Asian Journal of Pharmaceutical and Clinical Research

CHITOSAN COATED MUCOADHESIVE MULTIPARTICULATE DRUG DELIVERY SYSTEM FOR GLICLAZIDE.

PATIL D.A, PATIL G.B*, DESHMUKH P.K, BELGAMWAR V.S, FURSULE R.A.

The purpose of this research work was to develop optimized and systematically evaluate performances of mucoadhesive microcapsules of antidiabetic drug gliclazide. Alginate microcapsules coated with mucoadhesive polymer chitosan were prepared by ionotropic gelation technique utilizing calcium chloride ($CaCl_2$) as a cross linking agent, to take the advantage of swelling and mucoadhesive property of alginate beads for improving the oral delivery of gliclazide. Depending upon the variability in the concentration of alginate, percentage of cross linking agent, time of curing, the factors like particle size, incorporation efficiency and release rate of microcapsules varies. The microcapsules obtained were discrete, spherical and free flowing. The microcapsules coated with mucoadhesive polymer chitosan exhibited good mucoadhesive property in the *in vitro* wash off test and also showed high percentage drug entrapment efficiency. The swelling behavior was strongly depends upon chitosan concentration. The *in vitro* release study indicates that the swelling is the main parameter in controlling the release rate from microcapsules.

Keywords : Mucoadhesive microcapsules, Gliclazide, Entrapment efficiency.

INTRODUCTION

Multiparticulate system made up of natural biodegradable polymers have paid considerable attention for several years in controlling and sustaining of release rate of drugs. Recently, dosage forms that can precisely control the release rates and targets drugs to a specific body site have made enormous impact in the formulation and development of novel drug delivery systems. Oral multiunit dosage forms such as microcapsules and microspheres have received much attention as modified/controlled drug delivery systems [1, 2]. However the success of these Oral multiunit dosage forms is limited owing to their short residence time at the site of absorption. It will therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes [3]. This can be achieved by coupling the bioadhesive characteristics to microcapsules and develop bioadhesive microcapsules. Bioadhesive microcapsules have advantages such as efficient absorption and enhanced bioavaibality of drugs owing to high surface to volume ratio, a much more intimate contact to mucus layer and specific targeting of drugs to the absorption site. Alginate (polysaccharide) is obtained from marine brown algae, alginate can be considered as block polymers which mainly consist of mannuronic acid (M), guluronic acid (G) and mannuronic-guluronic (MG) blocks. The gelation of alginate is caused by forming an egg-box junction to associate divalent metal ions with the GC block of alginate polymer chain. The medicinal use of sodium alginate as a matrix material to achieve controlled release drug delivery is due to its hydrogel forming properties. Chitosan was selected as a polymer in preparation of mucoadhesive microcapsules because of their good mucoadhesive and biodegradable properties.

Gliclazide is one of the sulfonylurease in the treatment of type II diabetes (11). The conventional formulation required twice daily administration (11). A new once daily Gliclazide modified release formulation has been recently introduced (12). In a large randomized study on type II diabetic patients, once daily Gliclazide modified release 30-120 mg was found as effective as twice daily Gliclazide 80-320 mg in reducing glycosylated hemoglobin (HbA1C), with fewer side effects and less risk of hypoglycemia (12-15). Thus in this study an attempt was made to prepare oral controlled release coated chitosan microcapsules of gliclazide. The microcapsules were characterized by *in vitro* tests to optimize the variables.

MATERIALS AND METHODS

MATERIALS

Sodium alginate was purchased from loba chemie (Bombay), gliclazide was kindly gifted by Cadila pharma. (Ahmadabad), Chitosan (85% deacylated) of viscosity grade 200-800 cps, was kindly gifted by Central Institute of Fisheries Technology (Kochi., India). Calcium chloride was purchased from Loba Chemie (Bombay). All other chemicals and solvents were of reagent grade or higher.

