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RESEARCH ARTICLE

FORMULATION AND *IN VITRO* EVALUATION OF METOPROLOL TARTRATE MICROSPHERES

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The aim of this study was to prepare and characterize microspheres of a highly water soluble drug metoprolol tartrate by *w/o/o* double emulsion solvent diffusion method using ethyl cellulose polymer. A mixed solvent system consisting of acetonitrile and dichloromethane in a 1:1 ratio, and light liquid paraffin as a primary and secondary oil phase along with span 80 as a secondary surfactant for establishing the external oil phase were employed. The microspheres obtained were found to be spherical and free flowing in nature. The prepared microspheres were characterized by particle size analysis, entrapment efficiency, scanning electron microscopy and *in vitro* drug release studies. It was found that mean particle size and entrapment efficiency of the microspheres were enhanced with increasing drug-polymer ratio but reduced with increasing stirring speed, processing medium and surfactant concentration. SEM studies confirmed that the formulated microspheres were spherical and uniform in shape, porous and non aggregating in nature. Among all formulations, F5 (Drug:EC::1:1) was found to be the best as it released 91.40% of the drug at the end of 8 h following Higuchi matrix model (R^2 =0.987).

Key words: Metoptolol tartrate, Microspheres, w/o/o method, Controlled drug delivery.

INTRODUCTION

In the last few decades, several new techniques for delivery of drugs called controlled drug delivery systems have been developed. These delivery systems are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and targeting the delivery of drug to a specific site. Controlled release drug delivery systems offer both convenience and therapeutic benefits to the patients (Singh, 2000; Bhalerao *et al* 2001; Brayden, 2003). Though numerous routes and dosage forms have been explored in the development of controlled release drug delivery systems, oral controlled release systems have long been and still the most exploited route due to its flexibility in dosage form design. For drugs that are considered to be unsafe or which are rapidly absorbed, have short half-life and well absorbed along the gastrointestinal tract. Controlled release systems provide a useful means of presenting a safer dosage forms with prolonging drug action following a single oral dose (Atyabi *et al* 2004). The ideal release mechanism for controlled release systems should be at a constant rate (zero order). Controlled drug delivery may be achieved through the use of polymers as in the case of microcapsules, transdermal patches, hydrogels, matrix tablets or without polymer as in the case of liposome drug delivery systems in which the drug is encapsulated in vesicles formed by phospholipids, and erythrocytes, which may be impregnated with the drug using hypotonic saline solution and then to be administered

parenterally (Guyot and Fawaz, 1998). Rationale factors must be considered while designing controlled release systems avoiding irrational and inappropriate formulations. The release kinetics of drug from microspheres can be altered by modifying the ethyl cellulose polymer in order to achieve coating or polymer concentration. So, in present work an attempt was made to optimize the ethyl cellulose matrix system which can effectively control the drug release of highly water soluble drug metoprolol tartrate.

MATERIALS AND METHODS

Materials

Metoprolol tartrate was obtained as a gift sample from Ajanta Pharma Ltd. (Mumbai). Ethyl cellulose and dichloromethane was obtained from Loba Chemie, Mumbai. Light Liquid Paraffin and *n*-hexane were obtained from Ranbaxy Fine Chemicals, Delhi. Acetonitrile and span 80 were obtained from Merck India Ltd, Mumbai. All other chemicals used were of analytical grade. Double distilled water was used throughout the studies.

Methods

Characterization of the drug

The received gift sample of metoprolol tartrate was identified by melting point, infrared spectroscopy (IR), ultraviolet spectroscopy (UV).

Melting point

Melting point of the drug sample was determined by melting point apparatus and compared with the melting point of reference sample.

Ultraviolet spectroscopy

The spectrophotometric analysis of the drug was carried out in 0.1 M hydrochloric acid (pH 1.2) and phosphate buffer pH 7.4 in the range of 200 to 400 nm using the UV-spectrophotometer (Shimadzu 1700, Japan).

