www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.1, No.3, pp 905-913, July-Sept 2009

PREPARATION AND *IN VITRO* EVALUATION OF CHLORPHENIRAMINE MALEATE LOADED MICROSPHERES

Bhaskar Mazumder[1*], Sanjib Bhattacharya[2], Bibhash Mohanta[1], Sanjay Dey[1] Anindya Maity[3]

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

[2]Bengal School of Technology, Delhi Road, Sugandha, Hooghly 712102, West Bengal, India

[3]Department of Pharmaceutical Technology, Jadavpur Universirty, Kolkata 700032, West Bengal, India

E-mail: bhmaz@yahoo.co.in

ABSTRACT: The aim of this study is to prepare and characterize the microspheres of chlorpheniramine maleate (CPM) with the combination of cellulose derivatives: ethyl cellulose and cellulose acetate. Microspheres were prepared with the combination of ethyl cellulose and cellulose acetate using oil-in-oil emulsion solvent evaporation method. Microspheres were characterized by particle size analysis, percentage yield, and entrapment efficiency, scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy, *in vitro* release studies and release kinetics. The mean particle size, percentage yield were decreased significantly ($p < 0.05$) with decrease in drug-polymer ratio, surfactant concentration, stirring speed and volume of continuous phase. SEM and FT-IR studies revealed that the microspheres were spherical; non-aggregated, and porous in nature and drug polymer is compatible. It was found that *in vitro* release were decreased significantly ($p < 0.05$) with decrease in drug-polymer ratio and stirring speed but increased significantly ($p < 0.05$) with increase in surfactant concentration and volume of continuous phase. The analysis of dissolution kinetic data shows that it follows Higuchi model then zero order followed by first order. The result suggests that the combination of cellulose acetate and ethyl cellulose microsphere may be useful for the delivery of chlorpheniramine maleate.

Key-words: Chlorpheniramine maleate (CPM), ethyl cellulose, cellulose acetate, emulsion solvent evaporation technique.

INTRODUCTION

Chlorpheniramine maleate (CPM) is an inverse H_1 antagonist, commonly used in the treatment of asthma and other respiratory tract allergies. They are also common ingredients of the compounds preparation for symptomatic treatment of cough and cold[1]. It is absorbed relatively slowly from the gastrointestinal tract. The plasma concentration of 5.9 and 11 µg/ml are achieved in 2.5 to 6 hours after oral administration. The terminal half-life of CPM after single dosage in human subjects is in between 21 to 27 hours. Oral bioavailability of CPM is low; values are ranging from 25 to 50% [2,3]. Therefore, frequent administration of drug (4 mg for every four to six hours) is necessary to maintain the therapeutic drug level. CPM also has a bitter taste. Hence the development of controlled release therapeutic system for CPM that provides sustain release after single dosage, thereby minimizing the frequent administration, it also reduces the total dose require to elicit pharmacological activity and as well reduces side effect. Gastrointestinal irritation is the major problem due to sudden rise in the concentration of drug in GIT after administration of unit dosage. Micro particulate drug delivery due to the approach that can solve the problem associated with the unit dosage form, as they uniformly distributed through the GIT thereby reduce the total concentration of drug[4]. This type of drug delivery for CPM would be beneficial for an effective and safe therapy of asthma and also mask the bitter taste, thereby increase the oral bioavailability.

Ethyl cellulose and cellulose acetate is non-biodegradable and biocompatible polymers, which are

commonly used for the encapsulation of materials. It has been found that many researchers formulated controlled drug delivery system using ethyl cellulose and cellulose acetate by emulsion solvent evaporation method[5-8]. In our laboratory we already formulated microspheres drug delivery system of CPM using ethyl cellulose and cellulose acetate individually. The aim of this work was to prepare microspheres drug delivery system of CPM using combination of ethyl cellulose and cellulose acetate. The specific goal of the research is to evaluate the effect of drug-polymer combination (ethyl cellulose and cellulose acetate) ratio, surfactant concentration, stirring speed and volume of continuous phase on the particle size, percentage yield, percentage encapsulation efficiency and *in vitro* release of drug from the formulation.