Preparation of microcapsules by ionic gelation technique

Preparation of Coated chitosan microcapsules by ionic gelation technique [6]

The principle involved was the cation-induced gelation of alginate. Firstly sodium alginate is dissolved in purified water to form the homogenous polymer solution. The active substance gliclazide was then added to the solution and

*Corresponding author: *1 H.R.Patel Women's College of Pharmacy, Karwand Naka, Shirpur Dist- Dhule (MS)
e-mail: ganu16@gmail.com

stirred thoroughly to form the homogenous suspension. After thorough mixing, 20–30 min were allowed to elapse in order to make the solution bubble-free. The mixture was passed through 22 gauge syringe into the 50 ml of 5% calcium chloride solution containing 1% chitosan and the added droplets were retained in the calcium chloride (containing chitosan) solution for 25 minutes to complete the curing reaction and to produce spherical and rigid microcapsules. The microcapsules so prepared were collected by decantation technique and the product thus separated was washed repeatedly with water and dried at 45°C for 12 hours. The microcapsules were prepared using various alginate to mucoadhesive polymer ratios in order to sustain the release of the drug for 12 hours.

Physicochemical Characterizations[7-8]

Yields of production

The yields of production of microcapsules of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microcapsules and percent production yields were calculated as per the formula mentioned below.

$$\text{Production Yield} = \frac{Practical\ mass\ (microcapsules)}{Theoretical\ mass\ (Polimer + drug)} \times 100$$

Actual drug content and encapsulation efficiency

Actual drug content and encapsulation efficiency of the microcapsules prepared by ionic gelation technique was determined by following method, 100 mg of dried beads were crushed using mortar and pestle. The ground beads were placed in 100 ml of phosphate buffer of pH 7.4 and shaken for 24 h at 37±0.5 ^{0} C in mechanical shaker. The samples were then filtered to obtained clear solution and analysed for the drug content spectrophotometrically at 227 nm, it gives drug content for 100 mg of beads from that calculate drug content for total quantity of beads, from actual drug content, the value of encapsulation efficiency was determined using the formula given below.

Percent encapsulation efficiency =

$$= \frac{Actual\ drug\ Content\ (mg)}{Total\ mass\ of\ microcapsule} \times 100$$

Shape and size of microcapsules

The shape and size of the optimized batches of microcapsules were determined through Optical microscope with stage micrometer, and the surface morphology was studied by scanning electron microscopy.

Scanning electron microscopy (SEM)

The purpose of the SEM study was to obtain a topographical characterization of the beads. The beads were mounted on brass stubs using carbon paste. SEM photographs were taken using a scanning electron microscope (JSM-6390; Kochi) at the required magnification at room temperature. The acceleration voltage used was 5 kV, with the secondary electron image (SEI) as the detector.

Swelling determination of microcapsules

Swelling rate of microcapsules was measured as a function of percent water uptake. The beads were incubated in phosphate buffer of pH 7.4 at 37 ^{o} C at different time intervals the beads were removed and weighed after drying the excess water using filter papers. The swelling rate and the extent of swelling were determined by calculating the water uptake using the following equation.

$$\%\ water\ up\ take = 100 \times \left[\left(\frac{weight\ of\ wet\ microcapsule}{weight\ of\ dry\ microcapsule} \right) - 1 \right]$$

FTIR Spectroscopy

The interaction between the drug and various mucoadhesive polymers was studied by using the FTIR spectroscopy wherein infrared spectra of Gliclazide and various mucoadhesive polymers was taken individually first and then, were compared with the spectra of the formulations in which the drug was matrixed with various mucoadhesive polymers.

In vitro mucoadhesive strength determination of various polymers

Rotating cylinder method[9]

In this method 50 mg of the polymer was compressed into 5.0 mm diameter disc, keeping the compression pressure same for every polymer, then these discs so prepared were sticked to the freshly excised gastric mucosa of male Albino rats by just hydrating the discs with little amount of water and then the whole system was pasted on the stainless steel cylinder of USP XXVI apparatus (type 4) with the aid of the cyanoacrylate glue and the cylinder was immersed in the dissolution jar filled with phosphate buffer pH 7.4 at 37°C and was agitated at 125 rpm as shown in Fig.4 and the time for the detachment, disintegration or erosion of the test discs was monitored and recorded.

