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INNOVATIVE APPROACH FOR IMPROVED SUSTAINED DELIVERY OF METFORMIN HYDROCHLORIDE FOR ITS ANTI-HYPERGLYCEMIC ACTIVITY

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ABSTRACT
Metformin hydrochloride, an antidiabetic agent, is useful in reducing the blood glucose concentration in Type II diabetes. It is also finding its use as a repurposed drug. The formulations consisted of micro drug delivery systems prepared by emulsification method and were evaluated *in-vitro* and *in-vivo*. Process variables like amount of polymer, speed of agitation and stirring, presence or absence of surfactant and cross linker offered a versatile approach towards obtaining the formulation though affected physicochemical properties of formulations. Discrete, spherical, and free-flowing microspheres, in the size range and granularity of 250 to 700µ were used to control the drug release rate. Drug release was diffusion controlled as evident from the Higuchi kinetics. The physical characteristics of the formulations were reproducible. Healthy and alloxan induced hyperglycaemic male albino mice were used for *in-vivo* experimentation by evaluating plasma glucose level reduction and % reduction in the blood glucose level after administration of pure drug and formulations. The results indicate significant sustained fall in the blood glucose level for about 10 hrs following formulation administration as compared to the pure drug.

INTRODUCTION

While being the most preferred route of administration, oral route faces many challenges for drug delivery at different levels as compared to the systemic delivery of drugs through different dosage forms. The reasons that may result in poor bioavailability are linked to effects of food, excipients and poor localization of dosage form at the site of absorption. Controlled release systems during their course of gastrointestinal transit offer diverse yet repeatable kinetics. As microparticles move through the GIT and avoid stomach emptying, decrease inte subject variability and possibly low chances of dose dumping with a customizable release, microspheres are prefered to single unit dosage forms. Also, multi-particulate drug delivery systems overcome the 'all or nothing' disadvantage of single unit dosage forms. Metformin hydrochloride acts as an Insulin sensitizer and useful in controlling type II diabetes [1].

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Microspheres were prepared for the antidiabetic drug Metformin hydrochloride by emulsification denaturation method using egg albumin as the polymer. The drug is water soluble, bitter, white powder, given daily in doses of 1.5 to 2 grams in divided form and necessitates frequent administration due to its short half-life increasing patient noncompliance and chances of the side effects such as nausea, gastric disturbance etc. The formulation exhibited window of absorption in the stomach and upper small intestine, thus it will be useful to produce a controlled release formulation while floating on gastro-intestinal juices. The drug when given by oral route of administrations during its passage thorugh the gastro-intestinal tract undergoes alterations and thus make a good candidate for a sustained release dosage form [2]. The obtained microparticles were subjected to physicochemical characterizations as measurement of particle size, and release kinetics. *In-vivo* studies were carried out in mice to correlate the *in-vitro* release of the drug with the percent decrease in the plasma glucose level on administration of the pure drug and the prepared formulations.

MATERIALS AND METHODS

Materials: Metformin hydrochloride was gift sample by Sun Pharmaceuticals, Baroda, India. Egg albumin was obtained from Hi-Media, Mumbai, India. Liquid paraffin and petroleum ether were from Thomas Baker; Mumbai and glutaraldehyde (25% aqueous solution) from CDH laboratories, New Delhi, India. All other chemicals and reagents used were of the analytical grade.

Methods: Different solutions of egg albumin were prepared in distilled water containing Tween 80 (2% w/v) by stirring at 200 rpm using a magnetic stirrer. Varying quantities of finely powdered drug was added to the polymer solution to get different drug to polymer ratios (concentrations) and sonicated to obtain dispersion. This dispersion was added to liquid paraffin containing 1.0 ml of Span 80 while stirring at 1200 rpm for 10 minutes. Then, 10 ml of glutaraldehyde was added, and stirred for 3 hours. The formulated microparticles were filtered, washed and excess glutaraldehyde was removed using Sodium meta bi sulphite, dried in air and stored in desiccator until used [1].

