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Formulation, *in-vitro* and *in-vivo* evaluation of Ethyl cellulose microspheres of Glipizide

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ABSTRACT

Objective: The objective of the present study was to formulate sustained release glipizide loaded microspheres and evaluate the effect of various variables on their properties.
Materials and Methods: The microspheres were prepared by non-solvent addition coacervation method. Ethyl cellulose used as a polymer, petroleum ether (60-40º) for induced coacervation and n-hexane as non-solvent for microspheres preparation.
Results: Microspheres were characterized in term of percent yield, percent entrapment and release pattern of drug. The shape, color and particle size of microspheres were also evaluated. According the result of formulation, n-hexane used as non solvent resulted in enhances rigidization of coating and petroleum ether (60-40º) for induced coacervation. The maximum percent yield of GEN6 formulation was 75.7; particle size in the range of 50-450 µ, maximum entrapment was 89.8 ± 0.11% for GEN3 formulation. GEN1 formulation with low polymer ratio showed better *in-vitro* release between 95-100%. Formulation GEN4 showed 40-50% reduction in plasma glucose level than conventional dosage forms when tested *in-vivo*.
Conclusion: Result of the present study supported that the formulation showed sustenance release with the potential application of n-hexane for improving the physical properties as well as the release profile of this water insoluble drug.

Keywords: Glipizide, sustained release, ethyl cellulose, n-hexane, petroleum ether (60-40º), *in-vivo* & *in-vitro*.

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder affecting people worldwide, with significant morbidity and mortality caused by its micro-vascular and macro-vascular complications, affecting various vital organs and structures in humans [1]. It has been estimated that by year 2030, the diabetic population will rapidly increase in India. However, prevalence is much more, as many patients are asymptomatic and go undiagnosed.

The drug delivery systems that can precisely control the release rates or target drugs to specific body site have an enormous impact on the healthcare system. The last two decades in the pharmaceutical industry, have witnessed an avant-grade interaction among the field of polymer and material science, resulting in the development of novel drug delivery systems [2,3]. Microspheres acquire important features among the particulate drug delivery systems by virtue of their small size and efficient carrier characteristics. However, the success of microspheres delivery system is less due to their short residence time at the site of absorption.[2] The physicochemical characteristics of the active chosen vary considerably, so microspheres are often developed according to specific clinical needs [4,5]. Microspheres were prepared by Coacervation method for their ease and cost effectiveness [6].

Glipizide is an anti-diabetic drug (Fig 1) used to treat type II diabetes mellitus. It has a short half life of 2-3 hrs, and was easily converted in sustained release formulation to reduce dosing frequency. A model dosage form is one, which attains the desired therapeutic concentration of drug in plasma and maintains it for entire duration of treatment. The sustain release formulations of this drug results in constant plasma level for up to 24 hrs [7]. It is extensively metabolized by the liver (90%); and only 10% being excreted unchanged by the kidney [8].

Glipizide is an oral blood-glucose-lowering drug of the sulfonylurea class, chemical name is l-cyclohexyl-3-[[p-[2-(5-methyrpyrazine-carboxamido) ethyl]phenyl]sulfonyl]urea. It is a whitish, odorless powder, insoluble in water and alcohols, but soluble in 0.1 N NaOH, and freely soluble in dimethylformamide. Its

molecular formula is $C_{21}H_{27}N_5O_4S$, molecular weight is 445.55, melting point is 195-200° and pKa is 5.9.

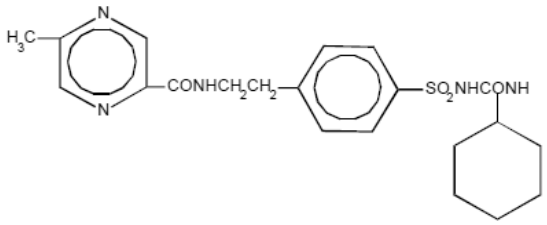

Figure 1: Chemical structure of Glipizide

In the present investigation, a formulation of glipizide, capable of providing detectable blood levels over 10 hrs was formulated using expandable, gelling, swellable hydrocolloid polymer.

