Available online at http://www.tjpr.org

Research Article

Characterization Of Glipizide-Loaded Polymethacrylate Microspheres Prepared By An Emulsion Solvent Evaporation Method

BC Behera*[a], SK Sahoo[a], S Dhal[a], BB Barik[a], BK Gupta[b]

[a]University Department of Pharmaceutical Sciences, Utkal University, Vani-Vihar, Bhubaneswar, Orissa. Pin-751004.
[b]Dept. of Pharmaceutical Technology, Jadavpur University Kolkata, India

Abstract

Purpose: The purpose of the present investigation was to formulate and evaluate microencapsulated glipizide produced by the emulsion – solvent evaporation method,

Method: Microspheres were prepared using polymethacrylate polymers (Eudragit® RS 100 and RL 100) by solvent evaporation method and characterized for their micromeritic properties and drug loading, as well as by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy. In vitro release studies were performed in phosphate buffer (pH 7.4).

Result: The resulting microspheres obtained by solvent evaporation method were white and free flowing in nature. The mean particle size of microspheres ranged from 420 - 660 μm and the encapsulation efficiencies ranged from 40.27 - 86.67 %. The encapsulation efficiency was also found to be dependant on nature of polymer used in the formulation. The infrared spectra confirmed the stable character of glipizide in the drug-loaded microspheres. Scanning electron microscopy revealed that the microspheres were spherical in nature. From the in vitro drug dissolution studies it was found that the sustaining effect of microspheres depended on the polymer concentration, amount of dispersant used and the type of polymer used in the formulation. The mechanism of drug release from the microspheres was found to be non-Fickian type.

Conclusion: Eudragit® microspheres containing glipizide could be prepared successfully by using an emulsion solvent evaporation technique using polymethacrylate polymers, which will not only sustain the release of drug but also manage the complicacy of the diabetes in a better manner.

Keywords: glipizide, Eudragit®, microspheres, controlled release, polymethacrylate.

---

*Corresponding Author: E-mail:: bhupenbehera2003@yahoo.com. Phone No: 09437149114

INTRODUCTION

Microspheres have been widely accepted as a means to achieve oral[1] and parenteral controlled release[2] drug delivery system. The microsphere requires a polymeric substance as a carrier and a core material. Among the various methods developed for formulation of microspheres, the solvent evaporation method has gained much attention due to its ease of fabrication without compromising the activity of drug. In the present investigation, Eudragit® RS 100 and Eudragit® RS 100 in combination with RL 100 microsphers were used as encapsulation materials. Eudragit ® RS 100 and Eudragit® RL 100 are referred to as ammoniomethacrylate copolymers, with the former having 5% functional quaternary ammonium groups and the latter having 10% functional quaternary ammonium groups. Eudragit® RS 100 is a water-insoluble polymer that is widely used as a wall material for sustained release microcapsules [3]. This is due to its biocompatibility, good stability, easy fabrication and low cost. The drug of choice, glipizide, is an effective antidiabetic drug particularly in Type II diabetes (Non-insulin dependent diabetes mellitus). It is a second-generation sulfonylurea that actually lowers the blood glucose level in human by stimulating the pancreatic cell and thereby releasing the insulin. It has a short biological half-life of 3.4 ± 0.7 h[2, 4] which make it more suitable to be designed as a controlled release formulation. The main purpose of the present research was to develop a controlled drug delivery system of glipizide for per-oral administration using biocompatible Eudragit® polymers in order to increase its biological half and to determine the influence of formulation and preparation variables on microparticle characteristics, such as drug incorporation and *in vitro* drug release rate.

MATERIALS AND METHODS
Materials
Glipizide was received as a gift from M/s Stadmed Private Ltd, Kolkata, India. Eudragit® RS 100 and RL 100 were obtained from Röhm Pharma, GmbH, Darmstadt, Germany. All other reagents and solvents used were of pharmaceutical or analytical grade.

