Research Article Arch Pharm Sci & Res Vol 1 No 1 66-74 July 2009

STUDIES ON FORMULATION AND CHARACTERIZATION OF CELLULOSE-BASED MICROSPHERES OF CHLORPHENIRAMINE MALEATE

Bhaskar Mazumder[1*], Sanjay Dey[1], Sanjib Bhattacharya[2], Sushanta Sarkar[1], Bibhash Mohanta[1]

[1]Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786004, Assam India.

[2]Bengal School of Technology (A College of Pharmacy), Delhi Road, Sugandha,

Hooghly 712102, West Bengal, India.

The aim of this study was to prepare and characterize microspheres of chlorpheniramine maleate (CPM) using ethyl-cellulose and cellulose acetate as polymeric retardant material. Microspheres were prepared by oil-in-oil emulsification method using ethyl-cellulose and cellulose acetate separately. The effect of process variables on mean particle size, percent yield and percent entrapment efficiency and *in vitro* release characteristics of microspheres in pH 7.4 phosphate buffer were studied. Fourier Transform-Infra Red (FT-IR) and Thermo Gravimetric Analysis (TGA) were performed to evaluate interaction between drug and polymer. Morphology of microspheres was characterized using Scanning Electron Microscopy (SEM). The prepared microspheres were white, free flowing and spherical in shape. The drug-loaded microspheres showed 79-93% and 80-89% of entrapment for ethyl-cellulose and cellulose acetate microspheres respectively. The FT-IR spectra and TGA showed the stable character of drug in the drug-loaded microspheres and revealed the absence of drug-polymer interactions. SEM study revealed that the microspheres were spherical, smooth surface and porous in nature. The prepared microspheres of ethyl-cellulose and cellulose acetate are therefore potential polymeric materials for the prolongation of release of chlorpheniramine maleate.

Key words: Chlorpheniramine maleate (CPM), Ethyl-cellulose, Cellulose acetate, Emulsification technique.

INTRODUCTION

Chlorpheniramine maleate (CPM), an inversely acting histamine H_1 receptor antagonist used in treatment of asthma, hay fever and other respiratory tract allergies[1]. The terminal half life of CPM after single dosage in human subjects is between 21 to 27 hours. It is absorbed relatively slowly from the gastrointestinal tract and weak plasma concentration of 5.9 and 11 µg/ml are achieved in 2.5 to 6 hours after oral administration. Its bioavailability is low; values between 25 to 50% have been reported, thus necessitating frequent administration of drug (4 mg for every four to six hours) to maintain the therapeutic drug levels [2, 3]. Such frequent drug administration may reduce patient compliance and therapeutic efficacy. Hence, the development of microsphere therapeutic system for CPM that provides prolonged release of single dose, thereby minimizing the frequent administration, it also reduced the total dose require to elicit pharmacological activity thereby reducing adverse effects.

In unit dosage form gastrointestinal irritation may occur on sudden increase in drug concentration in the GIT. Microparticulate system is the best an alternative to overcome the problem. Microparticulate drug delivery has more advantages in comparison to single unit dosage form as they are uniformly distributed through the GIT thereby reducing local concentration of drug[4]. The development of microspheres therapeutic drug delivery for CPM would be beneficial for an effective and safe therapy of bronchial asthma.

Ethyl-cellulose and cellulose acetate, the non-biodegradable and biocompatible polymers, are extensively studied in the encapsulation of material for controlled release of pharmaceuticals. Several researchers have investigated the utilization of ethyl-cellulose and cellulose-acetate as a polymer to prepare microparticulate drug delivery systems by solvent evaporation techniques, emulsification techniques etc [5-8].

The specific goal of the research is formulation of microspheres of CPM using ethyl cellulose and cellulose acetate individually and to evaluate the effect of various process variables on mean particle size, % yield, % encapsulation efficiency and *in vitro* release kinetics of drug from microspheres.

