*Available online at* www.pelagiaresearchlibrary.com

Pelagia Research Library

**Der Pharmacia Sinica, 2014, 5(4):32-39**

Der Pharmacia Sinica
**ISSN: 0976-8688**
**CODEN (USA): PSHIBD**

# Formulation and *in vitro* evaluation of oil entrapped floating beads of azithromycin for the effective treatment of *Helicobacter pylori*

**Sanjoy K Dey[1], Pintu K De[2], Tanmay Goswami[2], Arnab De[1]and Amalesh Samanta[1]***

*[1]Department of Pharmaceutical Technology, Jadavpur University, Kolkata*
*[2]Dr. B. C. Roy College of Pharmacy and Allied Health Science, Bidhan Nagar, Durgapur*

---

**ABSTRACT**

*The objective of this investigation was to develop an intra gastric floating drug delivery system of azithromycin in order to localize the antibiotic at the site of infection to achieve bactericidal concentration against Helicobacter pylori. In the present investigation oil entrapped floating beads of azithromycin was formulated and effect of polymer and oil concentration on physico chemicaland in-vitro drug release properties are evaluated. The formulation was developed by emulsion gelation technique using two polymers: Sodium alginate and Hydroxy propyl methyl cellulose (HPMC) and Soybean oil. The developed beads were evaluated in term of diameter, surface morphology, floating lag time, encapsulation efficiency, and in-vitro drug release. It was found that formulation variables such as amount of polymer (% w/v), amount of soybean oil (% v/v), the ratio of drug to polymer (w/w), affected the bead size, floating and encapsulation efficiency of the beads. The scanning electron microscope photograph indicated that prepared beads were spherical in shape. All batches of beads floated for 24 hours with a very short lag time of 175–210 seconds. The drug release profile was best fitted with Higuchi and first order kinetic model and the release exponent (n) values were 0.71 to 0.813 indicating anomalous transport mechanism. FTIR study confirms that drug was compatible with the polymers. The results provides evidence that formulated beads may be preferred to localize the antibiotic in the gastric region to allow more of the antibiotic to penetrate through the gastric mucus layer to act on H. pylori and thereby improve the therapeutic efficacy.*

**Keywords:** Floating beads, Azithromycin, *H. pylori*, Soybean oil.

---

## INTRODUCTION

Oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages. However, this approach is bedilled with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract (GIT) due to variable gastric emptying and motility. Therefore, control of placement of a drug delivery system in a specific region of the GIT offers advantages for a variety of important drugs characterized by a narrow absorption window in the GIT or drugs with a local action in the stomach. Floating drug delivery systems are among the several approaches that have been developed in order to increase the gastric residence time of dosage forms[1]. These systems, are more advantageous for the drugs that act locally in proximal gastro intestinal tract such as antibiotics[2], $H_2$blockers[3] etc. In that case the delivery system would serve dual purpose; the released drug would act locally in the lumen as well as systemically after absorption of drug through the basolateral membrane.

*H. pylori* is a small, spiral, gram negative organism which colonize on gastric mucosa of human stomach and produces a serious gastro duodenal disease- including peptic ulcers, gastric lymphoma and acute chronic gastritis[4].It can penetrate epithelial cells and survive intracellularly for at least 40 h which might account for persistent or recurrent infection[5]. The organism can be killed intracellularly only at high concentrations of antibiotic known to exert intracellular activity[6].

To overcome this problem in *H. pylori* treatment, a novel drug delivery system that localizes the antibiotic at the site of infection to achieve bactericidal concentration is highly desirable. The longer residence time of dosage forms can maintain a higher antibiotic concentration in the gastric region; will allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori* and thereby improve the therapeutic efficacy. In order to enhance the efficacy, we have made an attempt to develop oil entrapped floating multiple unit beads for stomach site specific delivery of azithromycin for effective treatment of *H. pylori* infection.

