

# Metformin HCl loaded mucoadhesive agar microspheres for sustained release

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In the recent past, a major interest in the control of blood sugar had been targeted to develop plenty of new formulations. The present work aims at the development of a low cost sustained release system of metformin hydrochloride embedded in microspheres of agar (*Gelidium cartilagineum*) to overcome the frequent dosing of the drug. Models were developed with respect to controlling variables ( $X_1$ , drug: Polymer,  $X_2$ , surfactant concentration, and  $X_3$ , pH of phosphate buffer). The most effective levels of parameters were found as  $X_1$  (1:2),  $X_2$  (1.25%),  $X_3$  (phosphate buffer pH 7.4). Instrumental analysis (Fourier transforms infra-red spectroscopy, differential scanning calorimetry, X-ray diffraction and scanning electron microscopy), mucoadhesion study, toxicity test and *in vivo* study were performed with the optimized product. The best batch (A2) exhibited a high drug entrapment efficiency of  $84.82 \pm 1.23\%$ , swelling index of  $3.84 \pm 0.38\%$  and 86% of mucoadhesion after 12 h. The *in vitro* release was also sustained for more than 12 h.

**Key words:** Agar, hypoglycemic, metformin hydrochloride, mucoadhesion, swelling index

## INTRODUCTION

Powdered agar is composed of two poly saccharides, agarose, and agaropectin. Agarose contains 1,3 linked D-galactose and 1,4 linked 3,6 anhydro L-galactose units, with very few hydroxyls being sulphated, useful as a material for the gel formation. Agaropectin is more complex structure than agarose, containing in addition to D-galactose and 3,6 anhydro galactose units, D-gluconic acid, pyruvic acid, and a much higher proportion of sulphate ester groups. Agar micro particulates can provide an intimate contact with the membrane for a long period. Drugs that are easily absorbed from the gastrointestinal tract (GIT) and having a short half-life are eliminated quickly from the blood circulation. To avoid this problem, the oral controlled release formulations have been developed as these will release the drug slowly into the GIT and maintain a constant drug concentration in the serum for a longer period of time. Therefore, prolonged gastric retention<sup>[1]</sup> is crucial in achieving the control over the gastric residence time (GRT) because this helps to retain the controlled release system in the intestine for a longer and predicted period of time. Type 2

diabetes mellitus is a heterogeneous disease associated with many health disorders, which necessitates discovering new medications improved dosage forms. Increased cardiovascular mortality owing to diabetes has drawn attention in the discovery of antidiabetic drug to limit the post meal excursion. Despite the availability of new agents, oral sulfonylurea remains a drug of choice by many medical practitioners because of its well-tolerance and less cost. Disadvantage of short biological half-life ( $3.4 \pm 0.7$  h) may be overcome by the development of sustained release dosage forms to improve patient compliance and to achieve better control over post meal hyperglycemic spikes. However, the development of mucoadhesive systems is one of such method by which the controlled release system adheres to the gastric mucosa to improve the GRT. Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have created enormous

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impact in the formulation and the development of novel drug delivery systems.<sup>[2-4]</sup> Microspheres form an important part of such novel drug delivery system. They have varied applications and are prepared using the various polymers.<sup>[5]</sup> However, the success of these microspheres is limited due to their short residence time at the site of absorption. It would therefore, be advantages to have means for providing an intimate contact of the drug delivery system with the absorbing membrane.<sup>[6-9]</sup> This can be achieved by coupling mucoadhesion characteristics to microspheres and developing mucoadhesive, microsphere which provides the advantages such as efficient absorption and enhanced bioavailability of the drugs due to high surface to volume ratio, much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site.<sup>[10]</sup>

## MATERIALS AND METHODS

Metformin Hydrochloride was a kind gift sample from M/s. Dey's Medical Pvt., Ltd., (Kolkata, India). Agar was from a commercial source (M/s. Ranbaxy fine chemicals Ltd. New Delhi, India). Liquid Paraffin provided by M/s. Loba Chemie Pvt. Ltd., (Mumbai, India). All other chemicals used were of analytical grade, obtained from a commercial source, and used without further purification.

