# INFLUENCE OF MICROENVIRONMENTAL pH OF ALGINATE FACILITATED ETHYL CELLULOSE MICROSPHERES ON ENTRAPMENT EFFICIENCY AND RELEASE CHARACTERISTICS OF FLUCONAZOLE

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## ABSTRACT

In this study, fluconazole-loaded ethyl cellulose microspheres were prepared by alginate facilitated water-in-oil-in-water (w/o/w) multiple emulsion solvent evaporation technique and the effect of micro-environmental pH on the properties of microspheres was investigated. The inclusion of aqueous alginate solution of pH 6 into internal aqueous phase of the multiple emulsion led to higher drug entrapment efficiency (72.79%) of the microspheres than those prepared with solutions of either pH 4 or 8. The mean diameter of microspheres ranged from 291 to 331µm and appeared spherical under scanning electron microscope at all pH conditions. Carr's index provided an indication of free-flowing nature of all the microspheres. The drug release rate in either pH 1.2 HCl solution or pH 7.4 phosphate buffer saline (PBS) solution varied depending upon the micro-environmental pH. The release profiles of microspheres having internal pH 4 always ran higher followed by pH 8 and pH 6 in either dissolution fluids. Irrespective of micro-environmental pH, the drug release in pH 1.2 HCl solution was slower than in pH 7.4 phosphate buffer saline (PBS) solution, when compared up to 2 h.  The drug release mechanism was unaffected by the micro-environmental pH of the microspheres and was found to be controlled by Fickian diffusion. Thus, the change of pH of inner aqueous alginate phase could be beneficial for the entrapment of slightly water soluble drugs like fluconazole into ethyl cellulose microspheres.

**Keywords:** multiple emulsion, microspheres, fluconazole, sodium alginate, entrapment efficiency

## INTRODUCTION

Microspheres are defined as homogeneous monolithic particles in the size range of about 0.1 to 1000 µm and are widely used as drug carriers for controlled release.[1]These multi-unit microparticulate systems provide many advantages over single-unit systems because of their small size. Studies have shown that multi-particulates are less dependent on gastric emptying, resulting in less inter- and intra-subject variability in gastrointestinal transit time.[2] These are also better distributed and less likely to cause local irritation.[3] Moreover, the drug dose in a multiple-unit system is divided over the multi-particulates that make up that system. As such, failure of a few units may not be as consequential as failure of a single-unit system.[4] Although a large number techniques such as coacervation phase separation,[5-7] spherical crystallization,[8] spray drying[9] have been developed for the preparation of multi-unit microparticulate dosage forms of drugs but the most common is the solvent evaporation technique.[1] However, the solvent evaporation technique based on the formation of water-in-oil-in-water (w/o/w) multiple emulsion is appropriate for the efficient incorporation of highly water soluble drugs.[10]Accordingly, several investigators have developed microparticulate carrier systems to encapsulate a variety of water soluble drugs using different polymers such as polylactide-co-glycolide,[11] eudragit RS,[12] poly methyl methacrylate,[13] and poly ε-caprolactone.[14] On the other hand, the use of w/o/w double emulsion solvent evaporation technique resulted in poor encapsulation efficiency (5-17%) of a slightly water soluble drug zidovudine into poly (lactide/glycolide) microparticles.[15] Recently, the complexation of melarsoprol with methyl β-cyclodextrin followed by the use of water-in-oil-in-water solvent evaporation method to prepare the poly (ε-caprolactone) microparticles has been reported[16] and such modification also led to poor incorporation of the poorly water soluble drug (2.89±0.20 µg mg$^{-1}$). Limited research reports are available regarding modification of the w/o/w multiple emulsion solvent evaporation technique to improve the properties of the drug-loaded microspheres, especially the entrapment efficiency of poorly water soluble drugs. Ethyl cellulose is a non-biodegradable and biocompatible polymer and has been extensively studied as an encapsulating material in the solvent evaporation techniques for the controlled release of drugs.[17-21]

However, the effectiveness of this hydrophobic polymer to prepare drug-loaded microspheres by w/o/w emulsion solvent evaporation method has been little studied. Recent studies have shown that the use of hydrophilic polymer, gelatin in the internal aqueous phase improved the drug entrapment efficiency of the polymeric microparticles.[22,23] In the present study we used a nontoxic, biocompatible, pH-sensitive hydrophilic polymer, sodium alginate in the inner aqueous phase of the multiple emulsion to improve the entrapment efficiency of a slightly water soluble drug into ethyl cellulose microspheres and evaluated the pH effect of inner alginate phase on the properties of microspheres such as drug entrapment efficiency, particle size, morphological characteristics, flowability, swelling behaviors and *in vitro* drug release characteristics in different dissolution media. A slightly water soluble drug fluconazole, widely used for the treatment of fungal infections of the vagina, mouth, throat and esophagus was tested as a model drug in this investigation.