Azithromycin, a new generation macrolide antibiotic is able to reduce the number of intracellular *H. pylori*[5]. The drug has been reported to accumulate in gastric tissue with concentrations five- to ten-fold greater than those found in gastric mucus where the concentrations exceed the MIC(minimum inhibitory concentration)[7].The polymer used sodium alginate is an inexpensive, nontoxic product extracted from kelp. Literature reports widespread use of sodium alginate for achieving sustained release of drugs[8], targeting gastric mucosa[9] and increasing the bioavailability of drugs[10]. Additionally it also reduces interfacial tension between an oil and water phase and is efficient for preparation of emulsion. Hydroxypropyl methylcellulose (HPMC) has been reported to enhance the sustained-release properties of alginate by providing a denser inner matrix[11].

## MATERIALS AND METHODS

### Materials:

Sodium alginate (low viscosity grade; 250 cp of 2% solution at 25°C) was purchased from Loba Chemie Pvt. Ltd., Mumbai. Azithromycin (drug) was donated by Martina Bio Genics Private Limited, Kolkata. Soybean oil (density 0.916-0.922 g/cm$^3$ at 25$^o$C) was purchased from Agro Tech Food Ltd., Secundrabad, India. Hydroxy propyl methyl cellulose, Calcium chloride dihydrate (CCD) and Hydrochloric acid (35% GR)were purchased from E. Merck India Ltd., Mumbai, India. All materials were used with no further purification. Deionized water was used throughout the study.

### Preparation of floating alginate beads:

Calcium alginate gel beads were prepared by emulsion gelation method. At first the aqueous slurry of sodium alginate, HPMC and azithromycin in deionized water was prepared. Soybean oil was added drop wise to the aqueous slurry with stirring at 2500 rpm by a PMDC stirrer (RQ-121/D, Remi, India)for 45 minutes to get a stable o/w type emulsion. Finally this emulsion was dropped through 18 Gneedle in to 10% w/v calcium chloride dihydrate (CCD) solution to get spherical beads. These beads were kept in contact with CCD solution for 15 minutes and then removed from CCD solution by filtering, washed, dried in hot air oven and stored in desicator for further study. The formulation variables are given in table 1.

**Table1: Formulation variables of the floating azithromycin beads**

| Batch No. | % w/v of polymer (Na alginate: HPMC=9:1) | Drug/polymer ratio (w/w) | Soybean oil (% v/v of demineralised water) | Calcium chloride dihydrate (% w/v of demineralised water) |
|---|---|---|---|---|
| $FB_1$ | 4 | 2:06 | 20% | 10% |
| $FB_2$ | 4 | 2:06 | 10% | 10% |
| $FB_3$ | 4 | 1:06 | 20% | 10% |
| $FB_4$ | 4 | 1:06 | 10% | 10% |
| $FB_5$ | 3 | 4:09 | 20% | 10% |
| $FB_6$ | 3 | 4:09 | 10% | 10% |
| $FB_7$ | 3 | 2:09 | 20% | 10% |
| $FB_8$ | 3 | 2:09 | 10% | 10% |

### Incompatibility study between drug and polymers by fourier transform infra-red spectroscopy (FTIR):

The pure drug (Azithromycin) and polymers were subjected to IR studies alone and in combination. About 3 mg of pure drug/pure polymer/combination of drug-polymer were grinded with 97 mg of potassium bromide in a smooth

mortar to affect through mixing. The mixtures were then placed in the sample holder of the instrument. These were analyzed by FTIRspectroscopy (JASCO-FTIR, Model-8300).

**Physicochemical evaluation of alginate beads:**

**Appearance**

Appearance of the prepared alginate beads are seen by placing them under triangular microscope (Magnus; Model MLX).

**Bead size**

The diameter of the dried beads and wetted beads (after 8 hr. wetting in 0.1 N HCl) were measured by Dial calipers [AEROSCAPE (150 X 0.2mm)].

**Weight uniformity study**

The weights of 100 beads of each were taken in three groups. Each group of 100 beads was taken randomly.