Drug loading, encapsulation efficiency and yield of microspheres

Drug content was determined using crushed microspheres and extraction in distilled water. Absorption max was 233 nm in UV Spectrophotometry and the readings were taken in triplicate. From the standard plot, the drug quantity was determined.

$$\text{Loading (\%)} = \frac{\text{Weight of drug}}{\text{weight of microspheres}} * 100$$

The following equation was used to calculate the encapsulation efficiency:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Calculated drug content}}{\text{Theoritical drug content}} * 100$$

Each batch's yield percentage was determined by dividing the weight of the prepared microspheres (M) by the total expected weight of drug and polymer (M_0):

$$Yield(\%) = \frac{M}{Mo} * 100$$

Table 1. Processing parameters Matrix

Experimental constants	Process variables
Polymer and solvent volumes	Stirring speed
Volume of cross linking agent	Temperature of formulation
Temperature	Concentration of polymer
	Drug concentration

Particle size and shape measurements [3]

Approximately 100 microspheres were taken on a glass slide and evaluated for surface characteristics and size using optical microscope and statistical analysis of the mean and the coefficient of variation was determined and values were recorded as ± S.D.

Buoyancy percentage and floating property

USP dissolution apparatus with 900 ml of 0.1 mol L-1 HCl was used for the study for minimum 10 hours at 100 rpm. Microspheres were retrieved separately, dried, and weighed to determine their floating and settled fractions. The mass of the microspheres that remained afloat was divided by the overall mass of the microspheres to determine buoyancy percentage. The data were reported as the S.D. after taking readings in triplicate [4].

Differential scanning calorimetry

The DSC- 60 (Schimadzu, Tokyo, Japan) calorimeter was used for calorimetric study, which consisted of a calorimeter, flow controller (FCL 60), thermal analyzer (TA 60), and operating software. Samples were heated at a scanning rate of 10°C/min

from 24° to 300°C in sealed aluminium pans with nitrogen flow (30 ml/min). Empty aluminium cans were used as reference [1].

Scanning electron microscopy

Samples were fixed in individual stubs and formulations sprinkled with gold dust. With a scanning electron microscope [3], (Jeal JSM 840A Scanning Microscopy, Jeol, Japan) operating at 15kv, all materials were analysed for surface morphological characteristics, size, and shape. Gold ion sputtering device used was JFC-1100E, Jeol, Japan.

***In-vitro* drug release and kinetics** [5]

Microspheres (100 mg) were submerged, and stirred with a thermally controlled system in 200 ml of buffer, at a temperature of 37 ±0.5°C. At regular intervals, samples (5ml) were removed, and the drug concentration determined at 233 nm and the system volume maintained with 5 ml of pre-warmed buffer. Statistical analysis was done via ANOVA and a p-value of <0.05 was considered as to be significant.

***In-vivo* studies in normal mice**

A crossover randomised block design was used for the studies (n=5) on male albino mice weighing about 35 grams with fasting blood glucose level of about 90 mg/dl. Following oral delivery of suspension in a 0.5% sodium carboxymethyl cellulose solution, equivalent to 60 mg of drug/kg body weight, plasma glucose levels were assessed following zero hour blood sample. The control group was given 0.5% sodium carboxy methyl cellulose solution. The mice's tail veins were routinely punctured for blood samples up to 12 hours. Plasma glucose levels and percentage reduction in plasma glucose levels were calculated. [6]

***In-vivo* studies in hyperglycemic mice**

Alloxan monohydrate was given intraperitoneally at a dose of 150mg/kg body weight as a single i. p injection (in sterile water) to induce hyperglycemia. After 7 days, mice with fasting blood glucose level ≈ 200 mg/dl were selected for the study and studies done.

RESULTS

Egg albumin microspheres, prepared by chemical denaturation of the protein at speed of 800 rpm yielded highest batch size of the microspheres. Upon decreasing the speed below 600 rpm, clumping of particles was observed having poor size uniformity and wide size distribution (Figure1). At over 1000 rpm, elliptical and irregularly shaped microspheres began to develop due to elastic and plastic deformation of the precipitating polymer with induced stress. The rate of agitation and other elements including the encasing material's density, viscosity, and interfacial tension all affected size of the microspheres going from size range 100 to 750 microns as shown is table 2 and figure 1.