## MATERIALS AND METHODS

### Materials

Fluconazole was received as a gift sample from East India Pharmaceutical Works Ltd., Kolkata, India. Ethyl cellulose (18-22 cps viscosity grade), sodium alginate (0.1%w/v aqueous solution exhibits a viscosity of 1.15 cp at 25°C), Span 80 were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Dichloromethane and Tween 80 were supplied by Merck Ltd., Mumbai, India. All other regents obtained commercially were of analytical grade and used as received.

### Preparation of fluconazole-loaded ethyl cellulose microspheres

Fluconazole-loaded ethyl cellulose microspheres were prepared by a modified w/o/w multiple emulsion solvent evaporation method. Eight milliliters of an aqueous alginate dispersion (pH 4, 6 and 8) containing 5.71% (w/w) fluconazole was first emulsified with 25 ml of 2% (w/v) solution of ethyl cellulose containing 0.5% Span 80 in dichloromethane using a homogenizer (Model RQ-127A, Remi Motors Ltd., Mumbai, India) at 1200 rpm for 5 min. The resulting water-in-oil (w/o) emulsion was then transferred into 100 ml water containing 0.6% (w/v) Tween 80 with continuous mechanical

stirring (Type-BL-433, Bio-Lab Instrument Mfg.Co., Mumbai, India) at room temperature to form w/o/w type multiple emulsion. The stirring speed was set at 700 rpm and was continued for a period of 1.5 h to allow evaporation of the organic solvent. Upon solvent evaporation, the polymer precipitated and the core of the microspheres solidified. The microspheres were then filtered off with the help of muslin cloth, washed with cold doubled distilled water (3×100 ml) and dried at 40°C for 24 h in an oven. The same procedure was adopted for the preparation of blank microspheres (without drug), and drug-loaded microspheres (without alginate).

**Yield of microspheres**

Microspheres recovered at the end of preparation were weighed and the yield was calculated as a percentage of the total amounts of polymers and drug added during the preparation of microspheres.

**Determination of drug entrapment efficiency**

Accurately weighed, 10 mg of fluconazole-loaded microspheres was dissolved in 2 ml dichloromethane; 30 ml of pH 7.4 phosphate buffer saline (PBS) solution was added, and stirred for 30 min with a magnetic stirrer. The mixture was heated at 50-55°C for 45 min in a thermostatic water bath to remove dichloromethane. After that the volume was adjusted to 50 ml with fresh PBS solution (pH 7.4) heated at 50-55°C. The solution was cooled, filtered, and an aliquot, after suitable dilution was analyzed spectrophotometrically (UV1, Thermo Spectronic, Great Britain) at 261 nm. Each experiment was carried out in triplicate. Reliability of the method was judged by conducting recovery analyses using known amounts of fluconazole with or without polymer, and recovery averaged 98.17 ± 0.61%.

**Measurement of viscosity**

The viscosity of the inner aqueous alginate phase was determined by a programmable Brookfield viscometer (Model DV-II+ Pro, Brookfield Engineering Labs., Inc. Middleboro, MA, USA) at 28°C. The spindle (spindle no. CPE 41) was rotated at 1 rpm.

**Partition co-efficiency**

Accurately weighed, 40 mg of fluconazole was dispersed in 10 ml of aqueous alginate phase of various pH or aqueous phase without alginate and then, 25 ml of organic polymer phase was added to the aqueous phase. The immiscible phases were held in contact with each other in a separating funnel and were shaken vigorously for 30 min to reach equilibrium at room temperature. The funnel was allowed to stand for 10 min. Two milliliters of organic phase was collected, evaporated to dryness and then it was dissolved in 50 ml of water. The sample was analyzed spectrophotometrically at $\lambda_{max}$ 261nm. The partition co-efficiency was determined by dividing the equilibrium concentration of drug in organic phase and aqueous phase. Each experiment was carried out in triplicate.