MATERIALS AND METHODS

DRUGS AND CHEMICALS: CPM was received

as a gift sample from Kon Text Chemicals Ltd., Kolkata; ethyl-cellulose (14 cps) and cellulose acetate (3 cps) were from Wilson Brothers, Mumbai; light liquid paraffin and Tween 80 were from Ranbaxy fine Chemicals Ltd... All other chemicals and reagents were of analytical grade obtained commercially.

MICROSPHERE PREPARATION METHOD: Microspheres were prepared by oil-in-oil emulsification technique using the formulations as shown in Table 1. Polymer and drugs were used in the ratio of 1:1, 1:1.5 and 1:3. Required amount ethyl-cellulose or cellulose acetate and CPM were dissolved in 15 ml of acetone using digital mechanical stirrer (Remi Motors, India) at 500 rpm for 5 mins. Then drug-polymer solution was added slowly in a thin stream into liquid paraffin oil (50 ml or 100 ml) containing 1, 1.5, 2% Tween 80 as surfactant with stirring (at 600 rpm/1200 rpm/1800 rpm). Stirring was carried out for 2.5 h to evaporate acetone. The mineral oil was decanted off and the collected microspheres were washed three times with 50 ml of *n*-hexane at room temperature, after which the microspheres were separated by filtration and air-dried for 12 hours.

PARTICLE SIZE ANALYSIS

The particle size was determined by optical microscopic method. For each batch of the microspheres 100 particles were counted and recorded in triplicate.

YIELD AND ENTRAPMENT EFFICIENCY

The calculation of percentage yield was done by

using the following formula:

Yield (%) = Amount of microspheres obtained / Theoretical content x 100 %

Drug entrapment efficiency was determined by crushing the microspheres using pastel and mortar. 50 mg of this powder were added to 50 ml phosphate buffer pH 7.4 followed by stirring of the solution at 1000 rpm for 3 h. Then the solution was filtered and diluted for spectrophotometric analysis of CPM at 264 nm. Drug entrapment efficiency was determined by using the following relationship.

$$\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} X 100$$

FT-IR STUDY

FT-IR spectra of blank and drug loaded microspheres were obtained at room temperature in KBr pellets by applying 6000 kg/cm^2 pressure using Perkin Elmer FT-IR model 883 (Pyris Diamond, USA) between the ranges of 500 to 4000 cm^{-1}

SCANNING ELECTRON MICROSCOPY (SEM)

The surface morphology of blank microspheres, drug loaded microspheres and microspheres collected after dissolution studies were examined by a scanning electron microscope (Hitachi, S-3600N, Japan). The samples were fixed on brass sub using double-sided tape and then gold- coated in vacuum by a sputter coater. The SEM pictures were then taken at an excitation voltage of 15 kV.

THERMOGRAVIMETRIC ANALYSIS (TGA)

Thermograms of pure CPM blank and drug loaded microspheres of ethyl cellulose and cellulose acetate were recorded on a Perkin Elmer (Pyris Diamond, USA) instrument. The samples were sealed in aluminium pans and heated at a rate of 8^0C/min from 32-410^0C in a nitrogen atmosphere with a flow rate of 400 ml/min.

***IN VITRO* DRUG RELEASE STUDY**

USP (type I) basket type dissolution test apparatus was used to carry out the *in-vitro* release studies of CPM from the combination of ethyl-cellulose and cellulose acetate microspheres in 900 ml phosphate buffer pH 7.4 at $37^0C \pm 1^0C$ at 50 rpm. Microspheres equivalent to the 100 mg of CPM was taken in the dissolution medium. A 5 ml aliquot was withdrawn at different time intervals up to 12 hours followed by filtration was carried out with a 0.45 µ nylon disc filter and replaced with 5 ml of fresh dissolution medium. The

filtered samples were diluted and analyzed for CPM. Absorbance was measured at 264 nm by using Hitachi U-2001 UV-VIS spectrophotometer. The experiments were conducted in triplicate.

The concentration of CPM in test samples was corrected for sampling effect using following formula:

$$C_n = M_n \ [V_T / V_T - V_S] \times [C_{n-1} / M_{n-1}]$$

Where C_n and C_{n-1} is the corrected concentration of n^{th} and $(n-1)^{th}$ sample respectively. V_T and V_S is the volume of dissolution medium and sample withdrawn respectively; M_n and M_{n-1} is the original concentration of the n^{th} and $(n-1)^{th}$ sample respectively.

