RESEARCH ARTICLE

FORMULATION AND EVALUATION OF CARBOPOL MICROSPHERES OF METFORMIN HCl.

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Abstract

The aim of the present work is to investigate the possibility of obtaining a prolonged, relatively constant effective level of Metformin loaded microsphere formulations using Carbopol^{940} carrier. In the present study carbopol based microspheres bearing metformin were prepared by emulsion–solvent evaporation technique. Three batches of microspheres with different concentration of drug and polymer (FS-1, FS-2 and FS-3) were prepared. The prepared microspheres were studied for drug loading, particle size distribution, *in vitro* release characteristics, *in vivo* tissue distribution study and stability studies. The microspheres were found to have diameters within the range of 50 to 110 µm and incorporation efficient of 41 to 69% was obtained. Percent drug release after 8 hours was 97.74%, 96.34% and 93.62% for FS-1, FS-2 and FS-3 respectively. *In vitro* release profile of all formulations shows slow controlled release up to 8 hrs. The *in vivo* result of microparticles showed preferential drug targeting to intestine followed by kidneys and spleen. Stability studies showed that maximum drug content and closest *in vitro* release to initial data was found in the formulation stored at 4ºC. In the present study Metformin loaded microparticles were prepared and targeted to various organs to a satisfactory level and were found to be stable at 4ºC.

Keywords: Metformin HCl, Carbopol^{940}, Microspheres, Emulsion-solvent evaporation, *In vivo* study.

Introduction

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration. However, a lack of understandings of anatomical and physiological barriers imposed impediments on the development of efficient delivery system. The drug delivery system should deliver a drug at a rate dictated by the needs of the body over a specified period of treatment [1-2]. These preparations provide an immediate dose required for the normal therapeutic response, followed by the gradual release of drug in amounts sufficient to maintain the therapeutic response for a specific extended period of time (usually between 8-12hrs). The major *advantage* of this category is that, in addition to the convenience of reduced frequency administration, it provides levels that are devoid of the peak and valley effect, characteristic of the conventional intermittent dosage regimen [3]. An ideal controlled drug delivery system is the one, which delivers the drugs at a predetermined rate, locally or systemically, for a specific period of time [4].

Microspheres can be defined as solid, approximately spherical particles ranging from 1 to 1000 um. They are made of polymeric, waxy or other protective materials, that is, biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. The natural polymers include albumin and gelatin; the synthetic polymers include polylactic acid and polyglycolic acid [5].

The idea of polymer microcapsules as delivery systems was prepared as early as the 1960s (Chang[15], 1964) and degradation was incorporated through the employment of a degradable polymer coating [6]. Various attempts have been made in the field of targeting but in past few years, pharmacists have focused their research in colloidal drug delivery system/ colloidal carriers like liposomes, microspheres, nanoparticles etc., as targeting carriers which has given selective targeting [7]. The rate of drug release from microspheres dictates their therapeutic action. Release is governed by the molecular structure of the drug and polymer, the resistance of the polymer to degradation, and the surface area and porosity of the microspheres [8].

Diffusion of drug through such a structure may involve transport not only through an isotropic medium, such as the drug in solution, but also through a polymeric membrane. Transport of drug through such a membrane involves dissolution of the drug in the polymer at the high-concentration side of the membrane interface and diffusion across the membrane in the direction of decreasing concentration [9]. In addition, the

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concentration difference across the membrane, which is taken as the driving force for drug transport, tends, to decrease as the solubility of the drug on the upstream side of the membrane decreases. Therefore, the dissolution rate of poorly soluble drugs can be an important factor in limiting drug release [10]. The coating material should be capable of forming a film that is cohesive with the core material; be chemically compatible and no reactive with the core material; and provide the desired coating properties, such as strength, flexibility, impermeability, optical properties, and stability. The coating materials used in microencapsulation methods are amenable, to some extent, to in situ modification [11].

Diabetes is a chronic metabolic disease characterized by hyperglycemia i.e. high blood sugar levels in the blood. Our blood always has some glucose in it because our body needs glucose for energy to keep going [12]. Insulin is a chemical (a hormone) made in pancreas. The pancreas release insulin into the blood. Insulin help the glucose from food get into our cells. If our body doesn't make enough insulin or if the insulin doesn't work the way should, glucose cannot get into our cells [13].

2. MATERIALS AND METHODS

Synthetic polymer carbopol[940] was collected from our laboratory. The pure drug Metformin HCl was obtained by Karanatak Antibiotices, Bangalore (Karnataka, India). HPMC-E15 obtained by Loba Chemie (Kerala, India). Alloxan the diabetes producing agent obtained by sigma chemicals & co, Bangalore, (Karnataka, India). All other chemicals used were of reagent grade.