**Particle size analysis**

Particle size analysis of the microspheres was done by sieving method. Several British standard sieves ranging 16 meshes to 120 meshes were arranged in a nest with the coarsest at the top. A weighed amount of samples were placed on the top and the sieve set was shaken with a mechanical sieve shaker (Geologists' Syndicate Pvt. Ltd., Kolkata, India) for 10 min. The samples retained on each sieve were collected and weighed. The arithmetic mean diameter was calculated following the method reported earlier,[24] and the percentage frequency was plotted as a function of mean particle size.

**Scanning electron microscopy**

The shape and surface morphology of microspheres were investigated using scanning electron microscope (Jeol, JSM-5200, Japan). Prior to examinations samples were mounted onto stubs using double-sided dried carbon tape and vacuum coated with gold-palladium film (thickness 2nm) to render them electrically conductive using sputter coater (Edward S-150, UK).

**Flow properties of the microspheres**

The flow properties of microspheres were investigated by measuring Carr's index (C). The bulk and tapped densities were measured in a 100 ml graduated measuring cylinder. The sample contained in the measuring cylinder was placed in a USP-I tap density test apparatus (Model: VTAP/ MATIC-II, Mumbai, India) and tapped mechanically by means of constant velocity rotating cam. Following initial taps of 500, additional 750 taps were applied and the tapped volumes were noted. From the initial bulk volume and final tapped volume, the respective densities were calculated. Carr's index was determined by the following formula:

Carr's index (C) = [(tapped density-bulk density) / tapped density] × 100

A Carr's compressibility index greater than 25% was considered to be an indication of poor flowability, and below 15%, of good flowability.

**In vitro drug release study**

In vitro release of fluconazole from the microspheres was carried out in enzyme free, gastric, and intestinal fluids using a digital USP type-II dissolution rate test apparatus (VDA-6D, Veego Instrument Corporation, Mumbai, India). About 50 mg of dried microspheres, accurately weighed, were suspended in 500 ml of either 0.1N HCl solution (pH 1.2) or PBS solution (pH 7.4). The paddle was rotated at 50 rpm and the temperature was set at 37 ± 0.5°C. At predetermined times 5 ml sample was withdrawn and replenished with fresh buffer solution. The aliquots were analyzed using a double beam spectrophotometer (UV1, Thermo Spectronic, Great Britain) at 261nm. Cumulative percentage of fluconazole released was plotted as a function of time. Dissolution efficiency (DE%) was calculated from the percentage between the area under the curve at time t and the area of the rectangle defined by 100% dissolution at the same time t.[25] The area under the % drug release vs. time curve was calculated using NCSS 2007 software.

**Swelling study**

Accurately weighed, 10 mg of dried microspheres were incubated in HCl solution (pH 1.2) and phosphate buffer saline solution (pH 7.4) for 2 h. At predetermined times; the microspheres were removed from the swelling media, blotted with a piece of tissue paper to absorb excess surface water and weighed (Metler Toledo, AB 204-S, Switzerland). The swelling ratios (Q) of the microspheres were calculated by the following equation: $Q = (w_2 - w_1)/w_1$, where $w_1$ is the weight of the dried sample and $w_2$ is the weight of the swollen sample. The swelling ratio was plotted as a function of time.

**Drug release kinetics**

To study release kinetics, the data obtained from *in vitro* drug release studies were plotted in various kinetic models: zero order as cumulative percentage of drug released vs time, first order as log cumulative percentage of drug remaining vs time, and Higuchi's model as cumulative percentage of drug released vs square root of time. The rate constants were calculated from the slope of the respective plots. The rate constants for zero-order ($K_o$), first-order ($K_1$) and Higuchi kinetics ($K_h$) have been presented in Table 2 with respective correlation coefficients ($R^2$).

**Drug release mechanism**

The mechanism of *in vitro* drug release from the microspheres was determined from the values of diffusional exponent (n) obtained by modeling the first 60% of the drug release into Korsmeyer-Peppas equation [26] $M_t / M_\infty = k\, t^n$, where $M_t / M_\infty$ is the fractional solute release at time t, $k$ is a constant which incorporates the structural and geometric characteristics of the device. The mechanism of drug release from spherical polymeric devices may be Fickian diffusion when the value of n = 0.43 or less, anomalous (non-Fickian) transport when the value of n lies between 0.43 and 0.85, and case II transport when n = 0.85. An exponent value of n greater than 0.85, signifies super case II transport mechanism.[27]

**Statistical analysis**

The differences in drug entrapment efficiency and drug release rate of the microspheres, prepared under different pH conditions were

evaluated by one-way analysis of variance (ANOVA) using GraphPad PRISM (version 3.00) software. Differences were considered significant when $p < 0.05$.