TABLE- 1 Formulation composition, production yield and encapsulation efficiency of mucoadhesive microcapsules prepared by Ionic gelation technique

Content (mg)	Formulations			
	F1	F2	F3	F4
Drug	80	80	80	80
Chitosan	100	100	100	100
Sodium alginate	100	500	700	900
Production yield (%)	76.2	82.4	86.1	89.7
Actual Drug content (%)	80.1	85.3	83.1	87.5
Encapsulation efficiency (%)	98.5	97.4	98.25	98.29

TABLE- 2 Shape and size of optimized formulations of microcapsules

Formulation	Size in µm	Shape
F2	1152.2-1160.1	Almost spherical
F4	1110.5-1112.5	Almost spherical

TABLE - 3 In vitro mucoadhesive strength determination of various polymers.

Polymer	Disk detachment time (hours) in pH 7.4 buffer ± S.D.
Chitosan	0.35 ± 0.52 (disk disintegrates)
Sodium alginate	9.00 ± 0.22

TABLE - 4 In vitro wash off test results of optimized batches

Formulation	No. of microcapsules adhered to mucosa initially	No. of microcapsules adhered to mucosa after 20 minutes			Percent Bioadhesion (Mean ± S.D)
F1	25	23	24	24	96%(0.25)
F3	25	25	25	25	100%(0.01)
F4	25	18	19	19	98%(0.42)

In vitro release study

The release of Gliclazide from mucoadhesive microcapsules was studied using USP XII dissolution test apparatus (Electrolab, India,) paddle type. Microcapsules equivalent to 50 mg of Gliclazide were taken and were enclosed in the muslin cloth and the cloth was tied with the paddle which was then immersed in the 900 ml of 0.1M HCl (pH 1.2) for initial 2 h and phosphate buffer (pH 7.4) for next 10 h at 37°C, and was rotated at the speed of 100 revolutions per minute. Sample aliquots of 5ml were withdrawn periodically and the withdrawn samples were estimated for its drug content through UV spectroscopy at 227 nm, every time a 5 ml of fresh medium maintained at 37 ^{0}C was added after the removal of each test aliquot to maintain the sink conditions. The results of the release rate were reported. The dissolution tests of all optimized batches were performed in triplicate and the dissolution profile of all the batches was fitted to zero-order, first-order, Higuchi, Korsemeyer and Peppas and the kinetic modeling of drug release.

In vitro wash off test for ionic gelation technique (Number method)10-11

In this technique male Albino rats (200-250g) were sacrificed and the intestine region was isolated, and from it the jejunum part was separated and cut longitudinally, then this separated portion was placed on the semi cylindrical Plexiglas support and was washed with saline for 30 minutes at the rate of 30 ml/minute, after that 25 number (No) of counted microcapsules were hydrated with little amount of water and were dispersed on the mucosal tissue and left on it for 20 minutes for interaction. During this period whole system was placed in a constant humidity chamber which was adjusted to 90% relative humidity. At the end of this period the system was washed with phosphate buffer pH 7.4 for 20 minutes at the rate of 22 ml/minute and the number of microcapsules detaching from the mucosal surface (N_S) were counted and their adhesive strength was determined using the formula given below.

