Delivery of repaglinide–cholestyramine complex loaded ethylcellulose microspheres to gastric mucosa for effective management of type-2 diabetes mellitus

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The main aim of this work is to develop and thoroughly evaluate resin-based multiparticle of repaglinide for effective management of type-2 diabetes mellitus. Repaglinide was complexed with cholestyramine resin; later the resin complexed drug was encapsulated in ethylcellulose microspheres. The microspheres were characterized for micromeritic properties, SEM analysis, entrapment efficiency, percentage yield, IR spectroscopy, buoyancy behaviour and in vitro drug release in simulated gastric fluid. Microparticles showed regular shape and spherical morphology with entrapment efficiency in the range 51–69%. Differential scanning calorimetry confirmed that there was no chemical interaction between the polymer and the drug. Resin-based microspheres showed good buoyancy behaviour (P < 0.05) due to the presence of bicarbonate ions and sustained release compared to plain drug microspheres (P < 0.05). Finally, the effectiveness of the formulations was evaluated in vivo for blood glucose lowering effect in both normal and streptozotocin-induced diabetic rats. Blood glucose lowering effect induced in diabetic rats by the repaglinide-loaded microspheres was significantly greater (P < 0.05) and prolonged (~8 h) compared to plain drug microspheres. In a nutshell, floating microspheres containing drug resin complex were able to sustain the drug release in an effective manner for prolonged periods of time and proven in vivo effectiveness by reducing blood glucose level for a longer duration.

Keywords: Cholestyramine, diabetes mellitus, ethylcellulose, microspheres, repaglinide.

The International Diabetes Federation estimates that more than 382 million people worldwide suffer from diabetes and the number is likely to rise to 592 million by 2035. More than 5.1 million deaths were reported in 2013 because of diabetes and this number is increasing day by day1. The situation is alarming in India where the number of diabetes patient is expected to cross the 100 million mark by 2030 (ref. 2). Current treatment of diabetes includes either use of oral anti-hyperglycaemic agents or parenteral administration of insulin. The inherent problems to parenteral delivery include poor patient compliance due to repeated injections and discomfort associated with pain. Hence, oral anti-hyperglycaemic agents are preferred as first-line therapy over parenteral administration. Conventional oral anti-hyperglycaemic agents do not target or adequately control postprandial glycaemia. The emergence of new classes of oral agents with a more specific mode of action provides, an opportunity to restore early-phase insulin release. Among the three main groups of oral anti-hyperglycaemic agents, insulin secretagogues (meglitinide analogues, i.e. repaglinide and nateglinide) have shown promising results for treatment of type-2 diabetes3.

Repaglinide, a fast and short-acting meglitinide analog has a short half-life (1 h), low bioavailability (50%) and poor absorption in the upper intestinal tract. Hence, it requires frequent dosing of 3–4 times a day4. All these characteristics make repaglinide an attractive choice for the development of a gastro-retentive dosage form.

Gastro-retentive systems can stay in the gastric region for several hours and therefore appreciably extend the gastric residence time of drugs. Prolonged gastric retention improves bioavailability, reduces wastage of drugs, and improves solubility of poorly soluble drugs in acidic environment. Such carriers also have applications for local drug delivery to the stomach and proximal small intestines. In addition, complexation of drug with ion exchange resin sustains the drug release pattern and further encapsulation of such complexes in gastro-retentive delivery system not only prolongs the transit time but also significantly enhances the duration of action and effectiveness of treatment. Our research group previously reported in vitro and in vivo characterizaion of calcium silicate-based floating microspheres and floating granular delivery system of repaglinide5–7.
Repaglinide has the potential to form a complex with ion exchange resin through its –COOH group and such a complex could be encapsulated in rate-controlling semi-permeable polymeric network. Anion exchange resin such as cholestyramine resin could be loaded along with bicarbonate ion in order to formulate an efficient gastro-retentive system. When such an encapsulated ion exchange resin–drug–bicarbonate system reaches the stomach, gastric HCl reacts with bicarbonate to release CO₂ which is locked inside the polymeric membrane leading to floating of carriers. Koukchak and Atyabi enrolled Amberlite® IRA900 resin complexed with diclofenac sodium and bicarbonate ions with a hydrophobic polymer (ethyl cellulose or Eudragit® RS100) and demonstrated its usefulness.

In this regard, we are prompted to study ion exchange resin-based sustained release gastro-retentive multiparticulate system of repaglinide for the effective management of type-2 diabetes mellitus. To achieve this goal, cholestyramine resin (an anion-exchange resin) was complexed with bicarbonate ions. The bicarbonated resin was further complexed with repaglinide and finally encapsulated within rate-controlling semi-permeable polymeric network in order to achieve the desired controlled and prolonged release profile.

Materials and methods

Materials

Repaglinide was obtained as a gift sample from M/s Torrent Pharmaceuticals Pvt Ltd, Ahmedabad. Cholestyramine resin (Tulsion 412) was a kind gift sample from Sigma-Aldrich GmBH (Munich, Germany), ethyl cellulose (EC) from HiMedia Laboratories Pvt Ltd, Mumbai and sodium bicarbonate. dichloromethane and polyvinyl alcohol were purchased from S. D. Fine-Chem. Ltd, Mumbai. All other chemicals used were of AR grade.