## RESULTS AND DISCUSSION

Briefly, when an aqueous solution of fluconazole was homogenized with ethylcellulose dissolved in dichloromethane, water-in-oil (w/o) type emulsion was formed in presence of an oil soluble emulsifier Span 80. Subsequent transfer of the primary emulsion into water containing Tween 80 under vigorous stirring resulted in the formation of w/o/w type multiple emulsion. The evaporation of organic solvent from the polymer/solvent droplets upon continuous stirring led to solid fluconazole-loaded ethyl cellulose microspheres. This conventional multiple emulsion solvent evaporation technique was used to prepare fluconazole-loaded ethyl cellulose microspheres and the effectiveness of this technique was judged primarily on the basis of morphological characteristics and drug entrapment efficiency of the microspheres. Although, the microspheres were spherical in shape and possessed smooth surface characteristics (Fig 1a), the fluconazole entrapment efficiency of the microspheres was only $17.35 \pm 2.17(\%)$. This result clearly indicated that fluconazole could not be successfully incorporated into ethylcellulose microspheres with the use of water alone in the internal aqueous phase.
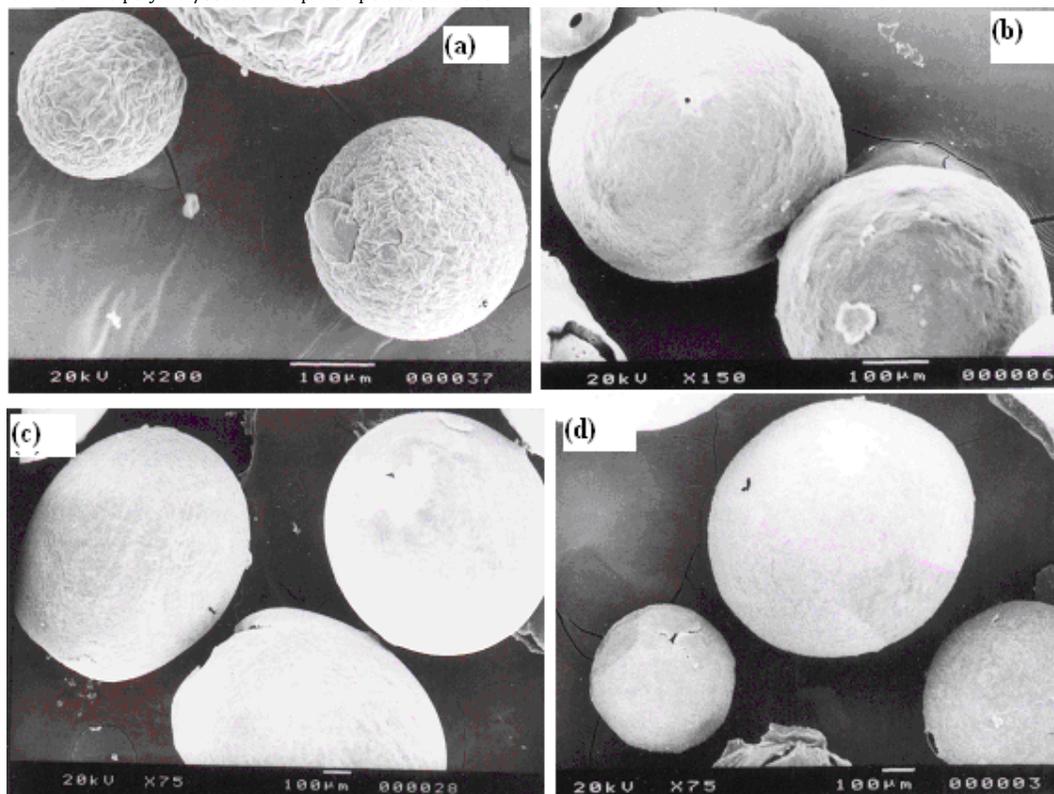