**Estimation of drug entrapment efficiency of prepared beads**

A 100 mg portion of dried beads were placed into 100 ml of 0.1 N HCl. It was shaken over night by mechanical shaker. On the next day the resulting dispersion was filtered through Whatman Filter Paper (0.45µm). The polymeric debris was washed twice with fresh buffer to extract any adhered drug. The filtrate was diluted with 2 ml Folin-Ciocalteuís phenol reagent (diluted to 1:2 with deionised water) and 2 mL of 20% sodium carbonate solution and 0.1 N HCl up to 10 mL and assayed spectrophotometrically at λ=760 nm in a double beam UV-visible spectrophotometer (Shimadzu UV 1700) against reagent blank. Entrapment efficiency was calculated by using equation:

Entrapment Efficiency = (Actual drug content / Theoretical drug content) x 100 %

**Surface morphology studies**

Morphological examination of the surface and internal structure of the beads was performed by using scanning electron microscope (JSM 6100 JEOL, Tokyo, Japan) operated at an acceleration voltage of 15 kV. For examination of the internal structure of the beads, they were cut in half with a steel blade.

***In-vitro* floating study:**

The in vitro buoyancy study for the beads was tested by visual observation in 0.1 N HCl using a USP dissolution apparatus II, Veego. Twenty beads of each batch were placed into 900 ml 0.1 N HCl maintained at 37±0.5°C and 100 rpm. The time between the introduction of the beads into the medium and its buoyancy to the upper one third of the dissolution vessel (buoyancy lag time) and the time for which the formulation constantly floated on the surface of the medium (duration of buoyancy) were measured simultaneously as a part of dissolution studies[12].

***In-vitro*drug release study:**

The in-vitro study of the floating beads was done in USP dissolution apparatus II (Veego, Mumbai) at 100 rpm and 37 ± 0.5 ˚C. The particular amount of beads (Which contained 100 mg of the drug) was placed in 900 ml of 0.1 N HCl. A 2 ml of the aliquot was withdrawn at predetermined intervals and replaced with fresh dissolution medium. Samples were suitably diluted with 2 ml Folin-Ciocalteuís phenol reagent (diluted to 1:2 with deionisedwater) and 2 mL of 20% sodium carbonate solution and 0.1 N HCl up to 10 mL and assayed spectrophotometrically at λ=760 nm in a double beam UV-visible spectrophotometer (Shimadzu UV 1700) against reagent blank. The results of in vitro drug release are fitted with zero-order, first-order, Higuchi and Korsmeyer & Peppas semi-empirical model for the analysis of the release kinetics. The criteria for selecting the most appropriate model were chosen on the basis of goodness of fit test.

## RESULTS AND DISCUSSION

**Fourier transform infra-red spectroscopy (FTIR) study:**

As per the describe method FTIR was carried out for pure azithromycin, pure HPMC, pure Na-alginate and physical mixture of azithromycin : HPMC : Na Alginate in 1:1:1 ratio. The spectrums are shown in fig. 1.