### Preparation of agar microspheres: By hot-cold congealing technique

Agar microspheres were prepared by hot-cold congealing method.<sup>[11]</sup> Aqueous (phosphate buffer pH 6.8-8.0) agar solution (4%) containing metformin hydrochloride (500 mg), was heated to  $37 \pm 1^\circ\text{C}$ , and slowly dispersed drop wise through a syringe (No. 20) into 100 ml light liquid paraffin containing span 85 (1-1.5% w/v, HLB value 1.8). This dispersion was stirred in a mechanical stirrer at a speed of 500 rpm. The vessel was surrounded by ice and left for 20 min without stirring. The formed microspheres were then recovered by decantation and washed twice with acetone followed by thrice with di-ethyl-ether. Then the microsphere was dried at room temperature. The prepared microspheres were stored in a desiccator for further use. Process control parameters for formulations were taken as per reported in Table 1.

### Drug incorporation efficiency

Microspheres (25 mg) were crushed in a glass mortar-pestle and the powdered microspheres were suspended in 50 ml phosphate buffer (pH 7.4). After 24 h, the solution

was filtered and the filtrate was analyzed by Ultraviolet spectrometer (U-2001 Hitachi, Japan) at 232 nm. The drug entrapment efficiency was calculated as per the following formula:<sup>[12]</sup>

Incorporation efficiency(%)=

$$\frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100 \quad (1)$$

### Determination of yield of the formulation

The microspheres yield was determined according to the formula:

$$\text{Yield}(\%) = \frac{\text{Mass of microspheres}}{(\text{Mass of drug} + \text{mass of polymer})} \times 100 \quad (2)$$

The drug content from the various formulations were determined by taking 25 mg of microspheres and crushed them to powder and dissolved in phosphate buffer of pH 7.4.<sup>[13]</sup> Then, the solution was filtered using the membrane filter and analyzed for the drug content by UV-VIS Spectrophotometer (U-2001 Hitachi, Japan) at 232 nm. The measured response with respect to the variables were reported in Table 2.

### Physical characterization of agar microspheres

Microspheres were characterized for their micromeritic properties such as particle size, shape, bulk density, tapped density, compressibility index, Hausner's ratio, and angle of repose. The prepared metformin loaded agar microspheres were sized using a Malvern 2600 laser diffraction spectrometer. The size of the microspheres was determined in isobutanol as a non-dissolving dispersion medium and particles were suspended mechanically by magnetic stirring during the measurement.

Bulk density/Tapped density is measured to determine "volume per unit mass" occupied by the microspheres at stationary state and under transportation state. It indicates free flow behavior of agar microspheres through the hopper. Angle of repose, Hausner's ratio, and Carr's index (% compressibility index) were determined to predict the flow

**Table 1: Process control parameters and their limits**

Parameters	Units	Notations	Limits		
			-1	0	+1
Drug to polymer ratio	w/w	$X_1$	1:2	1:3	1:4
Surfactant concentration	w/v	$X_2$	1	1.25	1.5
Phosphate buffer pH	ml	$X_3$	6.8	7.4	8.0

**Table 2: Design matrix and measured responses**

Variable levels in coded form				% yield	% DEE	% of drug release
Batch code	$X_1$	$X_2$	$X_3$			
A1	0	1	0	84.22±1.87	80.44±0.66	78.25±1.01
A2	-1	0	0	87.60±1.03	84.82±1.23	87.09±1.07
A3	-1	1	1	83.66±1.22	80.01±1.09	71.07±1.12
B1	0	0	-1	83.92±1.21	80.16±0.77	75.46±0.98
B2	1	1	-1	84.57±1.78	82.08±1.34	83.37±1.23
C1	1	-1	-1	83.78±1.11	80.08±0.98	73.29±1.13
C2	0	0	0	83.44±0.21	79.45±1.22	70.37±1.03

DEE: Drug entrapment efficacy

ability. A higher Hausner's ratio indicates greater cohesion between particles, although a high Carr's index<sup>[14]</sup> is indicative of the tendency to form the bridges within hopper.

### Determination of swelling index

Swelling of individual microsphere was carried out by measuring the percentage water of uptake<sup>[15,16]</sup> by the microspheres after 10 h, accurately weighed microspheres were incubated with 10 ml of phosphate buffer of pH 7.4 and 0.1 N HCl at  $37^\circ \pm 1^\circ\text{C}$ . The microspheres were then removed and weighted the final weight after drying the surface water. During this procedure, the swelling microspheres were handled carefully in order to avoid any mass loss due to the breaking or erosion of the microspheres. The water uptake was calculated in terms of percent water uptake as following.

$$\% \text{ Water uptake} = \frac{W_s - W_d}{W_d} \times 100 \quad (3)$$

Where  $W_d$  and  $W_s$  are the initial weight and final weight of microspheres.

