Microcapsules and Transdermal Patch: A Parallel Access for Improved Delivery of Antidiabetic Drug

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Abstract

Aim: Microcapsules and transdermal patch: A parallel access for improved delivery of antidiabetic drug. Materials and Methods: Different formulations were formulated employing sodium alginate (SA) alone or in combination with various coating polymers. Alginate coated microcapsules of gliclazide were prepared by dispersing the drug (15 mg) into aqueous solutions of SA under constant stirring. Preparation of transdermal patches (TDP): Glibenclamide (GL)-loaded TDP formulations were prepared by solvent casting method with slight modification. Hydroxypropyl methylcellulose was dissolved in a mixture of acetone/isopropyl alcohol/ethanol (50:30:20) with continuous stirring in a closed system at 25°C. Evaluation of the prepared microcapsules: Microscopical examination and particle size measurement of gliclazide microcapsules were done using an optical microscope. The prepared microcapsules were mounted in few drops of distilled water and examined under an optical microscope. Evaluation of TDP: Several steps such as moisture intake, moisture uptake, and weight variation are used for evaluation method. Conclusion: From the comparative study, it was concluded that the transdermal system of GL produced better improvement compared to oral microcapsule administration.

Key words: Diabetes, gliclazide, hydroxypropyl methylcellulose, oral microcapsules, transdermal delivery

INTRODUCTION

Diabetes is one of the major causes of death and disability in the world. The latest, WHO estimates for the number of people with diabetes worldwide, in 2000, is 171 million, which is likely to be at least 366 million by 2030. The focus of medical community is on the prevention and treatment of the disease, as is evident from the rising number of research papers every year on the subject. Diabetes mellitus is a group of metabolic diseases characterized by defects in insulin utilization, either from autoimmune destruction of insulin producing cells (Type I) or insulin resistance (Type II). The prevalence of Type II diabetes is rising dramatically worldwide. There are more than 171 million people with diabetes, and in the US, Canada, and Europe, over 80% of diabetes cases are Type II. It was the 6th leading cause of death due to the many complications associated with this disease, such as pulmonary hypertension and ischemia. Gliclazide is a second generation sulfonylurea that can acutely lower the blood glucose level in humans by stimulating the release of insulin from the pancreas and is typically prescribed to treat Type II diabetes. Its half-life is relatively short (25 h) which necessitates its administration in 2 or 3 doses of 2.5-10 mg/day. Therefore, it is a potential candidate for the development of extended release formulations.

The primary goal of therapy with many drugs is to achieve a steady state at the tissue level or in blood that is therapeutically effective and nontoxic for an extended period of time. Therefore, modified release drug delivery systems have been developed in an attempt to realize this goal.
goal.\textsuperscript{[9]} Microencapsulation process has been established as a technique to accomplish extended release and drug targeting.\textsuperscript{[9]} Microencapsulation using a variety of polymers and its applications has been previously depicted in standard textbooks and literatures.\textsuperscript{[10,11]} Microcapsule carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in extended drug delivery.\textsuperscript{[6,7]}

The purpose of this work was to microencapsulate gliclazide using certain hydrophilic polymers to control the release of this high water-insoluble drug. The polymers used in the microencapsulation process were alginate, alginate–chitosan, and alginate–carbopol 934P. Evaluation of the prepared microcapsules was performed by microscopical examination, determination of particle size, yield, and microencapsulation efficiency. The \textit{in vitro} release studies for the determination of gliclazide released from the microcapsule formulations were performed and analyzed. The effect of the storage at high temperatures, namely, 40, 50, and 60° for 12 weeks on the chemical stability of the selected microcapsules and prediction of the shelf life was also assessed. In addition, the effect of storage at these high temperatures on the release of the drug from the selected formulae was evaluated.