DRUG RELEASE KINETICS

To study the underlying mechanism of drug release, drug release data was computed by the use of following mathematical models; zero-order kinetics, first-order kinetics and Higuchi kinetics.

$$Q_t = k_0 t$$

$$\ln(Q_0 - Q_t) = \ln Q_0 - k_1 t$$

$$Q_t = K_h . t^{½}$$

The following plots were made; Q_t Vs t (Zero-order kinetic model), $\ln(Q_0-Q_t)$ vs t (First-order kinetic model) and Q_t vs $t^{½}$ (Higuchi model). Where Q_0 is the initial amount of drug present in the microspheres, Q_t is the amount of drug released at time t and k_0, k_1, and k_h are the constants of the above-mentioned equations. In order to define a model, which would represent a better fit for the formulation, dissolution data was further analyzed by Korsmeyer-peppas equation[9]:

$$M_t/M_\infty = k_t^{\ n}$$

Where M_t is the amount of drug released at time t and M_∞ is the amount of drug released at time ∞, thus the M_t/M_∞ is the fraction of drug released at time t. k is the kinetic constant and n is the diffusion exponent, a measure of the primary mechanism of drug release. r^2 values were calculated for the linear curves obtained by regression analysis of the above plots.

STATISTICAL ANALYSIS

All the means are presented with their standard deviation (mean ± S.D). An unpaired Student's t- test was used to compare the effect of different parameters on the mean particle size, percentage yield, percentage entrapment efficiency and percentage released of drug. A p value < 0.05 was considered significant.

Table 3. In-vitro release kinetic parameters of CPM loaded ethyl-cellulose and cellulose acetate microspheres.

Formulation (Drug: Polymer)		Zero-order		First-order		Higuchi Model		Korsemmeyer-peppas Model	
		r^2	K_0	r^2	K_1	r^2	K_h	r^2	N
Ethyl-cellulose	1:1	0.920	6.562	0.618	0.068	0.991	26.00	0.981	0.437
	1:1.5	0.965	6.382	0.759	0.069	0.977	24.52	0.959	0.486
	1:3	0.967	5.759	0.877	0.062	0.981	22.14	0.975	0.500
Cellulose acetate	1:1	0.881	6.907	0.588	0.069	0.988	27.93	0.985	0.459
	1:1.5	0.913	6.593	0.656	0.065	0.996	26.34	0.995	0.485
	1:3	0.962	6.559	0.861	0.068	0.988	25.43	0.986	0.573

r^2 is the coefficient of correlation; K_0, K_1 and K_h are the release constant for zero-order, first-order and Higuchi model respectively and n is the release exponent of Korsemmeyer-peppas Model.

RESULTS AND DISCUSSION

EFFECT OF DRUG-POLYMER RATIO

The mean particle size, % yield and % entrapment efficiency of microspheres containing various amounts of polymer were determined. The amount of CPM was kept constant and polymer (ethyl-cellulose and cellulose acetate) concentration was varied (1:1, 1:1.5 and 1:3). The mean particle size and % entrapment efficiency were decreased with decreasing in the amount of drug-polymer ratio. The % yield was decreased with decrease in drug-polymer ratio from 1:1 to 1:1.5. With further decrease in drug-polymer ratio from 1:1.5 to 1:3 statistically significant increase ($p < 0.05$, Student's t-test) was observed[10-12]. The results of effects of drug polymer ratio are given in Table 2.

EFFECT OF SURFACTANT CONCENTRATION

The effect of surfactant concentration on mean particle size (µm), % yield and % entrapment efficiency of microspheres were determined. The

Table 1. Formulation composition of ethyl-cellulose and cellulose acetate microspheres.

