

## FORMULATION AND EVALUATION OF GLIMEPIRIDE LOADED CELLULOSE ACETATE MICROPARTICLES.

BALAGANI. PAVAN KUMAR <sup>A\*</sup>, IRISAPPAN. SARATH CHANDIRAN <sup>B</sup>, KORLAKUNTA. NARASIMHA. JAYAVEERA <sup>C</sup>

<sup>a\*</sup> Asst. Professor, Dept. of Pharmaceutics, Gokula Krishna College of Pharmacy, Sullurpet-524121, A.P, India. <sup>b</sup> Prof & Principal, Dept. of Pharmaceutics, Gokula Krishna College of Pharmacy, Sullurpet-524121, A.P, India. <sup>c</sup> Director, International Science-Tech Research Institute, Anantapur - 515 001, A.P, India.

Email: pavan.gkcp@rediffmail.com

Received: 05 Mar 2014 Revised and Accepted: 20 Mar 2014

### ABSTRACT

**Objective:** The objective of the present investigation was to formulate and evaluate microencapsulated Glimepiride produced by the emulsion – solvent evaporation method.

**Methods:** Microparticles were prepared using Cellulose Acetate by emulsion solvent evaporation method and characterized for their micromeritic properties, encapsulation efficiency, particle size, drug loading, FTIR, DSC, SEM analysis. In vitro release studies were performed in phosphate buffer (pH 7.4). Stability studies were conducted as per ICH guidelines.

**Results:** The resulting microparticles obtained by solvent evaporation method were free flowing in nature. The mean particle size of microparticles ranges from 145.26 – 187.42  $\mu\text{m}$  and encapsulation efficiency ranges from 90.38 – 96.00%. The infrared spectra and differential scanning calorimetry thermographs confirmed the stable character of Glimepiride in the drug-loaded microparticles. Scanning electron microscopy revealed that the microparticles were spherical in nature. In vitro release studies revealed that the drug release was sustained up to 12 hrs. The release kinetics of Glimepiride from optimized formulation followed zero-order and peppas mechanism. The mechanism of drug release from the microparticles was found to be non-Fickian type.

**Conclusion:** Cellulose Acetate microparticles containing Glimepiride could be prepared successfully by using an emulsion solvent evaporation technique, which will not only sustain the release of drug but also manage complicity of the diabetes in a better manner.

**Keywords:** Microencapsulation, Emulsion Solvent Evaporation, Controlled Release, Diabetes Mellitus, Sulphonyl ureas, *In vitro* Evaluation, Release kinetics.

### INTRODUCTION

Glimepiride [1-4] is the only third generation sulphonyl urea, which lowers the blood glucose level in the healthy subjects as well as in patients with type II diabetes. Its biological half life is 2-3hrs. Due to its low biological half life, it requires frequent administration in order to maintain plasma concentration. This causes inconvenience to the patient and also leads fluctuations in plasma concentration. Therefore, development of controlled release dosage forms would clearly be beneficial in terms of decreased dosage requirements, thus increase patient compliance. Microencapsulation is a well known method for the preparation of microparticles for controlled release. Among the various methods developed for formulation of microparticles, solvent evaporation method is one of the mostly widely used one to formulate microparticles because of its ease of fabrication without compromising the activity of drug [5]. In the present investigation Cellulose Acetate<sup>6</sup> is used as a rate retardant polymer. It is widely used as a wall material for controlled release microparticles. In the present investigation solvent evaporation method was employed with an objective of developing microparticles for oral controlled release and subjected for evaluation in terms of drug content, encapsulation efficiency, size analysis, compatibility studies and *In-vitro* release studies.

### MATERIALS AND METHODS

#### Materials

Glimepiride was obtained as gift sample from Medley Pharmaceuticals Ltd., Daman Unit. Cellulose Acetate (Himedia; Mumbai, India), Acetone, liquid paraffin (Qualigens; Mumbai, India), tween 80 (Rankem, New Delhi, India), and the chemical reagents used were of analytical grade.