**Fig 1: Scanning electron micrographs of fluconazole-loaded microspheres; Key: (a) aqueous phase (without alginate); pH of alginate solution: (b) pH 4; (c) pH 6; (d) pH 8**

Such lowering in entrapment efficiency may be accounted for the partitioning out of the slightly water soluble drug from the aqueous dispersed phase into the external aqueous phase. Since dichloromethane has a limited solubility in water, it prolonged the solvent swollen conditions which involved a slower precipitation of polymer and consequently increased the drug leakage into the external aqueous phase.[28] Further, to explain such lowering of drug entrapment efficiency, the value of oil-water partition coefficient of the drug was determined and found to be $2.86 \pm 0.33$. Hence, the higher partition coefficient of the drug might be responsible for such lowering of drug entrapment efficiency. On the other hand, when an aqueous solution of sodium alginate (2%w/v) was used in the internal phase (pH 6), the efficiency of fluconazole entrapment into ethylcellulose microspheres was greatly improved ($72.79 \pm 2.11\%$). This result was consistent with the report that an increase in internal phase viscosity produced a significant increase in leuprolide acetate entrapment into poly (lactic-co-glycolic) acid microcapsules.[29] It could be anticipated that the use of hydrophilic polymer at a concentration of 2% (w/v) increased the viscosity of internal aqueous phase and held the active principle firmly from partitioning out into the external water phase. Above this concentration, the viscosity of alginate solution became higher, and it was difficult to formulate microspheres. Hence, the concentration of sodium alginate was kept constant at 2% (w/v).

To investigate the effect of the nature of aqueous alginate phase on the properties of the microspheres, the alginate solution was adjusted to different pH values: 4, 6 and 8, while keeping all other formulation and processing parameters fixed. The percentage yield of the microspheres was above 90% for all the formulations (Table 1). It was observed that the variations in pH of alginate solution significantly affected drug entrapment efficiency ($p < 0.05$). The drug entrapment efficiencies decreased at pH above or below 6 and as high as 72.79% fluconazole entrapment was noted at pH 6 (Table 1).

As sodium alginate solution is pH sensitive and tends to become insoluble at a pH value below 5,[30] the homogenous distribution of fluconazole in the aqueous alginate solution was difficult and therefore, the drug diffused to the external medium quite easily leading to lower entrapment efficiency at pH 4. Even, the adjustment of alginate solution to pH 8 led to lower drug entrapment efficiency of the microspheres. The study revealed that the viscosity of alginate solution having pH 8 was lower (872.2 cp) than that of the solution having pH 6 (1087 cp). Hence, it could be suggested that such lowering of viscosity of alginate solution having pH 8 caused easier diffusion of the incorporated drug from polymer/solvent droplets to the external processing medium and resulted in lower entrapment efficiency at pH 8. Furthermore, the oil-water partition coefficients were in the order of $1.11 \pm 0.13$, $0.07 \pm 0.01$ and $0.16 \pm 0.03$,

respectively for the aqueous alginate solutions having pH of 4, 6 and 8. Therefore, pH effect of inner alginate phase on drug entrapment efficiency could be attributed to the different partitioning between the inner alginate solution and the ethylcellulose solution in dichloromethane. Scanning electron micrographs of the microspheres prepared at different pH conditions have been displayed in Fig 1b-d. All the microspheres appeared to be spherical having smooth surfaces and no appreciable morphological distinctions were noted. Increase in pH of the aqueous internal phase from 4 to 6 induced a slight increase in mean particle diameter of the microspheres from 291 to 331 μm (Table 1); however their particle size distributions were monomodal in nature (Fig 2). Similar trends in mean particle diameter as well as drug entrapment efficiency were observed with bovine serum albumin (BSA)-loaded poly (ε-caprolactone) microparticles where the pH of the internal aqueous phase was changed from 3 to 7.[14]

**Table 1: Effect of pH of aqueous alginate solution on percentage yield, entrapment efficiency, particle size, flow properties and release characteristics of fluconazole-loaded ethyl cellulose microspheres**

| pH of alginate solution | % Yield | Entrapment efficiency* (%) | Mean diameter (μm) | Carr's index (%) | $t_{50\%}$ value (h)* | DE (%)* (pH 7.4) | DE (%)* (pH 1.2) |
|---|---|---|---|---|---|---|---|
| 4 | 90.54 | 35.22±1.12 | 291.29 | 12.53 | 4.90±0.08 | 46.35±0.79 | 22.13±0.54 |
| 6 | 95.87 | 72.79±2.11 | 331.62 | 11.13 | 7.90±0.07 | 31.69±0.41 | 13.05±0.21 |
| 8 | 92.35 | 51.91±3.71 | 315.57 | 10.36 | 6.40±0.05 | 39.80±0.40 | 13.64±0.23 |

