RESUMEN

Objetivo. El objetivo del presente estudio es diseñar y evaluar las microesferas mucoadherentes de liberación controlada para uso oral.

Material y métodos. Las microesferas de rosiglitazona maleato con una capa de alginato y polímeros mucoadherentes de carboximetilcelulosa de sodio, carbopol 934P e hidroxipropilmetilcelulosa fueron elaboradas por un proceso de gelificación iónica de orificio. Las microesferas se evaluaron mediante rayos infrarrojos con transformado de Fourier, se estudio la morfología, el tamaño de partícula, las propiedades «micromeritics», el porcentaje de eficacia de entrapamiento, la prueba in vitro de «wash-off» y estudios in vitro de liberación.

Resultados. Las microesferas que resultaban eran esféricas y de flujo libre. La eficiencia de captura fue de 68,2 a 85,6%. Las microesferas exhibieron buena propiedad mucoadherente en el ensayo in vitro de lavado. La liberación de rosiglitazona fue lenta y se prolongó más de 12 h dependiendo de la composición de la capa.

Conclusiones. Las microesferas preparadas con mucoadherentes son convenientes para la liberación oral controlada de rosiglitazona maleato y así ayudar en el tratamiento de la diabetes mellitus tipo II.

Palabras clave: microesferas mucoadherentes, rosiglitazona maleato, liberación controlada.

ABSTRACT

Aim: The objective of the present study is to design and evaluate mucoadhesive microspheres for oral controlled release.

Materials and Method: Rosiglitazone maleate microspheres with a coat consisting of alginate and a mucoadhesive polymer sodium carboxymethylcellulose, carbopol 934P and hydroxypropylmethylcellulose were prepared by an orifice-ionic gelation process. The microspheres were evaluated for FTIR studies, morphology, particle size, micromeritic properties, percentage entrapment efficiency, in-vitro wash-off test and in-vitro release studies.

Results: The resulting microspheres were spherical and free flowing. The percent entrapment efficiency was 68.2 to 85.6%. The microspheres exhibited good mucoadhesive property in the in vitro wash-off test. Rosiglitazone release from these mucoadhesive microspheres was slow and extended over 12 h duration of time depending on the composition of coat.

Conclusions: The prepared mucoadhesive microspheres are thus suitable for oral controlled release of Rosiglitazone maleate and thereby help in the management of type II diabetes mellitus.

Keywords: mucoadhesive microspheres, rosiglitazone maleate, sustained release.
INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, hypertriglyceridaemia and hypercholesterolaemia, resulting from defects in insulin secretion or action or both. Several attempts have been proposed to improve oral bioavailability, among them microencapsulation represents a promising concept. Microencapsulation has been employed to sustain drug release, providing a long lasting and more reliable release with gastrointestinal (GI) irritation reduced or eliminated.

Bioadhesive delivery of drugs has gained prominence in recent times as a means of drug administration. The intimate contact of the mucoadhesive polymer with the mucous surface can result in an increased drug retention time, increasing bioavailability and increasing contact time between drug and mucosa. When the mucoadhesive dosage form is administered in either tablet or capsule form, they may or may not adhere to the mucous surface due to the weight of the dosage form and the vigorous movement of the GI tract, resulting in a large variation. However, mucoadhesive microspheres have some advantages. These include a light weight and a smaller dose variation due to the large number of microspheres administered.

Rosiglitazone maleate (RSZ) is an antidiabetic drug for type II diabetes that improves insulin sensitivity in muscle and adipose tissues through activation of peroxisome proliferator-activated γ receptor (PPAR γ) that are involved in the transcription of insulin responsive genes responsible for glucose production, transport, and utilization. The aim of the present work is to characterize the rosiglitazone microspheres with biological half-life of 3-4 h and to evaluate the usefulness and feasibility of these microspheres for orally administered drug delivery system.