### Methods

#### Preparation of Microparticles

The microparticles were prepared by emulsion solvent evaporation technique<sup>7</sup>. Glimepiride microparticles were formulated by varying the drug and polymer ratios. Weighed amount of drug and polymer were dissolved in 10ml of acetone. The organic solution was then slowly added to 100ml of liquid paraffin containing 1% surfactant (tween 80) with constant stirring for 1h. The resulting microparticles were separated by filtration and washed with petroleum ether. The microparticles finally air dried over a period of 12 hrs and stored in a dessicator.

#### Characterization of Microparticles

##### Yield of Microparticles [8]

Microparticles recovered at the end of preparation were weighed and the yield was calculated as a percentage of the total amounts of polymer and drug added during the preparation of microparticles.

##### Flow properties [9]

##### Angle of Repose

Angle of repose is defined as the maximum angle possible between the surface of the pile of the powder and the horizontal plane. The flow characteristics of different microcapsules were studied by measuring the angle of repose employing fixed funnel method. The angle of repose was calculated by using the following formula.

$$\tan \theta = \frac{\text{Height of the pile}}{\text{radius of the base of the pile}}$$

where  $\theta = \tan^{-1}(h/r)$   $\theta$  = angle of repose.

### Bulk Density & Tapped Density

Bulk density and tapped density were measured by using 10 ml of graduated cylinder. The pre weighed sample was placed in a cylinder; its initial volume was recorded (bulk volume) and subjected to tapings for 100 times. Then the final volume (tapped volume) was noted down. Bulk density and tapped density were calculated from the following formula.

$$\text{Bulk Density} = \frac{\text{mass of microparticles}}{\text{bulk volume}}$$

$$\text{Tapped Density} = \frac{\text{mass of microparticles}}{\text{tapped volume}}$$

### Carr's Index

Compressibility index (CI) or Carr's index value of microparticles was computed according to the following equation:

$$\text{Carr's Index (\%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

### Hausner's Ratio

Hausner ratio of microspheres was determined by comparing the tapped density to the bulk density using the equation

$$\text{Hausner's Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

### Size Distribution and Size Analysis

For size distribution analysis, 250 mg of the microparticles of different sizes in a batch were separated by sieving, using a range of standard sieves. The amounts retained on different sieves were weighed. The mean particle size of the microparticles was calculated by the formula [10].

$$\text{Mean Particle Size} = \frac{\sum (\text{Mean Particle Size of the Fraction} \times \text{Weight Fraction})}{\sum (\text{Weight Fraction})}$$

### Estimation of drug content

An accurately weighed portion of microparticles equivalent to 5 mg of Glimepiride were weighed and transferred in to a mortar. Powdered and dissolved in 100 ml of pH 7.4 phosphate buffer, suitably diluted, the absorbance of the resulting solution was measured at 236 nm [11].

### Entrapment Efficiency [12]

Entrapment efficiency was calculated using the formula

$$\text{Entrapment efficiency} = \frac{\text{Estimated percent drug content}}{\text{Theoretical percent drug content}} \times 100$$

Estimated percent drug content was determined from the analysis of microparticles and the theoretical percent drug content was calculated from the employed core: coat ratio in the formulation of microparticles.

### Morphological Characterization by SEM

Morphology and surface characteristics were studied by Scanning Electron Microscopy. The samples for the SEM analysis were prepared by sprinkling the microparticles on one side of the double adhesive stub. The stub was then coated with fine gold dust. The microparticles were then observed with the scanning electron microscope (Leica Electron Optics, Cambridge, USA) at 10 kv.

### Fourier Transform Infrared Spectroscopy (FTIR) studies

The pure drug and optimized formulations were subjected for FTIR analysis. The samples were scanned over a range of 4000-400  $\text{cm}^{-1}$

using Fourier transformer infrared spectrophotometer. Spectra's were analyzed for drug polymer interactions.