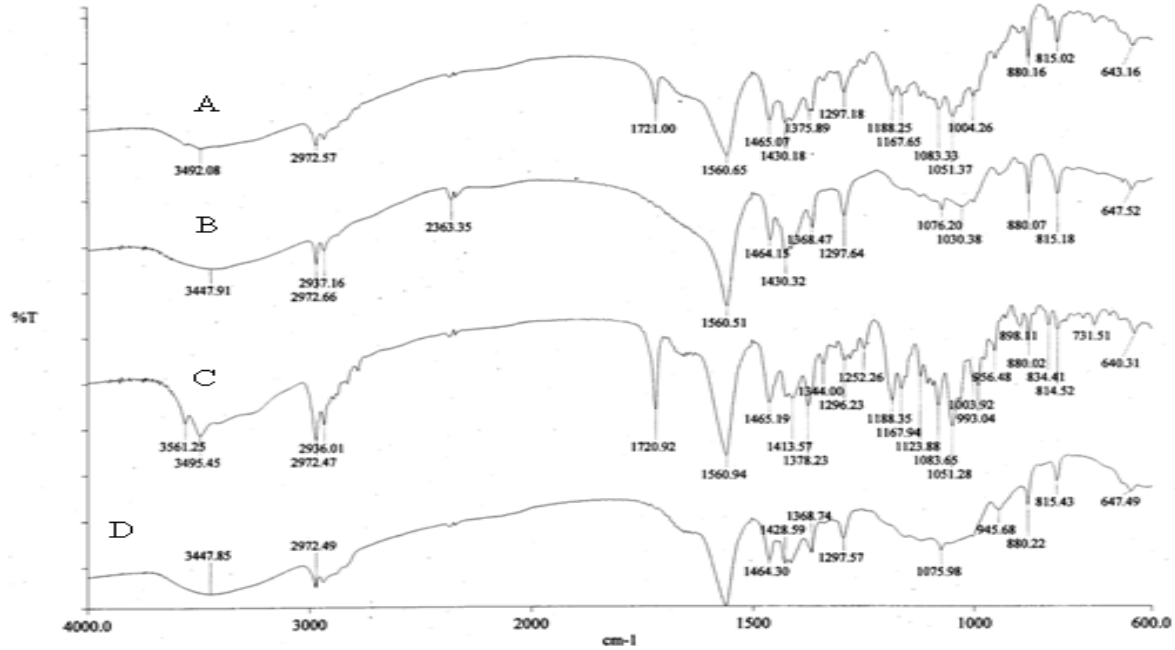


**Fig. 1. FTIR spectrums of physical mixture of azithromycin, HPMC and sodium alginate in 1:1:1 ratio(A), pure sodium alginate(B), pure azithromycin(C) and pure HPMC(D)**

From the figure it has been seen that there was no appearance or disappearance of any peak in the IR spectra. There were no major shifts in the absorption bands of the azithromycin in presence of polymers. Hence we can conclude that the drug polymers interactions were not present and azithromycin is fully compatible with the polymers.

**Formation of gel beads:**

The gel beads were easily manufactured without any sophisticated equipment. When emulsion of Na-alginate & HPMC with soybean oil was dropped into calcium chloride solutions, spherical gel beads were then formed instantaneously due to intermolecular cross-linking between the divalent calcium ions and the negatively charged carboxyl group of alginic acid.

**Appearance:**

The beads containing soybean oil were almost spherical, yellowish white, spherical in shape and hard. Yellowish white coloration was owing to original color of soybean oil. Upon drying the beads become small and dense(Fig. 2 and Fig. 3).

**Fig. 2. Photograph of freshly prepared beads**

**Fig. 3. Photograph of dried beads**

**Size:**
The average size of the dried spherical beads ranged from 1.84mm to 2.08mm considering the spherical shape of the beads. Size of the beads increases with the increase in the concentration of drug, polymer and soybean oil at 10% w/v fixed concentration of calcium chloride. This could be attributed due to the increase in the viscosity of polymer solution that in turn increases the droplet size during addition of the polymer solution to the cross-linking solution.The mean diameter of oil-entrapped gel beads containing different types and amounts of oil is shown in table 2.