The temperature of formulation played a major role in deciding the drug content (from 58.9 to 91%) and percentage yield (71 to 86%). The process constants and variables are given in table1. When the temperature was increased beyond 50°C, the percent entrapment efficiency decreased and the irregular sized microspheres were obtained. At low polymer concentration, the surface of the microspheres showed presence of drug crystals while smoother surface was obtained with increase in the polymer concentration. A depression was seen on the surface which was due to the rapid evaporation of the solvent with low concentrations of polymer (as evident from the scanning electron microphotograh of the formulation, figure 2).

Microspheres were yellowish in color and became darker with increase in denaturation time and volume of denaturating agent. The agent used was 25% w/v aqueous solution of glutaraldehyde to induce chemical cross linking which is considered safe for pharmacological purpose, although lesser the quantity to used better it would be. With increase in polymer concentration, percentage yield and percentage entrapment increased from 71.3± 0.8 to 88.3± 0.4 % and 58.9± 0.7 to 91.0± 1.5 % respectively (Table2). Large numbers of particles were in the range of 100 to 250µ. The SEM of the microspheres revealed that the egg albumin microspheres were porous and water permeable (Figure 2).

DISCUSSION

With the increase in the temperature, the number of depressions on the surface increased which was due to the rapid evaporation of the solvent. The increase in the temperature also caused for the faster and more denaturation of the polymer, thus, decreasing the release of the drug. It has been reported that the physiochemical properties of the drug and carrier may make it possible to predict the drug uptake by albumin microspheres. The exact amount of the cross linking agent required needs optimisation for getting the required sustained release [7]. Such predictions would be extremely valuable for evaluating the

acceptability of carriers, especially for medications that are very sensitive to the microspheres' microsphere formation conditions, such as heating and cross-linking agents. The small standard deviation and low coefficient of variation (<2.0) for entrapment values indicate good reproducibility of formulations and homogeneity among the batches (Table 3). The DSC profile exhibits no interaction (Fig 3). The release profiles of the formulations are given in figures 4 and 5.

Table 2: Properties of the formulated microspheres

Formulation	% Yield		% Entrapment		Shape	Color	Floating Property (%)
	±S.D	COV	±S.D	COV			
MEG_1	71.3± 0.8	1.2	58.9± 0.7	1.2	Spherical	Yellow	21.1± 0.2
MEG_2	74.7± 0.4	0.5	74.7± 0.5	0. 8	Spherical	Yellow	15.5± 1.1
MEG_3	8.66± 0.5	0.5	85.4± 0.5	0.6	Spherical	Yellow	14.7± 1. 5
MEG_4	88.3± 0.8	0.9	88.0± 0.7	0.8	Spherical	Yellow	16.7± 2.2
MEG_5	86.6± 0.5	0.5	91.0± 1.5	1.7	Spherical	Yellow	12.1± 0.9

Values are expressed as mean±SEM (Standard Error Mean); S.D = Standard deviation, COV = Coefficient of variance