Purification of cholestyramine resin

Purification of cholestyramine resin was carried out using the method described by Torres et al. Briefly, 5 g slurry of the resin was treated with 3 × 50 ml of deionized water, 2 × 50 ml of 95% ethanol, 1 × 50 ml of 50% ethanol, and finally, 1 × 85 ml of deionized water. Each stage of treatment was carried out for at least 1 h using a batch process with magnetic stirring (MAC, India). Purification was achieved by treating the resin twice between its protonated and sodium form followed by washing with distilled water. This procedure was repeated once again and finally the resin was recovered by vacuum filtration, dried until constant weight at 50°C in a hot-air oven and stored in a desiccator until further use.

Complexation of bicarbonate ions with cholestyramine resin

Briefly, purified resin powder (10 g) was mixed with 50 ml of 1 M sodium bicarbonate (NaHCO₃) solution and kept for 15 min followed by decantation. This step was repeated again with fresh 50 ml of NaHCO₃ solution. The resin powder was filtered, washed with deionized water and dried overnight at 40°C. The filtrate was subjected to titrimetric analysis as follows in order to determine bicarbonate complexation with resin.

The filtrate (10 ml) was titrated against 0.1 M HCl solution using methyl orange as indicator, which shows a colour change from orange to blue at end-point. Each millilitre of 0.1 M HCl is equivalent to 0.08401 g of NaHCO₃ and the amount of bicarbonate complexed with resin is determined by the difference between initial amount of NaHCO₃ taken and unbound bicarbonate.

Preparation of drug bicarbonated resin complex (DBRC)

Complexes of drug and bicarbonated resin in the weight ratios 1 : 0.5, 1 : 1, 1 : 2, 1 : 3 and 1 : 4 were prepared by batch process (Table 1). The required amount of bicarbonated cholestyramine resin was added to 20 ml ethanolic solution of repaglinide followed by stirring using a magnetic stirrer. After stirring for a predicted time-period the slurry was filtered using Whatman filter paper (#41), washed with ethanol to remove unbound drug, if any, and dried in a hot-air oven at 50°C for 24 h. The amount of drug bound to the resin was calculated as the difference between the initial and remaining amount of drug in the filtrate. The loading capacity was found using the following formula:

\[
\text{Drug loading capacity} = \frac{\text{Amount of drug bound}}{\text{Initial amount of drug}} \times 100.
\]

Formulation of ethyl cellulose floating microsphere

Microspheres were prepared according to the procedure described by Sriwongjanya and Bodmier. Microspheres were prepared according to the procedure described by Sriwongjanya and Bodmier. Microspheres were prepared according to the procedure described by Sriwongjanya and Bodmier.

<table>
<thead>
<tr>
<th>Code</th>
<th>Drug : bicarbonated resin</th>
<th>Drug loaded after 2 h stirring (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBRC1</td>
<td>1 : 0.5</td>
<td>39.8 ± 3.1</td>
</tr>
<tr>
<td>DBRC2</td>
<td>1 : 1</td>
<td>51.0 ± 2.2</td>
</tr>
<tr>
<td>DBRC3</td>
<td>1 : 2</td>
<td>59.4 ± 2.2</td>
</tr>
<tr>
<td>DBRC4</td>
<td>1 : 3</td>
<td>62.8 ± 2.1</td>
</tr>
<tr>
<td>DBRC5</td>
<td>1 : 4</td>
<td>63.7 ± 1.9</td>
</tr>
</tbody>
</table>

DBRC, Drug bicarbonated resin complex. Data are mean ± standard deviation (SD; n = 3).

Table 1. Ratio of cholestyramine resin to drug for DBRC preparation and drug loading
weighed quantity of DBRC was dispersed in 5 ml of ethyl cellulose solution in dichloromethane. The resulting dispersion was subjected to sonication (Frontline, India) for 10 min to remove the air bubbles and added drop-wise into an external aqueous phase (200 ml, 0.25% w/v polyvinyl alcohol) with continuous stirring at 500 rpm by mechanical stirrer (Macro Scientific Works, India). The stirring was continued for 2 h at 40°C, which allowed evaporation of dichloromethane, resulting in the formation of microspheres. The microspheres were collected by filtration and dried in a desiccator for at least 48 h (Table 2). Microspheres of plain repaglinide (MR) were also prepared by the same method using drug polymer ratio 1:5 and treated as control for comparative studies.

Characterization of DBRC and microspheres

Infrared spectroscopy: IR spectroscopy of DBRC was performed on Fourier transformed-infrared spectrophotometer (FTIR-8400S, Shimadzu, Japan). DBRC was mixed properly with KBr (95:5) and placed into the sample holder. The spectrum was collected by scanning over 3000–400 cm\(^{-1}\) at room temperature.

Differential scanning calorimetry (DSC): Differential scanning calorimeter (DSC-60, Shimadzu, Japan) equipped with an intra-cooler and a refrigerated cooling system was used to analyse the thermal behaviour. Drug, resin, DBRC and microspheres were placed in hermetically sealed flat aluminum crucibles. Indium standard was used to calibrate the temperature. The temperature was changed at the rate of 10°C/min and scanned over a range from 50°C to 350°C.