### In vitro wash-off test to determine mucoadhesivity

The mucoadhesive property of the microspheres was evaluated by *in vitro* wash-off test as reported by Lehr *et al.*<sup>[17]</sup> A 1 cm<sup>2</sup> piece of prepared goat intestine was tied onto a glass slide using the thread. Fifty Microspheres were spread on to the rinsed tissue specimen and the prepared slide was hung onto one of the grooves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated whereby the tissue specimen was given regular up and down movements in the beaker of the disintegration apparatus, which contained phosphate buffer of pH 7.4 and 0.1 N HCl (pH 1.2) at 37°C. At the end of 30 min, 1 h and at hourly intervals up to 10 h, the numbers of microspheres still adhering onto the tissue were counted.

$$\% \text{ Mucoadhesion} = \frac{W_a - W_b}{W_a} \times 100 \quad (4)$$

Where  $W_a$  and  $W_b$  were the number of beads applied and number of beads adhered to the tissue upto 10 h.

### Fourier transforms infra-red spectroscopy

Fourier transforms infra-red spectroscopy (FT-IR) measurements were taken at ambient temperature using a Jasco FT-IR 670 plus to investigate the possible chemical interactions between the drug and polymer matrix.<sup>[18]</sup> About 2 mg of the samples were ground thoroughly with KBr and pellets were formed under a hydraulic pressure of 600 kg/cm<sup>2</sup>. The scanning range was 4000-400 cm<sup>-1</sup>.

### Differential scanning calorimetry

The differential scanning calorimetry (DSC) analysis was carried using the Perkin Elmer Pyris Diamond thermo

Table 3: Degree of swelling, % mucoadhesion, physical characterization and release kinetics parameters of prepared formulations

Code no	Degree of swelling (%) at 37°C±1°C		% Mucoadhesion		Particle size, µm	Bulk density, g/ml	Tapped density, g/ml	Angle of repose (°)	Carr's index	Hausner's ratio	Zero order R <sup>2</sup>	First order R <sup>2</sup>	Higuchi R <sup>2</sup>	Koresmeyer-Peppas	
	pH 7.4		pH 1.2											R <sup>2</sup>	n
	pH 7.4	pH 1.2	pH 7.4	pH 1.2											
A1	3.18±0.89	2.50±0.77	78	40	782.45±1.06	0.728±0.033	0.814±0.007	26.02±1.16	12.67±1.03	1.13±0.075	0.985	0.915	0.951	0.981	0.656
A2	3.84±0.38	2.68±0.29	86	48	746.98±0.98	0.819±0.035	0.882±0.013	28.78±2.96	11.05±1.01	1.48±0.088	0.988	0.873	0.992	0.977	0.672
A3	3.01±0.72	2.01±0.30	64	32	834.56±1.45	0.665±0.009	0.783±0.018	24.56±2.56	12.96±0.56	1.31±0.007	0.974	0.947	0.951	0.993	0.727
B1	3.10±0.91	2.28±0.59	74	38	796.34±1.56	0.712±0.010	0.807±0.014	25.67±3.98	14.47±1.11	1.11±0.004	0.973	0.980	0.986	0.997	0.651
B2	3.44±0.63	2.56±0.39	80	42	768.22±1.02	0.788±0.003	0.832±0.012	26.11±1.26	12.67±1.22	1.31±0.007	0.982	0.946	0.957	0.991	0.72
C1	3.04±0.46	2.20±0.29	70	36	809.23±1.34	0.691±0.009	0.794±0.011	25.33±2.12	13.42±1.72	1.21±0.017	0.987	0.963	0.963	0.987	0.684
C2	2.98±1.23	1.93±0.88	68	30	868.19±0.98	0.655±0.021	0.767±0.016	23.98±1.56	14.56±1.35	1.45±0.015	0.986	0.986	0.951	0.981	0.682

gravimetric/differential thermal analyzer TG/DTA to evaluate any possible drug-polymer interaction.<sup>[19]</sup> The DSC studies on the samples were performed by heating samples at a heating rate of 8°C/min over a temperature range of 30°-350°C in closed aluminum pans under argon purge.