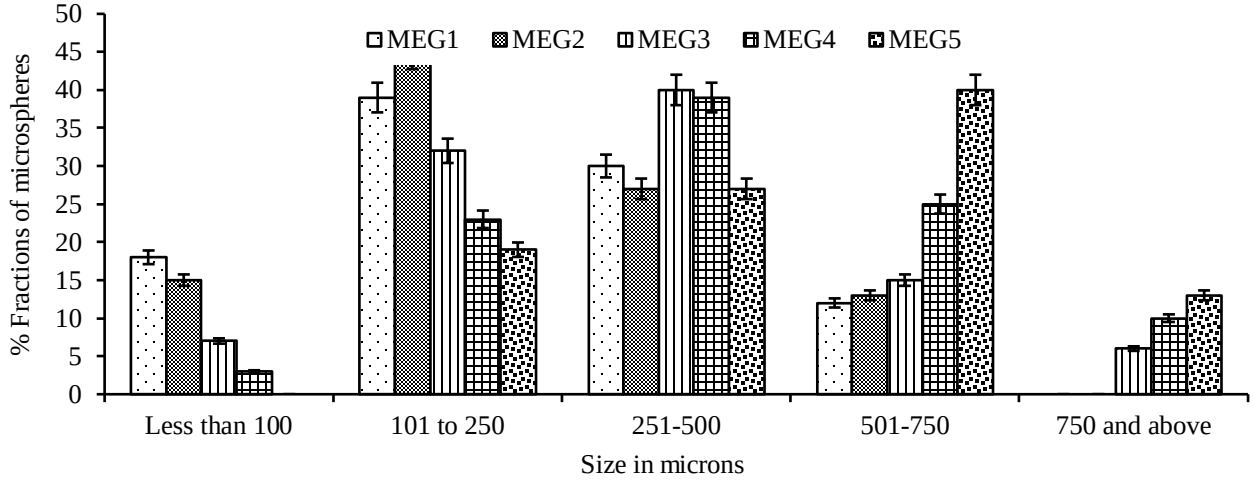

Figure 1: Size distribution of the microspheres

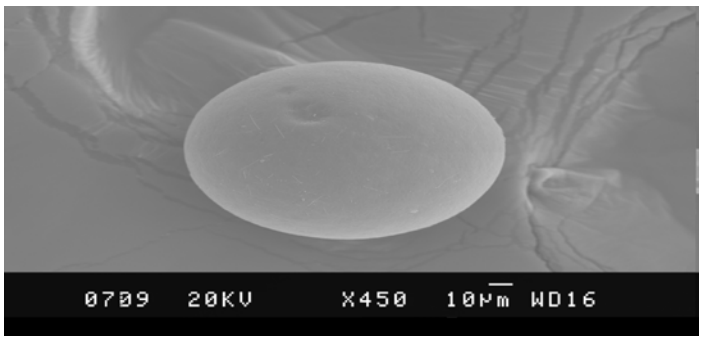

Figure 2: Scanning electron microscopic photograph

A suspension of egg albumin and drug in water containing Tween 80 was added to paraffin oil and cross linked using glutaraldehyde. The interaction between negatively charged egg albumin molecules and cationic Metformin hydrochloride was the basis for these formulations. Free flowing microspheres were obtained in all combinations. To prevent overusing chemicals, the minimum amount needed was established. Further, higher amount of the denaturating agent changes the structure of the polymer, with a loss of water molecule and tightens the core. The properties of the microspheres were greatly impacted by the encapsulating media's pH. As the pH of the microencapsulation media was increased, the incorporation efficiency, particle size, and flowability decreased, along with increase in drug release rate. Egg albumin also shows changes in solubility with pH, that may affect the drug release. This result was brought about by the microsphere matrix's insufficient cross connecting. High concentrations of albumin solution or the increase of albumin to drug weight ratio were accompanied with increase in incorporation efficiency and particle size with improved flowability and diffusion controlled slow drug release. With increase in agitation rate the size of microspheres decreased (from 750 to 150 µ) and then clumping was observed due to faster matrix formation but improper solvent evaporation. The release was slow and extended over longer duration of time.

The release kinetics exhibit that release of the drug follows Fickian diffusion across the polymeric matter. Albumin is a biodegradable polymer and is non toxic. It degrades in body as a response to the effect of diffusion media. At the same time the hydrophilic drug tends to dissolve in the media and leaves the matrix creating channels for the outward passage. This suggest a combination of drug release kinetics involving polymeric biodegradation and pores creation (anamolous transport as per Korsemeyer and Peppas kinetic model), which is controlled, sustained and depends on process variables like size, concentration of denaturating agent, duration of denaturation and drug polymer ratio. The surface of egg albumin microspheres was heterogeneous and hydrophilic in nature as compared to bovine egg albumin. The results are shown in table 4. The microspheres demonstrated the ability to regulate the release of the medication, and process variables had a significant impact on their characteristics.

The formulations utilized the biodegradable polymer which retained its hydrophilic nature. The incorporation of hydrophilic drug and the intended controlled release to impact its pharmacokinetic behaviour could be achieved by adjusting and careful selection of the method and the ingredients.