Formulation code	Ethyl-cellulose (mg)	Cellulose acetate (mg)	CPM (mg)	Light liquid paraffin (ml)	Tween 80(%)	Stirring Speed (rpm)
A1	900	-	900	50	1.5	1200
A2	900	-	600	50	1.5	1200
A3	900	-	300	50	1.5	1200
A4	-	900	900	50	1.5	1200
A5	-	900	600	50	1.5	1200
A6	-	900	300	50	1.5	1200
A7	900	-	600	50	1.0	1200
A8	900	-	600	50	2.0	1200
A9	-	900	600	50	1.0	1200
A10	-	900	600	50	2.0	1200
A11	900	-	600	50	1.5	600
A12	900	-	600	50	1.5	1800
A13	-	900	600	50	1.5	600
A14	-	900	600	50	1.5	1800

Table 2. Effect of drug and polymer ratio on mean particle size, %yield and entrapment efficiency.

Process variables	Polymers	Formulation code	Mean particle size(µm)	% Yield	% Entrapment efficiency
Effect of drug and polymer ratio	Ethyl-cellulose	A1(1:1)	1118.91±27.54	86.34±2.65	93.63±3.87
		A2(1:1.5)	815.32±22.86	82.78±5.86	87.42±6.11
		A3(1:2)	601.45±31.42	84.55±8.54	81.39±4.77
	Cellulose acetate	A4(1:1)	1205.23±20.73	87.01±3.43	89.64±5.97
		A5(1:1.5)	712.42±21.59	83.52±2.39	84.25±2.29
		A6(1:2)	657.33±37.82	86.32±8.54	80.79±3.06
Effect of surfactant concentration	Ethyl-cellulose	A7 (1%)	843.46±30.21	81.42±7.43	88.37±4.98
		A2 (1.5%)	815.32±22.86	82.78±5.86	87.42±6.11
		A8 (2%)	792.19±16.95	80.75±4.41	91.28±2.98
	Cellulose acetate	A9(1:1)	789.45±32.52	88.52±2.41	86.35±2.94
		A5(1:1.5)	712.42±21.59	83.52±2.39	84.25±2.29
		A10(1:2)	688.74±20.21	78.43±4.85	82.31±5.97
Effect of stirring speed	Ethyl-cellulose	A11(600)	875.91±28.45	87.42±2.96	79.47±4.09
		A2(1200)	815.32±22.86	82.78±5.86	87.42±6.11
		A12(1800)	730.48±29.65	80.98±1.07	89.23±5.96
	Cellulose acetate	A13(600)	822.20±27.75	86.23±2.98	81.52±4.21
		A5(1200)	712.42±21.59	83.52±2.39	84.25±2.29
		A14(1800)	670.46±28.31	81.21±5.03	88.33±7.01

amount of surfactant was varied (1%, 1.5% and 2%). The mean particle size was decreased and encapsulation efficiency was increased with increasing in surfactant concentration significantly ($p < 0.05$ Student's t-test). In ethyl-cellulose microspheres, maximum % yield was found by increasing in surfactant concentration 1% to 1.5%. The % yield decreased significantly ($p < 0.05$ Student's t-test) by increasing surfactant concentration from 1.5 to 2%. In cellulose acetate microspheres % yield was increased significantly ($p < 0.05$ Student's t-test) with increasing surfactant concentration[7]. The results of effects of surfactant concentration are given in Table 2.

EFFECT OF STIRRING SPEED

Effect of stirring speed on mean particle size (µm), % yield and % entrapment efficiency of microspheres were determined. The speed of stirrer was varied (600 rpm, 1200 rpm, 1800 rpm).The mean particle size and % yield decreased significantly ($p < 0.05$ Student's t-test) with increasing in stirring speed. The % encapsulation efficiency was increased significantly ($p < 0.05$ Student's t-test) with increasing stirring speed[13-15]. The result of effects of stirring speed is given in Table 2.