\textbf{Introduction to transdermal drug delivery system}

Introduction to transdermal drug delivery transdermal delivery represents an attractive alternative to oral delivery of drugs these therapeutic systems are defined as “self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at a controlled rate to the systemic circulation.” Thus it is anticipated that transdermal drug delivery system can be designed to deliver drug at appropriate rates to maintain suitable plasma drug levels for the therapeutic efficacy using skin as the port of entry of drugs.\textsuperscript{[12]} Scopolamine was the 1\textsuperscript{st} transdermal system to treat motion sickness which was approved for use in the United States in 1979. A decade later, nicotine patches became the first transdermal blockbuster, raising the profile of transdermal delivery in medicine and for the public in general. Between 1979 and 2002, a new patch was approved on average every 2.2 years. Over the past 5 years (2003-2007), that rate has more than tripled to a new transdermal delivery system every 7.5 months. It is estimated that more than 1 billion transdermal patches (TDP) are currently manufactured each year.\textsuperscript{[13,14]}

\textbf{MATERIALS AND METHODS}

\textbf{Materials}

Gliclazide was received from Sigma-Aldrich. Hydroxypropyl methylcellulose (HPMC), isopropyl myristate (IPM), and sodium alginate (SA) were obtained from SD Fine Chemicals, Mumbai, India. Calcium chloride (CaCl\textsubscript{2}) and alloxan were procured from Merck Limited, Mumbai. Other reagents were used of analytical grade.

\textbf{Methods}

\textbf{Formulation of gliclazide microcapsules}

Different formulations were formulated employing SA alone or in combination with various coating polymers as reported in Table 1. An orifice ionotropic gelation process that has been extensively used to prepare large alginate beads was employed to fabricate the microcapsules.\textsuperscript{[15,16]}

\textbf{Formulation of SA coated gliclazide microcapsules}

Alginate coated microcapsules of gliclazide were prepared by dispersing the drug (15 mg) into aqueous solutions of SA under constant stirring (500 rpm) at 25 ± 0.5° for 10 min. The microcapsules were formed by dropping the dispersion into gently agitated aqueous solutions of the counter ion 0.2 M CaCl\textsubscript{2} at a rate of 3 ml/min through a syringe with a nozzle of size no. 18. The ratio of alginic solution and CaCl\textsubscript{2} solution was adjusted to be 1:10. The mixtures were then stirred slowly for 10 min to cure alginic microcapsules and to produce spherical rigid microcapsules.\textsuperscript{[17]} The microcapsules were collected by decantation, rinsed with distilled water and air dried for 24 h, followed by drying at 40° for 4 h and stored in a desiccator until used.

\textbf{Formulation of SA-chitosan coated gliclazide microcapsules}

Drug alginate dispersions were prepared as previously mentioned. CaCl\textsubscript{2} solutions containing chitosan were prepared by adding 1% v/v glacial acetic acid containing chitosan to CaCl\textsubscript{2} solution with mild agitation at ambient temperature. The concentration of chitosan in CaCl\textsubscript{2} aqueous solution was 0.3% w/v, and two different molecular weights were employed.\textsuperscript{[17]} Then, the drug alginate dispersion was added dropwise to CaCl\textsubscript{2} solution. The formed microcapsules were retained in CaCl\textsubscript{2} solution for 10 min to complete the curing reaction to produce spherical rigid microcapsules and treated as previously discussed.\textsuperscript{[18]}

\textbf{Formulation of SA-carbopol 934P coated gliclazide microcapsules}

SA and carbopol 934P were dissolved in distilled water to form homogeneous polymer solution. The active substance, gliclazide was added to the polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion.\textsuperscript{[19]} The resulting dispersion was then added dropwise into CaCl\textsubscript{2} solution as mentioned before.