EXPERIMENTAL

DRUGS AND CHEMICALS

The following materials were used: cholrpheniramine maleate I. P. (Gift sample from Kon Text Chemicals Ltd. Kolkata); ethyl cellulose 14 cps (Wilson Brothers, Mumbai); and cellulose acetate 3 cps (Wilson Brothers, Mumbai); light liquid paraffin (Ranbaxy fine chemicals); Tween 80 (Ranbaxy fine chemicals). All the chemicals were of analytical grade.

MICROSPHERE PREPARATION METHOD

Microspheres were prepared by using oil-in-oil emulsion solvent evaporation method using the formulation as shown in Table 1. Drug and combination of polymers ethyl cellulose and cellulose acetate (1:1) were used in the ratio of 1:1, 1:1.5, and 1:3. Required amount of the combination of ethyl cellulose and cellulose acetate (1:1) and CPM were dissolved in 15 ml of acetone using digital mechanical stirrer (Remi Motors, India) at 500 rpm for 5 mins; this polymer solution was then added slowly in a thin stream into liquid paraffin oil (50 ml and 100 ml) containing 1, 1.5, 2% Tween 80 as surfactant while stirring (at 600 rpm/1800 rpm/1200 rpm). Stirring was carried out for 2.5 h to evaporate acetone. The mineral oil was decanted and the collected microspheres were washed thrice with 50 ml n-hexane at room temperature, after which the microspheres were separated by filtration using filter paper and air dried for 12 h.

PARTICLE SIZE ANALYSIS

The particle size was determined by microscopic method. For each batch of the microspheres 100 particles were counted and done 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 morter. 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 hours.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.

$$\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} X100$$

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 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.

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±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:

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

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.

$Q_t = k_0 t$

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

$Q_t = K_h . t^{1/2}$

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 $t^{1/2}$ (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 of < 0.05 was considered significant.

RESULTS AND DISCUSSION

EFFECT OF DRUG AND POLYMER RATIO

The mean particle size, percentage yield and percentage entrapment efficiency of microspheres containing the combination of polymers (as shown in Table 2) were determined. The amount of polymers (ethyl-cellulose and cellulose acetate at 1:1 ratio) was kept constant and the CPM was varied (1:1, 1:1.5 and 1:3). The mean particle size, percentage yield and the percentage entrapment efficiency were decreased significantly ($p < 0.05$, Student's t-test) with the decrease in the drug-polymer ratio. The reduction in microsphere size with the decrease in drug to polymer ratio might be due to increase in viscosity of the internal phase[10-12].

EFFECT OF SURFACTANT CONCENTRATION

The effect of surfactant concentration on mean particle size (µm), percentages yield and percentage entrapment efficiency of microspheres (as shown in Table II) was determined. The amount of surfactant was varied (1%, 1.5% and 2%). The mean particle size, percentage yield and percentage entrapment efficiency decreased significantly ($p < 0.05$ Student's t-test) with the increased surfactant concentration[7].

EFFECT OF STIRRING SPEED

Effect of stirring speed on mean particle size (µm), percentage yield and the percentage entrapment efficiency (as shown in Table 2) were determined. The mean particle size and percentage yield decreased significantly ($p < 0.05$ Student's t-test) with increased in the stirring speed of stirrer. The percentage encapsulation efficiency increased significantly ($p < 0.05$ Student's t-test) with increased in the stirring speed of stirrer. It might be due to increase in high shear results in decrease in the size of microdroplets of the emulsion, resulting formation of smaller size of microspheres[13-15].

EFFECT OF VOLUME OF CONTINIOUS PHASE

Mean particle size (µ) decreased significantly ($p < 0.05$ Student's t-test) with increased in the volume of continuous phase (as shown in Table 2). But percentage yield and percentage entrapment efficiency decreased significantly ($p < 0.05$ Student's t-test) with increase in the volume of continuous phase. This is due to as the volume of external phase increases; the emulsion droplets are more free to move with less chances of collision of emulsion droplets thereby yielding small and uniform size microspheres[16,17].