FT-IR ANALYSIS

The FT-IR spectra of pure CPM (Fig 1a) depicts triple characteristic peaks at 1580 cm^{-1}, 1476 cm^{-1} and 1352 cm^{-1} due to C=C stretching, C-H stretching and C-H bending respectively. Another two sharp peaks were seen at 864 cm^{-1} and 702 cm^{-1} which are due to C-C and C-Cl stretching vibration. The blank ethyl-cellulose microspheres (Fig 1b) showed a strong characteristic peak at 1105 cm^{-1}. When FT-IR spectra of CPM loaded ethyl-cellulose microspheres (Fig 1c) were studied there found all the characteristic peak of CPM (at 1588 cm^{-1}, 1471 cm^{-1}, 1358 cm^{-1}, 865 cm^{-1} and 701 cm^{-1}) and also a strong peak at 1093 cm^{-1} which was due to ethyl-cellulose polymer. It is expected that there was no interaction occurred between CPM and ethyl-cellulose polymer. The FT-IR spectrum of blank cellulose-acetate microspheres (Fig. 1d) showed two characteristic peaks at 1751cm^{-1} and 1248cm^{-1}. When FT-IR spectra of drug loaded cellulose acetate (Fig 1e) was studied, all the characteristic peaks of CPM were observed (at 1588 cm^{-1}, 1471 cm^{-1}, 1365 cm^{-1}, 864 cm^{-1} and 699 cm^{-1}) and another two characteristic peaks for cellulose-acetate were found at 1740 cm^{-1} and 1245 cm^{-1}. Therefore, It was expected that there is no interaction between CPM and cellulose acetate polymer.

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

From the scanning electron microscopy analysis it was found that microspheres prepared by emulsification method were spherical, non-aggregated and porous. The surface of the blank microspheres for both ethyl-cellulose and cellulose-acetate were smooth than of drug loaded microspheres and this might be due to crystalline nature of encapsulated drug which was present in the surface of microspheres (Fig. 2a, b, c). The study of drug loaded microspheres shows the presence of drug particles on the surface, might be responsible for the initial burst release of drug from the entire formulated microsphere. The surface study of microspheres (Fig 2d, e, f) shows a greater pore size suggested that drug might be released through these pores and mechanism of drug release was diffusion controlled.

THERMOGRAVIMETRIC ANALYSIS (TGA)

The TGA curve of CPM (Fig. 3a) shows that weight loss of CPM started at about 150^0C and around 80-88% of weight loss occurred at around 300^0C. The TGA curve of blank ethyl-cellulose microspheres (Fig. 3b) shows that weight loss of drug started at about 281.3^0C having weight loss about 5.5%, which might be due to hydration, and decomposes at around 382.31^0C with weight loss of 89%. The TGA curve of drug loaded ethyl-cellulose microspheres (Fig. 3c) shows two step of decomposition. The first stage started at around 167^0C and finished at around 250^0C with a total loss of nearly 28% which can be attributed to drug decomposition and the second decomposition started at around at 250^0C and up to 300^0C which was for the polymer decomposition. The TGA curve of blank cellulose acetate microspheres (Fig3d) indicates an initial weight loss of about 1 to 5%, might be due to dehydration. The decomposition started at about 170^0C. The TGA curve of drug loaded cellulose acetate microspheres (Fig. 3e) also shows two step decomposition curve, first occurred in between 150^0C to 200^0C, which might be due of the drug decomposition and next curve in between 250^0C to 300^0C which is for the cellulose acetate polymer. Therefore it can be concluded that no interaction occurred in between the polymer and drug.

***IN VITRO* DRUG RELEASE STUDY**

The influence of different processing condition were evaluated on in-vitro drug release and % drug released was found in the range of 76.28% to 92.45% and 77.21% to 100% for ethyl-cellulose and cellulose acetate microspheres at period of 12 hours respectively. A biphasic *in vitro* drug released profiles was observed with initial burst effect for all the formulation prepared. The initial burst release might be due to the presence of drug on the prepared

