Preparation and Evaluation of Starch Acetate Based Gliclazide Microcapsules

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ABSTRACT

Objective: The aim of the present research is to study the starch acetate (SA) as microencapsulating agent in the preparation of controlled release microcapsules containing gliclazide.

Methods: Gliclazide microcapsules were prepared by adopting an emulsion solvent evaporation technique. Various batches were prepared employing different core coat ratios.

Results: In vitro drug release studies revealed that, the drug release from the microcapsules was continued up to 24 hours and it was depended on the size of the microcapsules, thickness of coat and concentration of SA. From the in vivo pharmacokinetic studies, it was evident that the drug absorption was slow and constant for a longer period upon administering of drug in microcapsule form prepared using SA and in vivo pharmacodynamic studies showed the significant sustained hypoglycemic effect for a prolonged period of time with gliclazide microcapsules in comparison with pure form of the drug.

Conclusion: It is concluded that the synthesized starch acetate was successful in controlling the drug release and found suitable as microencapsulating agent.
Introduction

The objectives of designing of Controlled release drug delivery systems are to release the drug constantly for extended period of time to achieve constant prolonged therapeutic effect, reduction of dosing in frequency and improved patient compliance.\(^1\) Several technologies have been developing to design oral controlled release systems but, microencapsulation is still most widely used technique for designing of sustained release products and is getting significant attention fundamentally and commercially.\(^2,3\) Polymers are the novel and varied class of materials used in designing of controlled drug delivery systems. The polymers will affect the properties of dosage forms and also influence the mode of drug release from the dosage form.\(^4,5\) Modified starches have long been used in pharmacy for a variety of puposes.\(^6-8\) Acetylation is one of the imperative modification of starch, based on the acetate moiety content it posses disintegrating, the rate controlling, slow releasing and film forming properties.\(^9-11\) Though starch acetate has superior film forming properties it has not been investigated thoroughly in microencapsulation for controlled release application. In the present investigation studies were performed on microencapsulation with an objective of evaluating starch acetate as a new controlled release coat polymer in microencapsulation for controlled release of Gliclazide. In the present research, starch acetate was assessed as micro-encapsulating agent by preparing gliclazide controlled release microcapsules. Literature declared that high concentrations of Gliclazide causes severe hypoglycemia gastro intestinal complications like pain, nausea, vomiting and constipation. Designing of gliclazide controlled release dosage form is essential to enhance clinical efficacy to reduce GI disturbances and to enhance patient compliance.\(^12\)

Materials and Methods

Materials

Gliclazide was a gift sample obtained from M/s Ranbaxy Research Labs., Gurgaon, Haryana, India. Starch acetate (ds: 2.72) was synthesized in the laboratory. Sodium carboxy methyl cellulose (high viscosity grade 1500-3000 Cps, Loba-Chemie), chloroform GR (Merck) and Methanol (Qualigens) were purchased from commercial sources. All other materials used were of pharmacopoeial grade.

Preparation of starch acetate microcapsules

An emulsification – solvent evaporation technique\(^13\) was tried to prepare starch acetate microcapsules. Accurately weighed 2.0g of SA was dissolved in 50 ml of chloroform to form a clear solution. 800 mg of gliclazide was added to the 5 ml of above solution and mixed thoroughly. Further, the resulting mixture was added in a thin stream to 0.5% sodium CMC solution in water while stirring at 1000 rpm using mechanical stirrer to emulsify the added dispersion as fine droplets. The stirring was continued until evaporation of chloroform at room temperature for 3 hours and to obtain spherical microcapsules. The formed microcapsules were separated by vacuum filtration and then washed thoroughly with water. The collected microcapsules were then dried to get discrete microcapsules\(^14\). Different proportions of core: coat was selected to prepare microcapsules with varying coat thickness viz, 19:1, 9:1, 8:2, and 7:3 which were coded as GLIC MC1, GLIC MC2, GLIC MC3 and GLIC MC4.

