1). The results of the influence of preparation parameters on the characteristics of the Cefixime loaded were shown in Table 2. The PVA (0.5, 0.1 and 0.15% w/v) was employed as stabilizer for the preparation of liposphere.

Table1: Formulation variables in Cefixime Liposphere.

Formulation code	Drug (mg)	Cetyl alcohol (mg)	Cetostearylalcohol (mg)	Stearyl alcohol (mg)	PL 90H	EPC	PVA (%)	Water (ml)	Appearance
CFX1	100	200	-	-	-	-	0.1	100	DP
CFX2	100	500	-	-	-	-	0.1	100	DP
CFX3	100	1000	-	-	-	-	0.1	100	DP
CFX4	100	-	200	-	-	-	0.1	100	DP
CFX5	100	-	500	-	-	-	0.1	100	DP
CFX6	100	-	1000	-	-	-	0.1	100	DP
CFX7	100	-	-	200	-	-	0.1	100	DP
CFX8	100	-	-	500	-	-	0.1	100	DP
CFX9	100	-	-	1000	-	-	0.1	100	DP
CFX10	100	500	-	-	-	-	0.15	100	DP
CFX11	100	-	500	-	-	-	0.15	100	DP
CFX12	100	-	-	500	-	-	0.15	100	DP
CFX13	100	500	-	-	-	-	0.5	100	DP
CFX14	100	-	500	-	-	-	0.5	100	DP
CFX15	100	-	-	500	-	-	0.5	100	DP

Percentage yield

Percentage yield, EE and drug content have been exhibited in the Table 2. The presence of cetyl alcohol 500 mg (CFX2) showed increased percentage yield $92.33 \pm 2.05\%$, high entrapment efficiency $95.50\% \pm 1.23$ and drug content 53.03 ± 1.03

Particle size

Discrete spherical particle were obtained with 0.1% PVA at 1000rpm speed. The particle size ranged from 64.99 ± 1.83 to 88.22 ± 2.10 μm for cetyl alcohol lipid coat, 66.76 ± 2.01 to 88.28 ± 0.82 μm for cetostearyl coat, 79.09 ± 1.02 - 98.14 ± 1.52 μm for stearyl alcohol. Cetyl alcohol 500mg (CFX2) showed lowest particle size hence higher drug release.

Table 2: Evaluation characteristics of Cefixime loaded liposphere

Formulation code		Percentage yield(%)	Particle size (μm)	Entrapment efficiency (EE%)	Drug content (%)
Cetyl alcohol	CFX1	66.80 ± 1.42	70.50 ± 1.83	74.03 ± 1.24	42.20 ± 1.65
	CFX2	92.33 ± 2.05	64.99 ± 2.01	95.50 ± 1.23	53.23 ± 1.03
	CFX3	57.25 ± 1.03	74.20 ± 1.53	77.67 ± 1.86	11.36 ± 1.18
	CFX10	57.86 ± 1.5	88.22 ± 2.10	67.80 ± 1.35	17.87 ± 0.84
	CFX13	70.22 ± 1.77	75.99 ± 2.53	71.03 ± 1.30	32.98 ± 0.98
Cetostearyl alcohol	CFX4	30.25 ± 1.55	71.40 ± 1.50	60.24 ± 1.59	36.62 ± 1.25
	CFX5	85.44 ± 1.62	88.28 ± 0.82	83.11 ± 1.54	24.53 ± 2.11
	CFX6	65.65 ± 2.04	66.76 ± 2.01	85.42 ± 2.60	43.3 ± 0.88
	CFX11	67.25 ± 1.10	71.42 ± 2.30	65.67 ± 0.53	45.69 ± 0.73

	CFX14	63.85 ± 1.56	80.52 ± 2.40	65.66 ± 1.19	28.18 ± 0.48
Stearyl alcohol	CFX7	42.81 ± 2.28	79.09 ± 1.02	69.41 ± 1.57	42.37 ± 0.73
	CFX8	80.89 ± 1.48	91.49 ± 1.45	69.96 ± 2.52	47.66 ± 0.85
	CFX9	58.45 ± 0.93	98.14 ± 1.52	79.80 ± 1.60	25.84 ± 0.66
	CFX12	65.94 ± 2.24	71.17 ± 1.62	67.03 ± 1.59	31.40 ± 1.01
	CFX15	60.96 ± 1.13	83.32 ± 1.22	63.31 ± 1.93	27.38 ± 1.12