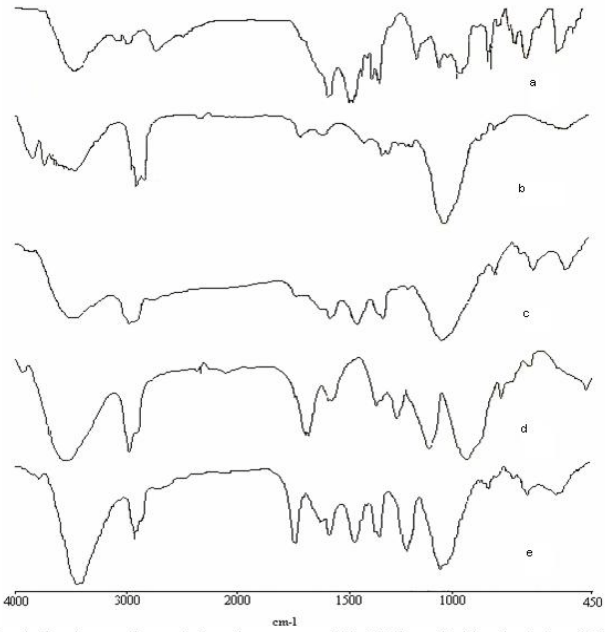

Fig. 1: Fourier transfor m infrared spectrums of (a) CPM; (b) bla nk ethyl -cellulose microspheres; (c) CPM load ed ethyl cellulose microspheres; (d) blank cellulose -acetate microspheres; (e) CPM loaded cellulose acetate microspheres.

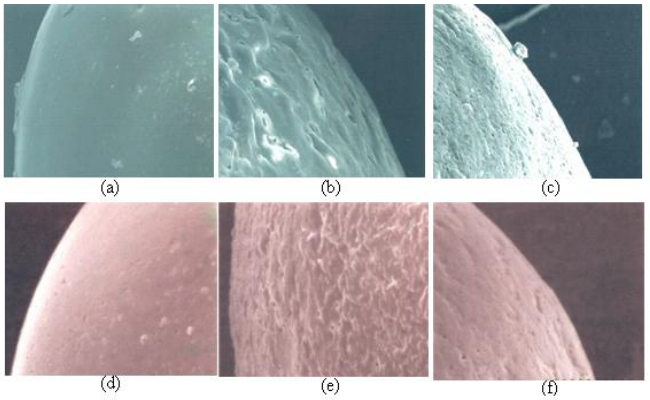

Fig. 2: SEM photograph (X300) of (a) blank ethyl cellulose microsphere before dissolution; (b) CPM loaded ethyl cellulose microsphere before dissolution; (c) CPM loaded ethyl cellulose microsphere after dissolution; (d) blank cellulose acetate microsphere before dissolution ; (e) CPM loaded cellulose acetate microsphere before dissolution; (f) CPM loaded cellulose acetate microsphere after dissolution.

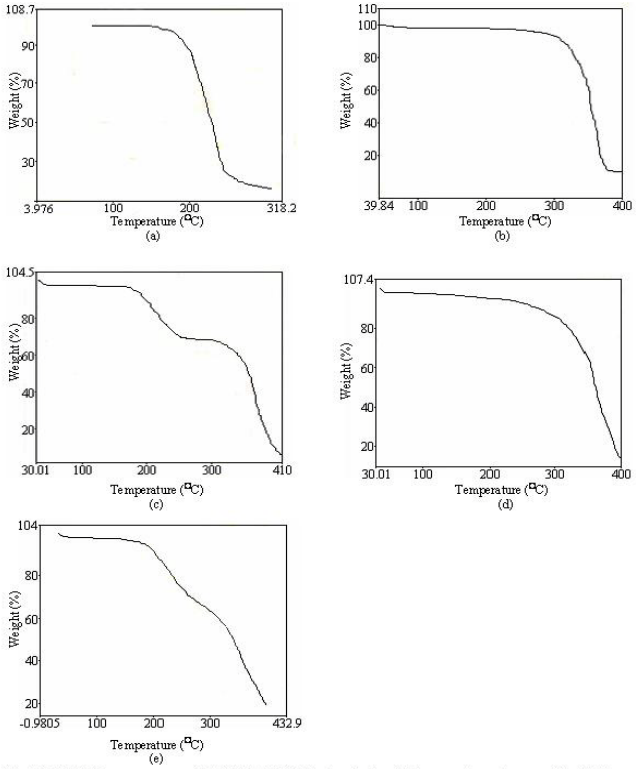

Fig.3: TGA thermograms of (a) CPM; (b) blank ethyl cellulose microspheres; (c) CPM loaded ethyl cellulose microspheres; (d) blank cellulose acetate microspheres; (e) CPM loaded cellulose acetate microspheres