FT-IR ANALYSIS

The FT-IR spectra of pure CPM (Fig 1a) depicts a triple characteristic bands 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 bands can be seen at 864 cm^{-1} and 702 cm^{-1}, which are due to C-C and C-Cl stretching vibration. The FT-IR spectra of blank ethyl cellulose and cellulose acetate (Fig 1b) combination microspheres show two bands at 1751 cm^{-1} and 1249 cm^{-1}, which attribute the presence of cellulose acetate and strong characteristic band for ethyl cellulose at 1107 cm^{-1} is also observed. The FT-IR spectra of drug (CPM) loaded polymers combination (ethyl cellulose and cellulose acetate) microspheres (Fig 1c) shows all the characteristic bands of CPM (i.e. at 1590cm^{-1}, 1468 cm^{-1}, 1359 cm^{-1}, 865 cm^{-1} and 702 cm^{-1} which are for C=C stretching, C-H stretching, C-H bending, C-C stretching and C-Cl stretching respectively) and also two characteristic bands of cellulose acetate at 1738 cm^{-1} and 1247 cm^{-1} and a strong band of ethyl cellulose at 1092 cm^{-1} can be seen. Therefore it can be concluded that there is no interaction occurred in between each of polymers and/or drug or in between

the polymer. A very slight shift in the bands was observed in combination of substances formulation, which may be due to the reduction in purity of substances.

SCANNING ELECTRON MICROSCOPIC (SEM) ANALYSIS

From the scanning electron microscopy analysis it was found that microspheres prepared by oil in oil emulsion solvent evaporation method were spherical, non-aggregated and porous. The surface of the blank microspheres and microspheres 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, 2b and 2c). The study of drug loaded microspheres showed 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 after dissolution exhibited a greater pore size suggested that drug might be release through these pores and mechanism of drug release was diffusion controlled.

***IN VITRO* DRUG RELEASE STUDY**

The influence of different processing condition was evaluated on *in vitro* drug release and percentage drug release was found in the range of 77.48 % to 96.44 % at period of 12 hours. A biphasic in-vitro drug release profile was observed with initial burst effect for all the formulation prepared. The initial burst release is due to the presence of drug on the surface prepared 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 indicated the release of drug diffusion through the polymer wall of microspheres. Through dissolution profiles it was observed that the decrease in drug to polymer ratio from 1:1 to 1:3 resulted a decrease in release rate. It is considered that the higher drug to polymer ratio in the microspheres, result in increase in coat thickness surrounding the drug particles thereby increasing the distance travelled by the drug through coat[18-20]. Dissolution profiles also indicate that the release rate of drug 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 decreased as the concentration of surfactant increased thereby free drug on microspheres surface is available for dissolution. Release curves indicate that with increase of stirring speed an increased in drug release significantly ($p < 0.05$ Student's t-test). This can be attributed to the fact that the drug migration is to be high for low stirrer speed and more amounts of drug remain in the microspheres surface but when stirring speed is increased drug migration is less due to collision of emulsion droplets[21].

It was also observed that an increased in release rate significantly ($p < 0.05$ Student's t-test) due to the increased volume of external phase from 50 ml to100 ml. It was due to higher migration of drug due to free movement of emulsion droplets, when the volume of external processing medium was increased[17].

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 combination microspheres were analyzed by using four mathematical model, i.e. zero order, first order, Higuchi model and Korsmeyer-peppas model and correlation coefficients for all the release kinetics were calculated from the graph. The results are shown in Table 3. 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 microspheres was diffusion type. The 'n' value from Peppas model was found 0.466, 0.524 and 0.519 for drug to polymer ratio of 1:1, 1:1.5 and 1:3 respectively indicating that first formulation follows Fickian diffusion controlled release while last two follows anomalous or non Fickian diffusion release.

CONCLUSION

Chlorpheniramine maleate (CPM) microspheres were prepared successfully by oil-in-oil emulsion solvent evaporation method using the combination of ethyl-cellulose and cellulose acetate polymers in the ratio of 1:1. It was found that the prepared microspheres were spherical, free flowing, high percentage entrapment efficiency and high percentage yielding capacity. It can be concluded from this study that CPM could be made into controlled-release drug delivery system using ethyl cellulose and cellulose acetate (1:1 ratio) as retardant materials in the ratio drug to polymer of 1:3, surfactant concentration of 3%, stirring speed of 1800 rpm and volume of continuous phase of 100 ml as optimum process parameter. The *in-vitro* controlled release of CPM from the prepared microspheres formulations have been established in this study. However, the *in-vitro* release characteristics of drug from the microspheres are subject to conformation in animal and human studies for coming into conclusion of enhanced bioavailability and reduced dose frequency to improve patient compliance.