Morphology: The external and internal morphology of the microspheres was studied by scanning electron microscopy (SEM; JSM 5600 JEOL, Japan). The microspheres were sprinkled on a double-adhesive tape, which was previously adhered to an aluminum stub. The stub was coated with gold to a thickness of about 300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The samples were randomly scanned and photomicrographs were taken.

| Table 2. Drug loading to 1:2 drug–resin ratio at various stirring times |
|---------------------------|-----------------------------|
| Time of stirring (h)      | Drug loading for 1:2 drug–resin combination (%) |
| 1                        | 36.39 ± 1.8                |
| 2                        | 59.11 ± 2.7                |
| 3                        | 60.87 ± 1.3                |
| 4                        | 61.53 ± 2.4                |
| 5                        | 61.97 ± 1.7                |
| 24                       | 64.88 ± 3.2                |

Data are the average of values (mean) ± standard deviation (SD; \(n = 3\)).

Micromeritic studies

The particle size of DBRC and microspheres was determined by an optical microscope equipped with a previously calibrated ocular microscope. The mean particle size was calculated by measuring at least 300 particles with the help of an ocular micrometer. The experiments were performed in triplicate.

The microspheres were characterized for their micromeritic properties, such as true density, tapped density, compressibility index and flow properties. The tapping method was used to determine the tapped density and percentage compressibility index. Formulation (500 mg) was poured into a 10 ml graduated cylinder via a funnel; volume was noted as bulk volume. The cylinder was tapped up to the constant volume and noted as tapped volume\(^{14}\). The tapped density and percentage compressibility index were calculated as:

\[
\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}}.
\]

\[
\% \text{ Compressibility index} = \left[1 - \frac{\text{Bulk volume}}{\text{Tapped volume}}\right] \times 100.
\]

True density was determined using a benzene displacement method and porosity (\(\varepsilon\)) was calculated using the equation\(^{14}\)

\[
\varepsilon = \left[1 - \frac{P_{t}}{P_{p}}\right] \times 100,
\]

where \(P_{t}\) and \(P_{p}\) are the true density and tapped density respectively.

Angle of repose (\(\theta\)) of the microspheres was determined by a fixed funnel method\(^{15}\). The formulation was poured on a graph paper through a funnel fixed at a particular height by means of a stand. The diameter (\(D\)) and height of the heap (\(H\)) formed were used to calculate the angle of repose:

\[
\tan \theta = \frac{2H}{D}.
\]

Percentage yield and drug entrapment

The prepared microspheres were collected by filtration, dried in desiccators, weighed and percentage yield was calculated using the following formula:

\[
\text{Yield (\%)} = \frac{\text{Total weight of microparticles}}{\text{Total weight of drug, polymer and other nonvolatile solids (if added)}} \times 100.
\]
Entrapment efficiency was calculated by a method suggested by Basu and Rajendran. The drug content of microspheres was determined by dispersing accurately weighed quantity in 10 ml of ethanol followed by stirring on a magnetic stirrer for 12 h to dissolve the polymer and extract out the drug. After filtration through Whatman filter paper (# 41), drug concentration in the ethanolic phase was determined by UV spectrophotometer at 244 nm. Each determination was carried out in triplicate and per cent drug entrapment was calculated as follows:

\[
\text{Drug entrapment (\%) = } \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100.
\]

**In vitro buoyancy studies**

Fifty milligrams of the microspheres was added to 100 ml of simulated gastric fluid (pH 2.0) containing 0.02% w/v Tween 20 and stirred at 100 rpm on a magnetic stirrer. After 8 h, the layer of buoyant microspheres was pipetted out and sedimented layer was separated by filtration. Both parts of the particles were dried in a desiccator until constant weight and the fractions were weighed.

\[
\text{Buoyancy (\%) = } \frac{W_f}{(W_f + W_s)} \times 100,
\]

where \( W_f \) and \( W_s \) are the weights of the floating and settled microspheres respectively. All the determinations were carried out in triplicate.

**In vitro drug release study and data analysis**

The drug release rate from floating microspheres was determined using USP XXIII basket-type dissolution apparatus (Jyoti Scientific Laboratories, Gwalior). A weighed quantity of floating microspheres equivalent to 20 mg of drug was filled into a capsule (# 3) and placed in the basket. Simulated gastric fluid (SGF, pH 2.0, 500 ml) containing Tween 20 (0.02% w/v) was used as the dissolution medium and maintained at 37 ± 0.5°C at a rotation speed of 100 rpm (ref. 11). Sample (5 ml) was withdrawn at each 30 min interval, passed through Whatman filter paper (# 41) and analysed spectrophotometrically at 244 nm for drug estimation. The initial volume of the dissolution fluid was maintained by adding 5 ml of fresh dissolution fluid after each withdrawal. All the experiments were performed in triplicate.