\textbf{Preparation of TDP}

Glibenclamide (GL)-loaded TDP formulations were prepared by solvent casting method with slight modification. HPMC
was dissolved in a mixture of acetone/isopropyl alcohol/ethanol (50:30:20) with continuous stirring in a closed system at 25°C. Dibutyl phthalate was added followed by the addition of IPM with continuous stirring. Calculated amount of GL was added to the solution of the polymer matrix and poured into the backing membrane. The solvent was allowed to evaporate overnight at room temperature. Three formulations were prepared with different HPMC concentrations as per Table 2.[20]

### Table 1: Composition of different formulas of gliclazide microcapsules

<table>
<thead>
<tr>
<th>Composition</th>
<th>Microcapsule coat</th>
<th>%w/v</th>
<th>Drug: polymer ratio</th>
<th>CaCl₂ (M)</th>
<th>H₂O (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug (mg)</td>
<td>Drug (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium alginate</td>
<td>30</td>
<td>1:2</td>
<td>0.2</td>
<td>30</td>
</tr>
<tr>
<td>C1</td>
<td>15</td>
<td>30</td>
<td>1</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>C2</td>
<td>15</td>
<td>15</td>
<td>1:1</td>
<td>0.2</td>
<td>7.5</td>
</tr>
<tr>
<td>C3</td>
<td>15</td>
<td>15</td>
<td>1:1</td>
<td>0.2</td>
<td>7.5</td>
</tr>
<tr>
<td>C4</td>
<td>Sodium alginate-chitosan (LMW)</td>
<td>30</td>
<td>1:2</td>
<td>0.2</td>
<td>30</td>
</tr>
<tr>
<td>C5</td>
<td>15</td>
<td>30</td>
<td>1:2</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>C6</td>
<td>15</td>
<td>15</td>
<td>1:1</td>
<td>0.2</td>
<td>7.5</td>
</tr>
<tr>
<td>C7</td>
<td>15</td>
<td>15</td>
<td>1:1</td>
<td>0.2</td>
<td>7.5</td>
</tr>
<tr>
<td>C8</td>
<td>Sodium alginate-chitosan (HMW)</td>
<td>30</td>
<td>1:2</td>
<td>0.2</td>
<td>30</td>
</tr>
<tr>
<td>C9</td>
<td>15</td>
<td>30</td>
<td>1:2</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>C10</td>
<td>15</td>
<td>30</td>
<td>1:2</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>C11</td>
<td>15</td>
<td>15</td>
<td>1:1</td>
<td>0.2</td>
<td>7.5</td>
</tr>
<tr>
<td>C12</td>
<td>15</td>
<td>15</td>
<td>1:1</td>
<td>0.2</td>
<td>7.5</td>
</tr>
<tr>
<td>C13</td>
<td>Sodium alginate: carbopol 934P (6:4)</td>
<td>30</td>
<td>1:2</td>
<td>0.2</td>
<td>30</td>
</tr>
<tr>
<td>C14</td>
<td>15</td>
<td>30</td>
<td>1:2</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>C15</td>
<td>15</td>
<td>15</td>
<td>1:1</td>
<td>0.2</td>
<td>7.5</td>
</tr>
<tr>
<td>C16</td>
<td>15</td>
<td>15</td>
<td>1:1</td>
<td>0.2</td>
<td>7.5</td>
</tr>
<tr>
<td>C17</td>
<td>Sodium alginate: carbopol 934P (8:2)</td>
<td>30</td>
<td>1:2</td>
<td>0.2</td>
<td>30</td>
</tr>
<tr>
<td>C18</td>
<td>15</td>
<td>30</td>
<td>1:2</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>C19</td>
<td>15</td>
<td>15</td>
<td>1:1</td>
<td>0.2</td>
<td>7.5</td>
</tr>
<tr>
<td>C20</td>
<td>15</td>
<td>15</td>
<td>1:1</td>
<td>0.2</td>
<td>7.5</td>
</tr>
</tbody>
</table>