ACKNOWLEDGEMENT

The authors are grateful to the AICTE, New Delhi for granting scholarship and other financial support for this study.

Table 1: Formulation composition of ethyl-cellulose and cellulose ac etate combination (1:1 ratio) microspheres.

Formulation code	Ethyl-cellulose	Cellulose acetate(mg)	CPM (mg)	Light liquid paraffin (ml)	Tween 80(%)	Stirring Speed (rpm)
A1	450	450	900	50	1.5	1200
A2	450	450	600	50	1.5	1200
A3	450	450	300	50	1.5	1200
A4	450	450	600	50	1.0	1200
A5	450	450	600	50	2.0	1200
A6	450	450	600	50	1.5	600
A7	450	450	600	50	1.5	1800
A8	450	450	600	100	1.5	1200

Table 2: Effect of various parameters on mean particle size, yield and entrapment efficiency.

Formulation code	Variables	Mean particle size (μm)	Percentage Yield	Percentage Entrapment efficiency
Drug to polymer ratio (Parameters kept constant: surfactant concentration- 1.5%, volume of processing medium-50 ml, stirring speed-1200 rpm)				
A1	1:1	1264.84±32.64	89.02±5.54	90.63±6.39
A2	1:1.5	869.92±29.35	85.63±9.09	84.16±7.84
A3	1:2	732.62±30.53	83.91±7.43	83.01±7.96
Surfactant concentration (Parameters kept constant: Drug to polymer ratio-1:1.5, Volume of processing medium-50 ml, Stirring speed-1200 rpm)				
A4	1%	898.44±31.43	86.52±3.1	87.08±3.67
A2	1.5%	869.92±29.35	83.91±7.43	83.01±7.96
A5	2%	815.82±19.43	82.05±6.31	78.35±9.50
Stirring speed (rpm) (Parameters kept constant: Drug to polymer ratio- 1:1.5, Surfactant concentration- 1.5%, Volume of processing medium- 50 ml)				
A6	600	822.20±27.75	86.23±2.98	81.52±4.21
A2	1200	712.42±21.59	83.52±2.39	84.25±2.29
A7	1800	670.46±28.31	81.21±5.03	88.33±7.01
Volume of continues phase (ml): (Parameters kept constant: Drug to polymer ratio- 1:1.5, Surfactant concentration- 1.5%, Stirring speed-1200 rpm)				
A2	50	869.92±29.35	83.91±7.43	83.01±7.96
A8	100	774.83±26.85	85.93±4.65	85.31±3.07

Table 3. Correlation coefficient (r^2) and constant (k) for drug to polymer ratio 1: 1, 1:1.5 and 1: 3 after fitting of dissolution results to the different kinetic models.

	Kinetic models							
	Zero order		First order		Higuchi model		Peppas model	
Formulation code	r^2	k	r^2	k	r^2	k	r^2	n
A1	0.9294	6.9138	0.6299	0.0705	0.9943	27.313	0.9873	0.466
A2	0.9633	6.7828	0.7642	0.0714	0.9858	26.206	0.9854	0.5241
A3	0.957	5.8771	0.843	0.0639	0.9907	22.837	0.9912	0.5197

Figures:

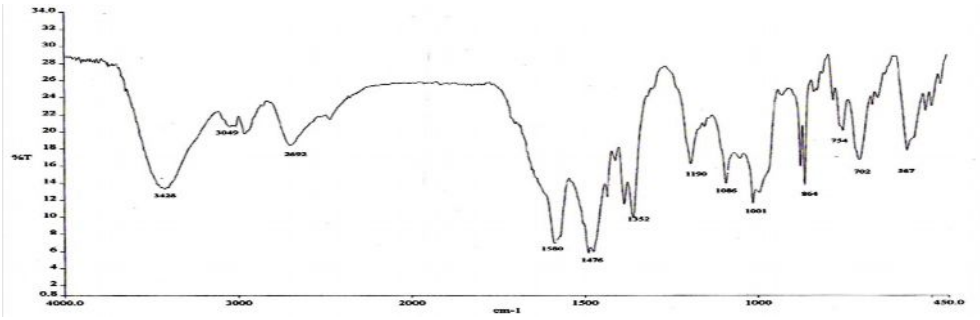

Fig. 1(a)