Five kinetic models, namely zero order (eq. 1), first order (eq. 2), Higuchi matrix (eq. 3), Peppas–Korsmeyer (eq. 4) and Hixon–Crowell (eq. 5) release equations were applied to process the in vitro release data to find the best fit equation using PCP Disso v3 software.

\[
R = k_i t,
\]

\[
\log UR = \frac{k_2 t}{2.303},
\]

\[
R = k_3 t^{0.5},
\]

\[
R = k_4 t^n,
\]

or

\[
\log R = \log k_s + n \log t,
\]

\[
(UR)^{1/3} = k_f t,
\]

where \( R \) and \( UR \) are the percentage released and unreleased drugs respectively, at time \( t \); \( k_1, k_2, k_3, k_4 \) and \( k_s \) are the rate constants of zero order, first order, Higuchi matrix, Peppas–Korsmeyer and Hixon–Crowell model respectively.

**Animal studies**

The protocol for animal studies was approved by the Institutional Animal Ethical Committee (IAEC) of SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur (Reg. No. 780/CPCSEA, Sept. 2006). Male Sprague–Dawley rats weighing 200–220 g were acclimatized in a new atmosphere for 1 week before initiation of experiments. The animals were housed under standard conditions and fed laboratory diet (regular rat chaw), and water ad libitum.

**Induction of diabetes:** Diabetes was induced by intraperitoneal administration of streptozotocin (solution in 100 mmol/l citrate buffer, pH 4.5, freshly prepared to avoid decomposition) at a dose level of 60 mg/kg to the rats fasted overnight but had ad libitum access to water. The degree of diabetes was assessed after 4 days by measurement of blood glucose levels using a glucometer (Accu-check). Rats having blood glucose level above 300 mg/dl were considered diabetic and were included in the experiments.

**Experimental procedure:** Diabetic rats were fasted overnight and randomly divided into three groups of six animals each. Plain PBS was administered orally to the diabetic control rats. Microsphere formulations (MR and FM-5) were dispersed as suspension (1% w/v gum acacia as suspending agent) and administered orally to other groups at a dose equivalent to 100 µg/kg of repaglinide. Efficacy of formulation was assessed both in diabetic as well as in normal rats. Normal rats were fasted overnight and randomly divided into three groups of six animals each. Also, plain PBS, pure drug microspheres (MR) and FM-5 were administered at a dose equivalent to 100 µg/kg of repaglinide to various groups of normal...
rats. Blood samples (approximately 0.1 ml) were withdrawn at scheduled intervals from the sepharose vein (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h) and estimated for blood glucose level as described previously.

Results and discussion

Purification of cholestyramine resin and incorporation of bicarbonate into the resin

Cholestyramine resin was supplied as industrial grade; hence it contains various impurities which might produce unwanted effect. Therefore, its purification was carried out by washing the resin with various solvents such as deionized water, 95% ethanol and 50% ethanol. The impurities get dissolved in these solvents and removed as the filtrate. Purification process activated the resin for complex formation with bicarbonate ion as well as the drug. Bicarbonated resin was prepared and subjected to titrimetric assay of NaHCO₃ in the filtrate in order to find out the bicarbonate content in it. Volume of 0.1 M HCl consumed during the titration to neutralize the free unbound bicarbonate was found to be 6.42 ml, which is equivalent to 0.5393 g of NaHCO₃. Total amount of bicarbonate loaded to 10 g of resin was 2.607 g (0.260/g of resin).

Preparation and optimization of DBRC

Repaglinide complexation with cholestyramine resin is essentially a process of diffusion of ions between the resin and the surrounding drug solution. As the reaction is an equilibrium phenomenon, maximum efficiency is best achieved in batch process (Figure 1). Drug complexation with cholestyramine resin was optimized for two parameters, namely stirring time and drug to resin ratio. Drug–resin w/w ratio was optimized for loading efficiency by preparing five batches of ratios 1 : 0.5, 1 : 1, 1 : 2, 1 : 3, 1 : 4 after 2 h of stirring (Table 1). DBRC with maximum drug binding efficiency was selected as the optimum and used for the preparation of floating microspheres. In order to optimize stirring time, six batches were prepared by stirring for different time-periods, i.e. 1, 2, 3, 4, 5 and 24 h respectively and drug loading at different stirring times (Table 2).

An increase in the amount of resin increases the amount of drug absorbed from the solution, but at the same time decreases the loading efficiency of DBRC. Moreover, there is no significant increase in drug loading with 1 : 3 and 1 : 4 drug–resin combinations compared to 1 : 2. More than 2 h of stirring did not increase drug loading significantly with respect to time. Only a small increment (~5%) in drug loading was observed after 24 h stirring. In view this, drug–resin ratio of 1 : 2 (i.e. DBRC3) showed optimum loading and was considered as optimized resin complexed drug.

Characterization of DBRC and microspheres

Infrared spectroscopy: Presence of characteristic peaks of repaglinide such as 1586 cm⁻¹ (due to N–H bending of 1°, 2° amine), 1149 cm⁻¹ (due to aliphatic C–N stretching) in the FTIR spectra of DBRC confirmed the presence of drug in the resin complex. It also revealed that drug binding to resin did not show any involvement of –NH₂ group of repaglinide as there was no change in the peak due to N–H bending as well as C–N stretching. The peak at 1688 cm⁻¹ due to aryl carboxylic acid of repaglinide vanished, which indicates the potential binding of resin at carboxylic group of the drug (Figure 2). Characteristic peak of cholestyramine resin at 1030 cm⁻¹ due to C–N stretching of quaternary amine was shifted to 1020 cm⁻¹. This might be due to replacement of Cl⁻ ion with drug –COO⁻ ion (ionic form of the drug), which has comparatively less electronegativity, causing a small decline in C–N stretching energy and resulting in a decrease in absorption wavelength. All these findings confirm complex formation between the drug and resin through carboxylic group.