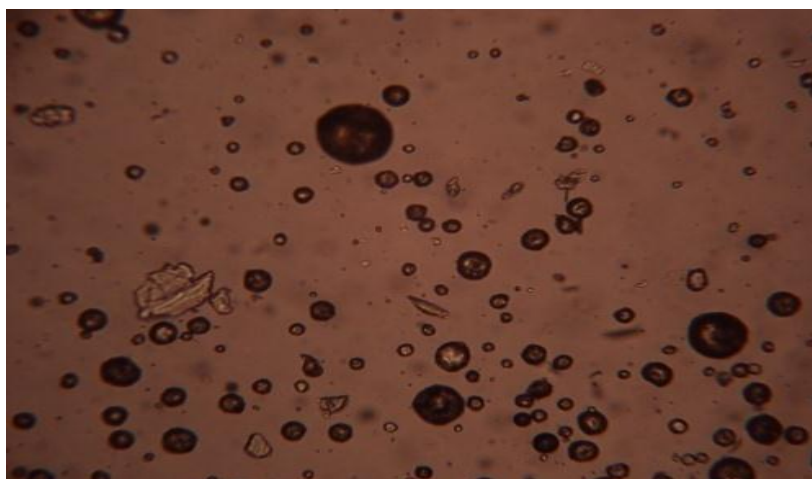


Figure 4 Photomicrographs of optimized formulation

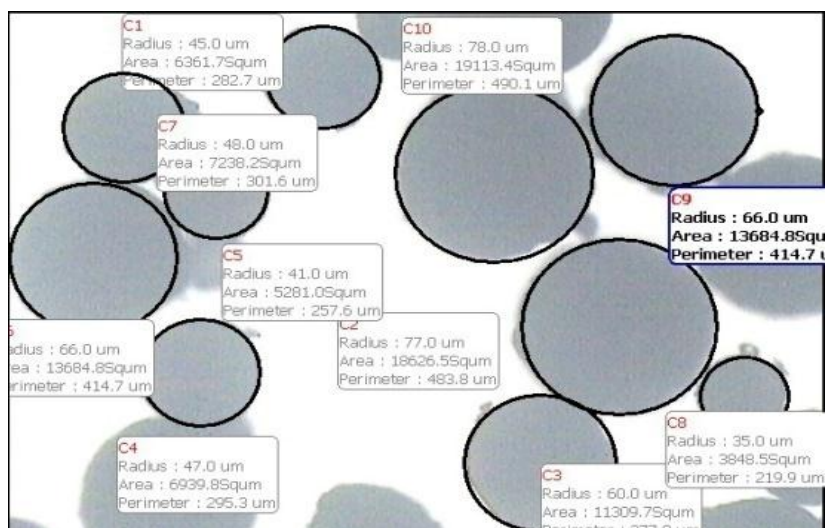


Figure 5 Photomicrographs of optimized formulation

Morphology

The surface morphology of the liposphere prepared by melt dispersion technique was studied and has been depicted in figure 6. The SEM results showed spherical particles with the evidence of less crystals of Cefixime on the carrier.