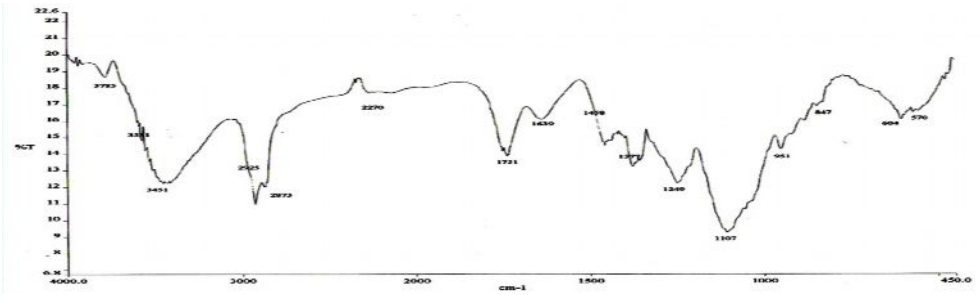

Fig. 1(b)

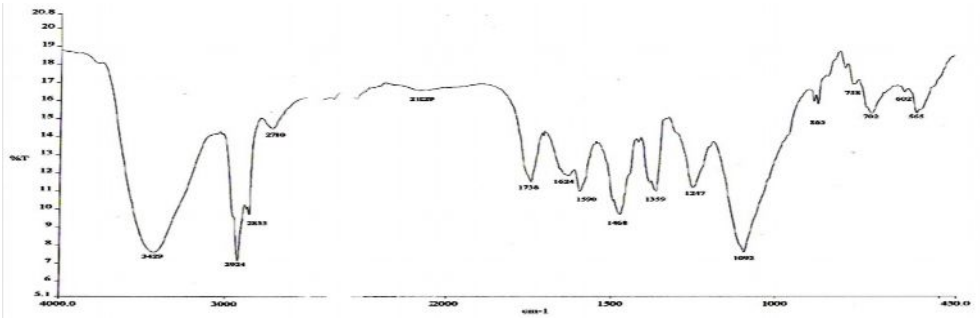

Fig. 1(c)

Figure 1: Fourier transform infrared spectrums of (a) CPM; (b) blank ethyl-cellulose and cellulose acetate combination microspheres; (c) CPM loaded ethyl cellulose and cellulose acetate combination microspheres.

KK

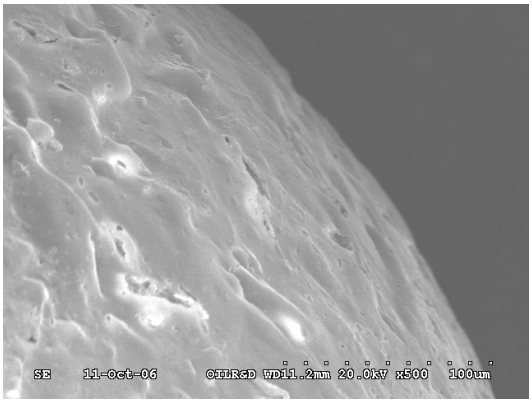

Fig. 2(a)

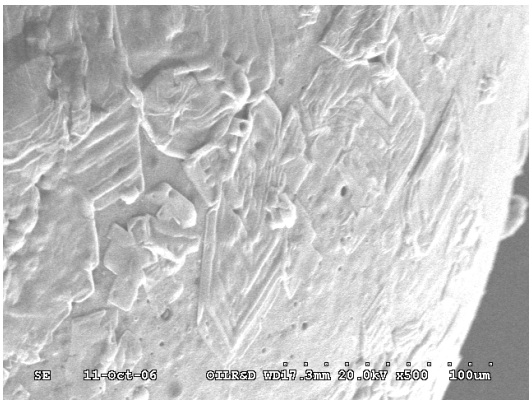

Fig. 2(b)