Figure 1. Schematic representation for the preparation of floating microspheres and the mechanism of floating. (HCO₃⁻, H⁺Cl⁻, DR⁻ represent bicarbonate ion, gastric acid and the drug respectively. DR⁻ is the drug in ionic form.)
increase in the polymer concentration using drug resin complex; slightly higher than all the resins. The marked difference in DEE of MR and FM:

A marked difference in DEE of MR and FM: n-microspheres (FM), Floating microspheres of repaglinide containing drug resin complex (DBRC3), ethyl cellulose, and DBRC3-based formulation. Absence of resin might have some positive effects on percentage yield due to higher size of resin-based microspheres. Percentage yield for FM formulation was in the range 64.67 ± 6.47% to 71.15 ± 3.42%, indicating the absence of any trend as well as no significant change with an increase in polymer content (Table 3). Drug entrapment efficiency of the FM formulations was found to be in the range 51.03 ± 2.40% to 68.72 ± 2.89%. The drug entrapment efficiency (DEE) also increased with an increase in the polymer concentration, which may be due to highly dense internal structure of cellulose matrix and effective encapsulation of DBRC particles. A marked difference in DEE of MR and FM-5 was observed irrespective of the same polymer concentration, which reveals that the resin interferes in the entrapment of DBRC (Table 3).

Morphology and micromeritic studies

Non-uniformity in particle size distribution of the prepared microspheres is clearly evident from the SEM photomicrographs (Figure 4). Fibres-like aggregates were marked on the surface which might be due to solidification of the polymer prior to droplets formation. The microspheres were found to be spherical in shape and

<table>
<thead>
<tr>
<th>Code</th>
<th>DBRC : polymer</th>
<th>Drug : polymer</th>
<th>Yield (%)</th>
<th>Drug entrapment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR</td>
<td>–</td>
<td>1 : 5</td>
<td>79.86 ± 1.26</td>
<td>81.38 ± 2.56</td>
</tr>
<tr>
<td>FM-1</td>
<td>1 : 1</td>
<td>–</td>
<td>69.40 ± 2.49</td>
<td>51.03 ± 2.40</td>
</tr>
<tr>
<td>FM-2</td>
<td>1 : 2</td>
<td>–</td>
<td>68.20 ± 4.23</td>
<td>59.67 ± 1.37</td>
</tr>
<tr>
<td>FM-3</td>
<td>1 : 3</td>
<td>–</td>
<td>71.15 ± 3.42</td>
<td>63.84 ± 5.56</td>
</tr>
<tr>
<td>FM-4</td>
<td>1 : 4</td>
<td>–</td>
<td>64.67 ± 6.47</td>
<td>64.61 ± 3.60</td>
</tr>
<tr>
<td>FM-5</td>
<td>1 : 5</td>
<td>–</td>
<td>67.78 ± 4.54</td>
<td>68.72 ± 2.89</td>
</tr>
</tbody>
</table>

MR, Microspheres of repaglinide without using drug resin complex; FM, Floating microspheres of repaglinide containing drug resin complex. Data are the average of values (mean) ± standard deviation (SD; n = 3).

Figure 2. IR spectra of optimized drug bicarbonated resin complex.

Differential scanning calorimetry: A differential scanning calorimeter (DSC; Mettler Toledo-821, Greifensee, Switzerland) was used to analyse the thermal behaviour of pure repaglinide, cholestyramine resin, drug–resin complex (DBRC3), ethyl cellulose, and DBRC3-loaded microspheres (FM-3) in the temperature range from 30°C to 350°C. Figure 3 displays the thermal behaviour of the pure drug showing endotherm at 137°C corresponding to the melting point of the pure drug. Pure cholestyramine resin shows two endotherms at 103.2°C and 270°C. The thermal behaviour of DBRC shows endotherm at 85.5°C and a small gradual endotherm at 210°C indicating onset (endothermic–exothermic inversion) and gradual decomposition of the optimized complex, thus confirming the presence of drug in a complex state with resin. Thermal behaviour of pure ethyl cellulose showed an endotherm at 195°C corresponding to the melting point of the ethyl cellulose. FM-3 shows small gradual endotherm at 89°C corresponding to the peak of DBRC3 and at 199°C corresponding to the endothermic peak of the polymer confirming the presence of drug and resin in the prepared formulation FM-3.

Percentage yield and drug entrapment efficiency

Percentage yield of MR formulation was found to be 79.86 ± 1.26, which is slightly higher than all the resin-based formulations. Absence of resin might have some positive effects on percentage yield due to higher size of resin-based microspheres. Percentage yield for FM formulation was in the range 64.67 ± 6.47% to 71.15 ± 3.42%, indicating the absence of any trend as well as no significant change with an increase in polymer content (Table 3). Drug entrapment efficiency of the FM formulations was found to be in the range 51.03 ± 2.40% to 68.72 ± 2.89%. The drug entrapment efficiency (DEE) also increased with an increase in the polymer concentration, which may be due to highly dense internal structure of cellulose matrix and effective encapsulation of DBRC particles. A marked difference in DEE of MR and FM-5 was observed irrespective of the same polymer concentration, which reveals that the resin interferes in the entrapment of DBRC (Table 3).
surface morphology of the microspheres clearly revealed the presence of many small pores on the surface (Figure 4). This might be due to the subsequent evaporation of the entrapped dichloromethane through the surface during the preparation of microspheres.