Characterization of microcapsules

The prepared microcapsules were characterized for the following.
Size and size distribution analysis
The method of sieving was adopted to separate the various sizes. Different sizes in a batch were separated by using set of standard sieves. The quantity retained on each sieve was weighed and the percentages retained were calculated.

Estimation of glilazide content in the microcapsules
A UV spectrophotometric method was used to determine the glilazide content of the microcapsules by measuring the absorbance at 229 nm in phosphate buffer of pH 7.4. Briefly the procedure, three samples of 25 mg each was collected from each batch of microcapsules and transferred into 25 ml volumetric flasks and to this add 20 ml of methanol. The mixture was warm in a hot water bath with continuous stirring for about 30 min to extract the glilazide from the microcapsules. The solution was then made up to volume with methanol. Afterward the methanolic solution was diluted suitably with phosphate buffer of pH 7.4 and assayed for glilazide by measuring absorbance at 229 nm. Gliclazide content of the microcapsules was calculated using the calibration curve.

Microencapsulation efficiency
Microencapsulation efficiency was calculated using the equation. 
Encapsulation efficiency = Estimated drug content (%) / Theoretical drug content (%) × 100.

Wall thickness
The average wall thickness of microcapsules was calculated using the equation proposed by Luu et al\textsuperscript{16}.
\[ h = \left( \bar{r} \right) \left( 1-p \right) d_1/3 \left[ p d_2 + \left( 1-p \right) d_1 \right] \]
Where, \( h \) is the wall thickness, \( \bar{r} \) is the mean radius of the microcapsules, \( d_1 \) is the density of the core material, \( d_2 \) is the density of the coat material and \( p \) is the portion of the medicament in the microcapsules.

Scanning electron microscopy (SEM)
The morphology and shape of prepared microcapsules were studied by Scanning Electron Microscopy (Jeol JXA 8100 Ltd., Tokyo, Japan). The samples were pressed on a brass stub using double sided sticking tape and then coated with gold in vacuum by a sputter coater. The SEM photographs were then taken at an excitation voltage of 29 KV.

In vitro drug release study on gliclazide microcapsules
Gliclazide release from microcapsules was studied by taking weight of microcapsules equivalent to 60 mg of drug in USP XXXII apparatus II (rotating paddle)\textsuperscript{17}, at a paddle speed of 50 rpm at 37 ±0.5º C. The dissolution medium used was 900ml of pH 7.4 phosphate buffer. At specified time intervals a 5ml of sample was withdrawn and the same quantity was replaced using fresh medium which was maintained at the same temperature. The collected samples were filtered through 0.45μ membrane filter and assayed them spectrophotometrically by measuring the absorbance at 229 nm. The cumulative amount of drug was calculated and the drug release profiles were constructed.

In vivo pharmacokinetic evaluation
In vivo pharmacokinetic evaluation was done on gliclazide microcapsules prepared by using SA as coat, Glic MC3, 20/35 in comparison to gliclazide pure drug in rabbits. A reverse phase HPLC method was used to estimate the blood concentration of drug\textsuperscript{18}, with a view to evaluate the release retarding and rate controlling efficiency of SA in vivo.
In vivo pharmacodynamic evaluation

For pharmacodynamic evaluation, the glucose concentrations in the rabbit blood samples collected were measured by ACCU-CHECK sensor (Roche Diagnostics).

Results and Discussion

In the present study starch acetate was evaluated as coat in microencapsulation by preparing gliclazide controlled release microcapsules. SA microcapsules of gliclazide could be prepared by the emulsification-solvent evaporation method as it is a method of choice to prepare microcapsules of poorly soluble drugs\textsuperscript{19,20}. The sizes in a batch of microcapsule could be separated by sieving and more uniform size range of microcapsules could readily be obtained. On size analysis it was evident that 28.0% of microcapsules were in the size range of -20+35 (670 µ) and 48.5% in the size range of -35 +50 (398.5 µ) mesh respectively. The drug content of microcapsules was in the range of 70.2 to 94.9%. The microencapsulation efficiency was from 99.37 to 101.0% and wall thickness is in the range of 14.6 to 45.0 µ.