$$\% \ adhesive \ strength = \frac{N_0 - N_s}{N_s}$$

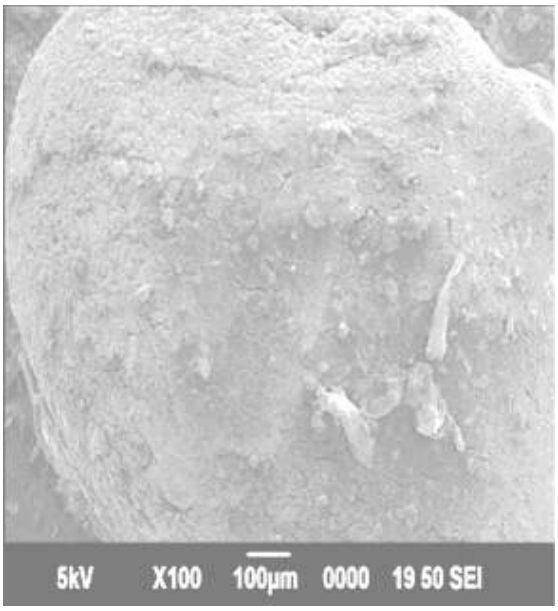

FIGURE - 1 SEM photographs showing drug loaded chitosan microcapsule (F4) and Entrapment of drug on the surface of microcapsules.

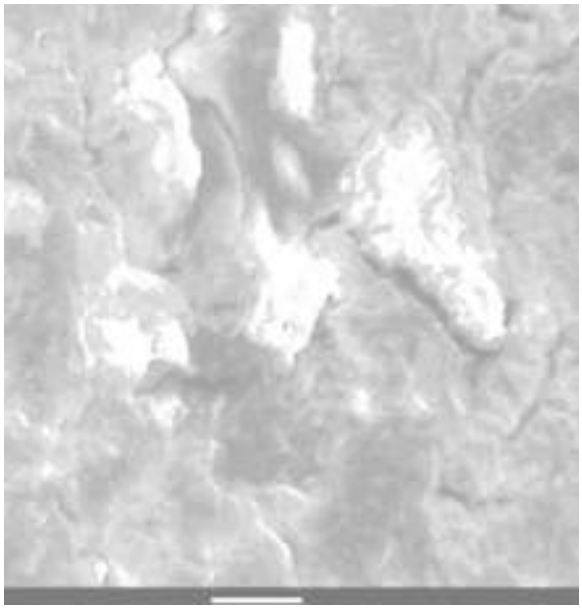

FIGURE - 2

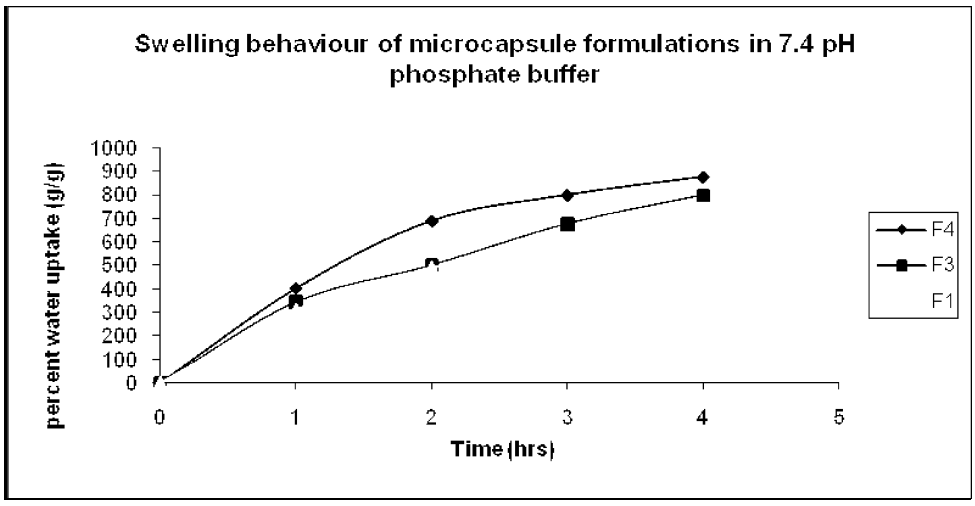

FIGURE- 3 Swelling studies of microcapsule formulations at different time intervals.