The average particle size of the optimized DBRC (i.e. DBRC3) was found to be 125 ± 13 \( \mu \)m, which was slightly higher than the size of pure resin, indicating the presence of drug. Microspheres of pure drug (MR) showed particle size of 160 ± 23 \( \mu \)m, which is significantly small \((P < 0.01)\) compared to microspheres containing DBRC; ranging from 233 ± 14 to 271 ± 17 \( \mu \)m. This higher size of DBRC microspheres is due to the presence of resin, which itself has a size of \( \approx 125 \mu \)m. In case of DBRC formulations, the microsphere size increases upon increasing the concentration of the polymer. The tapped density ranged from 0.54 and 0.84 g/cm\(^3\), while true densities ranged between 1.58 and 1.89 g/cm\(^3\) for all the formulations, which may be due to the presence of low-density ethyl cellulose (i.e. 50 cps). The porosity of all the formulations was found to be in the range 47%-70%. The compressibility index ranged from 24.0% to 34.0%. The formulations FM-3, FM-4 and FM-5 showed good flowability (i.e. \( \theta < 30^\circ \)) as expressed in terms of angle of repose (Table 4). Low compressibility index might be due to smooth surface of microspheres with higher polymer content, which decreases the friction during the flow. The better flow property indicates that the floating microspheres do not tend to aggregate. All the micromeritic properties were significantly improved \((P < 0.01)\) upon microspheres preparation using DBRC (FM-5) compared to pure drug microspheres (MR) irrespective of the same drug–polymer ratio.

**In vitro buoyancy studies**

The time taken for dosage form to emerge on the surface of the medium is called the floating lag time, which mainly depends on the rate of diffusion of HCl through the polymer coat into DBRC core and subsequent replacement of bicarbonate ions by Cl\(^-\) ions to release CO\(_2\) gas which is responsible for floating. Total time duration for which the dosage form constantly emerges on the surface of the medium is called the total floating time (TFT). All the FM formulations had floating lag time more than 10 min. However, FM-3, FM-4 and FM-5 have longer floating lag time (>20 min) compared to FM-1 and FM-2. This might be due to the fact that high polymer content increases diffusional path length by increasing polymer coat thickness and thus allows slow diffusion of HCl, resulting in increased floating lag time. More than 50% of microspheres was found to be floating for 8 h or more. Presence of ethyl cellulose provides an additive effect on floating behaviour\(^8\). After 8 h, FM-5 showed highest percentage of buoyancy (74.9 ± 4.1%), whereas, FM-1 showed lowest (51.4 ± 6.8%; Table 5). An increase in percentage of buoyancy was observed upon increasing polymer concentration (FM-1 to FM-5). The amount of entrapped DBRC and level of loaded bicarbonate resulted in strong force generated by released CO\(_2\) thus increasing percentage of buoyancy.

**In vitro drug release study**

Figure 5 clearly shows that formulation without DBRC (MR) released the drug rapidly compared to formulations containing DBRC, which released the drug in a controlled manner due to presence of drug in complex form. The
**Figure 5.** *In vitro* drug release profile of repaglinide from various formulations. MR, Microspheres of repaglinide without using drug resin complex, FM, Floating microspheres of repaglinide containing drug resin complex. Values are mean ± SD (n = 3). (Inset) Comparison of repaglinide release profile between MR and FM.

**Table 4.** Micromeritic studies of the prepared formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Particle size (μm)</th>
<th>Tapped density (g/cm³)</th>
<th>True density (g/cm³)</th>
<th>Porosity (%)</th>
<th>Compressibility index (%)</th>
<th>Angle of repose (θ; deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR</td>
<td>160 ± 23</td>
<td>0.84 ± 0.03</td>
<td>1.58 ± 0.14</td>
<td>46.9 ± 2</td>
<td>27.0 ± 2</td>
<td>54.0 ± 4</td>
</tr>
<tr>
<td>FM-1</td>
<td>233 ± 14</td>
<td>0.74 ± 0.08</td>
<td>1.79 ± 0.07</td>
<td>58.6 ± 7</td>
<td>24.0 ± 1.9</td>
<td>36.0 ± 4</td>
</tr>
<tr>
<td>FM-2</td>
<td>239 ± 21</td>
<td>0.70 ± 0.02</td>
<td>1.74 ± 0.06</td>
<td>59.7 ± 5</td>
<td>26.6 ± 1.3</td>
<td>32.8 ± 3</td>
</tr>
<tr>
<td>FM-3</td>
<td>243 ± 11</td>
<td>0.68 ± 0.05</td>
<td>1.82 ± 0.19</td>
<td>62.6 ± 6</td>
<td>32.1 ± 1.2</td>
<td>29.1 ± 5</td>
</tr>
<tr>
<td>FM-4</td>
<td>256 ± 19</td>
<td>0.64 ± 0.07</td>
<td>1.87 ± 0.12</td>
<td>65.7 ± 6</td>
<td>31.0 ± 0.7</td>
<td>28.2 ± 3</td>
</tr>
<tr>
<td>FM-5</td>
<td>271 ± 17</td>
<td>0.54 ± 0.03</td>
<td>1.89 ± 0.09</td>
<td>70.0 ± 3</td>
<td>34.2 ± 1.6</td>
<td>28.6 ± 4</td>
</tr>
</tbody>
</table>

Data are the average of values (mean) ± standard deviation (SD; n = 3).