Method
Glipizide microspheres were prepared by solvent evaporation techniques[5-7]. Different amounts of Eudragit® RS or Eudragit® RS / RL combination were dissolved in 8.5 ml acetone separately by using a magnetic stirrer (Remi Equipments, Mumbai, India, model 2MIH). The core material, glipizide, was added to the polymer solution and mixed for 15 minutes, followed by magnesium stearate (100mg) and then mixed thoroughly. The resulting dispersion was added in a thin stream to a mixture of 90 ml light liquid paraffin and 10 ml n-hexane contained in a 250 ml beaker, while stirring at 700 rpm using a mechanical stirrer (Remi Motors, Model No.RO-123R, Mumbai, India.). Stirring was continued for 3 h until the acetone evaporated completely. The microspheres formed were filtered using Whatman no.1 filter paper. The residue was washed 4-5 times with 50 ml portions of n-hexane. The product was then dried at room temperature for 24 hours. Formulations containing drug: polymer ratio 1:1.5,1:2 and 1:3 were coded F1, F2 and F3, respectively, while the formulation with a Eudragit® RS: RL combinations of 0.9:0.1 (drug: polymer ratio1: 3) was codeed F4. A Eudragit® RS formulation in a drug: polymer ratio of 1:3 and containing 50 mg magnesium stearate coded F_5 .All batches were prepared in triplicate.

Micromeritic properties of beads
The flow properties of the microspheres were investigated by measuring the bulk density, tapped density and Carr's index[1, 3]. The bulk and tapped densities were measured in a 10 ml graduated measuring cylinder. The sample contained in the measuring cylinder was tapped mechanically by means of constant velocity rotating cam. The initial bulk volume and final tapped volume were noted from which, their respective densities were calculated. Carr's index was determined by using the following formula:

Carr's index =[(Tapped density-bulk density) / Tapped density] X 100

Table 1: Yield, mean microsphere size, entrapment efficiency, Carr's Index and release mechanism of glipizide microspheres

Batch code	Yield (%)	Mean particle size (μm)	Entrapment efficiency (%)	Carr's Index	Korsemeyer-Peppas model	
					r^2	n
F_1	89.2	420	40.3±1.16	10.3	0.997	0.651
F_2	88.4	459	54.2± 0.74	9.6	0.995	0.587
F_3	80.8	513	83.4±1.46	8.5	0.985	0.782
F_4	79.6	505	78.1±1.66	12.8	0.983	0.764
F5	99.0	661	86.7± 1.49	16.0	*	*

Note: n = diffusion exponent and r^2 = correlation coefficient related to mechanism of drug release according to equation, $M_t / M\alpha = Kt^n$,
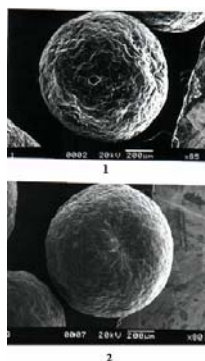**Value not determined.*

Fig 1: SEM photographs of Eudragit® RS 100 microspheres, before (1) and after dissolution
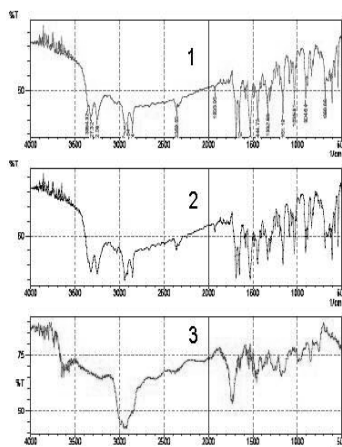

Fig. 2: FTIR Spectra of pure glipizide (1), Eudragit® RS 100 loaded microspheres (2), Eudragit® RS 100 blank microsphere (3).