The developed SA microcapsules were discrete, spherical and free flowing in nature. SEM (Fig. 1) indicated that the microcapsules were spherical with smooth surface and completely covered with the polymer starch acetate, coat. Size frequency distribution curves of four batches in each case were close to each other indicating that the method of preparation used to prepare the starch acetate microcapsules is reproducible with regard to size and size distribution of the microcapsules. Lower coefficient of variation in drug content (< 1.28) designated uniformity of drug content in microcapsules. Drug content of the microcapsules was found to be the same in different sieve fractions in each case. Microcapsules prepared with various ratios of core: coats were shown different wall thickness. Smaller microcapsules have thinner walls. (table-2)

Gliclazide release from the starch acetate coated microcapsules was tested in phosphate buffer of pH 7.4. The release profiles of various batches of microcapsules were shown in fig. 2 and fig. 3. Release data was analyzed as per different kinetic models and the correlation coefficient values ($r^2$) values were given in table-1 and the release parameters viz, $T_{50}$, $T_{90}$, $K_0$, $K_1$ and release exponent (n value of Peppas) were in table-2.

Gliclazide release from all the starch acetate microcapsules was slow and release was continued for > 24 hours and depended on polymer concentration, size of the microcapsule and thickness of the wall, fig-2,3. On substitution of release data in various kinetic models, it was evident that both first and zero order models are applicable to describe the data as they showed nearly equal $r^2$ values. The linearity ($r^2 > 0.970$) in the plots f drug release vs square root time\textsuperscript{21} indicates that the controlled release of drug from microcapsules follows diffusion mechanism. When release data were analyzed as per Peppas equation\textsuperscript{22}, the release exponent ‘n’ was> 0.5 with all the gliclazide microcapsules indicating anomalous non-fickian diffusion as the release mechanism from the microcapsules. Small size microcapsules exhibited higher drug release due to larger surface area. As the proportion of the coat was increased, wall thickness was increased and glimepiride release rate was decreased. Good linear relationships were observed between (i) wall thickness of the microcapsules and release rate ($K_1$) and (ii) wall thickness and $T_{50}$ values. As the starch acetate is insoluble in both acidic and alkaline fluids the mechanism of dissolution and erosion of the coat are not applicable.

Starch acetate is a biodegradable, biocompatible, non-toxic polymer having a
superior film forming properties.\textsuperscript{11,23} When the starch acetate microcapsules of gliclazide were administered orally at the same dose of 4 mg/kg to rabbits\textsuperscript{24,25} the plasma concentrations were found to be lower than those observed with gliclazide pure drug (Fig. 4) indicating slow absorption of gliclazide from the starch acetate microcapsules. The plasma concentrations were stabilized and maintained within a narrow range for longer periods of time in the case of SA microcapsules (Fig. 4). The mean residence time (MRT) was increased from 10.24 h for gliclazide pure drug to 14.75 h with the SA microcapsules. The MRT value indicated longer stay of drug in the body when administered as starch acetate microcapsules. Based on $\text{AUC}_{\infty}$ the relative bioavailability of gliclazide from starch acetate microcapsules was found to be 114.8 % when compared to gliclazide pure drug (100%). Summary of pharmacokinetic parameters are shown in table no 3.

When starch acetate microcapsules of gliclazide were administered at the same dose to rabbits, reduction in blood glucose occurred slowly and percent reduction in blood glucose values was lower than those observed with pure drug. The reduced glucose levels were sustained over longer periods of time with the SA microcapsules. A significant hypoglycemic effect was maintained during the period from 3.0 – 20.0 h with starch acetate microcapsules (Fig. 5).

