PREPARATION AND IN VITRO EVALUATION OF CHLORPHENIRAMINE MALEATE LOADED MICROSPHERES

Bhaskar Mazumder1*, Sanjib Bhattacharya2, Bibhash Mohanta1, Sanjay Dey1, Anindya Maity3

1Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786004, Assam, India
2Bengal School of Technology, Delhi Road, Sugandha, Hooghly 712102, West Bengal, India
3Department of Pharmaceutical Technology, Jadavpur University, 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 H₁ 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. 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%. 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. 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\textsuperscript{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 \emph{in vitro} release of drug from the formulation.

**EXPERIMENTAL**

**DRUGS AND CHEMICALS**

The following materials were used: chlortalpheniramine 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); Cholrpheniramine maleate I. P. (Gift sample from Kon Text Chemicals Ltd. Kolkata); ethyl cellulose and cellulose acetate by emulsion solvent evaporation method. 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:

\[
\text{Yield(\%)} = \frac{\text{Amount of microspheres}}{\text{Theoretical content}} \times 100
\]

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 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.

\[
\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100
\]

**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\textsuperscript{2} pressure using Perkin Elmer model 883 (Pyris Diamond, USA) between the ranges of 500 to 4000 cm\textsuperscript{-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 \emph{in-vitro} release studies of CPM from the combination of ethylcellulose and cellulose acetate microspheres in 900 ml phosphate buffer pH 7.4 at 37\textsuperscript{0}C±10C 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 \mu 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:

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

Where \(C_n\) and \(C_{n-1}\) is the corrected concentration of \(n\)\textsuperscript{th} and \((n-1)\textsuperscript{th}\) 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 \(n\textsuperscript{th}\) and \((n-1)\textsuperscript{th}\) 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:\[ M_t/M_\infty = k_t^n \]

Where \( M_t \) is the amount of drug released at time \( t \) and \( M_\infty \) is the amount of drug released at time \( \infty \), thus the \( M_t/M_\infty \) is the fraction of drug released at time \( t \). \( k_0 \) 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 increased in the volume of continuous phase (as shown in Table 2). But percentage yield and percentage entrapment efficiency increased 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 STIRRING SPEED**

Effect of stirring speed on mean particle size (\( \mu m \)), 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 CONTINUOUS PHASE**

Mean particle size (\( \mu m \)) 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 1590 cm\(^{-1}\), 1468 cm\(^{-1}\), 1359 cm\(^{-1}\), 865 cm\(^{-1}\) and 702 cm\(^{-1}\) which are for C=C stretching, C-H stretching, 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. 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.

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 to 100 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.

**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 yield 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 acetate combination (1:1 ratio) microspheres.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Ethyl-cellulose (mg)</th>
<th>Cellulose acetate (mg)</th>
<th>CPM (mg)</th>
<th>Light liquid paraffin (ml)</th>
<th>Tween 80(%)</th>
<th>Stirring Speed (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>450</td>
<td>450</td>
<td>900</td>
<td>50</td>
<td>1.5</td>
<td>1200</td>
</tr>
<tr>
<td>A2</td>
<td>450</td>
<td>450</td>
<td>600</td>
<td>50</td>
<td>1.5</td>
<td>1200</td>
</tr>
<tr>
<td>A3</td>
<td>450</td>
<td>450</td>
<td>600</td>
<td>50</td>
<td>1.5</td>
<td>1200</td>
</tr>
<tr>
<td>A4</td>
<td>450</td>
<td>450</td>
<td>600</td>
<td>50</td>
<td>1.0</td>
<td>1200</td>
</tr>
<tr>
<td>A5</td>
<td>450</td>
<td>450</td>
<td>600</td>
<td>50</td>
<td>2.0</td>
<td>1200</td>
</tr>
<tr>
<td>A6</td>
<td>450</td>
<td>450</td>
<td>600</td>
<td>50</td>
<td>1.5</td>
<td>600</td>
</tr>
<tr>
<td>A7</td>
<td>450</td>
<td>450</td>
<td>600</td>
<td>50</td>
<td>1.5</td>
<td>1800</td>
</tr>
<tr>
<td>A8</td>
<td>450</td>
<td>450</td>
<td>600</td>
<td>100</td>
<td>1.5</td>
<td>1200</td>
</tr>
</tbody>
</table>

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

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

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.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Kinetic models</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi model</th>
<th>Peppas model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$r^2$</td>
<td>k</td>
<td>$r^2$</td>
<td>k</td>
</tr>
<tr>
<td>A1</td>
<td></td>
<td>0.9294</td>
<td>6.9138</td>
<td>0.6299</td>
<td>0.0705</td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td>0.9633</td>
<td>6.7828</td>
<td>0.7642</td>
<td>0.0714</td>
</tr>
<tr>
<td>A3</td>
<td></td>
<td>0.957</td>
<td>5.8771</td>
<td>0.843</td>
<td>0.0639</td>
</tr>
</tbody>
</table>
Figures:

**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.
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.
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 from ethyl cellulose and cellulose acetate combination microspheres.

REFERENCES