2.1. METHOD
2.1.1. Formulation Deign of Microspheres

Carbopol microspheres of Metformin HCl was formulated by using emulsion-solvent evaporation technique. Carbopol polymer was dissolved in distil water to form a homogenous polymer solution. Core material, Metformin HCl was added to the polymer solution and mixed thoroughly. The resulting mixture was then added in a thin stream to of alcohol mucilage of HPMC contained in a 450 ml beaker, while stirring at 1000 rpm to emulsify the added dispersion as fine droplets. A Remi make medium duty stirrer with speed meter was used for stirring. The solvent, alcohol was then removed by continuous stirring at room temperature for 3 h to produce spherical microspheres. The microspheres were collected by vacuum filtration and washed repeatedly with water. The product was then air-dried to obtain discrete microspheres. Formulation design was shown in table

Ingredients	FS1	FS2	FS3
Drug	**500mg**	**500mg**	**500mg**
Carbopol	250mg	500mg	1gm
HPMC	250mg	500mg	1gm

Table 1 Formulation of Metformin HCl microspheres

2.1.2. Physicochemical studies

The overall objective of the investigation of physical and chemical properties of a microspheres substance is to develop stable and bio available dosage forms.

2.1.2.1. Loss on drying

About 1gm of spheres taken in the plate of digital moisture balance. Maintain $105^{o}C$ till constant weight obtained. The percentage loss on drying was calculated by using the formula.

% LOD = Practical wt / Theoritical wt * 100

2.1.2.2. Flow properties

A funnel was kept vertically in a stand at a specified height above a paper placed on a horizontal surface. The funnel bottom is closed and 10 gm of sample powder is filled in funnel. Then funnel was opened to release the powder on the paper to form a smooth conical heap, is found by measuring in different direction. The height of the heap was measured by using scale. The value of angle of repose is calculated by using the following formula;

$\tan\theta = h/r$, $\theta = \tan^{-1} h/r$, Where, h height of the heap, r radius of the heap

2.1.2.3. Bulk density

A known quantity of powder was poured into the measuring cylinder carefully level the powder without compacting, if necessary and read the unsettled apparent volume V_o, to the nearest graduated unit . Calculate the bulk density, in gm per ml, by the formula m / Vo.

2.1.2.4. Tapped density

Tapped density is achieved by mechanically tapping a measuring cylinder containing a powder sample. Cylinder dropping distance 14± 2 mm at a normal rate of 300 drops / minute. Unless otherwise specified, tab the cylinder 500 times initially and measure the tapped volume, Va, to the nearest graduated unit. Repeat the tapping an additional 750 times and measure the tapped volume, Vb, to the nearest graduated unit.

Tapped Density = $\dfrac{m}{Vf}$

2.1.2.5. Measurement of powder compressibility

The compressibility Index and Hausner Ratio are measures of the propensity of a powder to be compressed. As such, they are measures of the relative importance of inter particulate interactions. In a free flowing power, such interactions are generally less and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater inter particle interactions, and a greater difference between bulk and tapped densities will be observed. These differences are reflected in the Compressibility index and the Hausner ratio calculated by the formula:

$$\textit{Compressibility index} = 100\frac{(V_0 - Vf)}{V_0}, \textit{Hausner Ratio} = \frac{V_0}{Vf}$$

2.1.2.6. Melting point

A small quantity of powder was placed into a fussion tube. That tube is placed in the melting point determining apparatus containing liquid paraffin. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

2.1.2.7. Particle size (Sieving methods)

This test was performed with the help of sieves of different size. They were fitted in the platform of sieve shaker in such a way that the coarse sieve was placed on top corresponding to the finer sieves. Placed 10 gm of the metformin HCl on the top and runner the machine to separate out the powder and after some time off the machine and took the weight of the powder remain on the sieve(s). Finally, calculated the % of powder retained on each sieve by the following equation:

$$\% \text{ powder retained} = \frac{\text{amount of powder retained}}{10}X100$$

Stability Study

All the formulations were studied for stability profile for 1 month at different environmental conditions such as 4^oC, room temperature, 45° C and 82.5% RH. Keeping the microspheres in refrigerator to produce 4^oC environment, 45^oC environment was produced by keeping the microspheres in hot air oven. The change in drug relief when was stored at 4*c and room temperature. Stability study of formulated microsphere at various c temperature such as 4^*c, room temperature, 45*c were carried out and results were shown in table-6. There was no significant

There was a slight variation in drug release when it was stored at 45*c.