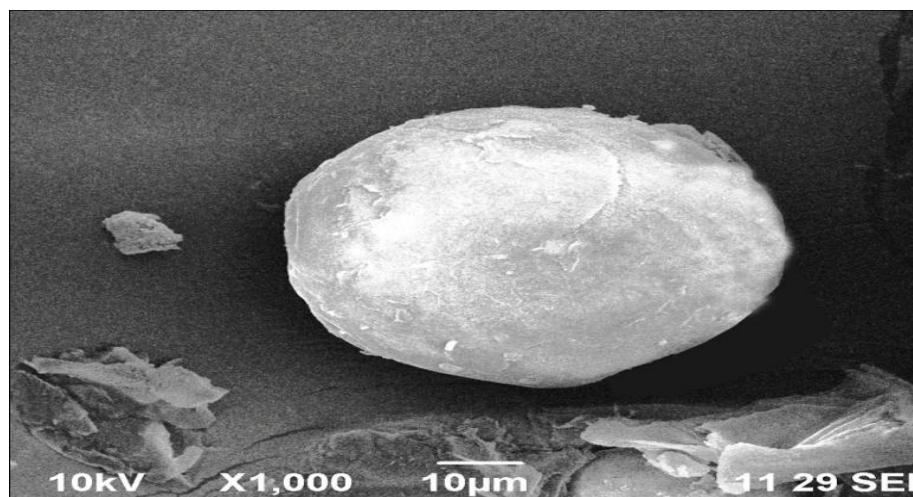


Figure 6: SEM image of optimized formulation CFX2

***In – vitro* dissolution characteristics**

The results of a 16 h *in-vitro* release study from formulations CFX1 to CFX15 were conducted in pH7.4 buffer solutions at $37\pm 0.5^\circ\text{C}$ are depicted in Table 3. 5 and 7. There was a controlled release of Cefixime from the liposphere without burst release. The best formulation CFX2 showed $82.95\pm 0.99\%$ in 16 h. From this study one could conclude that the extended release dosage form of Cefixime can be obtained through liposphere techniques. Cefixime is highly used in number of infections and showed good results. For instance in a recent study, liposome encapsulated ciprofloxacin was compared with conventional ciprofloxacin and ceftriaxone in rat model of pneumococcal pneumonia^[18], ciprofloxacin liposomes were superior to conventional one. In this study an attempt was made to form novel formulation of Cefixime and *in vitro* release of the formulations were studied for possible oral delivery of the API.

Table 3: Cumulative Percentage Drug Release of Formulation with Cetyl Alcohol

Time in hrs	Cumulative % of drug release from Cefixime loaded liposphere in cetyl alcohol				
	CFX1	CFX2	CFX3	CFX10	CFX13
1	10.92	10.62	16.52	9.14	6.88
2	16.96	18.82	18.16	10.52	10.56
3	18.14	22.14	22.14	21.78	14.78
4	22.96	26.36	26.32	28.82	18.52
5	25.85	30.05	28.55	36.42	22.63
6	30.48	36.62	33.92	44.36	26.26
7	35.52	41.08	35.00	46.24	30.16
8	40.75	45.76	37.57	48.54	36.82
9	43.97	50.72	41.01	50.56	43.82
10	46.93	55.86	44.31	54.53	49.95

11	51.52	60.96	47.67	58.26	56.62
12	55.35	64.94	51.12	60.48	62.24
13	60.16	68.24	58.58	61.56	68.52
14	65.12	72.51	65.12	62.52	73.84
15	72.18	76.46	70.42	62.82	78.52
16	80.29	82.95	79.12	63.02	79.02

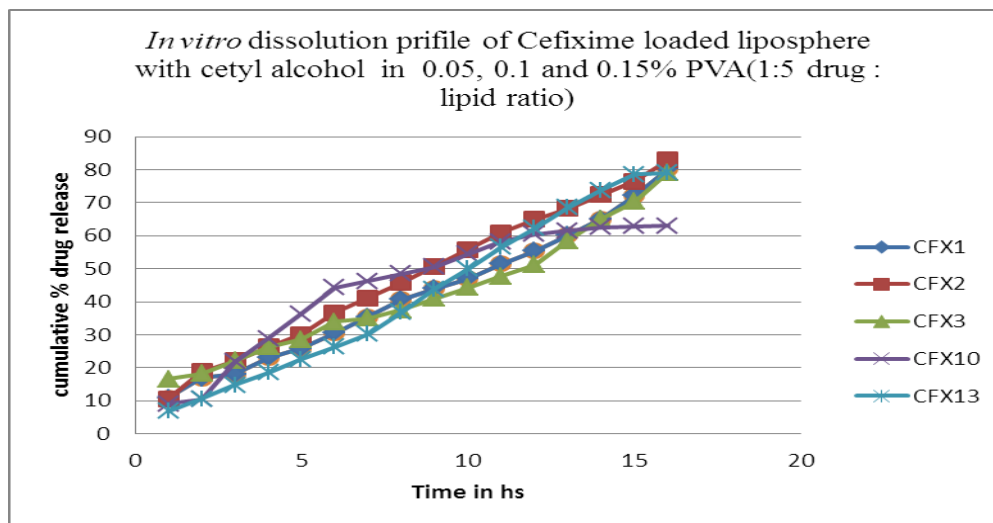


Figure- 7: *In vitro* dissolution profile of Cefixime loaded liposphere with cetyl alcohol in 0.05, 0.1 and 0.15% PVA(1:5 drug : lipid ratio)

Table4. Descriptive Statistics of Regression and parameters of the Mathematical models for dissolution data of formulation with Cetyl alcohol.