MATERIALS AND METHODS

Materials

Rosiglitazone (RSZ) was obtained from Zydus Cadila, Ahmedabad, India. Sodium alginate (CDH, New Delhi), Calcium chloride (Qualigen, Mumbai), Hydroxypropylmethylcellulose (HPMC), K4M, Sodium carboxymethylcellulose (SCMC) and carbopol-934P (CP) were procured from Central Drug House, Mumbai. All other reagents used were of analytical grade and purchased from their respective commercial sources.

Methods

Preparation of Microspheres

Rosiglitazone microspheres were prepared by ionotropic gelation method employing sodium alginate in combination with various polymers using sodium CMC, HPMC and carbopol 934P in different ratios (1:1, 1:9 and 9:1). Sodium alginate (1.0 g) was dissolved in purified water (50 ml) to form a homogeneous polymer solution with 1.0 g of each polymer. The drug RSZ (2.0 g) was added to the polymer solution and the resulting dispersion was added manually drop wise into 10% w/v of calcium chloride solution (about 40 ml) through a syringe with a needle size no. 18. The added droplets were retained in the calcium chloride solution for 30 min to complete the curing reaction. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with water and dried at 45° C for 12 hrs.

FT-IR Study

The Fourier transform infrared (FT-IR) spectra of pure drug (RSZ) and drug-polymer loaded microspheres were obtained using a Perkin-Elmer spectroscope. About 2-3 mg of samples were mixed with dried KBr of equal weight and compressed to form KBr disks. The samples were scanned from 400 to 4000 cm⁻¹.

Morphology and Particle size

Shape and surface morphology of microspheres were studied using Scanning Electron Microscope (SEM LEO 430, Leo Electron Microscopy Ltd., England). For determination of surface characteristics all the microspheres were coated uniformly with gold palladium by using sputter coater for 5 to 7 minutes, after fixing the sample in individual steps. All samples of microspheres were then randomly examined for surface morphology at different magnification ranges.

Micromeritic Properties of Microspheres

Accurately weighed microspheres were gently poured using a glass funnel into a graduated cylinder exactly to 10 ml mark. Initial volume was noted. Bulk density and tapped
density were noted using tapping method using measuring
cylinder (of 10 ml).

Angle of repose of prepared microspheres (n=3) was deter-
dined by fixed funnel standing method. The granules
were allowed to flow through funnel orifice on a plane paper
kept on the horizontal surface to form a pile of granules.
Angle of repose (θ), Hausner’s ratio (H) and Carr’s index
(\% C) were calculated to study the flow properties of micro-
spheres by using following formulas\(^\text{12}\).

\[
\theta = \tan^{-1} \frac{h}{r}
\]

where, h is height and r is radius of the pile, respectively.

\[
H = \frac{D_t}{D_b}
\]

\% C = \frac{D_t - D_b}{D_t} \times 100

where, Dt is tapped and Db is bulk density, respectively.

**Percentage Entrapment efficiency**

50 mg of microspheres were dispersed in 10 ml of 0.1 N
HCl for 30 min with occasional shaking. The suspension
was then centrifuged at 2000 rpm for 5 min and the super-
natant was kept aside. The drug concentration was deter-
mined spectrophotometrically by UV at 228 nm (Shimadzu
Pharmspec UV-1700, Japan). The entrapment efficiency
(n=3) was calculated by using following formula\(^\text{13}\).

\[
\% EE = \frac{D_{cal}}{D_{th}} \times 100
\]

where, Dcal is the calculated drug content and Dth is the
theoretical drug content, respectively.