**Table 2: Physicochemical properties of different batches of floating azithromycin beads(n=3)**

| Batch No. | Diameter of beads (mm) (Mean ± S.D.) | Weight of 100 beads (mg) (Mean ± S.D.) | % Entrapment efficiency | Lag time (seconds) | Floating Time (Hrs.) |
|---|---|---|---|---|---|
| $FB_1$ | 2.05 ± 0.04000 | 591.7 ± 4.726 | 98.85896581 | 188.3± 8.622 | >24 |
| $FB_2$ | 1.98 ± 0.0600 | 512.3 ± 4.163 | 96.13533761 | 201.7± 5.508 | >24 |
| $FB_3$ | 2.04 ± 0.05000 | 558.7 ± 4.726 | 98.53244729 | 176.3± 7.767 | >24 |
| $FB_4$ | 1.86 ± 0.0600 | 432.3 ± 4.933 | 94.20969231 | 210± 7 | >24 |
| $FB_5$ | 2.08 ± 0.06000 | 504.0 ± 6.557 | 97.72503419 | 175.7± 7.506 | >24 |
| $FB_6$ | 1.95 ± 0.07550 | 404.7 ± 5.508 | 93.69844658 | 196± 7.211 | >24 |
| $FB_7$ | 2.02 ± 0.05568 | 396.0 ± 5.568 | 92.89214815 | 182.7± 5.859 | >24 |
| $FB_8$ | 1.84 ± 0.04000 | 372.0 ± 4.583 | 90.14065385 | 195.3± 8.083 | >24 |

**Weight variation:**
Weight variation data(table2) shows that beads are uniform in weight. Slight variation of weight is due to the bead size variation and different composition of batches.

**Surface Morphology:**
SEM photograph shows that the drug loaded oil entrapped beads were almost spherical in shape and the presence of minor projections on the surface may be attributed to the presence of insoluble drug particles in the bead matrix (fig. 4). The section showed sponge-like structure which indicates that oil was entrapped (fig.5). The size of these oil filled pores visible on the section surface ranging from 25 to 150 µm. The uneven size of the pores could be due to the coalescence of the oil droplets during the gelling process[13].

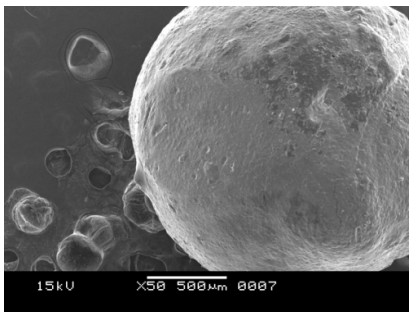


**Fig. 4. SEM photograph showing sphericity of the bead**

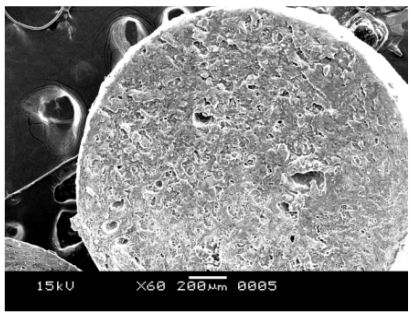


**Fig. 5. SEM photograph of cross section of beadshowing sponge like structure**

**Entrapment efficiency:**
Drug entrapment efficiency ranged from 90.14 % to 98.85% depending on the composition of the eight batches of polymeric beads of azithromycin (table 2). It was observed that an increase in the amount of polymer increases drug entrapment efficiency because of the formation of larger beads with denser internalstructure which are able to retain azithromycin more effectively. Also, there was a correlation observed between proportion of oil and drug entrapment efficiency of the beads. A higher % of oil in the formulation of beads increased the drug entrapment efficiency in different batches, some amount of drug diffused in surrounding oil pockets during gellification of beads.

***In-vitro* floating:**
It is evident from the result that all formulations floated within 210 seconds (table 2) after being placed into the acidic medium (0.1 N HCl) and remained buoyant in the medium throughout the study irrespective of polymer and oil concentration. It was found that beads swell very less in 0.1 N HCl. This is because in acidic pH alginate is protonated into insoluble form of alginic acid and have less swelling rate [14].

So swelling is not responsible for floating of the beads. The factors contributing to the floating appeared to be the porous structure(multiple tiny pockets) of the beads, low relative densities of soybean oil (0.916-0.922 g $mL^{-1}$) as compared to that of gastric media (1.004 g mL–1) and facilitation of air entrapment by the oils. It was also observed that increase of the amount of oil in the matrix decrease the lag time and increase the floating time. So the formulations containing oil 20% v/v e.g. BF1, BF3, BF5 & BF7 float comparatively faster than those formulations containing oil 10% v/v e.g. BF2, BF4, BF6 & BF8 (fig.6) irrespective of the amount of polymer included in the various batches.