Formulation code	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Korsemeyer–Peppas	
				(R^2)	n
CFX1	0.973	0.880	0.915	0.950	0.805
CFX2	0.993	0.963	0.978	0.998	0.831
CFX3	0.966	0.884	0.905	0.949	0.769
CFX10	0.901	0.962	0.956	0.942	0.531
CFX13	0.989	0.942	0.944	0.990	1.127

Table 5. Cumulative Percentage Drug Release of formulation with Cetostearyl Alcohol

Time in hrs	Cumulative % of drug release from Cefixime loaded liposphere in cetostearyl alcohol				
	CFX4	CFX5	CFX6	CFX11	CFX12
1	6.52	8.61	12.72	7.52	5.25
2	11.68	13.05	15.62	10.52	10.56
3	14.63	15.78	20.86	15.52	16.02
4	20.21	22.01	25.42	20.58	28.58
5	22.54	24.45	30.68	26.57	36.76
6	29.52	28.65	35.28	32.98	44.16
7	32.13	31.34	42.46	38.15	52.52
8	35.76	37.68	46.52	44.63	60.52
9	40.12	42.27	50.37	50.45	66.52
10	45.76	45.33	54.72	59.53	64.25
11	52.67	49.62	56.42	63.63	64.02
12	59.52	54.12	60.82	67.23	63.86
13	64.62	56.42	64.89	71.58	63.56
14	76.06	60.82	70.25	74.56	-
15	74.28	74.52	75.18	77.32	-
16	72.35	72.96	74.83	78.43	-

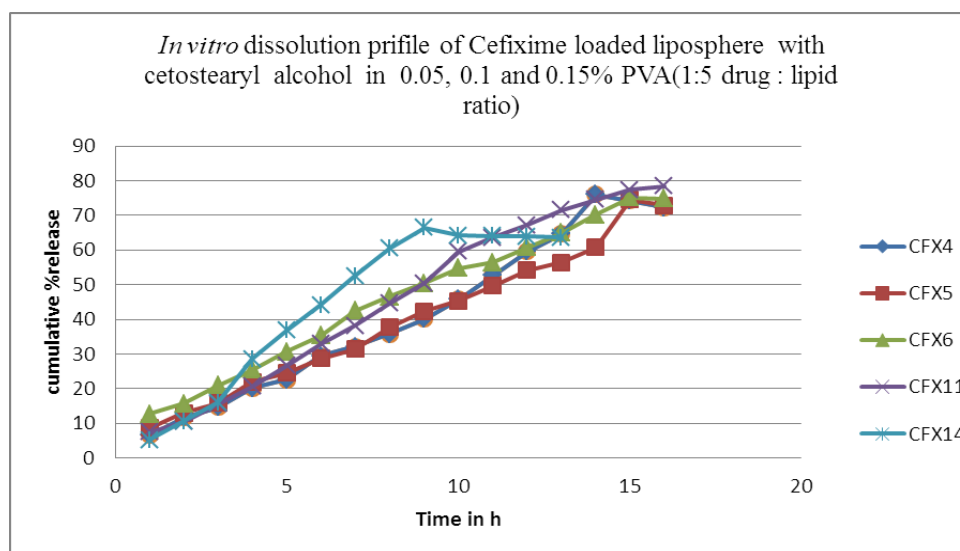
Figure- 8: *In vitro* dissolution profile of Cefixime loaded liposphere with cetostearyl alcohol in 0.05, 0.1 and 0.15% PVA(1:5 drug : lipid ratio)

Table 6: Descriptive statistics of regression and parameters of the mathematical models for dissolution data of formulations with cetostearyl alcohol.

Formulation code	Zero order (R ²)	First order (R ²)	Higuchi (R ²)	Korsmeyer -Peppas	
				(R ²)	n
CFX4	0.989	0.925	0.942	0.975	1.002
CFX5	0.991	0.942	0.948	0.985	0.938
CFX6	0.986	0.983	0.980	0.994	0.785
CFX12	0.988	0.980	0.975	0.985	0.995
CFX14	0.993	0.945	0.953	0.986	0.892

Table 7. Cumulative Percentage Drug Release of formulation with Stearyl Alcohol

Time in hrs	Cumulative % of drug release from Cefixime loaded liposphere in stearyl alcohol				
	CFX7	CFX8	CFX9	CFX12	CFX15
1	7.53	7.64	9.14	6.88	7.58
2	16.55	15.52	10.52	12.56	11.20
3	18.65	18.58	13.58	15.78	15.28
4	21.05	21.46	16.88	22.52	20.51
5	28.02	23.64	20.64	27.43	25.96
6	30.86	27.46	24.43	30.26	31.56
7	32.45	30.24	29.66	35.58	38.92
8	37.86	36.54	34.54	39.62	43.56
9	43.75	42.65	40.62	45.82	48.72
10	45.62	48.25	48.53	50.95	52.65
11	47.62	54.02	54.63	57.54	56.21
12	55.42	60.86	60.48	64.24	60.08
13	61.35	64.54	63.54	68.45	65.26
14	66.65	69.73	66.7	74.56	70.43
15	72.12	73.52	69.52	75.02	71.28
16	72.02	72.21	71.52	76.54	72.86