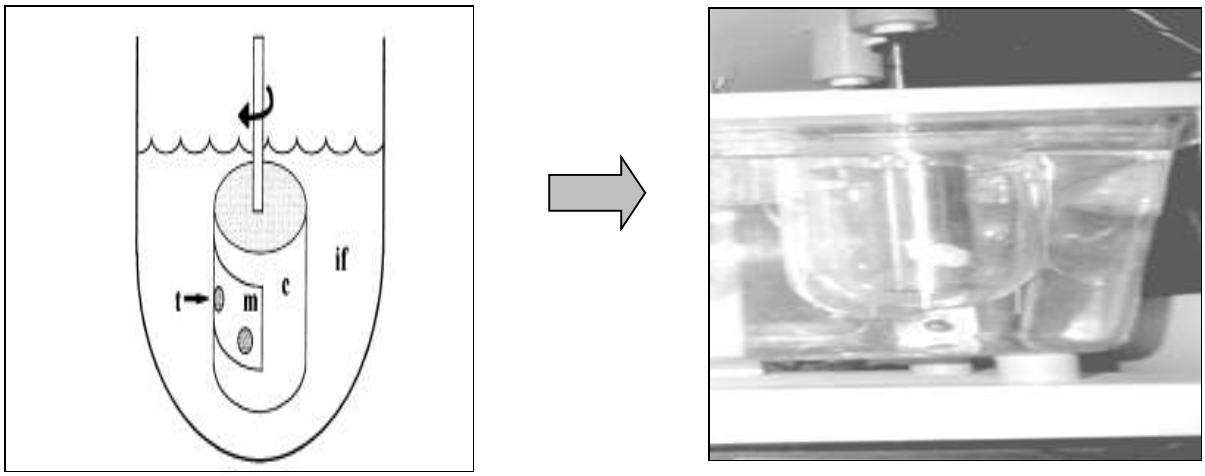

FIGURE- 4 Schematic presentation of the test system used to evaluate the mucoadhesive properties of tablets based on various polymers. C- Cylinder; if- intestinal fluid; m-rat mucosa; t-tablet.

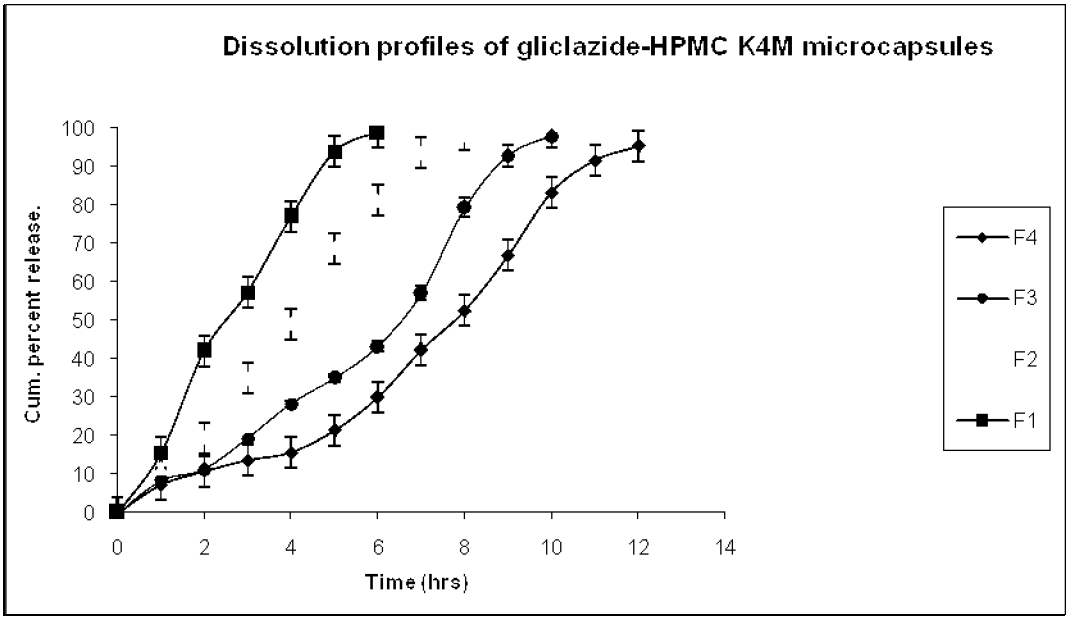

FIGURE - 4 Drug release profiles of gliclazide-Chitosan microcapsules

RESULT AND DISCUSSION

Microcapsules of gliclazide with coat consisting of various concentration alginate and mucoadhesive polymer chitosan in ratio of 1:1, 5:1, 7:1 and 9:1 (Alginate: Mucoadhesive polymer) could be prepared by ionotropic gelation technique. The microcapsules were found to be discrete, spherical, free flowing and of the monolithic matrix type. The microcapsules were completely covered with coat polymer.