### Scanning electron microscopy

Scanning electron microscopy (SEM) was used to evaluate the shape and surface characteristics of particles.<sup>[20]</sup> Scanning was carried out using the Carl Zeiss (Leo), model-S-440. Prior to examination, the sample was fixed on a brass stub and coated with a gold palladium layer under argon atmosphere by using a gold sputter module in a high vacuum evaporator. The instrument was set at an excitation voltage 20 kv.

### X-ray diffraction (X-RD) studies

It is a physical technique used in the present study to identify on substances and other types of analysis principally for crystalline materials in the solid state. It was carried out by using the Rigaku, Japan, model-Miniflex. The target material of the instrument was copper (Cu) and Kb was used as a filter and Voltage of 30 kv and a current of 15 mA. It was done at room temperature. The sample was mounted on to the diffractometer and ciliated the X-rays on to the powdered sample to get the diffraction peak of certain intensities and recorded. Scan speed and scan axis were 1.000°/min and 20/θ respectively.

### *In vitro* drug release profiles

The drug release study was carried out using the USP XIII-6 basket<sup>[21]</sup> apparatus (Thermonic, Cambell Electronics, Mumbai, India) at 37° ± 0.5°C at 100 rpm using 900 ml of phosphate buffer pH 7.4 as a dissolution medium (*n* = 3). A total of 50 mg of microspheres were used for this test. 5 ml of sample solution was withdrawn at a pre-determined time intervals up to 12 h and replenished with dissolution media kept at a constant temperature at 37° ± 0.5°C and spectrophotometrically analyzed (at  $\lambda_{\text{max}}$  = 232 nm) using Hitachi, model U-2001. The drug release rates were plotted as a function of time versus percent release.

### Kinetics of drug release

To identify release mechanism, it is usual practice to fit the % release data in various established equations. Drug release data were fitted to kinetic model equations, which include the zero order equation,  $M = k_0 t$ ; First order equation,  $\text{Log}(UM) = k_1 t/2.303$ ; Higuchi model equation,  $M = k_h \sqrt{t}$ ; Korsmeyer-Peppas model,  $M = k_{kp} t^n$ . Coefficient of correlation (*r*) values were calculated for the linear curves obtained by regression analysis. *M* is the fraction of drug released at time *t* with respect to the drug released at infinite time. *UM* represents a fraction of the drug unreleased at time *t*. In the present work, it was assumed that the whole quantity of the drug was released at infinite time. Percentage of cumulative drug release was used in place of *M*.  $k_0$ ,  $k_1$ ,  $k_h$  are the rate constants of zero order, first order, Higuchi model,

respectively.  $K_{kp}$  and *n* are the rate constant and exponent respectively in Korsmeyer-Peppas model. Different *n* values indicate different mechanisms of drug release. If the *n* value is around 0.5, then Fickian diffusion is apparent if the *n* value ranges from 0.5 to 1.0 it represents anomalous diffusion transport. For zero order release (case II transport), *n* = 1 and for super case II transport *n* > 1.

### Stability test

Optimized formulation (A2) of metformin hydrochloride-loaded agar microsphere was tested for stability studies. Three samples of A2 were stored at 4° ± 1°C, 25° ± 2°C (60 ± 5% RH) and 37° ± 2°C (65 ± 5% RH). After 90 days, % drug release was determined.

### Acute toxicity test

Acute toxicity test is mandatory to identify toxic effect of new optimized formulation if any, owing to pharmacological interaction between the drug and the pharmaceutical excipients and to ensure its safe administration to the human system. Acute oral toxicity of the formulation was conducted in Wistar rat according to the guidelines of "organization for economic co-operation and development-425)." Starting dose was selected to be 2000 mg/kg body weight and finally a dose of 5000 mg/kg body weight was evaluated for toxicity. Wistar rats (*n* = 6, average weight ~200 g) were under observation to check any change in behavior of animals for the first 2 h of administration, and mortality if any within 48 h.

### *In vivo* hypoglycemic activity of agar microspheres

A glucose tolerance test is conducted to find out how quickly it is cleared from the blood. *In vivo* efficiency of the optimized batch was performed in healthy normal Wistar rats weighing 200-250 g each, by measuring the hypoglycemic effect produced after oral administration of metformin.<sup>[22]</sup> The approval of Institutional Animal Ethical Committee was obtained before the start of the study, which was conducted in accordance with the standard institutional guidelines. Three groups of Wistar rats (*n* = 5) were kept on fast (with water) at least 12 h before starting the *in vivo* experiments. Pure metformin HCl (100 mg/kg of body weight) was administered orally to animals of Group I. Animals of Group II and Group III were administered with the same dose of marketed sustained release tablets (Diomet SR 500 mg, JB Chemicals, India) and mucoadhesive metformin microspheres respectively. Blood sample (1 ml) was collected from each rat from behind the eyeball through the angle of ocular cavity using small capillary tubes at a pre-determined time intervals at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 16 h and the blood glucose level was estimated by using the glucose oxidase-peroxidase reactive strips (Accu-chek, Roche Diagnostics, USA). Serum glucose levels were determined in the samples and percentage reduction in the glucose levels with respect to initial glucose level were calculated and plotted in Figure 1.