SA: Sodium alginate, HMW: High-molecular-weight, LMW: Low-molecular-weight

### Table 2: Formulation and drug content of transdermal patches

<table>
<thead>
<tr>
<th>Formulation</th>
<th>GL:HPMC</th>
<th>DBT (% w/w)</th>
<th>IPM (% v/v)</th>
<th>% Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDP 1</td>
<td>1:1</td>
<td>30</td>
<td>1.5</td>
<td>99.6±1.8</td>
</tr>
<tr>
<td>TDP 2</td>
<td>1:2</td>
<td>30</td>
<td>1.5</td>
<td>98.9±1.7</td>
</tr>
<tr>
<td>TDP 3</td>
<td>1:3</td>
<td>30</td>
<td>1.5</td>
<td>98.1±2.0</td>
</tr>
</tbody>
</table>

GL: Glibenclamide, SA: Sodium alginate, HPMC: Hydroxypropyl methylcellulose, DBP: Dibutyl phthalate, IPM: Isopropyl myristate, TDP: Transdermal patches

Evaluation of the prepared microcapsules

Microscopical examination and particle size measurement of gliclazide microcapsules were done using an optical microscope. The prepared microcapsules were mounted in few drops of distilled water and examined under an optical microscope (Leica Image, Germany) and photographed at a magnification of ×100, by means of a fitted camera (JVC, Japan). The particle size of the microspheres was also determined using the calibrated optical microscopy method, where approximately 100 microspheres were counted for particle size.[21]

The yield of gliclazide microcapsules was determined using the equation: % Process yield = (Recovered mass/Mass entered into the experiment) × 100
Chemical stability by high pressure liquid chromatography ultraviolet (HPLC UV)

To assess the chemical degradation of gliclazide microcapsules compared to the drug as received, HPLC UV was used. The powders were dissolved in the mobile phase in triplicate, and 20 µl of each sample was injected onto the HPLC column for analysis. To determine the degradation, the percentage area of the drug peak was compared against total area of all the peaks in the chromatogram. Any decrease in the percentage area of the drug peak in the microcapsule powder was considered as degradation. Before injecting the samples, a blank (without drug) was always injected onto the HPLC column under the same conditions. The chemical stability of gliclazide in the prepared formulations was determined by chromatographic analysis of the microcapsules compared to the drug as received.

HPLC analysis of gliclazide

A reverse-phase HPLC method was used for quantifying gliclazide samples (n = 3). The HPLC system consisted of a solvent delivery pump (Shimadzu L7110, Hitachi Ltd., Japan), a controller (SCL10A), and a UV/VIS detector (SPD10A). The peak areas were integrated using Shimadzu CR6A chromatopac, Hitachi Ltd., Japan. The drug was separated on a C18 column packed with Nucleosil 120 (250 × 4.6 mm, 5 µ), Teknokroma, Barcelona, and Spain. Standards and samples were prepared in Milli Q water. Mobile phase consisted of a mixture of acetonitrile: 2 mM phosphate buffer (50:50% v/v), adjusted to pH 3.5 with orthophosphoric acid. The drug was eluted isocratically at a mobile phase flow rate of 1.2 ml/min and monitored with a UV detector operating at 230 nm. GL was used as internal standard. The run time for the assay was 10 min, and the retention time for the drug was 3.9 ± 0.2 min, and glibenclamide retention time was 7.9 ± 0.3 min.

Preparation of gliclazide capsules

An appropriate amount of prepared microcapsules equivalent to 15 mg of gliclazide was filled into hard gelatin capsules size 3.

Content uniformity

A total of 20 capsules of each formula were individually analyzed for initial drug content by dissolved each capsule in 200 ml phosphate buffer pH 7.4 containing 0.1% polysorbate 80, using water bath sonicator for 30 min. The mixture was then filtered through Whatman filter membrane (0.45 µm) before drug analysis spectrophotometrically at 276 nm.