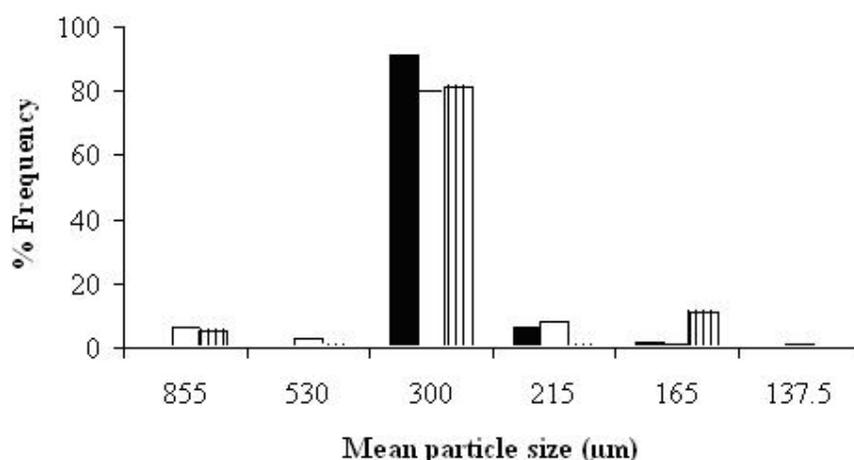* Data are the mean ± SD, n=3



Fig 2: Particle size distribution of fluconazole-loaded microspheres; Key: pH of alginate solution: pH 4 (closed block); pH 6 (open block); and pH 8 (striped block)

The flow properties of the microspheres have been expressed in terms of Carr's index (Table 1). The Carr's index for all formulations was less than 15, which indicated good flow properties and suggested that the microspheres could be easily handled during processing. Fig 3 and Fig 4 illustrate the *in vitro* release profiles of the formulations, by presenting the percentage of fluconazole released with respect to the amount of encapsulated fluconazole in pH 1.2 HCl solution and pH 7.4 PBS solution, respectively.
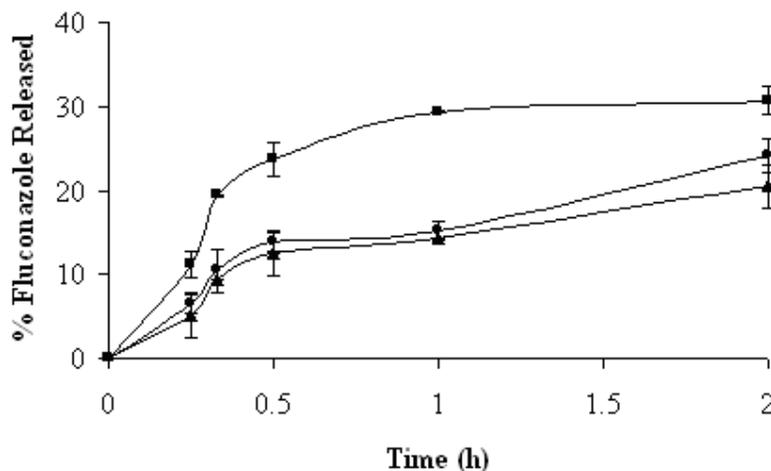