Ethyl cellulose is a derivative of cellulose in which some of the hydroxyl groups on the repeating glucose units are converted into ethylether groups. The number of ethyl groups can vary depending on the manufacturer. It is mainly used as a thin-film coaterial. Ethyl cellulose is used as a food additive as an emulsifier.

MATERIALS AND METHODS

Materials

Glipizide was obtained as a gift sample from by Kreative Organics, Hyderabad. Ethyl-cellulose was purchased from Loba Cheme, Mumbai, India. All other chemicals used were of analytical grade.

Method

Preparation of microspheres:

Microspheres were prepared by coacervation method. Ethyl cellulose was dissolved in warm toluene to get a homogenous solution. Drug added and dispersed thoroughly by the vertical mechanical stirrer for 30 min. Coacervation was induced by adding petroleum-ether (60-40º) slowly over a period of 20 min while stirring continuously at the same speed (800 rpm) until the coating is formed, then n-hexane (non solvent) was added to enhance rigidization of coating. The product was filtered, air dried and stored in amber colored bottles and kept in desiccator until used (Table 1).

Table 1: Composition for glipizide microspheres formulated by coacervation method

Glipizide Ethyl Cellulose coacervation microspheres						
Code	**Drug**	**Ethyl Cellulose**	**Toluene (350 ml)**	**Pet. Ether (250 ml)**	**N-Hexane**	**D:P**
GEN1	100 mg	+	+	+	100 ml	1:0.5
GEN2	+	+	+	+	+	1:1
GEN3	+	+	+	+	+	1:1.2
GEN4	+	+	+	+	+	1:1.4
GEN5	+	+	+	+	+	1:1.6
GEN6	+	+	+	+	+	1:1.8
GEN7	+	+	+	+	+	1:2

D:P = Drug to polymer ratio, + = present.

Characterization of microspheres

The prepared microspheres were characterized in terms of % yield, particle size distribution, entrapment efficiency, *in vitro* drug release, *in vivo* drug release etc.

Percentage yield

The percentage yield of the microspheres was calculated for each batch by dividing the weight of microspheres by the total weight of drug and polymer.

Percentage Yield= practical yield/theoretical yield x 100

Entrapment efficiency:

Microspheres were crushed and dissolved in 5 ml acetone. The suspention was suitably diluted with water and filtered to remove the shell fragments [9]. The amount of drug present in weighed samples was assayed spectrophotometrically (Shimadzu UV 1800) at λ max 276 nm in triplicate (Table 2).

Drug entrapment efficiency (%) = Calculated drug content/theoretical drug content x 100

Size distribution and size analysis

Glipizide-ethyl cellulose microspheres were fractionated into different sizes by sieving for 10 min using a mechanical shaker with standard sieves as per the specifications of The Indian Pharmacopoeia. The particle size distribution (Fig 2) was determined and means particle size of microspheres was calculated by the following formula.

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

Eqn.1

Differential scanning calorimetry (DSC)

Thermograms of drug, polymers and microspheres were obtained using Differential Scanning Calorimetry 822e (Mettler Toledo) calorimeter. It measures the amount of heat energy absorbed or released by a sample, as it is heated, cooled or held at a constant temperature (Fig 3). DSC was carried out to determine the possible interaction between drug and polymers. No interactions were observed and individual peaks of the polymers were present in DSC thermograms. Extra peaks were of the impurities or solvent residues in the formulation.

In vitro drug release

The in vitro release studies were carried out at 37 ± 0.5° and at 100 rpm by buffer change method using 200 ml pH 1.2 HCl (1 h), 4 pH (1 h), 6 pH (3 h), 6.8 pH (3 h) and 7.4 pH (2 h) phosphate buffers in sink conditions using a Diffusion cell. Accurately weighed sample of prepared microspheres were added to the donor cell. At pre-set time intervals, 5 ml of aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium [10-12]. The aliquots were

analyzed UV spectrophotometrically at λ max 276 nm after proper dilution (Fig. 4, Table 3).