Release studies

The release of gliclazide from the prepared capsules was performed according to the USP XXIV dissolution tester apparatus 1 (basket method; Hanson Research, SR 8 plus model, USA). Studies are conducted at 37 ± 0.5°C in 900 ml 0.1 N HCl for a period of 2 h followed by the release in phosphate buffer pH 7.4 containing 0.1% polysorbate 80 for 6 h. Rotation speed is 50 rpm. At predetermined time intervals, aliquots (5 ml) were withdrawn and replaced with fresh medium to maintain constant dissolution volume. Samples were filtered through Whatman filter membrane (0.45 µm), diluted appropriately and analyzed spectrophotometrically at 276 nm for the percent gliclazide released. All experiments were done in triplicate. The obtained data were subsequently analyzed to determine the order of release.

Accelerated stability testing

The accelerated stability testing was performed on the selected formulas (C9 and C19) which gave the most optimum results in all previous tests. The test was carried out by placing the capsules of each selected formula in sealed pouches and stored in thermostatically controlled ovens adjusted at different temperatures, namely, 40, 50, and 60°C ± 0.5 with relative humidity 75% (maintained using a saturated solution of NaCl) for 12 weeks. Three capsules from each formula were taken from the ovens after 1, 2, 4, 6, 8, and 12 weeks. The stored capsules were examined visually for any changes in color and/or appearance and analyzed for the determination of the amount of drug remaining in each formula using HPLC stability-indicating method as previously mentioned. The dosage forms were crushed and dissolved in 100 ml mobile phase. The solution was filtered and the first 20 ml of the solution was rejected, then 10 ml of the filtrate was diluted to 100 ml in a volumetric flask with mobile phase. 1 ml aliquot of the prepared solution was transferred to 10 ml volumetric flask, and the volume was completed with the mobile phase. 20 µl of the above solution was injected into the column for quantitation. The unknown concentration of gliclazide in each dosage form was calculated as follows: $Q = (R/A ± B) \times$ dilution factor, where, $Q$ is the gliclazide concentration, $R$ is the peak area ratio (drug/internal standard), $A$ is the slope of calibration curve, and $B$ is the $Y$-intercept.
Fourier transform infrared (FTIR) studies
Drug–polymer interaction studies were conducted by FTIR spectroscopy. The spectra were recorded for GL, HPMC, GL-loaded MC, and TDP in a FTIR spectrophotometer (SHIMADZU, Japan) using KBr pellets at 400-4000 cm⁻¹.

Powder X-ray diffraction (PXRD) studies
The PXRD studies were conducted in a PXRD (Philips, PW 1050/37) with a vertical goniometer using CuKα radiation with Ni filter at a voltage of 40 kV and a current of 20 mA. Powder XRD patterns for GL, SA, and GL-loaded MC formulations were obtained by scanning from 0° to 50° 20.

Particle size determination
Particle size analysis study of the prepared microcapsules was determined by sieving the beads on a mechanical shaker using a nest of standard sieves (BP test sieves) with a shaking time of 15 min. In this study, microcapsules with a mean diameter of 240 μm were used for further investigations.

Drug content studies
For drug content determination, 200 mg of GL-loaded MC and 1 cm² GL-loaded TDP were taken into a 100 ml volumetric flask and dissolved in ethanol, and the solution was filtered through a 0.45 μm membrane filter and adjusted with phosphate buffer, pH 7.4, before drug content analysis using UV-VIS spectrophotometer (Shimadzu, 1601, Japan) at 292 nm taking phosphate buffer, pH 7.4, with ethanol as blank.