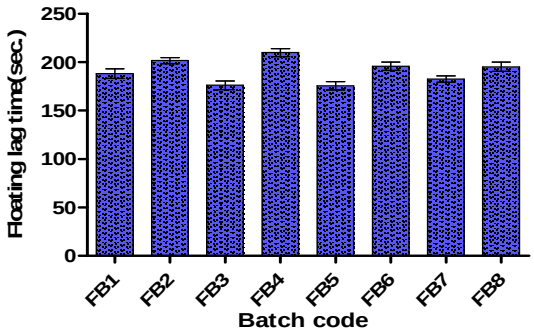


**Fig. 6.Comparison of lag time of different batches**

***In-vitro* drug release:**
The gel beads exhibited a biphasic release profile as an initial rapid drug release phase (burst effect) was followed by a slower, gradually declining drug release phase after one hour extending up to 7 hours to release up to about 92.53% drug (fig. 7). The initial burst release may be due to the release of adhering drug particle to the outer surface of the beads. From the release profiles of different of different batches it has been observed that release rate is inversely proportional with the polymer concentration. Comparison of cumulative % release vs. time profile between batch FB1 and FB5, FB2 and FB6, FB3 and FB7, FB4 and FB8 showed release rate is faster at 3 % w/v than that of 4% w/v polymers. Form the oil encapsulation it had been seen that as the concentration of oil increases (batch FB1, FB3, FB5 and FB7) drug release decreases to certain extent; it implies that the addition of oil in the formulation created an additional barrier and permit efficient control of the release of the drug.

It has been found from the correlation coefficients of different kinetics models that the kinetics of azithromycin from the system follows the Higuchi model kinetics (except the batches FB4 and FB6) proving that the release is by diffusion mechanism. From the results of release exponent data of different batches using the Korsemeyer–Peppas model (table 3), it was observed that the drug release from the polymer matrix system was non fickian (anomalous transport) type ($1<n<0.5$). Anomalous diffusion or non-fickian diffusion refers to combination of both diffusion and erosion controlled rate release.

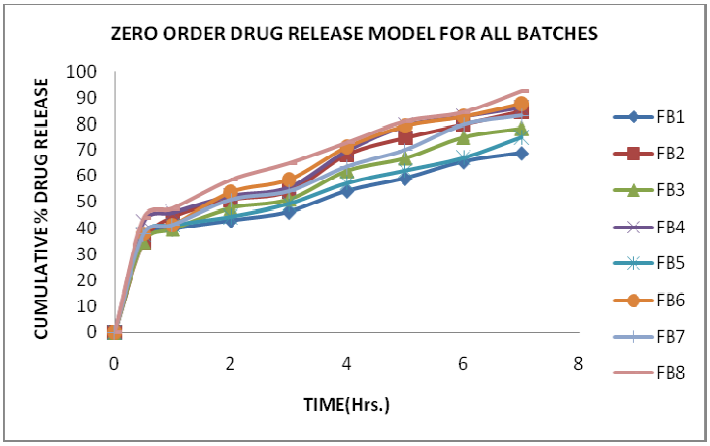


**Fig. 7. A plot of cumulative percentage release of azithromycin vs. times in hour**

**Table 3: Correlation coefficients of the drug release, model of best fit, release exponent and release mechanismd data of the azithromycin loaded floating beads(n=3)**