***In-vivo* Hypogycemic Activity**

***In-vivo* hypoglycemic activity of Glipizide formulation, in normal and hyperglycemic rats**

The *in-vivo* hypoglycemic activity was conducted in healthy and hyperglycemia induced male albino wistar rats of 175±25 gm body weight by measuring the hyperglycemic effect produced after oral administration of the sample dose equivalent to 5 mg/ kg body weight of glipizide in comparison to pure drug at same dose. The formulations were selected based on their in-vitro release profiles.

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal, (Reg. No. 1824/PO/ERe/S/15/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/PN-16042. Animals were housed in separate cages under controlled temperature (22 ± 2°) and provided standard diet (Golden feed, New Delhi) and water regularly [13-15]. They were exposed to alternating light and dark cycle of 12 hrs each. They were further divided into four groups with six animals in each group [16-19] (Table 4).

Experimentation:

Animals were divided into four groups with six animals in each group. Group I, received normal saline orally only and served as vehicle control, Group II made diabetic with streptozotocin (STZ). Group III received Glipizide (5 mg/kg) per day orally, group IV received formulation GEN4 (5 mg/kg) per day oral. Dosing of test samples were done orally throughout the experimentation (Table 5-6 & Fig 5).

RESULT AND DISCUSSION

The physiochemical characteristics of the prepared microspheres were showed in Table 2. Microspheres of glipizide were formulated using ethyl cellulose by coacervation method. Different concentrations of the polymer were used to entrap the drug and various process variable parameters were analyzed. According the result of formulation, n-hexane used as non solvent resulted in enhances rigidization of coating and petroleum ether (60-40º) for induced coacervation.

Table 2: Physicochemical properties of the glipizide ethyl cellulose coacervation microspheres

Ethyl cellulose non solvent Microspheres of Glipizide				
Code	**% yield**	**%entrapment ±S.D**	**Shape**	**Color**
GEN1	68.6	89.5 ± 0.37	spherical	White
GEN2	57.2	87.0 ± 0.55	spherical	White
GEN3	72.0	89.8 ± 0.11	spherical	White
GEN4	62.2	89.7 ± 0.26	spherical	White
GEN5	61.3	80.1 ± 0.15	spherical	White
GEN6	75.7	79.2 ± 0.10	spherical	White
GEN7	72.8	87.0 ± 0.11	spherical	White