### Statistical analysis

All the results were carried out in triplicate. Results are expressed as mean  $\pm$  SD. One-Way Student's *t*-test was used to determine statistical significance. Differences were considered to be significant for values of  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Polysaccharide characterization

The microspheres of agar were prepared by hot-cold congealing method. Microspheres did not form at high concentration (8%) of agar solution.<sup>[23]</sup> Same time low concentration (2%) solution ended the dropping process difficulties and microspheres could not readily be formed. The concentration of agar was given a best result at 4% w/v for preparation of microspheres.

### Physical characterization of agar microspheres

The particle size of the microspheres was found to be ranging between  $746.98 \pm 0.98 \mu\text{m}$  and  $868.19 \pm 0.98 \mu\text{m}$ . Bulk density and tapped bulk density of microspheres are determined to assess bulk volume. It is necessary to know this property for packaging purpose. The tapped bulk density gives an idea about its compactness and possibility of breakage of particles during the transportation. Bulk density and tapped bulk density varied in the range of  $0.655 \pm 0.021$  to  $0.819 \pm 0.035$  and  $0.767 \pm 0.016$  to  $0.882 \pm 0.013 \text{ g/ml}$  respectively. Bulk density depends on property of material and size of microspheres. Angle of repose of the microspheres varied from  $23.98^\circ$  to  $26.02^\circ$ . Carr's index and Hausner's ratio varied in the range of 11.05 to 14.56 and 1.11 to 1.48 respectively. These indicated that the particles are free flowing and data were tabulate in Table 3.

### Determination of swelling index

The swelling properties of metformin-loaded agar microspheres were studied by measuring the water uptake up to 10 h in 0.1N HCl (pH 1.2) and phosphate buffer (pH 7.4). Agar microspheres exhibited swelling properties that are

sensitive to the pH. The results of water uptake by the microsphere are summarized in Table 3. In 0.1 N HCl, the percentage of water uptake was low and independent of time relative to that obtained at pH 7.4. Maximum water uptake by 7 h in phosphate buffer (pH 7.4), after which no erosion and breakdown of microspheres occurred. The data in Table 3 suggest that, the dried agar gel particles may swell slightly in the stomach subsequently transferred to the intestine. The agar particles then may begin to swell more and behave as matrices for controlled release of incorporated drug.

### In vitro wash-off test to determine mucoadhesivity

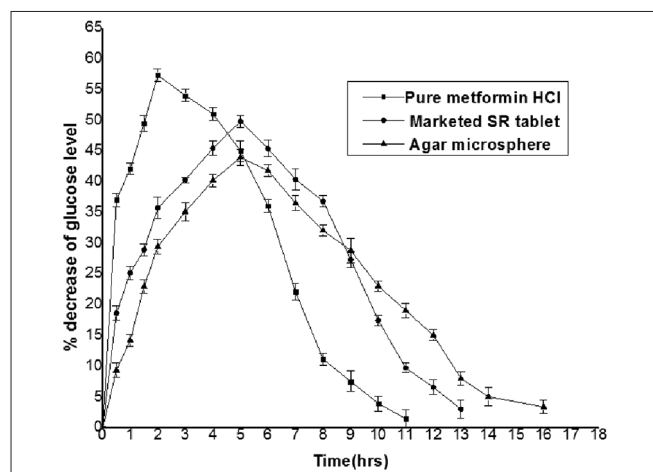
The polymers which showed the greatest swelling also appear to give the greatest work of adhesion in the system. Mucoadhesive performance is dependent on many parameters such as pH, state of hydration. pH influences the charge on the surface of both mucus and the polymers. Mucus has a different charge density depending on pH because of differences in dissociation of functional groups on carbohydrate moiety. Mucoadhesion of agar microspheres was dependent on pH (degree of ionization). Formulation A2 indicated best mucoadhesion strength due to gelation of phosphate buffer pH 7.4. It is fascinating to know that mucoadhesion increased between pH 6.8 and pH 7.4 and decrease at more alkaline pH level. Data were tabulated in Table 3.