Preparation of the calibration curve

10 mg of the drug (metoprolol tartrate) was dissolved in 10 ml of 0.1 M HCl and phosphate buffer pH 7.4 separately. One ml of the solution was withdrawn and diluted to 50 ml with 0.1 M HCl and phosphate buffer pH 7.4. The drug concentration in the stock solution was 20 μg/ml. The solution was diluted to make the concentration 2-20 μg/ml. Absorbance was measured spectrophotometrically at 274 nm using UV-spectrophotometer.

FT-IR analysis

Infrared spectra of metoprolol tartrate were recorded using IR spectrophotometer (Perkin Elmer-883) between ranges of 400-4000 $cm^{-1.}$ It was then compared with that of reference spectra. Drug-polymer interactions were studied by FT-IR spectroscopy. The spectra were recorded for pure drug by FTIR spectrophotometer (Jasco 4200). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400-4000 cm^{-1}.

Preparation of microspheres

Water-in-oil-in-oil (w/o/o) emulsion method:

The microspheres were prepared by water-in-oil-in-oil (*w/o/o*) double emulsion-solvent-diffusion method, using ethylcellulose. The preparation of microspheres was carried out by emulsifying an aqueous solution into a solution of drug and polymer in a mixed solvent system consisting of acetonitrile and dichloromethane in 1:1 ratio, followed by emulsification of the primary emulsion (*w/o*) into an external oil phase to form a water-in-oil-in-oil (*w/o/o*) prepared by adding 2 ml of water to the drug-polymer solution while using a mechanical stirrer (Remi motors, Mumbai) at 500 rpm for 5 min. This *w/o* primary emulsion was slowly added to 50 ml of light liquid paraffin, the secondary oil phase containing 0.5% span 80 were used as a surfactant. The whole system was then stirred for about 3 h. After stirring process is over the liquid paraffin (light) was decanted off and the microspheres formed were collected and washed with *n*-hexane to completely remove the remaining oil and air-dried at room temperature for 12 h and collected for further studies. **Figure 1** illustrates the scheme of preparation method. **Table 1** represents the formulation plan of microspheres.

Evaluation of microspheres

Particle size analysis:

The particle size of the microspheres was determined by using optical microscopy method. The prepared microspheres were mounted in light liquid paraffin, and the diameters of 100 particles were measured by means of an optical microscope fitted with a stage and an ocular micrometer. The mean diameter was calculated

by measuring the number of division of the ocular micrometer covering the microspheres.

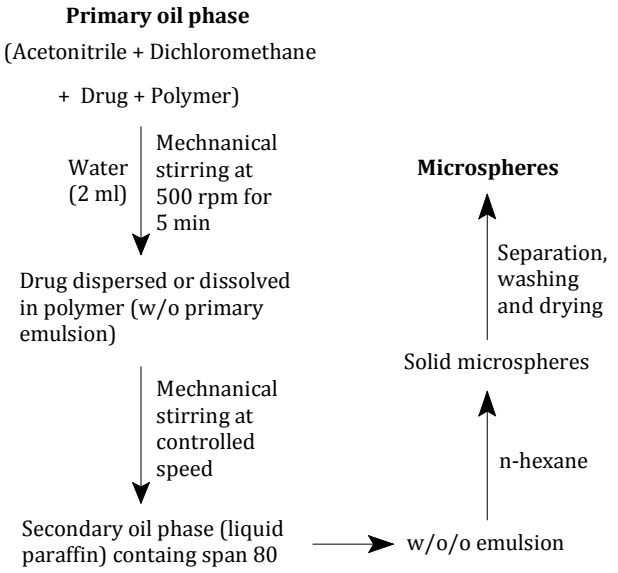


Figure 1. *w/o/o* type emulsion solvent evaporation method

Mean particle size = Σn.d / Σn

n is the number of counted particles and d the mean diameter at each measuring range.