Table 3: t test values of % entrapment of microspheres at 95% confidence interval

Parameters	**MEG_1**	**MEG_5**
Mean	58.9	91.0
S.D	0.728	1.58
Average absolute deviation from mean	0.480	1.20
T Value = -41.2		
S. Dev = 1.23		
Probability assuming null hypothesis = 0.0001		

S.D = Standard deviation

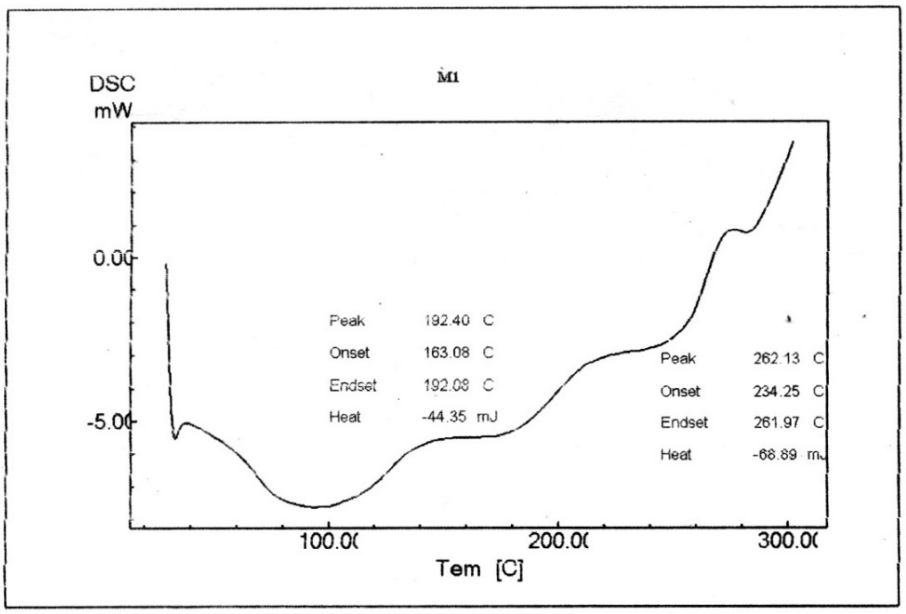

Figure 3: Differential Scanning Calorimetry (DSC) graph

Table 4: *In-vitro* release kinetics of microspheres

Formulation	**Zero Order**			**First order**			**Higuchi**		
	R_0^2	**C_0**	**M_0**	**R_1^2**	**C_1**	**M_1**	**R_H^2**	**C_H**	**M_H**
MEG_1	0.9594	21.1	07.6	0.9874	02.0	-0.1	0.9826	-10.14	32.5
MEG_2	0.9445	23.9	07.6	0.9718	02.0	-0.1	0.9786	-08.87	33.4
MEG_3	0.8974	32.5	06.7	0.9946	01.9	-0.1	0.9657	-02.49	29.8
MEG_4	0.9879	10.5	07.5	0.8846	02.1	-0.1	0.9616	-19.48	31. 8
MEG_5	0.9960	04.3	07.4	0.9899	02.0	-0.1	0.9809	-25.98	31.7

R_0^2, R_1^2 and R_H^2 = Regression coefficient values of zero, first order and Higuchi equations respectively

C_0, C_1 and C_H = Intercept values of zero, first order and Higuchi equations respectively

M_0, M_1 and M_H = Slope values of zero, first order and Higuchi equations respectively