**Table 5.** *In vitro* floating behaviour of the prepared formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Floating behaviour</th>
<th>Buoyancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>FM-1</td>
<td>&gt;10 min</td>
<td>51.4 ± 6.8</td>
</tr>
<tr>
<td>FM-2</td>
<td>&gt;10 min</td>
<td>57.3 ± 5.7</td>
</tr>
<tr>
<td>FM-3</td>
<td>&gt;20 min</td>
<td>64.8 ± 5.1</td>
</tr>
<tr>
<td>FM-4</td>
<td>&gt;20 min</td>
<td>72.2 ± 3.8</td>
</tr>
<tr>
<td>FM-5</td>
<td>&gt;20 min</td>
<td>74.9 ± 4.1</td>
</tr>
</tbody>
</table>

Data are the average of values (mean) ± standard deviation (SD; n = 3).

**Table 6.** One-way ANOVA (Dunnett’s multiple comparison) test for *in vitro* drug release of repaglinide in simulated gastric fluid (SGF) (pH 2.0)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean difference</th>
<th>q</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR vs FM-1</td>
<td>4.258</td>
<td>0.852**</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>MR vs FM-2</td>
<td>5.298</td>
<td>1.060**</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>MR vs FM-3</td>
<td>8.644</td>
<td>1.730**</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>MR vs FM-4</td>
<td>10.911</td>
<td>2.183**</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>MR vs FM-5</td>
<td>15.255</td>
<td>3.035**</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

**Significant; **Non-significant.

Release of repaglinide from the prepared formulations followed the order: MR > FM-1 > FM-2 > FM-3 > FM-5. Release pattern also indicates the effect of polymer content on drug release, i.e. higher the polymer content, lesser the drug release. When MR formulation (without DBRC) was compared with formulations containing DBRC by one-way ANOVA (Dunnett’s multiple comparison) test, the *in vitro* release in SGF (pH 2.0) from...
FM-5 was found to be significantly less \((P < 0.05)\). Based on this result, FM-5 is considered as optimized formulation on the basis of release point of view (Table 6). Release profile of MR versus FM-5 was compared to study the effect of resin on the drug release as these two formulations have the same polymer to drug ratio. Figure 5 shows that the release of drug from FM-5 is remarkably slower than MR and hence confirms that resin is the key factor in sustaining the drug release. These results are in line with previous reports\(^1,2\).

The data were subjected to treatment according to zero order, first order, Higuchi matrix, Hixon–Crowell’s cube root and Peppas–Korsmeyer to determine the drug release kinetics from the prepared formulations. The formulation MR had maximum \(r\) value (i.e. 0.993) close to 1 for Hixon–Crowell model and all other FM formulations (i.e. FM-1, FM-2, FM-3, FM-4 and FM-5) had maximum \(r\) value for Higuchi matrix kinetics (Table 7). This indicates that the release of drug from formulation containing pure drug follows Hixon–Crowell model and behaves as a matrix system, whereas formulations containing DBRC follow Higuchi mode and release the drug by diffusion.

The Peppas–Korsmeyer model was employed to distinguish between the competitive drug release mechanisms by comparing the value of diffusional exponent \((n)\)\(^23,24\). The diffusional exponent specifies the mechanism of release. Polymeric controlled delivery systems of spherical geometry have \(n = 0.43\), indicating Fickian diffusion; \(0.43 < n < 0.85\) indicates both diffusion-controlled drug release and swelling controlled drug release (anomalous transport or non-Fickian transport); and \(n > 0.85\) indicates case-II transport, i.e. polymer relaxation during gel swelling. The \(n\) value of all the formulations was in the range 0.43–0.85 irrespective of the presence or absence of resin, showing that the release of drug from all the formulations followed predominantly diffusion pathway as well as polymer swelling mechanism up to some extent. It was also noticed that upon increasing the polymer concentration, the value of \(n\) also increases, i.e. swelling mechanism plays a greater role and this might be due to the increased polymer thickness resulting in enhanced swelling. Formulation FM-5 was selected as optimized formulation due to its better entrapment efficiency, buoyancy behaviour and controlled release as compared to other prepared formulations and it was subjected to further \textit{in vivo} studies.