EVALUATION OF MICROSPHERES

Determination of % yield of microspheres

The dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formulae given below.

$$\% \text{ yield} = \frac{\text{microspheres obtained}}{\text{Total weight of drug and polymer}} x\ 100$$

Determination of drug content

Microspheres were estimated by an UV spectrophotometric method based on the measurement of absorbance at 233nm in phosphate buffer of pH 6.8. The method obeyed Beer's law in the concentration range of 5-50 µg/ml. when a standard solution was assayed repeatedly (n=6), the mean error (accuracy) and relative standard deviation (Precision) were found to be 0.6% and 1.2 % respectively. Weighed quantities of microspheres were first treated with phosphate buffer pH 6.8 and then it was diluted to 100 ml with the same. From this 5 ml of the sample was taken and mixed with 5 ml of picric acid solution and then the drug content was assayed spectrophotometrically at 405 nm using medium as blank.

$$\% \text{ loading} = \frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}} x\ 100$$

Particle size determination

For size distribution analysis, different sizes in a batch were separated by sieving using a range of standard sieves. The amounts retained on different sieves were weighed. Optical microscope was used to determine the size of the particle that lies within a range from 0.2 µm to 100 equal divisions and hence, each division is equal to 10 µm and the particles are measured along an arbitrarily chosen fixed line across the center of the particle. The particle size is a factor to be considered important in formulation of microspheres.

Scanning electron microscopic study

The microspheres were observed under a scanning electron microscope. The instrument used for this study was Hitachi S-450 scanning electron microscope. The microspheres were mounted directly on to the SEM sample stub, using double-sided sticking tape, and coated with gold film (thickness 200 nm) under reduced pressure (0.001 torr).

In-vitro release study

100 mg equivalent weight of the Metformin HCl was taken in a dialysis tube and placed in 500 ml of phosphate buffer. The medium was stirred by using the magnetic stirrer and the temperature was maintained at 37±0.5°C. Periodically 1 ml of the samples were withdrawn and diluted to 10 ml by using phosphate buffer. After each withdrawal the same quantity of the fresh medium was replaced immediately. Then the samples were assayed spectrophotometrically, Systronics UV spectrophotometer 116 at 233nm using, medium as blank. The release was compared with marketed Metformin HCl tablets.

In-vivo studies

Male Wister rats (150-175gm) were used for present study. They were maintained under standard environmental conditions and were fed with standard pellet diet water ad-labium. Animals were allow to fast 24 hrs prior to injection with a freshly prepared alloxane (50mg/kg I.P.) dissolved in distill water. After 48 hours rats with marked hyperglycemia (fasting blood glucose> 275mg / dl) were used for the study.

Experimental design

Four groups of rats were used to study the effect of drug and microspheres.
They were divided in to four groups each Consisting of six rats.
Group 1 - Normal rats
Group 2 - Diabetic control
Group 3 - Normal rats treated with 50mg/kg Metformin HCl.
Group 4 - Normal rats treated with 50mg/kg of Carbopol microspheres.
After an overnight fast, the pure drug and micro spheres suspended in Phosphate buffer (pH 4.5) was fed by gastric intubations with a syringe. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 2 and 3 hrs. The blood glucose level was determined via an electronic glucometer.

3. RESULTS AND DISCUSSION

In the present work an attempt was made to formulate & evaluate Metformin HCl microspheres of various concentrations of Carbopol[940] as carrier. The confirmation of formulations of FS1, FS2, and FS3 were shown in table 1. Carbopol microspheres size distribution, percentage of entrapment, micromeritics, flow properties, drug stability in various stress conditions and in vitro& in vivo release studies were evaluated.

Percentage of entrapment

The percentage of entrapment was determined for all the batches, the results were shown according to formulation. The encapsulation efficiency was found in the range of 49.76%, 68.4%, and 54.5% for FS-1, FS-2, and FS-3 respectively

Content uniformity

The percent of drug content indicated the uniformity of drug content in each batch of microspheres. All the batches of FS1 to FS3 were tested thrice to confirm the content uniformity and it was found uniform.