### Differential Scanning Calorimetry (DSC) studies

The pure drug and optimized formulation were subjected to differential scanning calorimeter equipped with an intra cooler (NETZSCH, Japan.). Indium/zinc standards were used to calibrate the DSC temperature and enthalpy scale. The sample were sealed in aluminum pans and heated at a constant rate 20°C/min over a temperature range of 20-250°C. An inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 ml/min.

### Drug Release Studies [13]

Release of Glimepiride from the microparticles, was studied in phosphate buffer of pH 7.4 (900 ml) using Eight Station Dissolution Rate Test Apparatus (M/s. Electrolab) with a paddle stirrer at 100 rpm and at 37°C  $\pm$  0.5°C. A sample of microparticles equivalent to 5 mg of Glimepiride was used in each test. Samples were withdrawn through a filter (0.45) at different time intervals and were assayed at 228 nm for Glimepiride using Shimadzu double beam UV spectrophotometer. The drug release experiments were conducted in triplicate.

### Dissolution kinetics [14]

The rate and the mechanism of release of Glimepiride from the prepared microparticles were analyzed by fitting the dissolution data into, zero-order equation,  $Q = Q_0 - k_0 \cdot t$  (1), where Q is the amount of drug released at time t, and  $k_0$  is the release rate. First order equation,  $\ln Q = \ln Q_0 - k_1 \cdot t$  (2), where  $k_1$  is the release rate constant and Higuchi's equation,  $Q = k_2 \cdot t^{1/2}$  (3) where Q is the amount of the drug released at time t and  $k_2$  is the diffusion rate constant. The dissolution data was further analyzed to define the mechanism of release by applying the dissolution data following the empirical equation,

$$\frac{M_t}{M_0} = \frac{1}{\alpha} \times K t^n \quad (4),$$

where  $M_t/M_0$  is the fraction of drug released at time t. K is a constant and n characterizes the mechanism of drug release from the formulations during dissolution process.

### Stability study

The formulation was subjected to accelerated stability studies as per ICH (The International Conference of Harmonization) guidelines. The optimized formulation was sealed in an aluminum foil and stored at 25  $\pm$  2°C, 60  $\pm$  5% RH and at 40  $\pm$  2 °C, 75  $\pm$  5% RH for 3 months [15]. Microparticles were periodically removed and evaluated for physical characteristics and in-vitro drug release.

## RESULTS AND DISCUSSION

Glimepiride loaded Cellulose Acetate microparticles were successfully formulated by emulsion solvent evaporation method. In these formulations, tween 80 is used as surfactant and the optimum concentration of tween 80 is 1% w/v. A total number of four batches were formulated by varying the polymer concentration. The detailed composition of microparticles was shown in Table 1.

These microparticles were evaluated for their percentage yield, flow properties, size analysis, percent drug content, percent encapsulation efficiency and morphological characterization, FTIR studies, DSC analysis, Invitro release studies and stability studies. The angle of repose values of all the formulations were found to be in the range of 20.67 – 26.50, i.e. less than 30, which shows their free flowing nature of the prepared microparticles. Bulk density and tapped density showed good packability of the microparticles. Carr's index ranges from 13.23 % to 16.72%, indicating excellent compressibility. Hausner's ratio ranges from 1.13 to 1.21, i.e. all the formulations showed that they had good flow properties. The results were depicted in Table No. 2. The particle size analysis reveals that, with the considerable increase in the concentration of Cellulose Acetate, the mean particle size of microparticles also increased. The results were shown in Table No. 2.

Table 1: Composition of Glimepiride Microparticles.

Ingredients	Formulations			
	F 1	F 2	F 3	F 4
Glimepiride (gm)	1	1	1	1
Cellulose Acetate (gm)	1	1.5	2	3
Acetone (ml)	10	10	10	10
Tween 80 %	1	1	1	1
Liquid Paraffin(ml)	100	100	100	100

Table 2: Micromeritic Properties of Glimepiride Microparticles.