As such starch acetate exhibited good release retarding and rate controlling effect in both \textit{in vitro} and \textit{in vivo} pharmacokinetic and pharmacodynamic evaluation. The plasma concentrations of gliclazide as well as hypoglycemic effect were sustained over longer periods of time with the starch acetate microcapsules of gliclazide.

**Conclusion**

Starch acetate microcapsules of Gliclazide could be successfully prepared by Emulsion Solvent Evaporation technique. This method is industrially feasible as it involves emulsification and removal of the solvent, which can be controlled precisely. Obtained microcapsules were spherical and discrete with good microencapsulation efficiency and drug content. Drug release was slow and continued for more than 24 hours, which was further confirmed by slow absorption in \textit{in vivo}. Pharmacodynamic studies showed the gliclazide administered in the form of microcapsules could successful in achieving constant hypoglycemic effect for an extended period of time when compared with pure drug. Hence, the SA was found successful as a microencapsulating agent to retard the drug release.

**Author contributions**

Prof. K.P.R. chowdary is a guide, who designed the protocol of the project work. Sowmya, who was the student and performed the entire work.

**Acknowledgements**

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**Conflicts of interest**

None.

**References**

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7. Chen L, Li X, Li L and Guo S. Acetylated starch based biodegradable material with potential biomedical applications as drug delivery systems, Current applied Physics, 2007, 7(supplement 1); e90-e93.


**Table 1.** Correlation coefficient (r) values in the analysis of release data of starch acetate microcapsules of gliclazide as per various kinetic models

<table>
<thead>
<tr>
<th>Microcapsules</th>
<th>Size</th>
<th>Zero order</th>
<th>First Order</th>
<th>Higuchi</th>
<th>Peppas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glic MC1</td>
<td>20/35</td>
<td>0.9357</td>
<td>0.9876</td>
<td>0.9723</td>
<td>0.9882</td>
</tr>
<tr>
<td></td>
<td>35/50</td>
<td>0.9741</td>
<td>0.9791</td>
<td>0.9726</td>
<td>0.9895</td>
</tr>
<tr>
<td>Glic MC2</td>
<td>20/35</td>
<td>0.9442</td>
<td>0.9861</td>
<td>0.9776</td>
<td>0.9880</td>
</tr>
<tr>
<td></td>
<td>35/50</td>
<td>0.9703</td>
<td>0.9768</td>
<td>0.9707</td>
<td>0.9848</td>
</tr>
<tr>
<td>Glic MC3</td>
<td>20/35</td>
<td>0.9961</td>
<td>0.9469</td>
<td>0.9875</td>
<td>0.9887</td>
</tr>
<tr>
<td></td>
<td>35/50</td>
<td>0.9938</td>
<td>0.9303</td>
<td>0.9902</td>
<td>0.9836</td>
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<tr>
<td>Glic MC4</td>
<td>20/35</td>
<td>0.9945</td>
<td>0.9800</td>
<td>0.9886</td>
<td>0.9899</td>
</tr>
<tr>
<td></td>
<td>35/50</td>
<td>0.9953</td>
<td>0.9533</td>
<td>0.9875</td>
<td>0.9839</td>
</tr>
</tbody>
</table>