Drug entrapment efficiency:
About 50 mg of accurately weighed drug-loaded microspheres were crushed in a glass mortar and pestle, and the powdered microspheres were suspended in 50 ml in phosphate buffer (pH 7.4) the resulting mixture was kept shaking on mechanical shaker for 4 h. Then, after the solution was filtered (Whatman filter paper no. 1), 1 ml of this was appropriately diluted to 25 ml using phosphate buffer (pH 7.4) and analyzed spectrophotometrically at 274 nm using the UV-spectrophotometer (n=3). The drug entrapment efficiency was calculated using the following formula:

Entrapment efficiency = (Practical drug content / Theoretical drug content) × 100

Table 1. Formulation plan of metoprolol tartrate loaded microspheres

Formulation	Drug: polymer ratio	Volume of processing medium (ml)	Surfactant (span 80 %)	Stirring speed (rpm)
F1	1:1	50	0.5	500
F2	1:2	50	0.5	500
F3	1:3	50	0.5	500
F4	1:1	50	0.5	1000
F5	1:1	50	0.5	1500
F6	1:1	100	0.5	500
F7	1:1	200	0.5	500
F8	1:1	50	1.0	500
F9	1:1	50	2.0	500

Scanning electron microscopy analysis:
The surface topography of the microspheres were examined by scanning electron microscopy (Hitachi, S-3600) prior to examination, samples were gold sputter-coated to render them electrically conductive.

In vitro drug release study:
The drug release study was performed using USP dissolution test apparatus paddle type (VDA-6D USP, Mumbai) at 37 ± 0.5°C and at 100 rpm using 900 ml of phosphate buffer pH 7.4, as dissolution medium for 8 h. Microspheres equivalent to 10 mg of metoprolol tartrate were used for the test. Five ml of sample solution was withdrawn at predetermined time intervals, filtered, diluted suitably, and analyzed spectrophotometrically at 274 nm. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample (n=3).

Release kinetics:
Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism of drug release from ethylcellulose microspheres .The kinetic models used were zero order model, first order model, higuchi model and korsemeyer-papas model.

Zero order kinetics
For this model, a graph o percent drug released versus time will be linear. The following relation can in a simple way express this model.

$Q_t = Q_0 + K_0 t$

where Q_t is the amount of drug released in time t, Q_0 is the initial amount of drug in the solution (most times $Q_0 = 0$) and K_0 is the zero order release rate constant.

First order kinetics
This model can be expressed as:

$$\ln Q_t = \ln Q_0 - K_1 t$$

where Q_t is the amount of drug released at time 't', Q_0 is the initial amount of drug in the solution and K_1 is the first order release rate constant.

Higuchi model
Higuchi model may be expressed as:

$$Q = K_h t^{1/2}$$

where Q is the amount of drug released in time 't' per unit area, K_h is the Higuchi dissociation constant. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms.

Korsemeyer – Peppas model
A simple, semi empirical model, relating exponentially the drug release to the elapsed time (t) was developed (Korsemeyer et al 1983), which can be described as:

$$M_t / M_\alpha = K t^n$$

where M_t / M_α is the fraction of drug released at time't' and 'k' is the rate constant and 'n' is the release exponent. For the determination of exponent n the portions of the release curve where $M_t / M_\alpha < 0.6$ should only be used. This model can be used to analyze the release of pharmaceutical dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved.