Formulation code	Angle of repose ( $\theta$ )	Bulk Density ( $\text{g}/\text{cm}^3$ )	Tapped Density ( $\text{g}/\text{cm}^3$ )	Carr's Index	Hausner's Ratio	Average Particle Size ( $\mu\text{m}$ )
F 1	26.50	0.784	0.941	16.72	1.21	145.26
F 2	24.62	0.796	0.939	15.24	1.18	159.34
F 3	22.80	0.912	1.069	14.71	1.16	172.48
F 4	20.67	0.927	1.189	13.23	1.13	187.42

Table 3: Percentage yield, percent drug content and percent encapsulation efficiency of Glimepiride Microparticles.

Formulation code	% Yield	% Drug Content	% Encapsulation Efficiency
F 1	86.5	45.19	90.38
F 2	94.4	36.62	91.55
F 3	96.33	31.40	94.29
F 4	97.75	24.01	96.00

Table 4: Release Kinetics of Glimepiride Microparticles.

Formulation Code	Correlation Coefficient Values ( $R^2$ )				Release Rate Constant ( $\text{mg}/\text{hr}$ ) $K_0$	$t_{50\%}$	$t_{90\%}$	Wall Thickness ( $\mu$ )	n value
	Zero Order	First Order	Higuchi Model	Peppas Model					
F 1	0.9997	0.8721	0.9296	0.9998	0.98	4.08	8.46	26.42	0.9817
F 2	0.9997	0.8535	0.9276	0.9990	0.94	4.25	7.65	37.28	0.9943
F 3	0.9998	0.8160	0.9223	0.9990	0.84	4.76	8.57	46.14	1.0301
F 4	0.9997	0.7916	0.9214	0.9942	0.72	5.55	10	48.52	1.1035

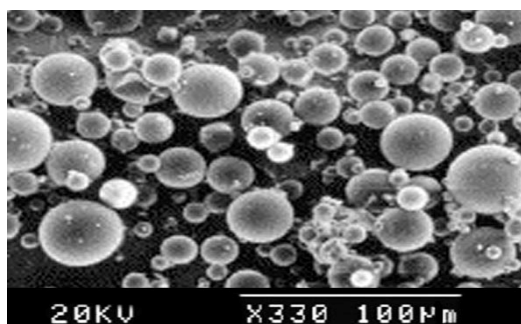


Fig. 1: SEM Photograph of Glimepiride Microparticles.

It was identified that, as the polymer ratio and the product yield directly proportional to each other. The % yield varies from 86.5 to 97.75%. The % drug content in the microparticles was found to be 24.01 to 45.19. The % drug content decreases with increase in polymer concentration. The % of encapsulation efficiency ranges from 90.38 to 96.00. The encapsulation efficiency increases with the increase in polymer concentration. The yield of microparticles, % drug content and encapsulation efficiency data were shown in Table 3. The SEM studies clearly showed that the obtained microparticles exhibit good spherical nature. Scanning electron microscopic photographs of microparticles are shown in Fig. 1. Glimepiride shows prominent peaks at wave numbers were 3323.07 (N-H), 2941.73 (C-H), 2853.82 (O-H), 1441.17 and 1524.46 (N=O), 1156.37 (C-N), 1031.83 (C-O). The spectra of optimized microparticles exhibited all the principle peaks present in the Glimepiride pure drug which indicates the stable nature of the drug during encapsulation. The FTIR Spectra's are shown in Fig. 2.