COMPATIBILITY STUDY

IR spectral study was carriedout to find the authenticity of drug & compatibility of polymer with drug. The IR spectra shown in graph no 1. It concludes that the drug was compatible with the carbopol[940].
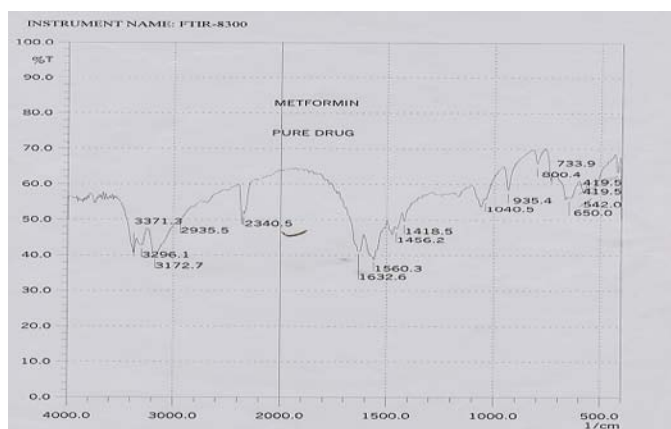

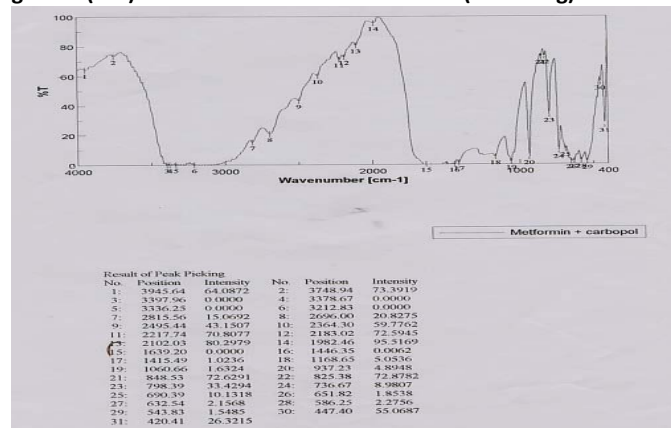**Fig. -1 IR (KBr) SPECTRUM OF METFORMIN HCl (Pure drug)**

Fig.-2 IR (KBr) SPECTRUM OF MICROSPHERES OF METFORMIN HCl

Size and shape of microspheres

Scaning electron photograph of all the formulation were shown in fig no-7.Different magnification uses while taking the photo micrograph.particle size of Metformin microsphere found to be range from 250 – 400 micrometer for FS1, FS2 and FS3 respectively. Particles of all the formulation were found smooth & discrete, where as formulation FS2 was found to be more spherical,

smooth and discrete. The size analysis of different batches of microspheres showed that about 65% were in the size range of 325 μm. The size distribution of the microspheres was found to be normal in all the batches.

S.No	Formulations	Particle size ±SEM μm
1	FS 1	289.9±9.1
2	FS 2	342.6±9.5
3	FS 3	435.1±10.3

Table 2 The particle sizes of microspheres of Metformin HCl
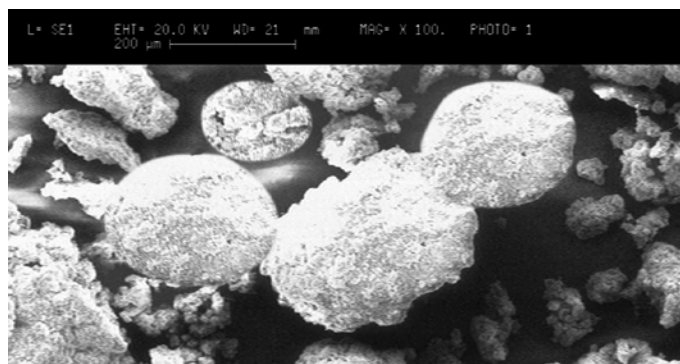
Fig. 1. FS-1 The scanning electron microscopic photo of microspheres
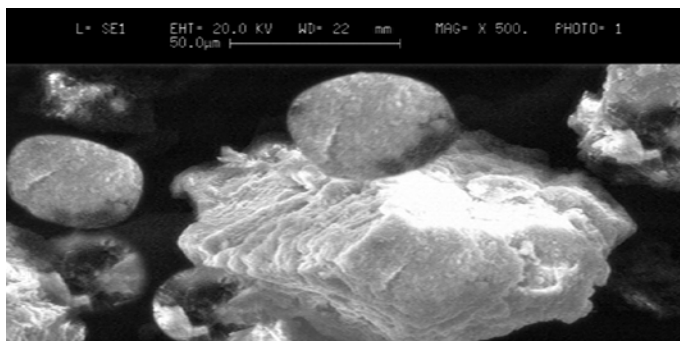
Fig.2. FS- 2 The scanning electron microscopic photo of microspheres
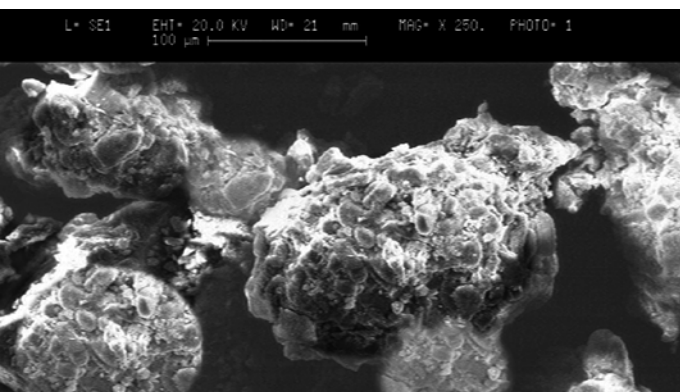
Fig.3. FS-3 The scanning electron microscopic photo of microspheres