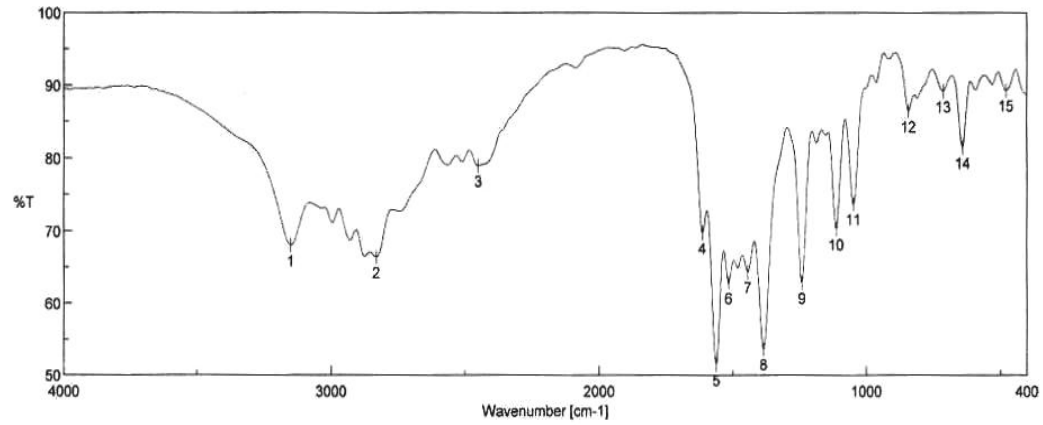
RESULT AND DISCUSSION

The received sample of metoprolol tartrate was identified by infrared spectroscopy (IR), Ultraviolet spectroscopy (UV), and melting point. Melting point of the drug sample was found to be 121.8°C and it matched with the literature confirming the identity of the received drug sample. The λ_{max} useful for quantitative analysis was found to be 274 nm when scanned between 200-400 nm. The calibration curve was found to be linear in the concentration range 2-20 μg/ml with r^2 value of 0.998 in case of both HCl pH 1.2 and phosphate buffer pH 7.4.

The FT-IR spectrum of metoprolol tartrate showed sharp band at 3300 cm^{-1} for –OH stretching vibration. The absorption at about 3151 cm^{-1} might be due to –NH stretching vibration. The presence of aliphatic –CH may be confirmed by the stretching vibration at 2960-2850 cm^{-1}. The appearance of absorption peak at 3030 cm^{-1} was indicative of aromatic –CH stretching vibration. An aromatic system was probably present because of low intensity of $-CH_2$ vibration. This was confirmed by the absorption at 1613, 1534 and 842-714 cm^{-1}. The absorption at 1613 cm^{-1} was very strong for an aromatic compound but it can be explained as being enhanced by the presence of a polar substituent. The absorption at 1240 cm^{-1} was certainly in the C–O stretching region of aromatic ether which absorbs strongly in this region. Some confirmation for the group was given by band at 1049 cm^{-1}. Peak at 1180 cm^{-1} indicated the presence of isopropyl group. Aliphatic ether and secondary alcohol absorption were most likely account for the band at 1112 cm^{-1}. Aromatic absorption at 842 and 714 cm^{-1} indicated 1, 4-disubstitution of benzene ring. So, spectral assignments for major absorption were consistent with the structure of metoprolol tartrate. The spectral similarity confirmed the identity of the sample. A comparison was made between the peak positions of pure drug and its physical mixture with ethyl cellulose (1:1). It was observed that major peak positions for the drug in physical mixture was totally in compliance with that of the pure drug indicating the absence of drug polymer interactions. (**Figure 2, 3**).

Morphology of various batches of microspheres prepared was found to be discrete and more or less spherical in shape. The mean particle size of the formulations were between 310 ± 2.50 μm to 565 ± 1.68 μm (**Table 3**). The mean diameter of the prepared microspheres was marginally increased with an increase in drug to polymer ratio (F1, F2, F3) (Das and Rao, 2006).