### Animal studies

The diabetic rats were treated with repaglinide at a dose level of 100 μg/kg to control blood glucose level in experimental animals. It is reported that repaglinide is not effective at low concentrations such as 30 μg/kg. A marked initial decrease in blood glucose levels with a maximum (~ 70% of initial) was observed up to 6 h after treatment with microspheres of pure drug (MR) and it started receding thereafter (Figure 6). In contrast, microspheres of DBRC (FM-5) showed a clear long-lasting effect \((P < 0.05\), compared to pure drug) on blood glucose levels for 8 h (~60% of initial) and minute effect till 10 h. The sustained blood glucose lowering effect observed for longer period of time in case of formulation FM-5 is due to the existence of drug in complex form with resin and also due to floating behaviour of the formulation, which causes slow release and absorption of repaglinide over an extended period of time. Repaglinide has a short half-life.

### Table 7

Comparison of different dissolution kinetics models for the release of repaglinide from different formulations in SGF (pH 2.0)

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order (r)</th>
<th>First order (r)</th>
<th>Higuchi matrix (r)</th>
<th>Hixon–Crowell (r)</th>
<th>Peppas–Korsmeyer (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR</td>
<td>0.981</td>
<td>0.964</td>
<td>0.988</td>
<td>0.993</td>
<td>0.975</td>
</tr>
<tr>
<td>FM-1</td>
<td>0.947</td>
<td>0.974</td>
<td>0.986</td>
<td>0.966</td>
<td>0.981</td>
</tr>
<tr>
<td>FM-2</td>
<td>0.954</td>
<td>0.979</td>
<td>0.987</td>
<td>0.972</td>
<td>0.975</td>
</tr>
<tr>
<td>FM-3</td>
<td>0.972</td>
<td>0.986</td>
<td>0.987</td>
<td>0.983</td>
<td>0.971</td>
</tr>
<tr>
<td>FM-4</td>
<td>0.974</td>
<td>0.990</td>
<td>0.994</td>
<td>0.985</td>
<td>0.990</td>
</tr>
<tr>
<td>FM-5</td>
<td>0.956</td>
<td>0.975</td>
<td>0.985</td>
<td>0.969</td>
<td>0.975</td>
</tr>
</tbody>
</table>

\(r\), Correlation coefficient.

### Table 8

One-way ANOVA followed by Dunnett’s multiple comparison test for comparison of blood glucose level (% of initial value) in diabetic rats

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean difference</th>
<th>(q)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug vs MR</td>
<td>26.380</td>
<td>4.248</td>
<td>*&lt;0.01</td>
</tr>
<tr>
<td>Pure drug vs FM-5</td>
<td>29.855</td>
<td>4.807</td>
<td>*&lt;0.01</td>
</tr>
</tbody>
</table>

*Very significant.

### Table 9

One-way ANOVA followed by Dunnett’s multiple comparisons test for comparison of blood glucose level (% of initial value) in normal rats

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean difference</th>
<th>(q)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug vs MR</td>
<td>15.463</td>
<td>3.259</td>
<td>*&lt;0.01</td>
</tr>
<tr>
<td>Pure drug vs FM-5</td>
<td>18.357</td>
<td>3.869</td>
<td>*&lt;0.01</td>
</tr>
</tbody>
</table>

*Very significant.
Figure 6. Blood glucose lowering effect of floating microspheres containing repaglinide administered orally to diabetic rats. Group-I, Control; Group-II, Non-floating microspheres of pure drug (MR), i.e. formulation without DBRC; Group-III, Optimized floating microspheres of pure drug (FM-5) containing DBRC. Values are mean ± SD (n = 6).

Figure 7. Blood glucose lowering effect of floating microspheres containing repaglinide administered orally to normal rats. Group-IV, Control; Group-V, Non-floating microspheres of pure drug (MR), i.e. formulation without DBRC; Group-VI, Optimized floating microspheres of pure drug (FM-5) containing DBRC; Values are mean ± SD (n = 6).

(~1 h) and also the developed formulations releases the drug up to 8–10 h. In addition, blood glucose levels were returned closer to initial level and hence experimental animal were followed for blood glucose lowering effect up to 10 h. Generally, 25% reduction in blood glucose levels is considered to be a significant blood glucose lowering effect25.

The study was also conducted in normal healthy animals at a same dose level to know the effect of drug. MR formulation was able to reduce the blood glucose level in normal rats but to a lower extent (~60% of initial) as well for shorter duration (4 h). DBRC formulation (FM-5) showed maximum effect of ~70% of the initial in 6 h and marginal effect till 10 h (Figure 7). One-way ANOVA followed by Dunnett’s multiple comparison tests were used to interpret the results. MR and FM-5 formulations showed significantly (P < 0.05) better result than control in terms of blood glucose reduction (Tables 8 and 9). In summary, results obtained vindicate that optimized DBRC formulation (FM-5) is effective therapeutically and has a prolonged duration of action not only in diabetic rats but also in normal rats.

Conclusion

In the present study, the floating microspheres containing DBRC formulation were able to sustain the drug in an effective manner for prolonged duration of time compared to free drug microspheres. These effects are presumed to be the result of CO₂ generation due to presence of bicarbonate ions and existence of drug in complex form with resin. In vivo, these floatable microspheres demonstrated the reduced blood glucose level for a longer duration compared to both non-floating microspheres and pure drug in streptozotocin-induced diabetic rats.

2. 44 Lakh Indians don’t know they are diabetic, Public Health Foundation of India; http://ceebdm.org/news.php
RESEARCH ARTICLES


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