STABILITY STUDIES

Physical stability and effect of aging on the drug release were studied in the control as well as microspheres. All microsphere formulations were kept in a small air tight glass containers and stored at different

Sr. No.	Formulation	Bulk Density	True density	Porosity %	Angle Of repose	Consolidation index %
1	FS 1	0.58	0.78	22.63	14.5	13.0
2	FS 2	0.63	0.82	26.85	14.7	10.4
3	FS 3	0.69	0.74	18.50	15.9	9.7

Table 3 True density, bulk density, void porosity of microspheres of

Metformin HCl

temperatures of $4\pm1^{o}C$ in a refrigerator and $45\pm2^{o}C$ in the oven and in a desiccators containing saturated solution of conc.H_2SO_4 (13.1 ml to100 ml with distil water) to obtain 85% RH, effect of aging on their release characteristics was studied after 4th week using dissolution method.

Formulation	$4^{o}C$	Room temperature	$45^{o}C$
FS 1	91.11	86.26	83.30
FS 2	83.03	87.14	88.90
FS 3	94.33	90.21	95.16

Table 4 The stability of microsphere formulations at various temperatures

INVITRO DISSOLUTION STUDIES

The *invitro* studies of carbopol microspheres was determined by using dialysis technique containing dissolution medium as phosphate buffer (pH-6.8). Table No. 8 shows the release of Metformin HCl from marketed tablets. The marketed Metformin HCl tablet releases 99.18% of the drug within 60 minutes. The microsphere formeulation of Metformin HCl using carbopol retards the release of Metformin HCl from the microsphere and produces the sustained action. The formulated microspheres of FS1, FS2, and FS3 released 93.62%, 96.34%, 97.4% of drug after 8hrs respectively. The increase in concentration of carbopol causes the decreasing the rate of release. The increasing concentration of HPMC was slightly retarding the release rate.


**Standard curve**

y = 0.051x - 0.009
R² = 0.999
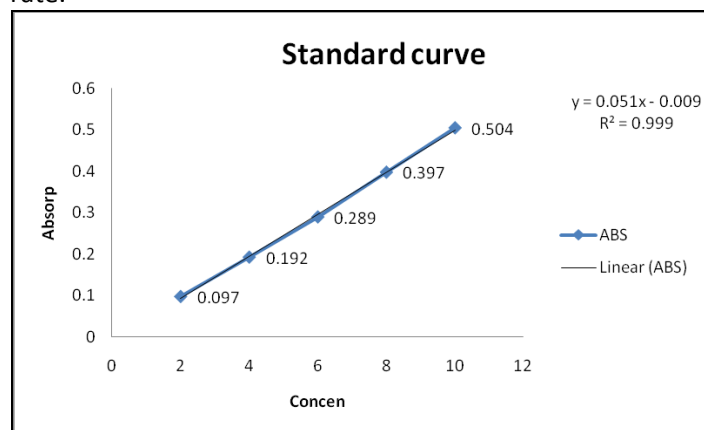
Graph –3

Sr. NO.	Design of Control	Drug	Blood glucose level mg/dl					
			0 hr	1 hr	2 hr	3 hr	4hr	5hr
1.	Control	–	78.50±0.92	80.83±0.79	79.50±0.76	80.50±0.80	79.0±0.79	80.80±0.76
2.	Diabetic Control	–	239.33 ±0.88	250.17 ±0.48	259.50 ±0.76	233.50 ±0.56	236.56±0.61	248.44±0.54
3.	Metformin	50 mg/kg	96±0.58*	111.50 ±0.76*	105.33 ±0.69*	100.17 ±0.40*	103.21±0.65*	107.52±0.48*
4.	Microsphere	50 mg/kg	127.83±0.48*	140.33 ±0.49*	134.50 ±0.76*	130.17 ±0.68*	132.74±0.74*	128.33±0.65*