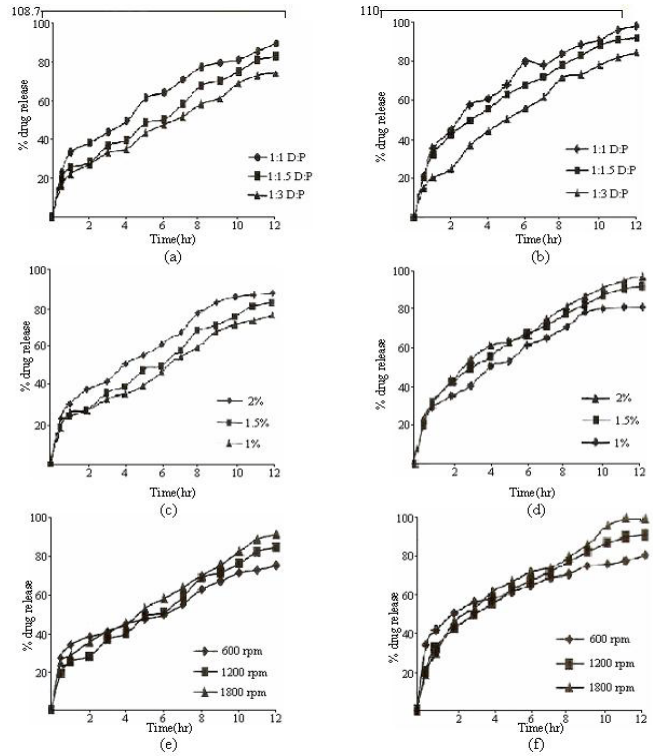

Fig 4: In-vitro release profile of (a) effect of drug-polymer ratio of ethyl-cellulose microspheres; (b) effect of drug-polymer ratio of cellulose acetate; (c) effect of surfactant concentration of ethyl-cellulose microspheres; (d) effect of surfactant concentration of cellulose acetate microspheres; (e) effect of stirring speed of ethyl-cellulose

microspheres. The initial burst release can be attributed as desired effect, which ensures the quick initial plasma therapeutic concentration of drug. All the formulated microspheres retained their shape and size even after dissolution which indicates the release of drug was diffusion through the polymer wall of microspheres. The release patterns are shown in Fig. 4.

Dissolution profiles indicate that when drug to polymer ratio decreased from 1:1 to 1:3 a decreased in release rate was observed. It is considered that higher the drug to polymer ratio in the microspheres, result in increased in coat thickness surrounding the drug particles thereby increasing the distance traveled by the drug through coat[16-18]. From the comparison of dissolution profiles studies found that drug release from cellulose acetate microspheres is faster than ethyl-cellulose microspheres. This could be due to high affinity to water for cellulose acetate than ethyl-cellulose.

Dissolution profiles indicated that the release rate from the microspheres increased significantly ($p < 0.05$ Student's t-test) as the concentration of surfactant increased. This might be attributed to the fact that average size of microspheres increased as the concentration of surfactant increased thereby free drug on microsphere surface is available for dissolution.

Release curves (Fig. 4) indicated that drug release was increased significantly ($p < 0.05$ Student's t-test) with increasing in stirring speed. when the stirring speed decreased from 1200 rpm to 600 rpm a initial burst release of around 38.45 % to 50.25 % occurred within 2 hours. This can be attributed to the fact that the drug migration will be high for low stirrer speed and more amount of drug will remain in the microspheres surface but when stirring speed was increased drug migration will be less due to collision of emulsion droplets [19].

It was found that when the volume of external phase was increased from 50 ml to 100 ml, an increased in release rate significantly ($p < 0.05$ Student's t-test). This is due to higher migration of drug due to free movement of emulsion droplets, when the volume of external processing medium was increased.