Fig 3: Release profiles of fluconazole-loaded microspheres in pH 1.2 HCl solution; Key: pH of sodium alginate solution: (■) pH 4; (▲) pH 6; (●) pH 8
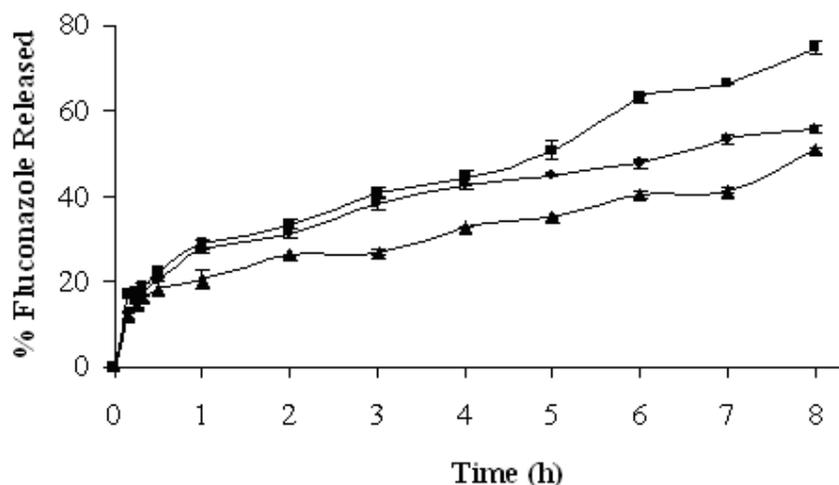
**Fig 4: Release profiles of fluconazole-loaded microspheres in pH 7.4 PBS solution; Key: pH of sodium alginate solution: (■) pH 4; (▲) pH6; (●) pH 8**

In order to keep the total surface area of the microspheres constant and thus to get comparable results, the release studies were carried out with same size fractions of the microspheres (retained on 60 mesh sieve) containing equivalent amount of fluconazole. It was evident that the microspheres prepared with the solution having pH 6, exhibited a comparatively slower release profile in either dissolution medium. The time point approach was adopted for the interpretation of dissolution data, obtained in pH 7.4 PBS solution. Table 1 clearly indicated that the $t_{50\%}$ value (i.e. the time required for the release of 50% encapsulated drug) was much higher with pH 6 than those prepared with alginate solutions of pH 4 and pH 8. Such differences in $t_{50\%}$ values were statistically significant (p<0.05). The dissolution efficiency (%) of the fluconazole-loaded microspheres in pH 1.2 HCl solution was found higher at a micro-environmental pH of 4, followed by pH 8 and pH 6 (Table 1). The dissolution efficiency (%) of the microspheres in pH 7.4 PBS solution followed similar trend to that observed in acidic dissolution medium (Table 1). However, the drug release rate in alkaline medium was higher than in acidic dissolution medium when compared up to 2h. The eventual modifications in the release of entrapped fluconazole following pH variations could be explained by the pH-dependent aqueous solubility of the drug and pH-dependent swelling behaviors of the microspheres. The antifungal has been reported to be sparingly soluble in aqueous 0.1N HCl solution (14 mg/ml at 23°C) and slightly soluble (5 mg/ml at 23°C) in both water and aqueous 0.1M NaOH solution.[31] Though the aqueous solubility of fluconazole is slightly higher at low pH, sodium alginate tends to precipitate at low pH condition.[32] This may cause inhomogeneous distribution of the drug in alginate solution (pH 4) during preparation and thus create a possibility for the drug particles to be present on the surface of the microspheres. As soon as the acidic fluid (pH 1.2) penetrates the microspheres, fluconazole gets easily dissolved and diffuses back to the external dissolution medium initially at a much faster rate. This was reflected in the release profiles of the microspheres having micro-environmental pH 4 (Fig 3) where a burst release of fluconazole pH 1.2 HCl solution (releasing about 29.37% drug within 1 h) was followed by a very slow release phase (releasing only 1.28 % drug in next 1 h). This hypothesis was further supported by the fact that the microspheres having micro-environmental pH 4 swelled more slowly than those having micro-environmental pH 6 and pH 8 (Fig 5). It has been reported that acidic fluid can not hydrate sodium alginate.[33,34] On contrary, the tendency of PBS solution (pH 7.4) to hydrate alginate matrix was higher than acidic fluid and hence, induced more swelling of the microspheres having internal pH 4 (Fig 5) and consequently, resulted in faster drug release in pH 7.4 PBS solution. Although, the release profiles of the fluconazole-loaded microspheres having internal pH 6 and 8 were more or less identical in pH 1.2 HCl solution, the difference in release patterns was evident in pH 7.4 PBS solution.
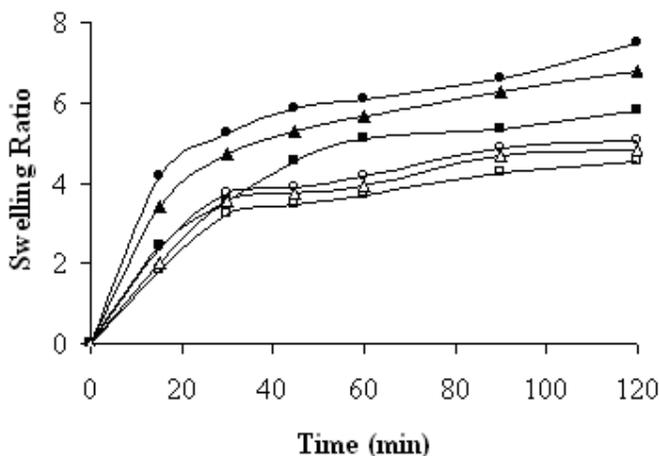