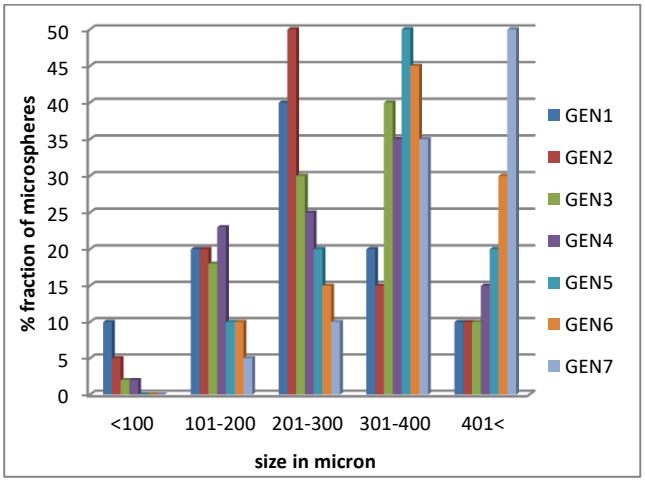

Figure 2: Size distribution of glipizide microspheres

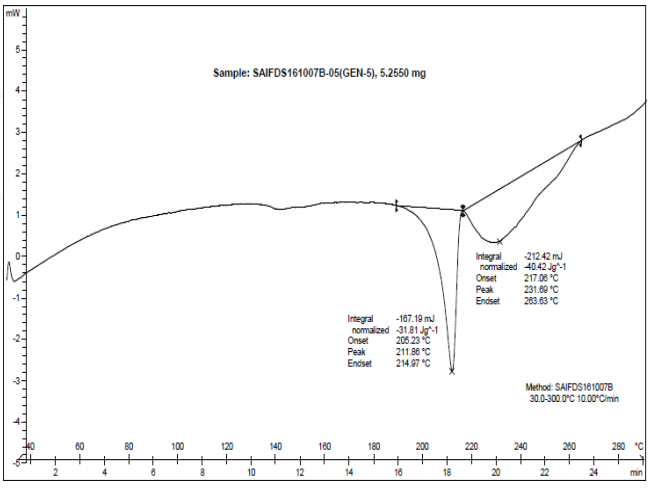

Figure 3: DSC graph of glipizide microspheres (Sample GEN-6

For preparation of microspheres, a constant speed of 800 rpm was chosen which aided in the evaporation of solvent from polymer solution. Change in temperature did not affect the size of microspheres, but the surface characteristics were greatly affected. The polymer concentration directly affected the morphological and release characteristics of formulations. Low polymer concentration lead to uneven while high polymer concentrations lead to smooth surfaces and even sized microspheres.

The release of drug from microspheres was fast in case of low concentration of polymer and drug release was slow when concentration was high. The formulations were white, spherical and small in size. Polymer ethyl cellulose is insoluble in all physiological pH values but swells at all pH conditions.

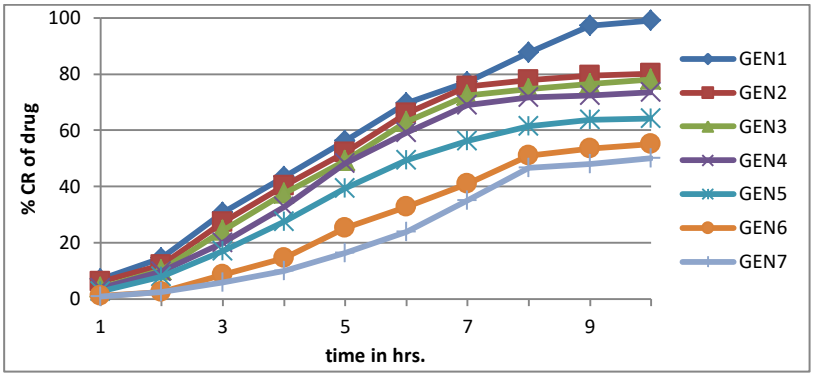

Figure 4: In-vitro release graph GEN1-GEN7

Table 3: *In vitro* release kinetic equation data of glipizide microspheres

Sample Code	Zero order	First order	Higuchi	Korsmeyer Peppas	Hixson Crowell
	R_0	R_1	R_H	R_K	R_{HC}
GEN1	0.983	0.848	0.981	0.760	0.962
GEN2	0.932	0.975	0.930	0.752	0.973
GEN3	0.938	0.970	0.938	0.777	0.965
GEN4	0.934	0.959	0.931	0.794	0.959
GEN5	0.955	0.970	0.950	0.813	0.974
GEN6	0.977	0.975	0.977	0.888	0.971
GEN7	0.958	0.938	0.953	0.931	0.946

R= regression coefficient value

The polymer ethyl cellulose produced water insoluble layer of around the microspheres. It is chemically resistant to alkali but in acidic conditions shows high sensitivity. The dissolution of microspheres carried out in simulated gastrointestinal media showed an initial burst effect due to migration of drug towards periphery. A homogenous solution of the polymer in toluene was added continuously to petroleum ether with stirring. Since the solvents are in compatible in nature; hence precipitation of polymer takes place on drug particle. The combination produced a rigidized film after solvent evaporation by continuous stirring. The coat was further hardened by addition of non-solvent, n-hexane. The release from microspheres was a function of polymer wall thickness. The complete drug released within 10 hrs.