### FT-IR

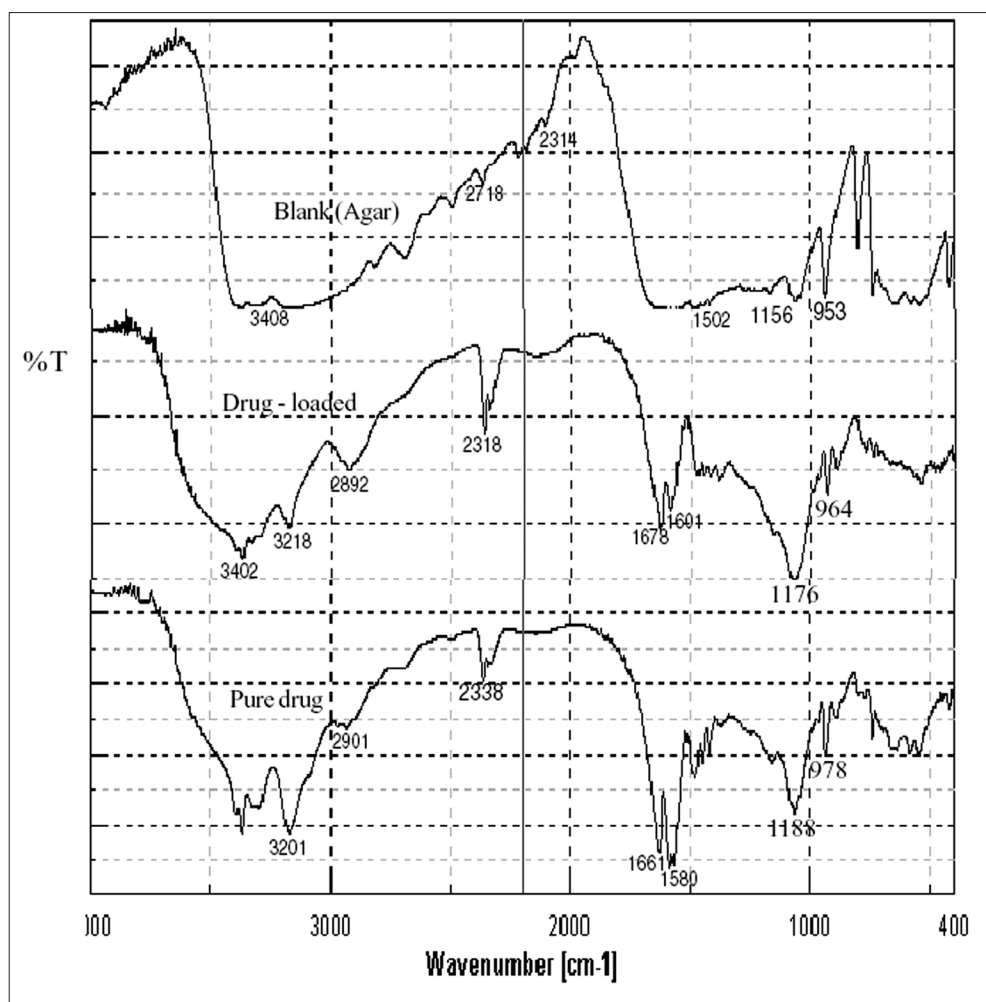
The FT-IR spectra as shown in Figure 2 were obtained from KBr disk. When recorded at 4 cm resolution, it showed vibration feature in  $4000\text{cm}^{-1}$  to  $400 \text{ cm}^{-1}$  region. The spectra of the free metformin hydrochloride and formulated metformin hydrochloride microspheres containing mucoadhesive agents showed similar peaks. In case of drug and drug-loaded device a broad band, which appeared at  $3201 \text{ cm}^{-1}$  and  $3218 \text{ cm}^{-1}$  were attributed to the presence of N-H bond due to stretching vibrations respectively. The peaks appearing at  $2892 \text{ cm}^{-1}$  for drug-loaded and  $2718 \text{ cm}^{-1}$  for blank device were due to C-H aliphatic stretching vibrations. In case of blank device, a broad band, which appeared at  $3408 \text{ cm}^{-1}$  is due to the stretching vibrations hydroxyl group (-OH) of 1,3 linked D-galactose and 1,4 linked 3,6 anhydro L-galactose units. As shown in Figure 2, there were no significant differences in the FT-IR spectra revealed that no significant interaction between drug and polymer.