**In-vitro wash-off test**

Freshly excised pieces of intestinal mucosa (5 x 2 cm) from
sheep were mounted onto glass slides (3 x 1 inch) with ad-
hesive material\(^\text{14}\). About 50 microspheres were spread onto
each wet rinsed tissue specimen, and immediately thereaf-
ther the support was hung onto the arm of a U.S.P. tablet dis-
integrating test machine. By operating the disintegrating
test machine the tissue specimen was given a slow, regular
up and down movement in the test fluid at 37ºC contained
in a 1 l vessel of the machine. At the end of one hr, and
at hourly intervals up to 12 hrs, the machine was stopped
and the number of microspheres still adhering to the tis-

**In-vitro drug release of microspheres**

A USP XXIII basket type dissolution apparatus was used to
study in-vitro drug release from microspheres. A weighed
amount of microspheres (50 mg) was used for the study. 900
ml of dissolution medium (0.1N HCl, pH 1.2) maintained at
a temperature of 37±0.5°C and rotation speed of 100 rpm
was used for the study\(^\text{15}\). The in-vitro release studies were
performed for 12 h. At predetermined time intervals, 5 ml
sample was withdrawn, passed through a 0.45 µm mem-
brane filter (Millipore). After appropriate dilutions, the
concentration of drug in samples were analysed spectro-
photometrically at 228 nm using Shimadzu, Pharmspec
UV-1700 series, Japan. The initial volume of dissolution
medium was maintained by adding 5 ml of fresh dissolu-
tion medium after each withdrawal. Perfect sink conditions
prevailed during the in-vitro drug release studies.

**Statistical analysis**

Experimental results were performed in triplicate (n=3)
and expressed as mean ± S.D. One-way analysis of variance
(ANOVA) was applied to check significant difference in
drug release from different formulations. Differences were
considered to be significant at P<0.05.

**RESULTS AND DISCUSSION**

**FTIR Study**

Potential chemical interaction between drug and polymer
may change the therapeutic efficacy of the drug. To inves-
tigate the possibility of chemical interaction between drug
and polymer, FTIR spectra of pure RGL and drug-loaded
formulations containing different polymers were analyzed
over the range 400-4000 cm\(^{-1}\). The IR spectra of pure RGL
showed strong absorption bands attributable to carbonyl
stretching at 1703 cm\(^{-1}\) and C=N stretching of pyridine moi-
ety at 1512 cm\(^{-1}\). FTIR spectra of the drug loaded formula-
tions displayed all the characteristic bands of drug without
any significant spectral shift. This suggested that there was
no potential chemical interaction between the components
of the microspheres (Spectras not shown).

**Physical characterization of microspheres**

The microspheres were found to be discrete, spherical and
free-flowing. The effects of alginate concentrations and
polymer ratios on the average particle size and % drug entrapment of microspheres are shown in Table 1. The mean particle size increased with increase in polymer concentration which might be due to the fact that as polymer concentration increases it produces a significant increase in the viscosity, leading to an increase of the emulsion droplet size and finally a higher microsphere size.

The % entrapment efficiency for the different formulations significantly increased with increasing polymer content (p<0.05). The alginate-CP microspheres (9:1) produced the highest % entrapment efficiency of 85.6±3.2. An increase in polymer concentration resulted in formation of larger microspheres entrapping greater amount of drug16.

The size of the microspheres increased with increase in the alginate concentration which may be due to the increase in viscosity, resulting in increase in droplet size during addition of the polymer dispersion to the harvesting medium. The SEM of prepared microspheres (F1) is shown in Figure 1. Formation of cracks on the surface of the microspheres were observed which may be due to the penetration of the dissolution medium into the microspheres and the subsequent dissolution of the drug and hence its diffusion through the polymer matrix.