Mean particle size

The mean particle sizes of the beads were determined by sieving method[1,3] Microspheres were separated into different size fractions by sieving for 10 minutes using mechanical sieve shaker (Cuprit Electrical Co. India) containing standard sieves having apertures of 1000, 710, 500, 355, 250 & 180 μm (Indian Pharmacopoeia, 1996). The particle size distribution of the microspheres for all the formulations was determined and mean particle size of microspheres was calculated by using the following formula.

$$\text{Mean Particle size} \ = \ \frac{\Sigma \ (\text{Mean particle size of the fraction X weight fraction})}{\Sigma \ \text{Weight fraction}}$$

Determination of glipizide

About 50 mg microspheres were accurately weighed and added to 50 ml of phosphate buffer (pH 7.4)**.** The resulting mixture was agitated on a mechanical shaker for 24 h. The solution was then filtered and the drug content was measured at 275.2 nm spectrophotometrically (Systronic 2101 UV-Visible double beam spectrophotometer, India) after suitable dilution.

FTIR Study

FTIR spectra of glipizide, blank microspheres and drug-loaded microspheres were obtained in KBr pellets using a Perkin-Elmer model 883 Spectroscope in the ranges, 500 to 4000 cm^{-1}.

Scanning electron microscopy (SEM)

A scanning electron microscope (JEOL, JSM-6360) was used to characterize the surface topography of the microspheres after gold coating.

Drug release behavior [8]

The *in vitro* dissolution studies were carried out in 500 ml of phosphate buffer, pH 7.4, maintained at 37 ± 0.5° and 100 rpm by using United States Pharmacopoeia basket type dissolution test apparatus (LABINDIA, Disso-2000, Mumbai, India) under sink conditions. Accurately weighed samples of the microspheres were added to the dissolution medium and at preset time intervals, 2 ml aliquots were withdrawn and replaced with an equal volume of fresh dissolution medium. After suitable dilution, the samples were analyzed spectophotometrically at 275.2 nm. The concentration of glipizide in test samples was corrected and calculated using a regression equation of the calibration curve. The dissolution studies were carried out in triplicate and the mean values were plotted as percentage cumulative release versus time.

Kinetics of drug release

In order to understand the mechanism drug release, the first 60 % of drug release was fitted to the Korsmeyer-Peppas model [9].

$$M_t / M_\alpha = k \ t^{\ n}$$

Where $M_t / M\alpha$ is the fraction of the drug released at time, t, k is the rate constant and 'n' is the release exponent. The n value is used to characterize different release mechanisms and is calculated from the slope of the plot of log of fraction of drug released vs log of time.

RESULTS

The resulting microspheres formulated by solvent evaporation method was found to be spherical and free flowing in nature. The mean particle size of microspheres ranged from 420 - 660 μm (see Table 1). It was noticed that mean particle size increased with increase in polymer concentration and decrease in magnesium stearate concentration. The encapsulation efficiencies ranged from 40.27 - 86.67 %. The encapsulation efficiency was also found to be dependant on nature of polymer used in the formulation. From the in vitro drug dissolution studies it was found that the sustaining effect of microspheres
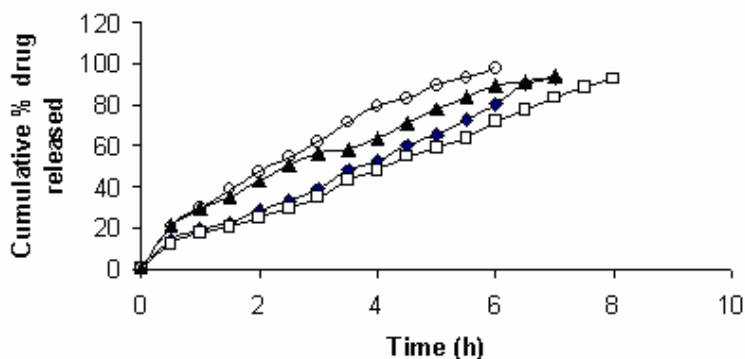
Fig. 3: Effect of polymer concentration on cumulative % drug release. Drug release profile from formulation F1 (—◇—), F2 (—▲—), F3 (—□—), F4 (—◆—).
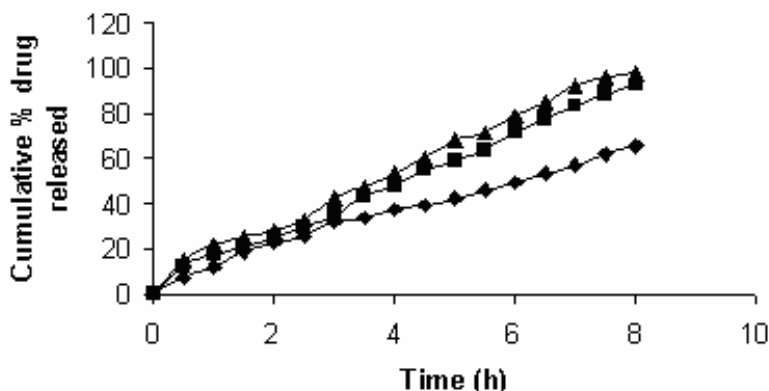