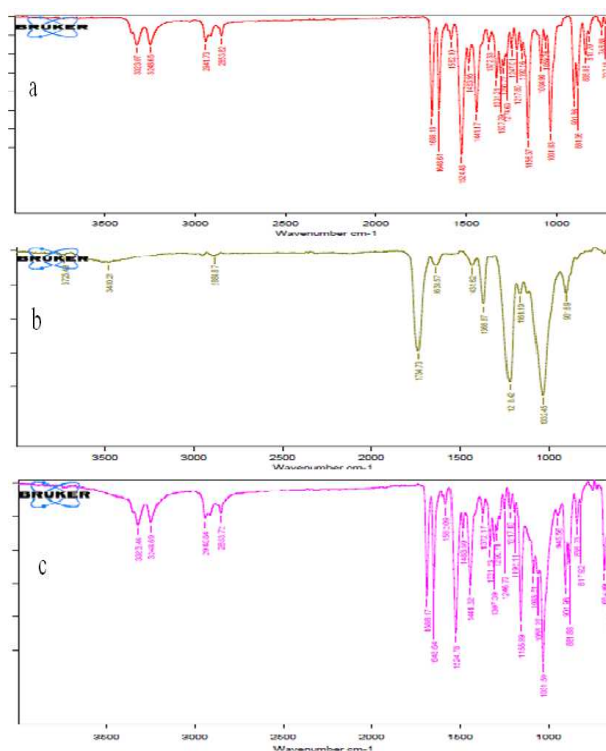


Fig. 2: FTIR Spectra of (a).Glimepiride, (b). Cellulose Acetate, (c). Glimepiride Microparticles

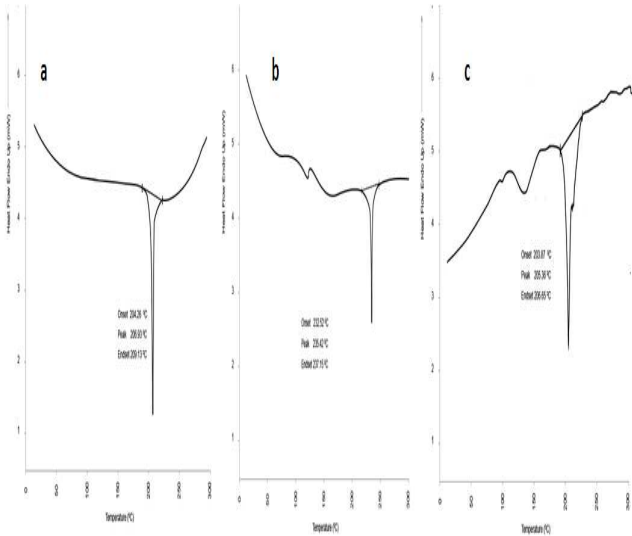


Fig. 3: DSC thermograms of (a).Glimpiride, (b). Cellulose Acetate, (c). Glimpiride Microparticles