In vitro dissolution studies
Dissolution studies of GL-loaded MC formulations were conducted in USP XXI dissolution apparatus Type II. GL-loaded MC was suspended in 900 ml of phosphate buffer of pH 7.4 as dissolution medium stirring at 100 rpm and maintained at constant temperature (37 ± 1°C). At predetermined time intervals, 5 ml aliquots were withdrawn and replaced by an equal volume of fresh pre-warmed dissolution medium. After suitable dilution, the samples were analyzed at 292 nm using UV-Vis spectrophotometer. The concentration of the GL released at different time intervals was determined. In vitro release (flux) study of the TDP was evaluated for drug release using Keshary–Chien type glass cells. Cellophane sheets treated with 5% (v/v) of glycerol were mounted between the donor and receptor compartments.[24] The patch was placed on the cellophane sheet and the compartment clamped together. The cell was placed in a water bath maintained at 37 ± 1°C. The receptor compartment (75 ml capacity) was filled with phosphate buffer, pH 7.4, and the hydrodynamics in the receptor compartment was maintained by stirring with a magnetic bead at 100 rpm. At predetermined time intervals, samples were withdrawn, and an equal volume of pre-warmed buffer was replaced. The samples were analyzed after appropriate dilution for GL content at 292 nm using UV-VIS spectrophotometer taking phosphate buffer pH 7.4 as blank. The in vitro skin permeation studies of TDP were conducted in a similar manner as that of in vitro release studies except that the membrane barrier used in this study was the dorsal section of full-thickness skin from Swiss albino mice (weighing between 25 and 30 g) whose hair has been removed on the previous day using an electric clipper.

Evaluation of TDP
1. Film thickness: The thickness of the patches was determined using digital Vernier calipers and was measured at three different places on the film. The average of the five values was calculated.[25]
2. Weight variation: Five films from each batch having an area of 2 cm × 2 cm (4 cm²) were weighed individually in a digital balance and average weight was calculated.[25]
3. Folding endurance: A strip of 2 cm × 2 cm (4 cm²) was subjected for folding endurance by folding the patch at the same place repeatedly several times until a visible. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance.[25]
4. Moisture uptake: The films were weighed accurately and placed in the desiccator containing 100 ml of saturated solution of aluminum chloride, which maintains 79.50% RH. After 3 days, the films were taken out and weighed.[26]
5. Moisture loss: The films were weighed accurately and kept in a desiccator containing anhydrous calcium chloride, after 3 days, the films were taken out and weighed.[26]
6. Drug content: The films of 2.4 (cm²) area were cut into small pieces and taken in a 100 ml volumetric flask and dissolved in 25 ml methanol. The solution was filtered, and the drug was determined spectroscopically at λmax 228 nm after suitable dilution.[26]
7. In vitro drug release studies: A modified Franz diffusion cell was fabricated to study the in vitro release profile as well as the permeation of GL from the matrix films. For this study, the patches were stuck to an aluminum foil which was slightly larger than a patch, fixed using water impermeable adhesive to ensure that the receptor fluid could not come in contact with the sides of the film. The faces with lower concentration were placed in contact with the receptor fluid pH 7.4 buffer with 20% w/v PEG-400 to maintain sink condition. Before placing the patch fixed on aluminum foil onto the diffusion cell, the mouth of the cell was coated with a thin layer of silicon grease to prevent leakage of receptor fluid.[27] 5 ml of the receptor fluid was withdrawn at an interval of 1 h up to 12 h. It was immediately replaced with 5 ml of a fresh buffer solution containing 20% w/v PEG-400 to maintain a constant volume. The removed volume fluid, after suitable dilution, was analyzed spectrophotometrically.
CONCLUSION

From the comparative study, it was concluded that the transdermal system of GL produced better improvement compared to oral microcapsule administration. The transdermal system showed comparative slow and continuous supply of GL at a desired rate to systemic circulation avoiding fast pass metabolism, which improves day-to-day glycemic control in diabetic subjects and might reduce manifestations such as sulfonylurea receptor downregulation and the risk of chronic hyperinsulinemia. The present study shows that the transdermal system of GL exhibited better control of hyperglycemia besides more effectively reversing the complications associated with diabetes mellitus than oral administration in mice. The significantly higher area under the curve values observed with transdermal systems also indicates increased the bioavailability of drug from these systems compared to oral route.

REFERENCES


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