The entrapment efficiency increased progressively with the increasing polymer concentration (F1, F2, F3) (**Table 3**). Increase in polymer concentration (1:1, 1:2, 1:3) resulted in the formation of larger microspheres entrapping greater amount of drug. (Pachuau *et al* 2008). The mean particle size and entrapment efficiency was increased from F1(435), F2(510), F3(565) with increasing polymer concentration (1:1,1:2,1:3), this may be due to increasing



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3151.11	67.9675	2	2830.99	66.3528	3	2449.15	78.6781
4	1613.16	69.6978	5	1562.06	51.4137	6	1514.81	62.7584
7	1442.49	64.2768	8	1382.71	53.5912	9	1240	62.7732
10	1112.73	70.231	11	1049.09	73.6587	12	842.74	86.3698
13	714.497	89.1282	14	642.179	81.4078	15	478.26	89.2715

Figure 2. FTIR spectrum of metoprolol tartrate

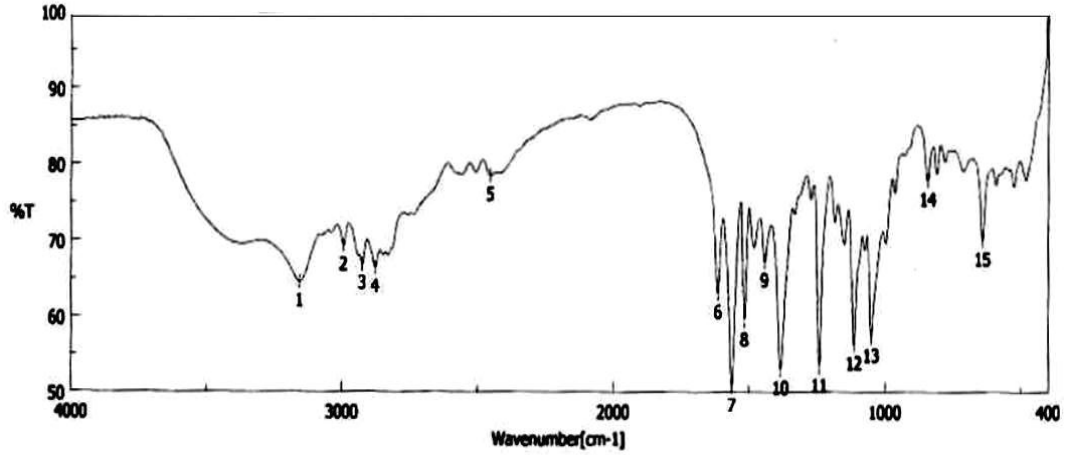


Figure 3. FTIR spectrum of drug and polymer (1:1) physical mixture

Table 2. Mean particle size distribution of metoprolol tartrate loaded microspheres

Formulation code	Mean particle size (m) (Mean ± SD, n=3)
F1	435.94 ± 1.54
F2	510.12 ± 2.63
F3	565.86 ± 1.68
F4	375.21 ± 6.50
F5	310.08 ± 2.50
F6	425.48 ± 3.08
F7	378.00 ± 4.78
F8	418.15 ± 2.21
F9	398.14 ± 1.33

polymer conc. that produced significant increase in the viscosity, leading to an increase of emulsion droplet size and a larger microspheres size (Mazumder and Bhattacharya, 2009).

Table 3. Entrapment efficiency of metoprolol tartrate loaded microspheres

Formulation	Entrapment efficiency (% *w/w*) (mean±SD, n=3)
F1	76.12 ± 3.14
F2	84.40 ± 3.16
F3	90.42 ± 2.77
F4	71.28 ± 4.87
F5	62.00 ± 2.89
F6	72.00 ± 4.25
F7	67.34 ± 4.11
F8	69.46 ± 1.04
F9	67.04 ± 3.03

The effect of stirring speed 500 rpm (F1), 1000 rpm (F4), 1500 rpm (F5) increased the size of particle decreases from 435, 375, 310 μm respectively. As increase in high shear results in decrease in size of microdroplets of the emulsion, resulting formation of smaller size microparticles (**Figure 4**). A more uniform particle size was seen at 1000 rpm compared to 500 and 1500 rpm. As the volume of the processing medium was increased from 50ml to 100 ml and 200 ml, the particle size and entrapment efficiency significantly decreased from 435, 425, 378 μm and 76%, 72%, 67%, respectively (**Figure 5**). When the volume of the processing medium was increased, the emulsion droplets can be moved freely in the medium, and they had very less chance to collide with each other, thereby yielding small and uniform microspheres. (Saravanan *et al* 2003)