**Fig 5: Swelling behaviors of microspheres having different micro-environmental pH in different dissolution media; Key: HCl solution (pH 1.2): (○) pH 8; (Δ) pH 6; (□) pH 4, and PBS solution (pH 7.4): (●) pH 8; (▲) pH 6; (■) pH 4**

As sodium alginate is highly soluble in alkaline medium, it attracts alkaline dissolution medium, then induces more swelling of microspheres having internal pH 8 (Fig 5), and causes diffusion of the drug easier. Thus the data suggested that a slower and uniform drug release from reasonably high drug-loaded ethyl cellulose microspheres could be obtained following adjustment of the internal aqueous alginate solution to pH 6. Kinetic analysis of the dissolution data stated that the drug release from these microspheres could be best described by Higuchi equation, followed by first and zero order equations (Table 2).

**Table 2: Mathematical modeling of *in vitro* release data for fluconazole-loaded ethyl cellulose microspheres obtained in pH 7.4 PBS solution**

| Alginate solution | Zero order | | First order | | Higuchi model | | Korsemeyer-Peppas model | | |
|---|---|---|---|---|---|---|---|---|---|
| | $R^2$ | $K_0$ (%/h) | $R^2$ | $K_1$ ($h^{-1}$) | $R^2$ | $K_h$ (%/$h^{-1/2}$) | $R^2$ | n | k |
| pH 4 | 0.9480 | 7.6337 | 0.9653 | 0.1416 | 0.9738 | 23.1402 | 0.9897 | 0.3297 | 0.2826 |
| pH 6 | 0.9010 | 4.5794 | 0.9389 | 0.0645 | 0.9653 | 14.1761 | 0.9754 | 0.3194 | 0.2184 |
| pH 8 | 0.8879 | 5.7268 | 0.9450 | 0.0877 | 0.9826 | 18.0189 | 0.9950 | 0.3653 | 0.2560 |

Hence, the drug release from the microspheres was expected to be controlled by micropore diffusion. Coefficients of correlation ($r^2$) were used to evaluate the accuracy of the fit. The n-value obtained after modeling the *in vitro* dissolution data (pH 7.4 PBS solution) into Korsmeyer-Peppas equation exhibited Fickian diffusion mechanism for drug release (Table 2). That is, when the drug-loaded microspheres come in contact with the dissolution medium, the aqueous medium penetrates the matrix and it begins to swell, decreasing the glass transition temperature of the polymer and reaching a rubbery state, which increases the permeability of the polymer to the drug and allows it to spread to the exterior.

## CONCLUSION

This study revealed that spherical, discrete, free flowing and high-yielded fluconazole-loaded ethyl cellulose microspheres could be prepared by the alginate facilitated w/o/w emulsion solvent evaporation technique. The use of alginate solution (pH 6) as internal aqueous phase was found to be highly effective for the incorporation of fluconazole into the microspheres. The variations in pH of alginate solution either below or above 6 resulted in poor drug encapsulation efficiency. Instead of pH 4 or pH 8, the adjustment of micro-environmental pH to 6 not only improved the drug entrapment efficiency of the microspheres, but also provided comparatively slower drug release profiles in both acidic and alkaline dissolution media. However, the drug release was found to be controlled by Fickian diffusion mechanism irrespective of the micro-environmental pH of the microspheres. Thus, the use of hydrophilic polymers in the internal aqueous phase and variations of micro-environmental pH could be useful for the successful incorporation of slightly water soluble drugs like fluconazole into ethyl cellulose microspheres. Further studies are required to optimize the various processing variables which could influence the properties of the fluconazole-loaded ethyl cellulose microspheres.

## ACKNOWLEDGEMENTS

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