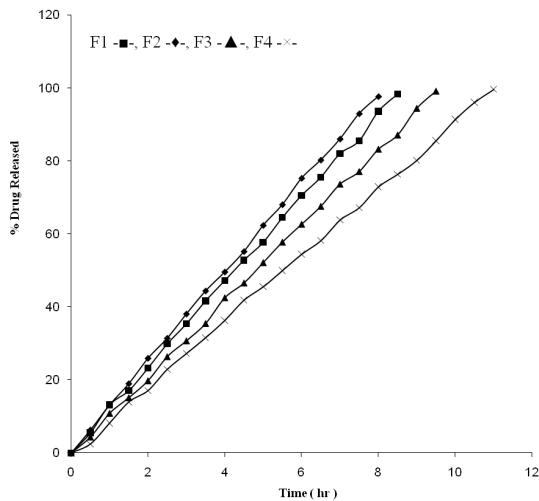


Fig. 4: Release Profiles of Glimpiride Microparticles

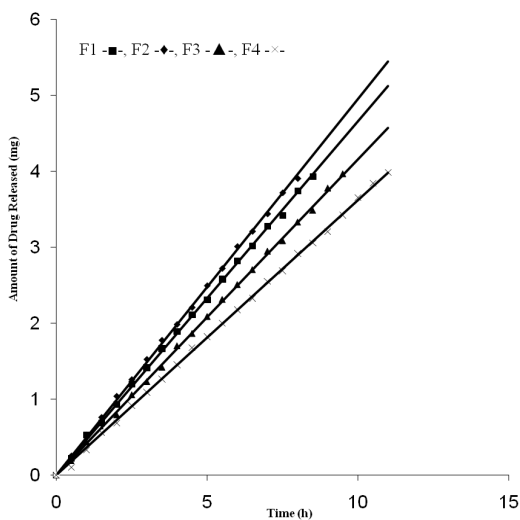


Fig. 5: Zero order Plots of Glimpiride Microparticles

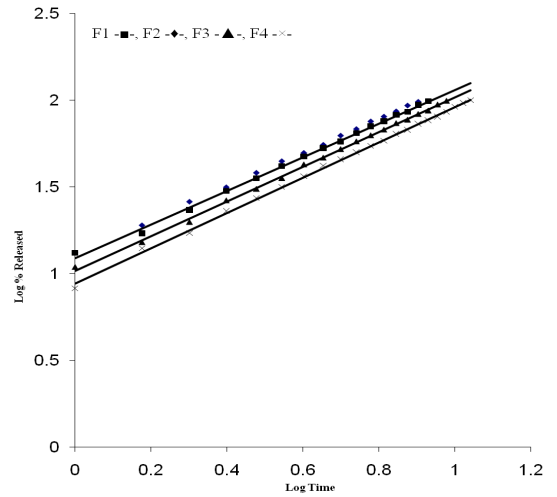


Fig. 6: Peppas Plots of Glimpiride Microparticles

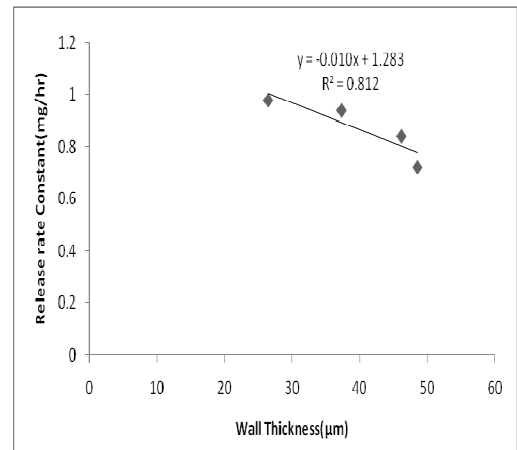


Fig. 7: Correlation ship between Wall Thickness and Release Rate Constant of Glimpiride Microparticles Prepared with Cellulose Acetate.

The Glimpiride thermal curve shows a sharp peak at 206.93°C which corresponds to its melting point. The optimized formulation showed an endothermic peak at 205.36°C, thus the results revealed that there were no major interactions between the drug and the polymer during microencapsulation process. The DSC thermograms were shown in Fig. 3.

The Glimpiride microparticles were subjected to In-vitro release studies by employing 7.4 pH phosphate buffer and the drug release profiles were shown in Fig. 4. When the amount of drug release values are plotted against time straight lines were obtained in all the cases indicating that the rate of drug release from these microparticles followed zero order kinetics and the graphs are shown in Fig. 5. To ascertain the mechanism of drug release from various microparticles plot of log % released vs log time (peppas plots) were drawn. The plots were found to be linear with all formulations. The peppas plots are shown in Fig. 6. Release Kinetic studies of Glimpiride microparticles were shown in Table 4. The exponential coefficient (n) values were found to be in between 0.9817 to 1.1035 indicating non fickian mechanism. These results indicated that the release rate was found to decrease with increase in concentration of coating material applied. The wall thickness of microparticles was found to be increased with the increase in concentration of coating material applied. A good correlation ship sustained in between wall thickness and release rate constant and the graphs were shown in Fig. 7.

The stability studies were carried out for the prepared microparticles. After 3 months storage of formulations at  $30 \pm 2^\circ\text{C}$ ,  $65 \pm 5\%$  RH and  $40 \pm 2^\circ\text{C}$ ,  $75 \pm 5\%$  RH, values of all parameters like percentage of drug content and encapsulation efficiency were evaluated and found to be almost similar to the initial values. The drug dissolution profile was similar to the initial profile. There was no significant change in any value and also no changes in the physical appearance. So it could be concluded that Glimepiride microparticles prepared with Cellulose Acetate is stable.

#### CONCLUSION

Cellulose Acetate microparticles containing Glimepiride was prepared successfully by using an emulsion solvent evaporation method. By varying the drug: polymer ratios, is found to influence the size, entrapment efficiency and release characteristics of the microparticles. The assessment of the release kinetics revealed that drug release from microparticles was found to be non-Fickian type. Controlled release without initial peak level achieved with these formulations may reduce frequency and improves patient compliance.