Table 5 Effect of Carbopol microspheres of Metformin on Alloxan induced diabetic Rats

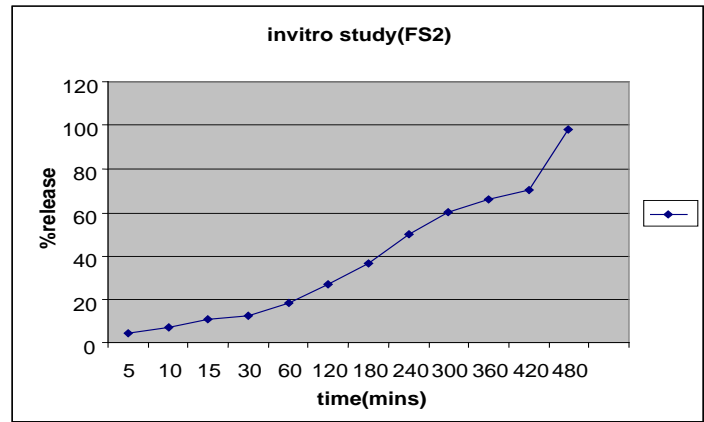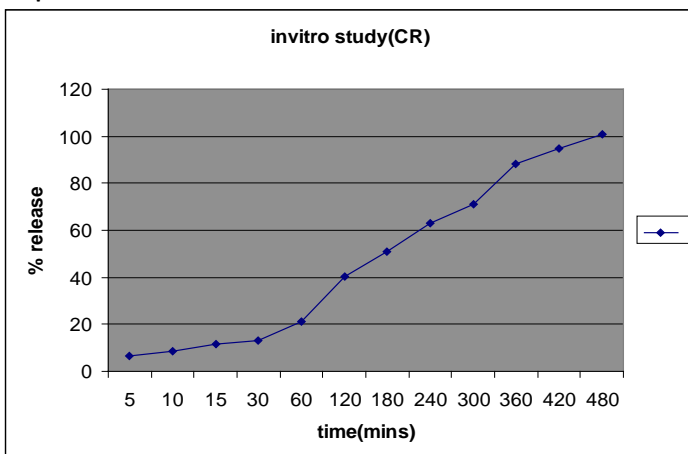N = 6, * $P < 0.001$ vs control, Values are expressed as mean ± SEM.