Table 4: For *In-vivo* grouping of animals

Group No.	Treatment	Dose	No. of animals
1	Normal Saline	5 ml/kg p.o.	06
2	Streptozotocin (STZ control)	60 mg/kg i.p.	06
3	Glipizide	5 mg/kg p.o.	06
4	GEN4	5 mg/kg p.o.	06

Statistical analysis of data:

All data were analyzed by One way ANOVA followed by Bonferroni test. $p<0.05$ was considered as level of significance. All data are presented in mean±SD.

Glipizide showed significant decrease in plasma glucose levels in both normal and hyperglycemic rats.

A 25% reduction in glucose is considered a significant hypoglycemic effect. The sustained hypoglycemic effect with microspheres is due to the slow release and absorption of glipizide over extended periods of time.

The plasma glucose level of diabetic controlled rats increased significantly from day 3 to day 7 of STZ injection. Hyperglycemic rats were selected for the study as discussed above. The plasma glucose data obtained indicates that the drug is entrapped in microspheres & produced consistent anti-hyperglycemic effect. This effect was pronounced in case of hyperglycemic rats whereas normal rats showed comparatively lesser alterations in plasma glucose level after formulation administration.

Table 5: Blood Glucose Level (mg/dl)

S. No.	Treatment	0 Day	3 Day	5 Day	7 Day
1	Normal Saline (5 ml/kg)	87.90±5.626	89.60±5.873	90.48±6.485	89.20±6.429
2	STZ Control	284.35±11.734	289.43±12.438	293.92±12.020	296.73±11.853
3	Glipizide (5 mg/kg)	282.93±8.574	222.65±8.025*	166.90±8.773*	119.43±9.211*
4	GEN4 (5 mg/kg)	284.42±6.205	256.73±6.757*	210.33±7.839*	156.58±9.295*

Values are expressed as MEAN±SD at n=6, One way ANNOVA followed by Bonferroni test, *P<0.050

significant compared to the diabetic control group

Table 6: Reduction in plasma glucose levels after administration

Treatment	Day 1	Day 3	Day 5	Day 7
STZ Control group Glipizide	18.101	21.013	40.211	50.874
Glipizide (5 mg/kg)	18.213	23.072	43.215	59.751
GEN4 (5 mg/kg)	06.758	11.298	28.439	47.231

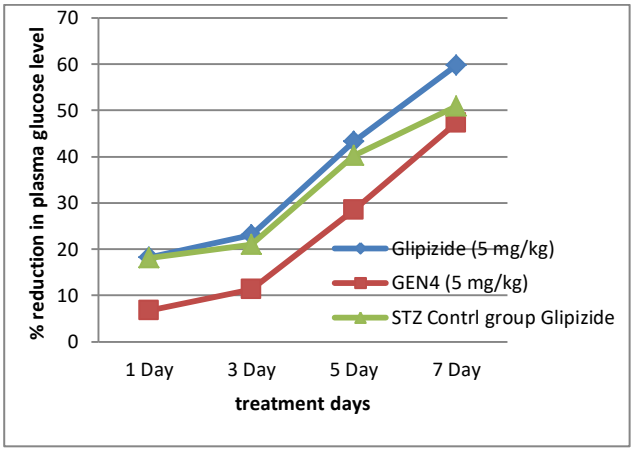

Figure 5: Percent reduction in plasma glucose level in hyperglycemic rats

CONCLUSION

A new sustained release system of glipizide microspheres of ethyl cellulose were designed and formulated by coacervation method. It's morphological and release characteristics were studied. The microspheres were easy to prepare and the mean diameter of microspheres increased with increase in the amount of the polymers increase. The pore size of microspheres was affected by concentration of the polymer. Stirring at high speed above 800 rpm causes destruction of microspheres. The microspheres showed excellent in vivo activity and sustained release characteristics as compared to the conventional oral dosage forms. Thus, drug entrapment technique is a useful tool for the development of multi-particulate system even for a water-insoluble drug.

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