### X-RD studies

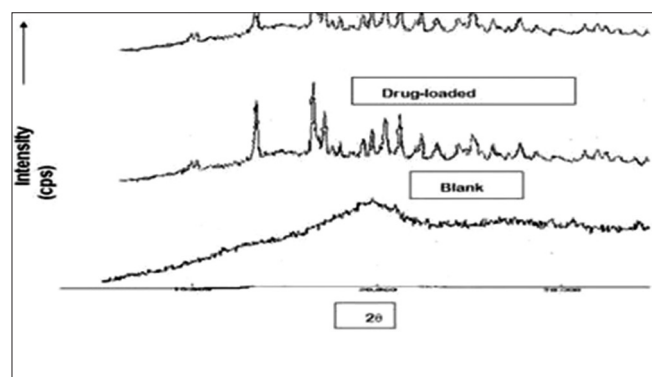
The X-RD spectra recorded for pure drug, drug-loaded microspheres and blank device were presented in Figure 3. These studies are useful to investigate the crystallinity of metformin in the microspheres. Metformin has shown characteristics intense peaks between  $2\theta$  of  $12.10^\circ$  and  $39.30^\circ$ , but in the case of blank device, no intense peaks were observed between  $2\theta$  of  $12.10^\circ$  and  $39.30^\circ$ . However, in drug-loaded microspheres, intense peaks were observed between  $2\theta$  of  $12.10^\circ$  and  $39.30^\circ$ , indicating the crystalline nature of the drug after entrapment into the microspheres.



**Figure 1:** *In vivo* glucose tolerance test of metformin hydrochloride in Wistar rat model



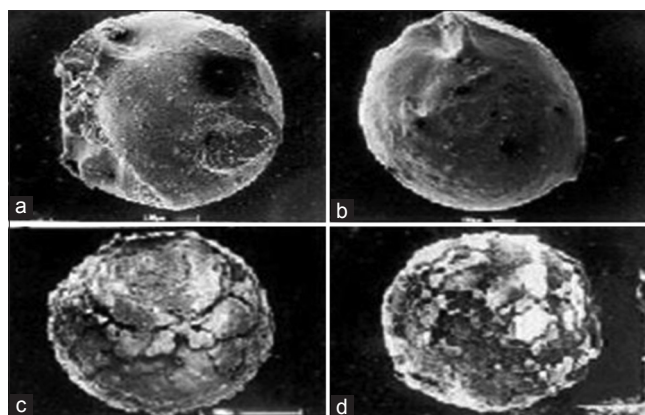
**Figure 2:** FTIR spectra of agar powder (top), metformin loaded microspheres (middle) and pure metformin hydrochloride (bottom)



**Figure 3:** X-ray powder diffraction spectra of pure drug, agar and drug loaded with microspheres

### SEM

The surface texture agar microspheres were observed by SEM as shown in Figure 4. The scanning electron micrograph indicated there was shrinkage over the surface after oven drying (40°C) and erosion. On to the surface after the dissolution in 0.1N HCl due to ion-exchange with HCl.<sup>[24]</sup>



**Figure 4:** A scanning electron microscopy (SEM) micrographs of agar microsphere: (a) SEM micrographs of agar microsphere before dissolution (A2), (b) microsphere of hot air oven dried, (c) upper surface after dissolution (pH7.4), (d) upper surface after dissolution (pH 1.2)

### DSC

The drug could be either dispersed in crystalline/amorphous form or dissolved in the polymeric matrix during the process of microencapsulation. Furthermore, any abrupt

or drastic change in the thermal behavior<sup>[25]</sup> of either the drug or polymer may indicate a possible drug-polymer interaction. A sharp endotherm (T peak 228°C) was observed for metformin at the temperature corresponding to its melting point. In the case of drug-loaded microspheres same endothermic peak is shown like pure drug. The same thermal behavior was observed in blank device showing thermal peak at 228°C. This may indicate that most of the drug was uniformly dispersed at the molecular level in the microspheres and there is no interaction between the drug and the polymer.