The concentration of surfactant/dispersing agents in light liquid paraffin also affects the particle size. For this types of surfactants used, the higher concentration of surfactant resulted in production of smaller particle size for span 80. This is due to better stabilization of internal droplets with increase of surfactant concentration preventing coalescence. As the volume of surfactant increased from F1 (0.5%), F8(1%), F9(2%) the particle size decreased from 435, 418, 398 μm (**Figure 6**) (Sarisuta *et al* 1999).

For the exploration of surface morphology, the scanning electron microscopy (SEM) was performed for prepared formulations and used throughout the study while evaluating the effect of other variables over product characteristics. The prepared microspheres were observed under 250x different magnifications to analyze surface morphology (**Figure 7a**). Studies revealed that particles were spherical in shape and had a rough surface due to higher concentration of drug molecules dispersed in the microspheres. Magnification of microspheres to 5000 times (**Figure 7b**) indicated that the surface possessed some crystals deposited on it, which probably was surface associated drug. The size and number of pores determined the rate and extent or release from the microspheres.

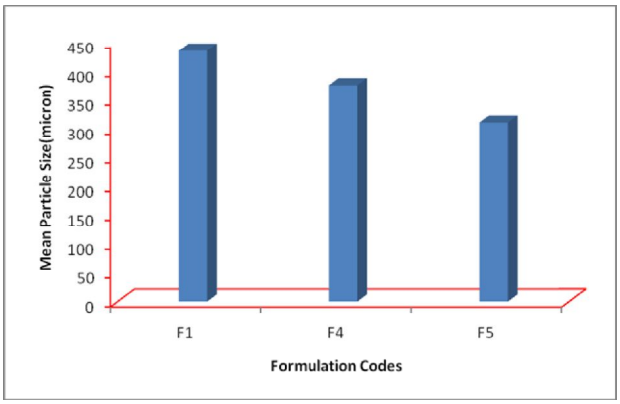


Figure 4. Mean particle size distribution of the metoprolol tartrate loaded microspheres showing effect of stirring speed

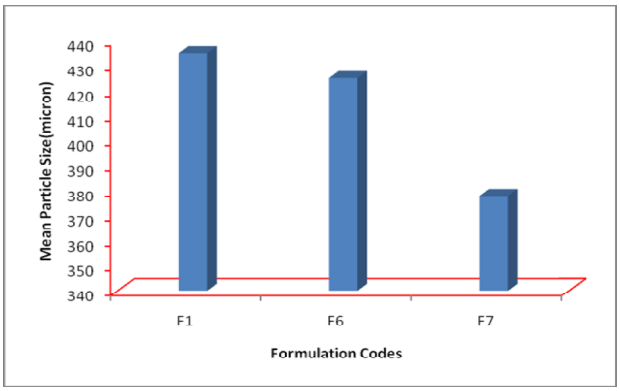


Figure 5. Mean particle size distribution of the metoprolol tartrate loaded microspheres showing effect of volume of processing medium showing effect of surfactant concentration

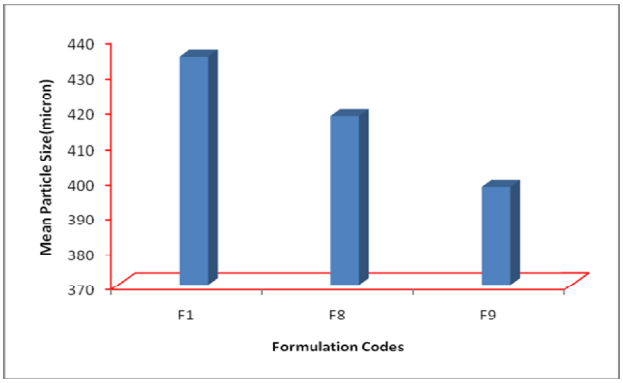


Figure 6. Mean particle size distribution of the metoprolol tartrate loaded microspheres