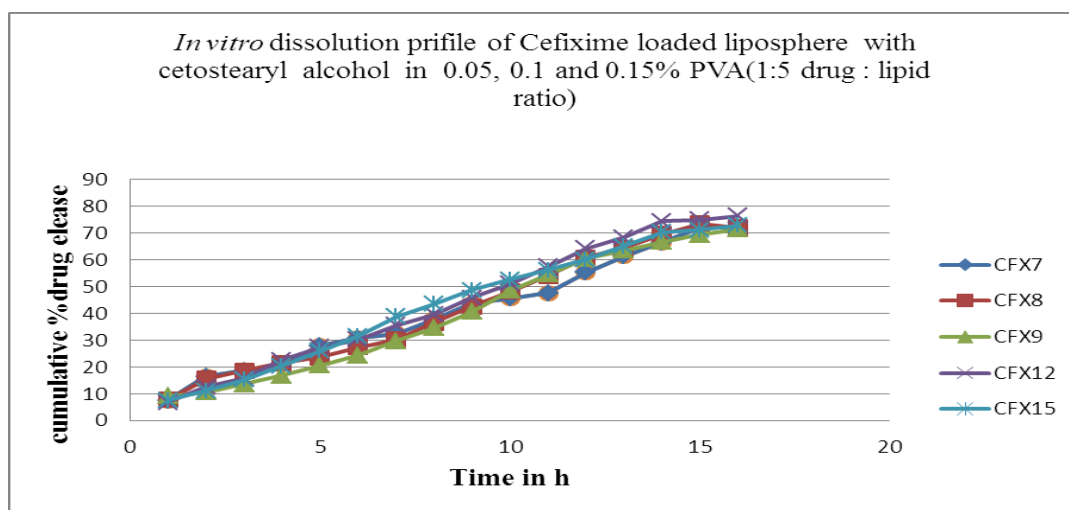


Figure- 9: *In vitro* dissolution profile of Cefixime loaded liposphere with stearyl alcohol in 0.05, 0.1 and 0.15% PVA(1:5 drug : lipid ratio)

Table 8: Descriptive statistics of regression and parameters of the mathematical models for dissolution data of formulations with stearyl alcohol

Formulation code	Zero order (R ²)	First order (R ²)	Higuchi (R ²)	Korsmeyer -Peppas	
				(R ²)	n
CFX7	0.989	0.954	0.957	0.985	0.883
CFX8	0.987	0.955	0.945	0.983	0.998
CFX9	0.987	0.963	0.941	0.990	1.125
CFX12	0.994	0.964	0.968	0.991	0.949
CFX15	0.990	0.987	0.982	0.988	0.924

CONCLUSION

Cefixime loaded liposphere were successfully incorporated in to cetyl alcohol, cetostearyl alcohol and stearyl alcohol by melt dispersion method. The prepared liposphere were evaluated for morphology, particle size percentage yield, entrapment efficiency, drug content, *in-vitro* drug release and *in-vitro* release kinetics. The best formulation CFX2 showed 92.33 ±2.05 percentage yield, 64.99 ±2.01 mean particle size, 95.50 ± 1.23, EE (%) and 53.23 ±1.03. The *in-vitro* drug release of the optimized formulation showed 82.95 % of drug release in 16 hrs in controlled manner. The release kinetics of Cefixime loaded liposphere confirmed that the formulation follows zero order kinetics. The high R² value 0.998 and the 'n' value ≥ 0.83 in Korsmeyer and Peppas confirms non Fickian type of diffusional release. The proposed Cefixime loaded lipospheres illustrates an efficient way to deliver the drug in controlled manner. Liposphere is considered as a promising delivery system for oral delivery of lipophilic drug to exhibit better therapeutic effect. Easy availability of formulation ingredients makes the delivery system more feasible and attractive for the formulation on industrial scale. Owing to the finer particle size of lipospheres and presence of surface stabilized by emulsifier particles, the bioavailability of several problematic drugs was found to increase. But further studies in terms of pharmacokinetics, toxicology and animal studies are required for the clinical utility of the formulation. The encouraging results obtained in this result could propose liposphere for future *in-vivo* studies.

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