Yield of production

The production yield of mucoadhesive microcapsules prepared by ionic gelation technique varied with different concentration of alginate and chitosan. It was observed that production yield of coated chitosan microcapsules were in the range of 76.2 - 89.1 percent. The result are shown in Table no. 1

This high yield of production is may be due to all the polymer is available for gelation into cross linking agent. Encapsulation efficiency and Actual drug content

It was observed that, due to water insoluble nature of gliclazide almost all the drug is entrapped in the polymer matrix resulted in higher drug content and encapsulation efficiency. Also the encapsulation efficiency of the microcapsules was dependent on mainly the concentration of sodium alginate, it was found that by increasing the concentration of sodium alginate, the encapsulation efficiency of the microcapsules also increases. Encapsulation efficiency of chitosan microcapsules it was found to be in the range of 97.12-98.56 %, as shown in Table no.1.

Shape and size of microcapsules

The microcapsules were uniform in size, with a size range of 1115-1118.3 µm for chitosan (determined by optical microscopy using stage micrometer) as shown in **Table no.2.**

Scanning electron microscopy (SEM)

Surface morphology of chitosan microcapsules is presented in **Fig no.1**. The difference in the shape of microcapsules is observed, representing that microcapsules containing higher amount of alginate (F4) are more spherical and regular as compared to that of microcapsules having lower percent of alginate (F2). Such results may be due to as the polymer (alginate) concentration increases the spherical nature of microcapsules also increases; this explains the dependence of the polymer concentration on the spherical nature of microcapsules. Insufficiency of ideal spherical morphology probably developed during drying process.

Swelling studies

From the swelling study, it was concluded that microcapsules prepared with highest alginate concentration (F4) shows highest swelling rate followed by (F3), (F2) and finally (F4) formulation. The probable reason behind this may be due to the percent water uptake of alginate increases with increase in the concentration as shown in **Fig no 2.**

In-vitro Mucoadhesive strength determination of various polymers by rotating cylinder method

Though the time based technique which was used for mucoadhesive strength determination, it was found that sodium alginate had greater mucoadhesive strength than

that of chitosan, this may be due to the greater swelling rate of alginate, which results in large surface of polymer that is expand to the mucosal layer, resulting in the increase in no. of hydrogen bonding between the polymer and mucosal layer and thus increase in the mucoadhesive strength of the polymer. The result of the *in vitro* mucoadhesive strength of various polymers determined by rotating cylinder method is shown in **Table no.3**. The schematic presentation of test system used to evaluate mucoadhesive strength of various polymers is shown in **Fig no 3.**

In vitro release studies

The *in vitro* release profile of Gliclazide from microcapsules is shown in **Fig no.4**. It was observed that with the increase in the concentration of sodium alginate the release of the Gliclazide from the polymer matrix was retarded, the less is the concentration of sodium alginate in the formulation, the faster is drug released from microcapsules, this may be due to the swelling property of sodium alginate. Hence all polymers show retardation of drug release upto 12 h with nine parts of sodium alginate, as seen from Fig. 5.

In case of chitosan coated formulation (F4), containing one part of chitosan and nine parts of sodium alginate, retardation of drug release seen up to 12 h and also has good production yield. As seen with HPMC K4M, in formulation (F3) also the release rate of the drug was found to be retarded upto 9h with one part of chitosan and seven part of alginate, seen from dissolution profile of formulation (F3). Similarly, in case of microcapsule formulations (F2) and (F1), the percent release of gliclazide is fast, but retardation is possible up to 6h and 4h respectively.