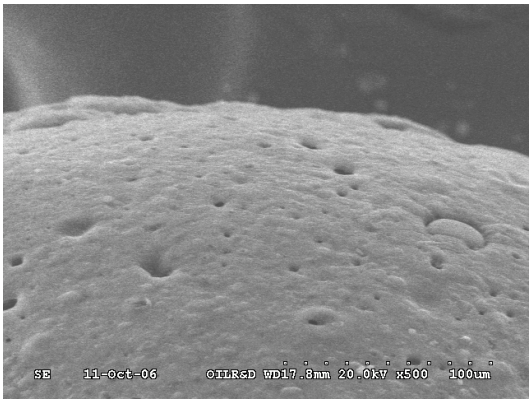

Fig. 2(c)

Figure 2: **SEM photograph (X500) of (a) blank ethyl cellulose and cellulose acetate combination microsphere before dissolution; (b) CPM loaded ethyl cellulose and cellulose acetate combination microsphere before dissolution; (c) CPM loaded cellulose acetate and cellulose acetate combination microsphere after dissolution.**

99999

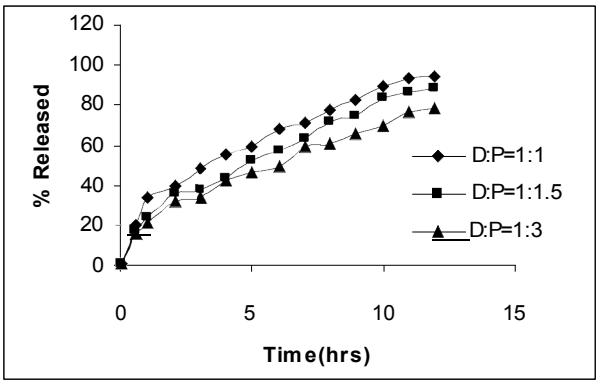

Fig. 3(a)

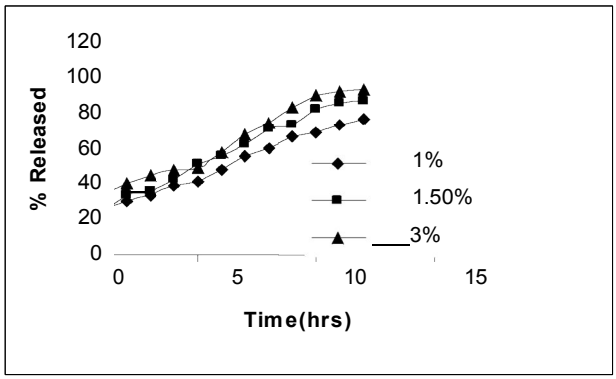

Fig. 3(b)

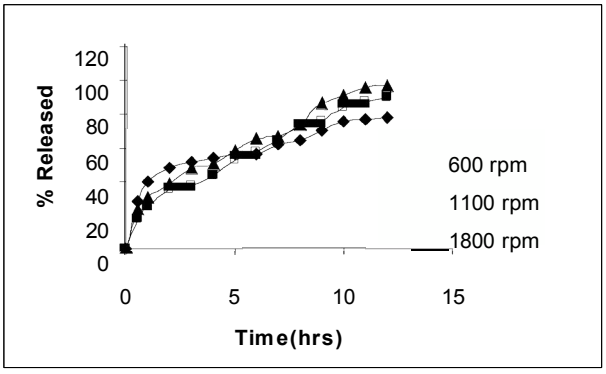

Fig. 3(c)

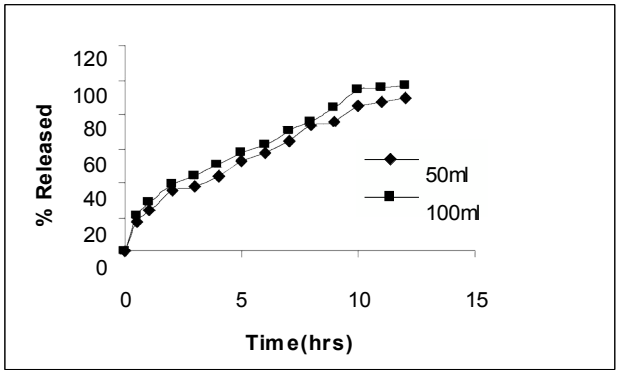

Fig. 3(d)

Figure 3: ***In-vitro*** **drug release profile (a) Effect of drug to polymer ratio; (b) Effect of surfactant concentration; (c) Effect of stirring speed; (d) Effect of volume of external phase fromethyl cellulose and cellulose acetate combination microspheres.**