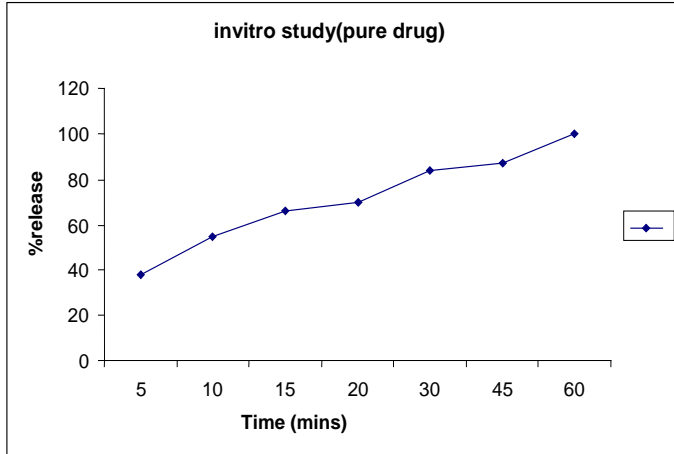
Graph -4
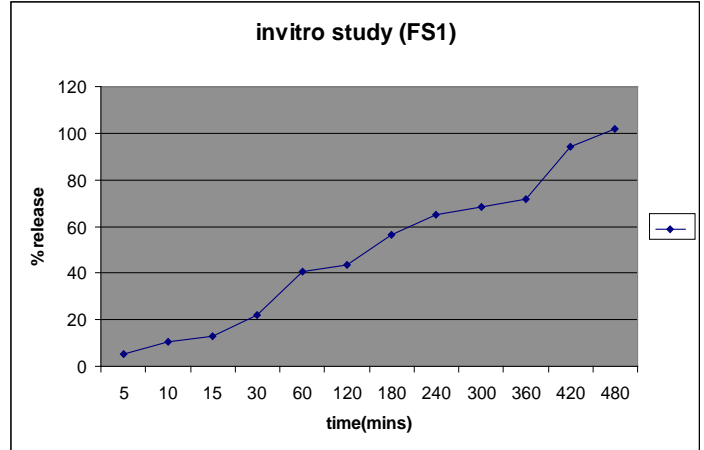
Graph -7
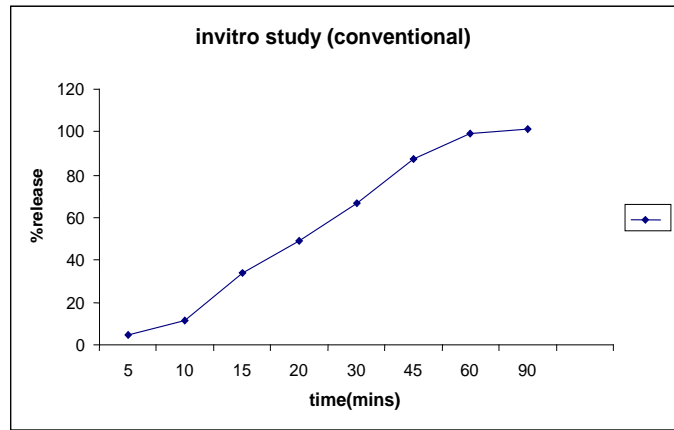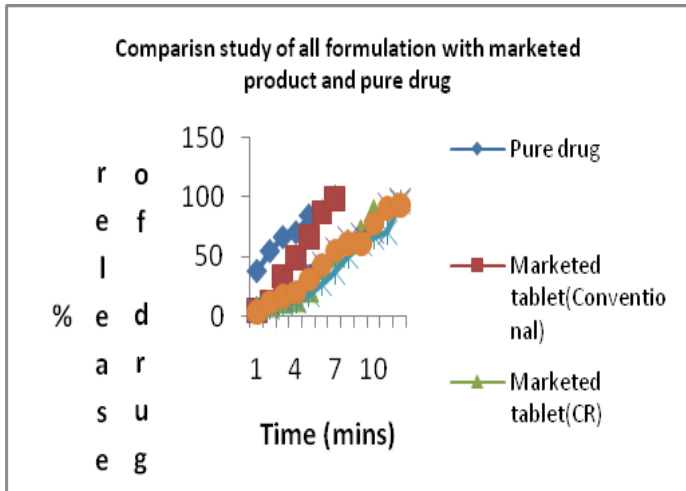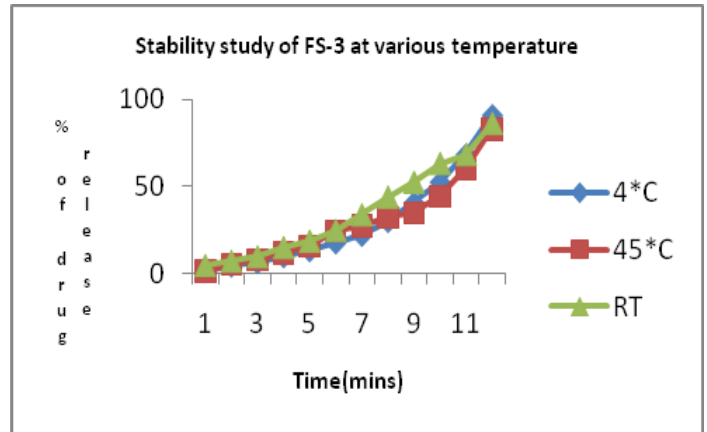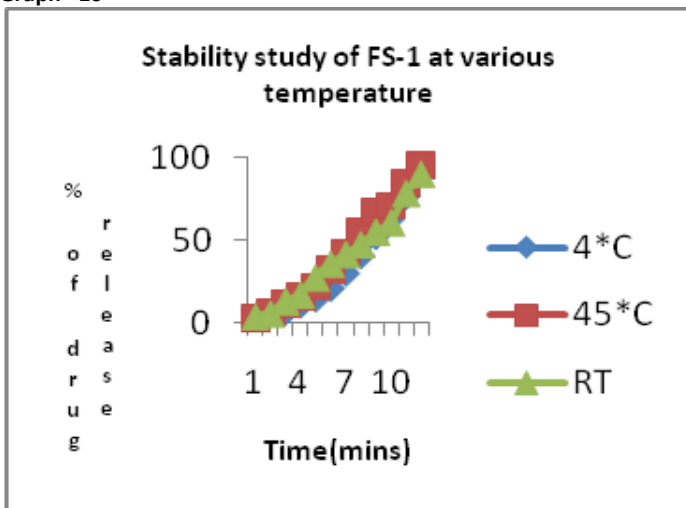