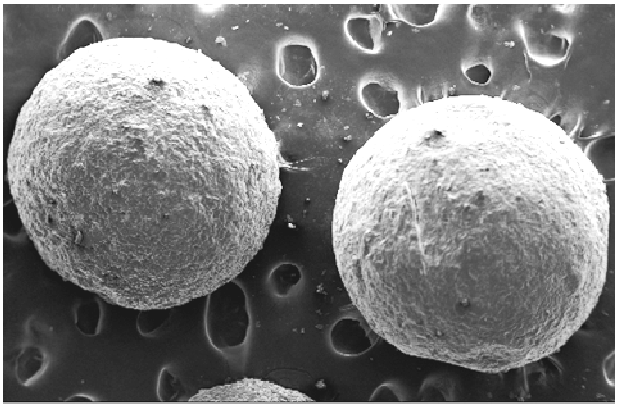


Figure 7a. Ethyl cellulose containing metoprolol tartrate microspheres at 250x

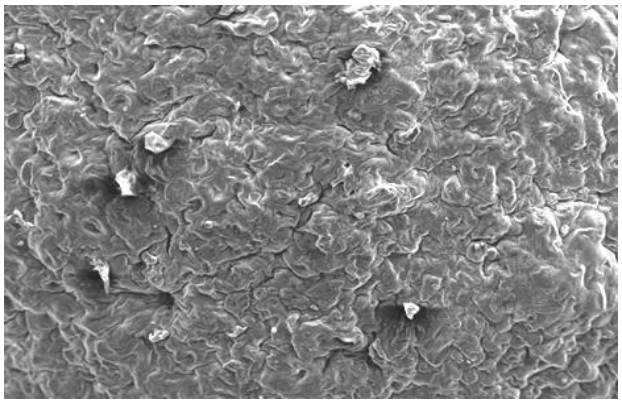


Figure 7b. Ethyl cellulose containing metoprolol tartrate microspheres at 5000x

The results of *in vitro* release studies are depicted in **Table 4-6**. It was evidenced from results that all the formulations retard the drug release as compared to pure drug. Factors such as microsphere size, drug loading, polymer composition and molecular weight govern the drug release from microspheres. The rate of drug release from the microspheres depends on the polymer concentration of the prepared devices, which indicated that the release rate decreased with increasing the amount of polymer. This can be explained by a decreased amount of drug present close to surface. The effect of stirring speed 500 rpm (F1), 1000 rpm (F4), 1500 rpm (F5) on drug release suggested that the rate and extent of drug release was significantly increased with the increase in stirring speed. This might be due to the fact that the drug migration is to be high for low stirrer speed and more amount of drug remained in the microspheres surface but when stirring speed is increased drug migration is less due to collision of emulsion droplets (Rao *et al* 2005). It was also observed that an increased in release rate significantly due to the increased volume of external phase 50 ml (F1), 100 ml (F2), 200 ml (F3). It may be due to the higher migration of drug due to free movement of emulsion droplets with increasing volume of processing medium (Amperiadou and Georgarakis, 1995). The effect of surfactant concentration 0.5% *w/v* (F1), 1.0% *w/v* (F8), 2.0% (F9) revealed that as the concentration of surfactant (span 80) increased, the faster drug release was observed which may be due to the presence of more free drug on the surface of the microspheres with increasing the concentration of span 80 for the secondary emulsification process.

Various kinetic models were employed to investigate drug release mechanism of the formulations using *in vitro* dissolution data. The in vitro release data were fitted to models representing Zero-order, First-order, and Higuchi's square root of time to determine the correlation coefficient, slope, and intercepts values. From the values of the correlation coefficients, the best fitted data can be predicted. The curve fitting of the release data was carried out mainly by regression analysis. In spherical matrices, if $n \leq 0.43$, a Fickian (case-I), $0.43 \leq$ 0:85, a non-Fickian, and $n \geq$ 0:85, a case-II (zero order) drug release mechanism dominates (Rout *et al* 2009).