| Batch No. | Correlation Coefficients ($R^2$ value) | | | | Model of Best Fit | Release exponent(n) | Release mechanism |
|---|---|---|---|---|---|---|---|
| | Zero order | First order | Higuchi | Korsemeyer–Peppas | | | |
| FB1 | 0.772±0.012 | 0.9± 0.032 | 0.92± 0.013 | 0.246± 0.017 | Higuchi | 0.71± 0.067 | Anomalous transport |
| FB2 | 0.844±0.014 | 0.9670± 0.015 | 0.968± 0.020 | 0.289± 0.090 | Higuchi | 0.807± 0.051 | Anomalous transport |
| FB3 | 0.839±0.021 | 0.956± 0.045 | 0.964± 0.037 | 0.282± 0.031 | Higuchi | 0.782± 0.095 | Anomalous transport |
| FB4 | 0.815±0.11 | 0.955± 0.041 | 0.945± 0.020 | 0.262± 0.015 | First order | 0.774± 0.069 | Anomalous transport |
| FB5 | 0.786±0.023 | 0.914± 0.017 | 0.926± 0.043 | 0.249± 0.024 | Higuchi | 0.723± 0.039 | Anomalous transport |
| FB6 | 0.85± 0.018 | 0.977± 0.033 | 0.974± 0.073 | 0.292± 0.041 | First order | 0.819± 0.034 | Anomalous transport |
| FB7 | 0.838± 0.041 | 0.955± 0.020 | 0.96± 0.023 | 0.275± 0.042 | Higuchi | 0.782± 0.039 | Anomalous transport |
| FB8 | 0.806± 0.020 | 0.9520± 0.024 | 0.954± 0.036 | 0.261± 0.020 | Higuchi | 0.783± 0.028 | Anomalous transport |

## CONCLUSION

Oil entrapped azithromycin floating beads were prepared by the emulsion gelation method showed satisfactory results for various physical parameters like size, weight variation, drug entrapment, *in vitro* floating and i*n-vitro* drug release. Resulted suitable floating property of the beads may be able to localize the antibiotic at the site of *H. pylori* infection to achieve higher bactericidal concentrationand thereby may improve the therapeutic efficacy.

**Acknowledgements**
Authors are thankful to Martina Bio Genics Private Limited, Kolkata for supplying azithromycin as gift sample.

## REFERENCES

[1] Seth PR, Tossounian J, *Drug Dev Ind Pharm*, **1984**, 10, 313-339.
[2] Tripahi GK, Singh S, Nath G, *Der Pharmacia Sinica*, **2010**, 1 (3): 245-255.
[3] Dave BS, Amin AF, Patel MM, *AAPS Pharm Sci Tech*, **2004**, 5(2), 77-82.
[4] Deshpande AA, Rhodes CT, Shah NH, Malick AW, *Drug Dev Ind Pharm*, **1996**, 22(6), 531-539.
[5] Hultén K, Cars O, Hjelm E, Engsrrand L, *Journal of Antimicrobial Chemotherapy*, **1996**, 37, 483-489.
[6] Al-Assi MT, Ramirez FC, Lew GM, Genta RM, Graham DY, *American Journal of Gastroenterology*, **1994**, 89(8), 1203-1205.
[7] Harrison JD, Jones JA, Morris DL, *European Journal of Clinical Microbiology and Infectious Diseases*, **1991**, 10, 862-864.
[8] Badwan AA, Abumalooh A, Sallam E, Abukalaf A, Jawan O, *Drug Dev Ind Pharm.*, **1985**, 11, 239-256.
[9] Murata Y, Kofuji K, Kawashima S, *J Biomater Sci Polym Ed*, **2003**, 14, 581-588.
[10] Stops F, Fell JT, Collett JH, Martini LG, Sharma HL, Smith AM, *Int J Pharm*, **2006**, 308(1-2), 14-24.
[11] Giunchedi P, Gavini E, Moretti MDL, Pirisino G, *AAPS Pharm Sci Tech*, **2000**, 1(3), 31-36.
[12] Choi BY, Park HJ, Hwang SJ, Park JB, *Int J Pharm*, **2002**, 239(1-2), 81-91.

[13] Murata Y, Sasaki N, Miyamoto E, Kawashima S, *Eur J Pharm Biopharm*, **2000**, 50, 221–226.
[14] Yotsuyanagi T, Ohkubo T, Ohhashi T, Ikeda T, *Chem Pharm Bull*, **1987**, 35, 1555-1563.