### *In vitro* drug release profiles

In the *in vitro* drug release studies, it was found that, the drug was released by diffusion from the matrix after hydration and swelling of the microspheres in the dissolution medium. The release metformin from the agar microspheres was depended upon pH of the gelation medium. It is inferred from Figure 5, that 1:2 ratio and gelation with phosphate buffer pH 7.4 (A2) showed better sustained release. It is noted that drug release increased with increasing with pH 6.8 to pH 7.4.

### Kinetics of drug release

The values of co-efficient of correlation ( $R^2$ ) and release constants ( $k_0$ ,  $k_1$ ,  $k_H$  and  $k_{kp}$ ) were calculated and data were best fitted to Higuchi model and good regression co-efficient was ( $R^2 < 0.992$ ) shown in Table 3.

### Stability test

The stability of formulation A2 was tested at various conditions. Agar microspheres (A2) stored at  $4^\circ \pm 1^\circ\text{C}$ ,  $25^\circ \pm 2^\circ\text{C}$  ( $60 \pm 5\%$  RH) and  $37^\circ \pm 2^\circ\text{C}$  ( $65 \pm 5\%$  RH) for 90 days showed drug release of 87.07%, 87.15%, and 87.03% respectively. Initially, the same formulation showed  $87.09 \pm 0.34\%$  release of drug. The result indicated that the formulation A2 ensured stability during its shelf life.

### Acute toxicity test

The oral administration of new formulations at a dose of

5 g/kg of body weight of rats did not produce any significant sign of toxicity or change in gross behavioral pattern during the specified period. It showed no lethal effect to any animal. It is suggested that the new formulation A2 is effective as sustained release dosage form and it did not cause any acute toxicity to the rats under experimental conditions.

### *In vivo* hypoglycemic activity of agar microspheres

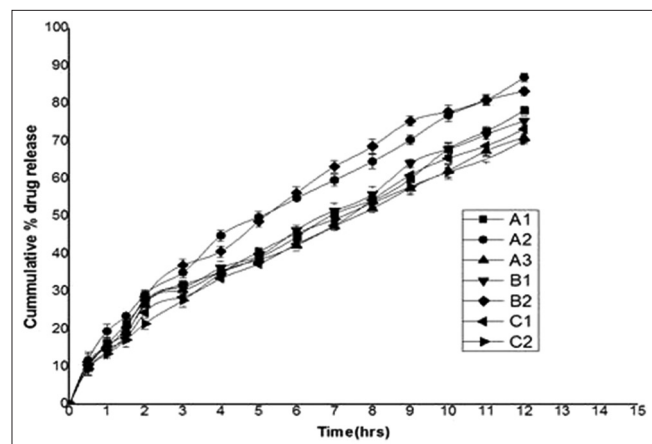
When pure metformin hydrochloride was Administered, a rapid reduction in blood glucose levels was observed, and maximum reduction of 57.42% was observed at 2 h [Figure 1]. In the case of marketed SR tablet, the lowering of glucose level reached the maximum level of 49.89% at 5<sup>th</sup> h. In the case of agar microspheres (A2), the lowering of glucose level was gradual and reached maximum reduction of glucose level of 44.03% at the 5<sup>th</sup> h and sustained for a long time. A 25% reduction in glucose level is considered significant for hypoglycemic effect.<sup>[26]</sup> From the results of Student's *t*-test, significant difference was observed between the hypoglycemic activity of pure metformin hydrochloride, marketed SR tablet ( $P < 0.05$ ) and agar microspheres ( $P < 0.01$ ). The results indicated that agar microsphere is more efficient in lowering glucose level when compared with that of pure metformin hydrochloride and marketed sustained release tablet.

### CONCLUSION

The interest is renewed in developing sustained release drug delivery system of antidiabetic drug, metformin hydrochloride with an aim to utilize the mucoadhesivity of carrier agar in the present study. The method used to form the microsphere with low cost agar was very easy and less time consuming. The optimized formulations showed mucoadhesion (86% in 10 h) for a longer period and drug release was 87.09% in 12 h period in phosphate buffer saline pH 7.4; its drug entrapment efficacy, yield were 84.82%, 87.60% respectively and it proved to be superior than marketed sustained release formulation when drug release and hypoglycemic effect were compared. Its stability study and toxicity test were also checked. Metformin hydrochloride from the optimized formulation A2 was slow, sustained, and depend on process variables. It is concluded that the new formulation is a potential sustained release dosage form of metformin hydrochloride.

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**Figure 5:** Drug release profile from mucoadhesive microspheres loaded with metformin hydrochloride

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