Table 4. *In vitro* release profile of metoprolol tartrate in phosphate buffer pH 7.4

Time (h)	Percentage drug release
1	97.51
2	98.10
3	98.10

Table 5. Summary of *in vitro* release studies of microspheres in phosphate buffer pH 7.4 (F1 to F5)

Time (h)	Cumulative Percentage of Drug Release				
	F1	F2	F3	F4	F5
0.5	14.36 ±1.04	11.21±0.55	8.34±0.26	22.34±1.26	28.34±1.24
1	19.77±0.83	16.21±0.28	14.15±0.99	31.15±0.99	36.15±0.93
2	26.21±1.55	23.17±0.22	21.44±0.05	38.44±0.05	44.44±0.05
3	33.54±0.48	30.34±1.27	28.77±0.28	46.77±0.28	52.77±0.22
4	41.24±0.08	38.34±0.24	35.43±1.86	53.43±0.86	59.43±0.8
5	48.05±0.07	45.77±0.06	42.09±0.21	61.09±0.21	66.09±0.04
6	55.34±0.11	52.46±0.35	48.13±0.76	68.13±0.76	74.13±0.76
7	66.46±0.21	59.22±1.70	53.23±0.46	74.23±1.46	83.23±1.41
8	68.37±0.22	64.02±0.45	58.40±1.44	79.40±1.44	91.40±1.59

Table 6. Summary of *in vitro* release studies of microspheres in phosphate buffer pH 7.4 (F6 to F9)

Time (h)	Cumulative Percentage of Drug Release			
	F6	F7	F8	F9
0.5	16.34±0.26	19.21±0.55	21.34±1.24	24.36 ±1.04
1	22.15±0.99	26.21±0.28	27.15±0.93	32.77±0.83
2	29.44±0.05	32.17±0.22	34.44±0.05	39.21±1.55
3	37.77±0.28	39.34±1.27	41.77±0.22	47.54±0.48
4	44.43±1.86	46.34±0.24	48.43±0.8	55.24±0.08
5	52.09±0.21	54.77±0.06	57.09±0.04	62.05±0.07
6	59.13±0.76	63.46±0.35	65.13±0.76	71.34±0.11
7	65.23±0.46	69.22±1.70	71.23±1.41	77.46±0.21
8	71.40±1.44	75.02±0.45	77.40±1.59	81.37±0.22

The maximum correlation coefficient has been considered statistical parameter to designate the function with the best fit to the data. The examination of correlation coefficient values indicated that the drug release followed the diffusion control mechanism from the microspheres. The data were supportive to the findings that a water soluble drug incorporated in the swellable matrix device is mainly released by diffusional mechanism (Salomon and Doelker 1980).

A more stringent test was applied to distinguish between the mechanisms of the drug release. Release data were analyzed by Korsmeyer-Peppas model expressed as:

$$Q(t) = a\ t^n$$

where Q(t) is a fraction of drug released after time 't' and 'a' is a coefficient and 'n' is release exponent. The values for 'n' were in the range of 0.329–0.490, further indicative of the drug release following a diffusion controlled mechanism.

CONCLUSION

Metoprolol tartrate microspheres were prepared successfully using double emulsion solvent diffusion method. The particle size, entrapment efficiency and release characteristics of microspheres were found to be affected by drug: polymer ratio, stirring speed, volume of continuous phase and concentration of surfactant. The assessment of release kinetics revealed that the drug release from the microspheres followed Higuchi matrix model with diffusion controlled release mechanism. The study concluded that drug : ethyl cellulose at 1:1 ratio was sufficient to retard the drug release of a highly water-soluble drug metoprolol tartrate which could be used as an approach for formulating the sustained release dosage form.

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