**Table 2.** Wall thickness and release characteristics of starch acetate microcapsules of gliclazide

<table>
<thead>
<tr>
<th>Microcapsules</th>
<th>Size</th>
<th>Wall Thickness (µ)</th>
<th>T$_{50}$ (h)</th>
<th>T$_{90}$ (h)</th>
<th>K$_0$ (mg/h)</th>
<th>K$_1$ (h$^{-1}$)</th>
<th>'n' in Peppas equation</th>
<th>Gliclazide content (%)</th>
<th>Microencapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glic MC1</td>
<td>20/35</td>
<td>19.2</td>
<td>4.3</td>
<td>10.1</td>
<td>3.4797</td>
<td>0.2488</td>
<td>0.61</td>
<td>94.8</td>
<td>99.84</td>
</tr>
<tr>
<td></td>
<td>35/50</td>
<td>14.6</td>
<td>3.8</td>
<td>7.8</td>
<td>4.6030</td>
<td>0.3983</td>
<td>0.55</td>
<td>94.9</td>
<td>99.89</td>
</tr>
<tr>
<td>Glic MC2</td>
<td>20/35</td>
<td>25.1</td>
<td>5.3</td>
<td>12</td>
<td>3.2440</td>
<td>0.2206</td>
<td>0.66</td>
<td>90.3</td>
<td>100.3</td>
</tr>
<tr>
<td></td>
<td>35/50</td>
<td>19.4</td>
<td>5.0</td>
<td>9.1</td>
<td>4.1370</td>
<td>0.3398</td>
<td>0.58</td>
<td>90.05</td>
<td>100.05</td>
</tr>
<tr>
<td>Glic MC3</td>
<td>20/35</td>
<td>39.0</td>
<td>10.2</td>
<td>21.4</td>
<td>2.2452</td>
<td>0.1146</td>
<td>0.63</td>
<td>79.75</td>
<td>99.68</td>
</tr>
<tr>
<td></td>
<td>35/50</td>
<td>36.1</td>
<td>8.0</td>
<td>20.1</td>
<td>2.2868</td>
<td>0.1348</td>
<td>0.55</td>
<td>79.5</td>
<td>99.37</td>
</tr>
<tr>
<td>Glic MC4</td>
<td>20/35</td>
<td>45.0</td>
<td>10.8</td>
<td>&gt;24</td>
<td>2.1044</td>
<td>0.0836</td>
<td>0.66</td>
<td>70.7</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>35/50</td>
<td>40.4</td>
<td>9.0</td>
<td>22.1</td>
<td>2.1370</td>
<td>0.1046</td>
<td>0.57</td>
<td>70.2</td>
<td>100.2</td>
</tr>
</tbody>
</table>
### Table 3. Summary of pharmacokinetic parameters estimated following the oral administration of gliclazide (A) and starch acetate microcapsules Glic MC3 size 20/35 of gliclazide (B) in rabbits (n = 6)

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (µg/ml)</td>
<td>4.82</td>
<td>2.78</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>3.0</td>
<td>6.0</td>
</tr>
<tr>
<td>$K_a$ (h$^{-1}$)</td>
<td>0.1246</td>
<td>--</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>5.56</td>
<td>--</td>
</tr>
<tr>
<td>$(AUC)_0^{24}$ (µg.h/ml)</td>
<td>55.86</td>
<td>54.89</td>
</tr>
<tr>
<td>$(AUC)_0^{\infty}$ (µg.h/ml)</td>
<td>60.74</td>
<td>69.25</td>
</tr>
<tr>
<td>$K_d$ (h$^{-1}$)</td>
<td>0.8174</td>
<td>0.156</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>10.24</td>
<td>14.75</td>
</tr>
<tr>
<td>BA (%)</td>
<td>100.0</td>
<td>114.8</td>
</tr>
</tbody>
</table>

**Figure 1.** SEM of starch acetate microcapsules of gliclazide, Glic MC3 (20/35)
Figure 2. Release profiles of starch acetate microcapsules of gliclazide (Size 20/35)

Figure 3. Release profiles of starch acetate microcapsules of gliclazide (Size 35/50)
Figure 4. Plasma concentrations of gliclazide following the oral administration of gliclazide (A) and starch acetate microcapsules Glic MC3 Size 20/35 of gliclazide (B) in rabbits (n = 6)

Figure 5. Percent reduction in blood glucose level following the oral administration of gliclazide (A) and starch acetate microcapsules Glic MC3 Size 20/35 of gliclazide (B) in rabbits (n = 6)