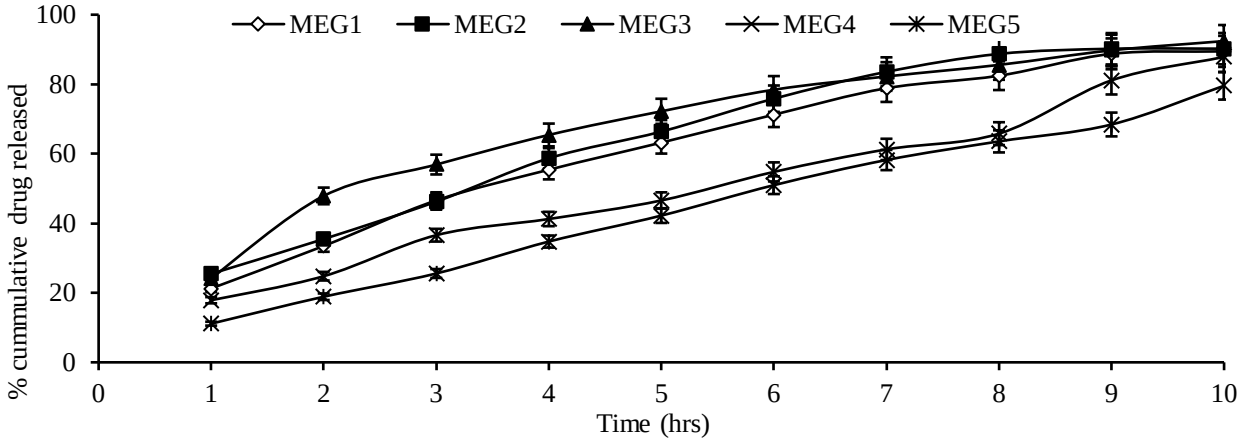

Figure 4: *In-vitro* drug release from microspheres

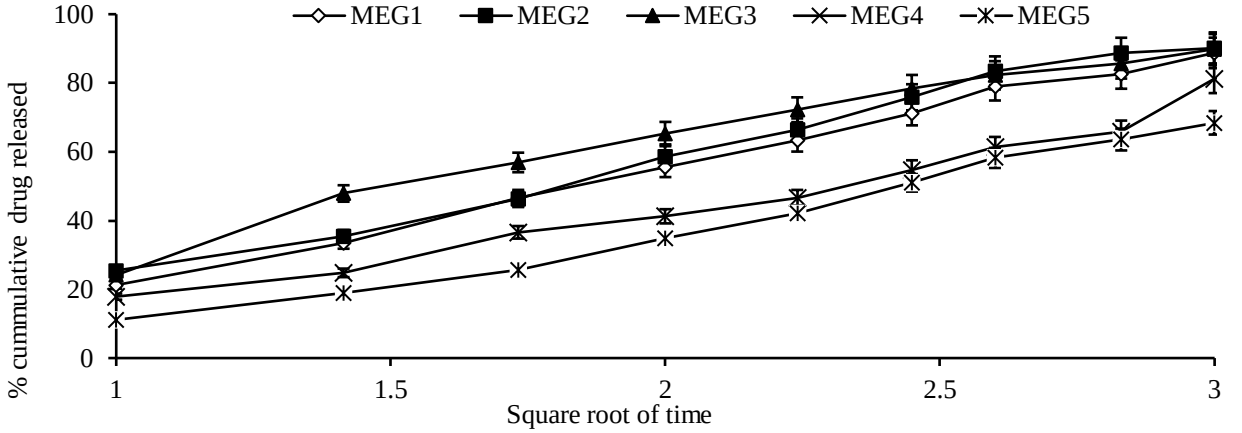

Figure 5: Higuchi kinetic drug release from microspheres

Table 5: % Reduction in plasma glucose levels in normal mice

Group/ Treatment	Absolute plasma glucose level (mg/dL)	% Reduction in plasma glucose levels						
		1hr	2 hr	3 hr	4 hr	6 hr	8 hr	10 hr
Control (0.2 ml CMC Solution)	88± 3.56	5.47± 0.73	10.49± 1.31	10.18± 0.93	11.59± 1.06	11.24± 0.79	6.12± 0.32	1.82± 0.11
Pure drug (60 mg/kg)	90± 5.82	8.57± 1.08	14.00± 0.93	23.33± 2.14	15.55± 1.16	10.00± 1.04	2.22± 0.06	2.22± 0.19
MEG_3	100± 6.89	10.36± 1.01	19.14± 1.60	28.63± 1.92	32.85± 2.38	32.36± 3.97	29.49± 3.14	19.73± 2.13

By comparing the hypoglycemic response to oral administration of Metformin hydrochloride at a dose equivalent to 60 mg per kg of body weight to pure medication at the same dose, the *in-vivo* hypoglycemic activity was tested in healthy and hyperglycemia-induced male albino mice. When using pure medication, the plasma glucose level dropped significantly within 30 minutes and was then recovered two hours later. When using microspheres, the glucose level was reduced more gradually; the largest decreases were felt four hours after treatment, and the reductions persisted for roughly 10 hours. One considered a substantial hypoglycemia effect is a 25% drop in glucose. A survey on related literatures and research work indicates a % plasma glucose level reduction of about 19% using microspheres [5,6].

When the pure medication was administered, the hypoglycemic effect persisted for 0.5 to 2 hours, but with the produced formulations, it persisted for more than 8 to 10 hours. The prolonged slow release and absorption of Metformin hydrochloride from the microspheres results in a sustained

hypoglycemic impact. The time intervals for sampling were based on *in-vitro* results and preliminary *in-vivo* studies. The release and *in-vivo* data were compared with recently reported research works incorporating hydrophilic polymer and Metformin hydrochloride as drug for oral administration of a multi-particulate systems [6-8].