In-vitro Mucoadhesive strength determination of microcapsules by in vitro wash off test (Number method) From the *in vitro* wash off test for mucoadhesive strength determination of various formulations, it was observed that microcapsules formulation (F4) exhibits greater mucoadhesive strength than other formulations and also retard the drug release up to 12 h as shown in **Table no 4.**

CONCLUSIONS

Thus, large spherical microcapsules with coat consisting of alginate and chitosan could be prepared by ionotropic gelation technique. The microcapsules exhibit good mucoadhesive property in an *in- vitro* mucoadhesion test. Gliclazide release from these mucoadhesive microcapsules was slow and extended over longer period of time and depends on the composition of coat. Drug release was diffusion control and followed zero order kinetics. These mucoadhesive microcapsules are thus suitable for oral control release of gliclazide.

Acknowledgments

The authors would like to thanks Central Institute of Fisheries Technology, Kochi (India), for providing Chitosan as a gift sample. We also give thanks to Dr. S. J. Surana, Principal of R.C.Patel Institute of Pharmaceutical Education and Research, Shirpur, for his so much cooperation with us.

REFERENCES

1. Woo B, Jiang G, Jo Y, DeLuca P, Preparation and characterization of composite PLGA and Poly (acryloyl hydroxyl methyl starch) microsphere system for protein drug delivery, Pharm.Res.18 (2001)1600-1606.
2. Gohel M, Amin A, Formulation and optimization of controlled release diclofenac sodium microspheres using factorial design, J.Control Release, 51(1998), 115-122.
3. Ikeda K, Murata K, Kobayashi M, Noda K, Enhancement of bioavailability of dopamine via nasal route in begal dogs, Chem. Pharm. Bull. (Tokyo), 40 (1992), 2155-2158.
4. Palmer K, Brodge R, Gliclazide-an update of its pharma cological properties and therapeutic efficacy in non-insulin dependant diabetic mellitus, Drugs, 46(1993), 9125.
5. Hong, S, Lee S, Lee Y, Chung S, Lee M., Shim C, Accelerated oral absorption of gliclazide in human subjects from a soft gelatin capsule containing a PEG 400 suspension of gliclazide, J. Control. Release, 51 (1998)185-192.
6. Gonzalez- rodriguze, M., Holgado M., Sanchez- L, et al, Alginate / chitosan particulate system for sodium diclofenac release, International Journal of Pharmaceutics,232 (2002), 225-34.
7. Chowdary K.P.R, Rao Y.S, Design and *in vitro* and *in vivo* evaluation of mucoadhesive microcapsules of glipizide for oral controlled release: a technical note. AAPS PharmSciTech, 4 (2003), 39.
8. Patel J.K, Patel R.P, Amin A.F, Patel M.M, Formulation and Evaluation of Mucoadhesive Glipizide Microspheres, AAPS PharmSciTech, 6(1) (2005) Article 10.
9. Smart J.D, An *in vitro* assessment of some mucosa-adhesive dosage forms, Int. J. Pharm, 73(1) (1991), 69-74.
10. Antonios G, Nicholas A, Bioadhesive analysis of controlled release systems: An experimental method for testing the adhesion of microparticulates with mucus, Journal of Controlled Release, 12 (1990), 31-37.
11. Lehr C, Bowstra J, Tukker J, Junginger H. Intestinal transit of bioadhesie microspheres in an in situ loop in the rat, J Control release 13 (1990), 51-62.
12. Tuncel T, Bergisadi N, Akin , Otuk G, Kuscu I , In vitro and In vivo studies on microcapsules and tabletted microcapsules of cephradine, pharmazie 51 (1996),168-171.

13.Nammi S, Boini M, Lodagala S, Behra R, The juice from fresh leaves of catharantyus roseus linn, reduces blood glucose in normal and alloxan diabetic rabvbits,2003 BMC Complement. Altern. Med. 3 (2003), 1-4.