Fig. 4: Effect of dispersant concentration and particle size on release of glipizide from formulation F3-50 (—◆—), F3 # 30 (—■—) and F3 # 44(—▲—).

depended on the polymer concentration, amount of dispersant used and the type of polymer used in the formulation.

DISCUSSION

Increase of mean particle size with increase in polymer concentration may have occurred due to the fact that as polymer concentration increases it produces a significant increase in the viscosity in a fixed volume of solvent, thus leading to an increase of the emulsion droplet size and finally a higher microsphere size. [10-11]

The flow properties of the microspheres are expressed in terms of Carr's Index (Table 1.). The Carr's index for all formulations was less than 16, which indicate excellent flow properties and suggests that the microspheres can be easily handled during processing

The encapsulation efficiency of the drug depended on the solubility of the drug in the solvent and continuous phase. An increase in the concentration of polymer in a fixed volume of organic solvent resulted in an increase in encapsulation efficiency[12]. Generally, the encapsulation efficiencies of the microspheres prepared with Eudragit® RS 100 were higher than those of microspheres prepared with the Eudragit® RS/RL combination. This can be attributed to the high content of the ammonium group in Eudragit® RL, which

might have facilitated the diffusion of some of the entrapped drug to the surrounding medium during formation of the microspheres. Higher entrapment of drug was noticed when a lower concentration of magnesium stearate was used. This may be due to the formation of large-size microspheres at lower magnesium stearate levels, which provided less surface area for drug escape to the external processing medium.

Scanning electron microscopic photographs of microspheres are shown in Fig. 1. The surface of the microspheres appeared spherical but rough. The microsphere were not agregated.

Glipizide showed prominent peaks at 1651, 2943, 3354, 1529 and 1689 due to the presence of C = N aliphatic group, C-H$_2$ aliphatic, N-H stretching of NH$_2$, C-H aliphatic and C=O stretching (Fig. 2). The same peaks were also observed in the formulation indicating the stable nature of the drug during encapsulation.

Fig. 3 shows that the release of glipizide from the microspheres and it illustrates that the rate of drug release from the microspheres depended on the polymer concentration and the type of polymer used. The decrease in release rate with increasing content of the polymer can be explained by a decreased amount of drug present close to the surface and also by the fact that the amount of uncoated drug decreases with increase in polymer concentration[13]. When Eudragit® RL was used in combination with Eudragit RS, the drug released at a faster rate compared to Eudragit® RS alone. This is due to the fact that the amount of quaternary ammonium groups of Eudragit® RS is lower than that of Eudragit RL, which renders Eudragit® RS is less permeable[14]. The effect of magnesium stearate on drug release rate is shown in Fig. 4 .The faster drug release dispersing agent concentration rose may be due to decrease in particle size, which provided a larger surface for drug release. The effect of particle size on the drug release was also studied using microspheres of different size fractions (355 and 500 µm, respectively) as shown in Fig. 4. The release profile was in line with the theory of the effect of the particle size on dissolution

rate [1]. As microsphere size decreased, the drug release increased as result of higher surface area.