#### ACKNOWLEDGMENT

The authors are thankful to Shri C. Srinivasa Baba, Shri G. Brahmaiah and Shri M.M. Kondaiah Management of Gokula Krishna College of Pharmacy, Sullurpet, SPSR Nellore Dist, A.P, India for availing the laboratory facilities during the course of research studies.

#### CONFLICT OF INTEREST

We declare that we have no conflict of interest

#### REFERENCES

1. Indian Pharmacopoeia, Vol I, Govt. of India, Ministry of Health & Family Welfare, New Delhi, The controller of Publications, 2007; 1418-19.
2. Hamaguchi T, Hirose T, Asakawa H. "Efficacy of glimepiride in type 2 diabetic patients treated with glibenclamide". *Diabetes Res. Clin. Pract.* 2004, 66 (1): S129-32.
3. Davis SN. "The role of glimepiride in the effective management of Type 2 diabetes". *J. Diabetes Complicat.* 2004, 18 (6): 367-76.
4. Nissen SE, Nicholls SJ, Wolski K. "Comparison of pioglitazone vs glimepiride on progression of coronary atherosclerosis in patients with type 2 diabetes: the periscope randomized controlled trial". *JAMA*, 2008, 299 (13): 1561-73.
5. Behera. BC, Sahoo. SK, Dhal. S, Barik. BB, Gupta. BK., Characterization of Glipizide-Loaded Polymethacrylate Microspheres Prepared By an Emulsion Solvent Evaporation Method. *Tropical Journal of Pharmaceutical Research*, March 2008; 7 (1): 879-885.
6. Daugherty PD, Nause RG. Cellulose acetate. In, Ray C Rowe, Paul J Sheskey, Paul J (ed). *Hand Book of Pharmaceutical excipients*, 6<sup>th</sup> edition, London, Pharmaceutical press, 2009; 141-43.
7. Chowdary. KPR, Srinivasa Rao.Y. Design and in vitro and in vivo evaluation of mucoadhesive microcapsules of glipizide for oral controlled release: A technical note. *AAPS PharmaSciTech* 2003, 4(3): 87-92.
8. Hazedar S, Dortunc B. Preparation and in vitro evaluation of Eudragit® microspheres containing acetazolamide. *International journal of pharmaceutics* 2004; 269: 131-140.
9. Parul Trivedi, AML Verma, N Garud. Preparation and characterization of Aceclofenac Microsphere. *Asian Journal of Pharmaceutics*. 2008; 2(2); 110-115.
10. Gohel MC, Amin AF. Formulation and optimization of controlled release diclofenac sodium microspheres using factorial design. *J Control Release*, 1998; 51: 115-122.
11. Pandey Abhishek, Bhadoria Vivek singh. Formulation development & optimization of Glimepiride microspheres using ionotropic gelation technique. *Pharmacia*. 2011; 1:67-72.
12. Lin WJ and Wu TL. Modification of the initial release of a highly water soluble drug from ethyl cellulose microspheres prepared by emulsion solvent evaporation method. *Journal of Microencapsulation*. 1999; 16: 639-646.
13. Sai Kishore V, Gopala krishna murthy TE, Pavan Kumar A, Satyanaryana J. Formulation and evaluation of mucoadhesive microcapsules of Glimepiride. *Res J Pharm Tech*. 2011; 4: 739-743.
14. Salomon C J, Bravo S A, Lamas M A. In-Vitro Studies of Diclofenac Sodium Controlled-release from Biopolymeric Hydrophilic Matrices. *Journal of Pharmacy and Pharmaceutical Sciences*. 2002; 5(3): 213-218.
15. Kamlesh D, Shailendra S, Swarnlata S. Effect of processing variables and in-vitro study of microparticulate system of nimesulide. *Brazilian J of Pharm Sci*. 2007, 43: 555-562.