Graph – 5

Graph - 8

Graph - 6
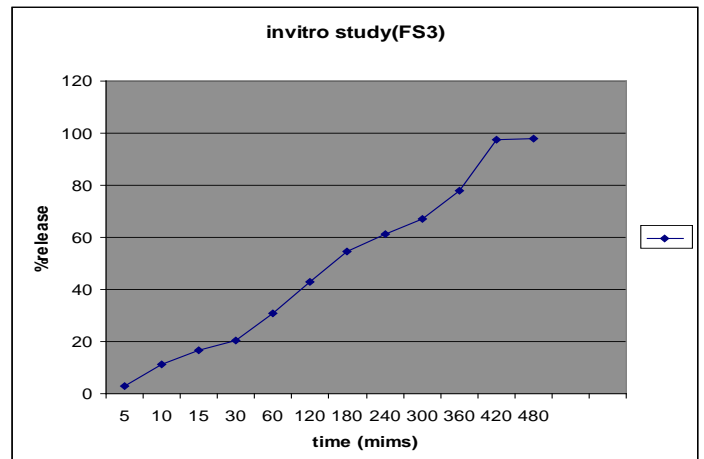
Graph – 9

Graph –10

Graph –11
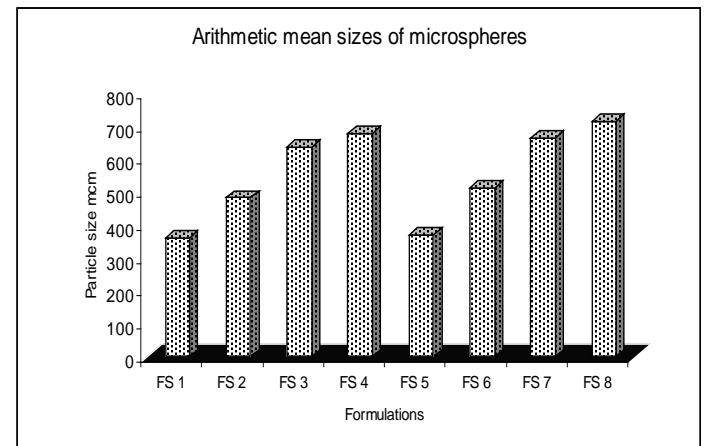
Graph –12

Graph –13

Fig. 4 Arithmetic mean sizes of microspheres

In vivo Study

The antidiabetic activity of formulated microsphere was carried out on four groups of male wister rats for 6 days, blood samples were collected from 0 to 5 hrs. The Blood glucose level determined and the results were shown in the table no 23.The intial blood glucose level of diabetic control rat was in the range 259.50 to 233.50. For the Metformin treated wister rat, the blood glucose levels were decreased from 1st to 5 hr .In the microsphere treated rat, the blood glucose level was stadily decreased from 1st to 5 hr and it was 140.33 to 127.83. It concludes the effect of diabetic activity microsphere was shown sustained action. Statistical analysis of all values were expressed as mean + SEM. The data were statistically analyzed by student t-test.

SUMMARY AND CONCLUSION

The present work an attempt was made to formulate and evaluate carbopol microsphere of Metformin to obtained controlled release there by proper duration of action at a particular site. The nature of distribute, chemical stability of the drug a compatibility of drug with excipience were evaluated by various physical methods and IR studies. Metformin microsphere formulations such as FS-1, FS-2, and FS-3 were prepared by solvent evaporation technique and were evaluated for physicochemical characteristic, analytical characteristic, SEM , stability study , *in vitro* dissolution study and *in vivo* (anti diabetic activity) studies. Formulated microsphere was evaluated for encapsulation efficiency, content uniformity, size & shape, micromeretics parameter. It

found that all micromeretics parameters comply with official specification. The encapsulation efficiency was good for (68.4%) FS-2 formulation. Size and shape of microspheres were confirmed by SEM. It found that microsphere were uniform in size with smooth surface. The stability study of formulated microsphere at various temperature such as 4*c, room temperature, 45*c were carried out and results were shown in table 5. There was no significant change in drug relief when was stored at 4*c and room temperature. There was a slight variation in drug release when it was stored at 45*c. All the formulations were carried out for *in vivo* antidiabetic activity on wister male rat. In the microsphere treated rat, the blood glucose level was steadily decreased from 1st to 5 hr and it was 140.33 to 127.83. It was concluded that the effect of antidiabetic activity of microspheres were in sustained action. It was possible to prepare carbopol microspheres of Metformin HCl by solvent evaporation technique, for prolonged activity with increased stability without losing its therapeutic activity.

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