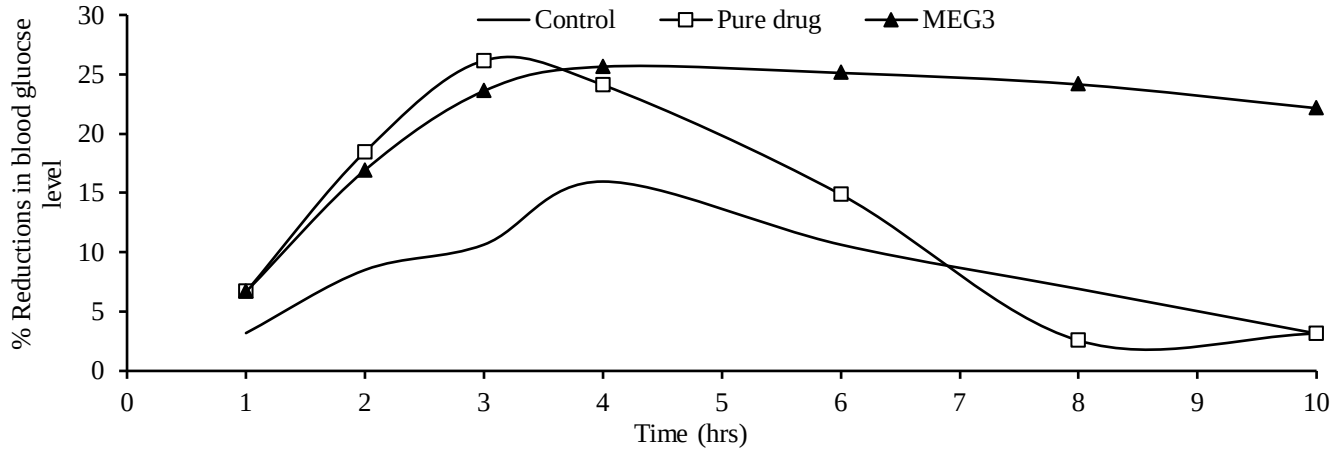

Figure 6: % reduction in plasma glucose level in normal mice

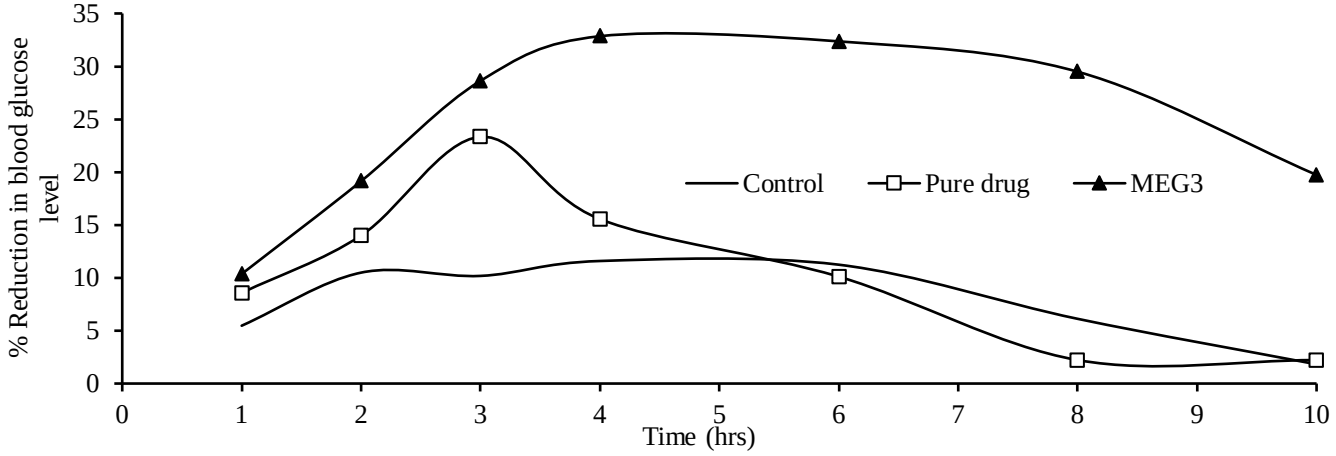

Figure 7: % Reduction in plasma glucose level in hyperglycemic mice

Table 6: Results of ANOVA statistical tests at 95% confidence level on percent reduction of plasma glucose level in normal and hyperglycaemic mice