DRUG RELEASE KINETICS

In order to investigate the mechanism of CPM release from the microspheres the release data of different drug to polymer ratio for ethyl-cellulose and cellulose acetate microspheres were analyzed using four mathematical model i.e. zero order, first order, Higuchi model and Korsmeyer-peppas model and correlation coefficient for all the release kinetics were calculated from the graph. The results are presented in Table 3. For ethyl-cellulose microspheres the highest correlation coefficient was obtained in Higuchi model than zero order followed by first order. From the Higuchi plot it was found that release from ethyl cellulose microspheres was diffusion type. The 'n' value from Korsmeyer-peppas model was found 0.437, 0.486, 0.500 for drug to polymer ratio of 1;1,1:1.5 and 1:3 respectively indicating that first two formulation follows Fickian diffusion controlled release while last follows anomalous or non fickian diffusion release. Similarly for cellulose acetate microspheres the highest correlation coefficient was obtained in Higuchi model than zero order followed by first order. The 'n' value was found 0.459, 0.485, and 0.573 for drug to polymer ratio 1:1, 1:1.5 and 1:3 which indicates that first two follows Fickian diffusion whereas last follows non fickian diffusion.

CONCLUSION

Chlorpheniramine maleate microspheres were prepared successfully by emulsification technique using ethyl-cellulose and cellulose acetate. It was found that the prepared microspheres were spherical, free flowing, high % entrapment efficiency and % yielding capacity. The release kinetic study revealed that the release of drug from microspheres was diffusion controlled process. From FT-IR and TGA study it was found that their was no interaction between drug and polymer. The release study indicated that the drug released from the microspheres over an extended period of time. Therefore, ethyl-cellulose and cellulose acetate both are the potential polymeric

retardant material for the effective controlled release of chlorpheniramine maleate..

ACKNOWLEDGEMENT

The authors are thankful to the AICTE, New Delhi for granting scholarship and other financial assistance for the current study.

REFERENCES

1. Tripathi KD. Histamine and antihistaminics. In: Essentials of medical pharmacology. New Delhi: Jaypee brothers; 2004, 140-141.
2. Rumore MM. Drug Intell Clin Pharm, 1984; 18, 701-707.
3. Paton DM, Webster DR. Clinical Pharmacokinetics, 1985; 10, 477-497.
4. Li SP, Kowarshi CR, Fled KM, Grim MW. Drug Dev Ind Pharm, 1988; 14, 353-376.
5. Chowdary KPR, Prasad KSR. Indian J Pharm Sci, 1994; 56, 138 -141.
6. Ramakrishna N, Mishra B. Drug Dev Ind Pharm, 2002; 28, 403-412.
7. Jones DS, Pearce KJ. Int J Pharm, 1995; 118, 119-205.
8. Yang CY, Tsay SY, Tsiang CC. J Microencapsulation, 1999; 17 (3), 269-277.
9. Korsmeyer RW, Gurny R, Doelker EM, Buri P, Peppas NA. Int J Pharm, 1983; 15, 25-35.
10. Amperiadou A, Georgarakis M. Int J Pharm, 1995; 115, 1-8.
11. Pongpaibul Y, Sayed HAM, Whitworth CW. Drug Dev Ind Pharm, 1989; 15, 2547-2558.
12. Arshady R. J Controlled Release, 1990; 14, 111-131.
13. Lee JH, Park TG, and Choi HK. Int J Pharm, 2002; 196, 75-78.
14. Babay D, Holfman A, Benita S. Biomaterials, 1988; 9, 482-488.
15. Kawashima Y, Niwa T, Handa T, Takenchi H, Iwamoto T, Itoh Y. Chem Pharm Bull, 1989; 37, 425-429.
16. Jalsenjek I, Nicolaidou CF, Nixon JR. J Pharm Pharmacol, 1976; 28, 912-914.
17. Mortada SM. Pharmazie, 1982; 37, 427-429.
18. Kim CK, Kim MJ, Oh KH. Int J Pharm, 1994; 106, 213-219.
19. Mostafa S, Shahbazi M, Shafiee A. Nature, 2008; 1544, 1-5.