In order to determine the mechanism of drug release the data obtained were fitted to the Korsemeyer-Peppas model in order to determine the 'n' value, which describes the drug release mechanism[9]. The 'n' value of all the formulations was between 0.5 and 1 indicating that the mechanism of drug release was non-Fickian type diffusion.

CONCLUSION
Eudragit® microspheres containing glipizide can be prepared successfully by using an emulsion solvent evaporation technique. The surface structure of the microspheres was spherical and rough. The encapsulation efficiencies were over 40 % and the mean size was in the range of 420 - 660 µm. The release rate of Eudragit® RS 100 microspheres was much slower than that than those prepared with the combination of Eudragit® RS/RL 100. The release pattern of the microspheres was found to be of the non-Fickian.

ACKNOWLEDGEMENT

REFERENCES
1   Sahoo SK, Mallick AA, Barik BB, Senapati PC. Preparation and in vitro evaluation of ethyl cellulose microspheres containing stavudine by the double emulsion method. Pharmazie 2007; 62: 117-121
2   Chowdary KPR, Koteshwara RN, and Malathi K. Ethyl cellulose microspheres of gliplizide: characterization, in vitro and in vivo evaluation. Indian Journal of pharmaceutical sciences 2004; 66: 412- 416.
3   Sahoo SK, Mallick AA, Barik BB, Senapati PC. Formulation and in vitro Evaluation of Eudragit® Microspheres of Stavudine. Tropical Journal of Pharmaceutical Research 2005; 4: 369-375
4   Kahn CR, Shechter Y. Oral hypoglycemic agents and the pharmacology of the endocrine

pancreas. In: Theodore WR, Alan SN, Taylor P, Gilman AG (eds) Goodman and Gilman's The Pharmacological Basis of Therapeutics. ed 8. New York, McGraw-Hill, 1991,pp1484.

5   Hazedar S, Dortunc B. Preparation and in vitro evaluation of Eudragit® microspheres containing acetazolamide. International journal of pharmaceutics 2004; 269: 131-140.

6   Barkai A, Pathak V, Benita S. Polyacrylate (Eudrugit retard) microspheres for oral controlled release of nifedipine. I. Formulation design and process optimization. Drug Dev Ind Pharm 1990; 16: 2057-2075.

7   Jones DS, Pearce KJ. Investigation of the effects of some process variables on microencapsulation of propranolol HCl by solvent evaporation method. Int J Pharm 1995; 118: 99 -2005.

8   Badri VN, Thomas PA, Pandit JK, Kulkarni MG, Mashelkar RA. Preparation of non-porous microspheres with high entrapment of proteins by a (water-in-oil) –in –oil emulsion technique. Journal of controlled release 1999; 58: 9-20.

9   Korsmeyer RW, Gurny R, Doelker EM, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. Int. J. Pharm 1983; 15: 25-35.

10 Rodriguez M., Jose LVJL, Torres D. Design of a new multiparticulate system for potential site-specific and controlled drug delivery to the colonic region. Journal of Controlled Release 1998; 55: 67-77.

11 Lee JH, Park TG, Choi HK. Effect of formulation and processing variables on the characteristics of microspheres for water soluble drugs prepared by w/o/o double emulsion solvent diffusion method. International Journal of Pharmaceutics 2000; 196: 75-83.

12 Youan BBC, Jacson TL, Dickens L, Hernandez C, Ababio GO. Protein release profiles and morphology of biodegradable microcapsules containing an oily core. Journal of Controlled Release 2001; 76: 313-326.

13 Alex R, Bodmeier R. Encapsulation of water-soluble drugs by a modified solvent evaporation method I. Effect of process and formulation variables on drug entrapment. Journal of Microencapsulation 2001; 7: 347-355.

14 Das SK, Das NG. Preparation and in vitro dissolution profile of dual polymer (Eudragit® RS 100 and RL 100) microparticles of diltiazem hydrochloride. Journal of Microencapsulation 1998; 15: 445-452.