Parameters	**Control**		**Pure**		**MEG$_3$**	
	N*	**H****	**N***	**H****	**N***	**H****
Mean	8.13	8.42	10.81	10.85	20.37	17.74
Standard deviation	3.70	4.55	7.56	9.12	10.8	8.35
Median	10.18	8.51	10.00	6.68	19.73	22.14
Absolute deviation from median	2.84	3.43	5.70	7.10	8.30	6.22
Probability assuming null hypothesis						
Normal mice	0.024					
Hyperglycemic mice	0.086					
F value						
Normal mice	4.608					
Hyperglycemic mice	2.823					

N= Normal mice* *H** = Alloxan induced Hyperglycemic mice*

The mean plasma glucose levels in control and test animals after oral administration of the microspheres (suspension in 0.5% Sodium CMC) is shown in table and figures 6 and 7. The anti-hyperglycemic effect was formulation dependent and reached a maximum level as shown in table for the formulations. The plasma glucose level of diabetic control increased significantly from 95± 5.8 to 200± 15.2 mg/dL on 5^{th} to 7^{th} day of alloxan injection. Hyperglycemic mice were selected for the study and prepared microspheres were administered as discussed above. The data on plasma glucose show unequivocally that the medication contained in the microspheres consistently provided an antihyperglycemic effect. When the formulation was administered to hyperglycemic mice, this impact was more significant; nevertheless, normal mice showed relatively little change in plasma glucose level. Microspheres with Metformin hydrochloride do not show marked decrease in plasma level in normal mice, but significant decrease for more than 8 hrs in hyperglycemic mice. The egg albumin microspheres showed a maximum reduction in plasma glucose level at 5 hrs with about 33 % reduction of plasma glucose level and maintains the low plasma sugar for more than 10 hrs (Figure 6 and 7) in normal mice and a maximum reduction of about 25% at 4 hrs in hyperglycaemic mice and this effect was sustained for about 10 hrs. It has been discovered that alloxan induces the production of free radicals and damages tissue. Alloxan is concentrated in the liver and Islets of Langerhans after injection, where it is converted to dialuric acid. Normal mice can develop insulin resistance, hyperinsulinemia, and disturbances in glucose metabolism by consuming a diet high in fructose or by inducing hyperglycemia using drugs like streptozocin/ alloxan monohydrate [9].

CONCLUSION

Microspheres of Metformin hydrochloride utilising egg albumin as the polymer included measurements of their physical properties as well as their *in-vitro* and *in-vivo* features. The physical measurements were reliable, consistent and offer reproducibility for further research. The *in-vitro* release statistics imply a constant and controlled release for the duration of the investigation. Egg albumin can be employed as a polymer for the controlled delivery of Metformin hydrochloride since the microspheres on oral administration could successfully lower the plasma glucose level significantly for extended periods of time. The major limitation of the study was in identifying the presence of the formulations GIT, as the formulation properties would change with change in the pH of the medium for the polymer. The obtained results of the study indicate techniques that can be used for formulation and need of the desired dosage form and route of delivery. Further, it explains the *in-vivo* effects of the drug and polymer and can act as a guideline for formulations utilising similar approaches in general. Further research may be required for analysing the process variables and the resulting pharmacokinetic data to improve the drug delivery as microspheres. Future research may address the importance of hydrophilic polymer alone or in combination with other hydrophilic polymers for drug release at a predetermined rate and prolonged duration without significantly affect the polymer solubility as per pH. This will also help to understand and improve the pharmacokinetics and pharmacodynamics of the system. The undertaken research work is versatile in nature and useful for varied categories of drugs. The repurposed use of the present drug offers a great range to explore use in treatment of different conditions as obesity, cardiovascular disorders, cancer etc.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

Mousumi Kar Pillai did the formulation and research. Sujit Pillai contributed to the analysis and data preparation. Sanjay Jain was involved in drafting the article. All the authors read the final manuscript and did the corrections

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