REFERENCES

1. Tripathi KD. Histamine and antihistaminics. In: Essentials of medical pharmacology. New Delhi: Jaypee brothers; 2004. p. 140-141.
2. Rumore MM. Clinical pharmacokinetics of chlorpheniamine. Drug Intell Clin Pharm 1984; 18:701-707.
3. Paton DM, Webster DR. Clinical pharmacokinetics of H1-receptor antagonist (antihistamine). Clinical Pharmacokinetics 1985; 10: 477-497.
4. Li SP, Kowarshi CR, Fled KM, Grim MW. Recent advances in microencapsulation technology and equipment. Drug Dev Ind Pharm 1988; 14: 353-376.
5. Chowdary KPR, Prasad KSR. A comparative evaluation of permeability and drug release from cellulose acetate microcapsules prepared by two complex emulsion methods. Indian J Pharm Sci 1994; 56: 138 -141.

6. Ramakrishna N, Mishra B. Plasticizer effect and comparative evaluation of cellulose acetate and ethylcellulose-HPMC combination coating as semipermeable membrane for oral osmotic pumps of naproxen sodium. Drug Dev Ind Pharm 2002; 28: 403-412.
7. Jones DS, Pearce KJ. An investigation of the effect of some process variables on the microencapsulation of propanolol hydrochloride by solvent evaporation method. Int J Pharm 1995; 118; 119-205.
8. Yang CY, Tsay SY, Tsiang CC. An enhanced process for encapsulating aspirin in ethylcellulose microcapsules by solvent evaporation in an o/o emulsion. Journal of Microencapsulation 1999; 17(3): 269-277.
9. Korsmeyer RW, Gurny R, Doelker EM, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. Int J Pharm 1983; 15: 25-35.
10. Amperiadou A, Georgarakis M. Controlled release salbutamol sulphate microcapsules prepared by emulsion solvent evaporation technique and study on release-affected parameters. Int J Pharm 1995; 115: 1-8.
11. Pongpaibul Y, Sayed HAM, Whitworth CW. Effect of process variables on drug release from microparticles containing a drug- resin complex. Drug Dev Ind Pharm 1989; 15: 2547-2558.
12. Arshady R, Albumin microsphere and microcapsule; methology of manufacturing techniques. J Controlled Release 1990; 14: 111-131.
13. Lee JH, Park TG, and Choi HK. Effect of formulation and processing variables on the characteristics of micro sphere for water-soluble drugs prepared by w/o/o double emulsion solvent diffusion method. Int J Pharm 2002; 196: 75-78.
14. Babay D, Holfman A, Benita S. Design and release kinetics pattern evaluation of indomethacin microsphere intended for oral administration. Biomaterials 1988; 9: 482-488.
15. Kawashima Y, Niwa T, Handa T, Takenchi H, Iwamoto T, Itoh Y. Preparation of prolonged- release spherical micromatrix of ibuprofen with acrylic-polymer by emulsion solvent diffusion method for improving bioavailability. Chemical Pharmaceutical Bulletin 1989; 37: 425-429.
16. Patrick BOD, Iwata M, McGinity JW. Properties of multiphase microspheres of poly (D, L-lactic-co-glycolic acid) prepared by potentiometric dispersion technique. Journal of Microencapsulation 1995; 12: 155-163.
17. Lee J, Park TG, Choi H. Effect of formulation and processing variables on the characteristics of microspheres for water soluble drugs prepared by w/o/o double emulsion solvent-diffusion method. Int J Pharm 2000; 196: 75-78.
18. Jalsenjek I, Nicolaidou CF, Nixon JR. The in-vitro dissolution of phenobarbitone sodium from ethyl cellulose microspheres. J Pharm Pharmacol 1976; 28: 912-914.
19. Mortada SM, Preparation of ethylcellulose microcapsules using the complex emulsion method. Pharmazie 1982; 37: 427-429.
20. Kim CK, Kim MJ, Oh KH. Preparation and evaluation of sustained release microspheres of terbutaline sulfate. Int J Pharm 1994; 106: 213-219.
21. Mostafa S, Shahbazi M, Shafiee A. Formulation and in-vitro evaluation of eudragitL100® microspheres of piroxicam. Nature 2008; 1544: 1-5.