Table 1: Composition and evaluation of drug loaded microspheres

<table>
<thead>
<tr>
<th>Code</th>
<th>Coat composition</th>
<th>% entrapment efficiency</th>
<th>Particle size</th>
<th>Angle of repose</th>
<th>Carr’s index</th>
<th>Hausner’s ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Alg-SCMC (1:1)</td>
<td>68.2±2.3</td>
<td>404±3.5</td>
<td>42°54’</td>
<td>9.8</td>
<td>1.04</td>
</tr>
<tr>
<td>F2</td>
<td>Alg-SCMC (1:9)</td>
<td>76.4±1.6</td>
<td>412±5.5</td>
<td>42°15’</td>
<td>10.2</td>
<td>1.08</td>
</tr>
<tr>
<td>F3</td>
<td>Alg-SCMC (9:1)</td>
<td>84.5±2.4</td>
<td>438±2.8</td>
<td>41°72’</td>
<td>11.2</td>
<td>1.12</td>
</tr>
<tr>
<td>F4</td>
<td>Alg-HPMC (1:1)</td>
<td>71.4±1.5</td>
<td>426±5.2</td>
<td>42°24’</td>
<td>10.7</td>
<td>1.06</td>
</tr>
<tr>
<td>F5</td>
<td>Alg-HPMC (1:9)</td>
<td>75.8±1.8</td>
<td>435±4.5</td>
<td>42°26’</td>
<td>10.8</td>
<td>1.11</td>
</tr>
<tr>
<td>F6</td>
<td>Alg-HPMC (9:1)</td>
<td>82.2±2.1</td>
<td>459±3.8</td>
<td>41°58’</td>
<td>11.5</td>
<td>1.14</td>
</tr>
<tr>
<td>F7</td>
<td>Alg-CP (1:1)</td>
<td>74.5±2.4</td>
<td>488±4.2</td>
<td>41°24’</td>
<td>11.7</td>
<td>1.09</td>
</tr>
<tr>
<td>F8</td>
<td>Alg-CP (1:9)</td>
<td>78.4±1.6</td>
<td>500±2.8</td>
<td>40°68’</td>
<td>12.2</td>
<td>1.12</td>
</tr>
<tr>
<td>F9</td>
<td>Alg-CP (9:1)</td>
<td>85.6±3.2</td>
<td>510±3.4</td>
<td>40°17’</td>
<td>12.8</td>
<td>1.16</td>
</tr>
</tbody>
</table>

![Figure 1. SEM photograph of prepared microspheres (F1)](image)
Micromeritic properties of microspheres

The rheological parameters like angle of repose, tapped density, bulk density and packing properties (Table 1) confirms better flow and packing properties of the prepared microspheres. All the formulation showed angle of repose value within the range of 30° to 45° (n=3), which is an appreciable limit for microspheres to show flow property while formulating in the dosage form.

In-vitro wash-off studies

The mucoadhesion test was performed on both simulated gastric pH (0.1N HCl, pH 1.2) and simulated intestinal pH (phosphate buffer solution, PBS, pH 6.8) for 12h. The results are given in Table 2. Ch’ng et al. (1985) observed that the pH of the medium was critical for the degree of hydration, swelling and mucoadhesion of the polymers. The wash-off was found to be faster at intestinal pH than at gastric pH. The rapid wash-off observed at simulated intestinal pH 6.8 may be due to ionization of carboxyl acid group and other functional groups present in the polymers at this pH, which increases their solubility and reduces adhesive strength.

The adhesion time of microspheres at pH 1.2 was ranked, HPMC > carbopol 934P, whereas the adhesion time for the microspheres at pH 6.8 was ranked, carbopol 934P > HPMC. Poor mucoadhesion of HPMC microspheres at alkaline pH may be due to its non-ionic nature possessing low hydrogen bonding capability with mucus glycoproteins. Although carbopol 934P microspheres had negative charge repulsion with mucus, numerous hydrophilic functional groups such as carboxyl groups in carbopol molecules could form hydrogen bonds with mucus molecules, thus producing some adhesive force of this polymer. The microspheres produced using alginate: SCMC coat did not adhere to the mucosa for more than 6 h showing poor mucoadhesion in either of the pH mediums used for the study.

<table>
<thead>
<tr>
<th>Code</th>
<th>Percent of microspheres adhering to tissue after 12 h duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 N HCl, pH 1.2</td>
</tr>
<tr>
<td></td>
<td>1   2   4   6   8   12</td>
</tr>
<tr>
<td>F1</td>
<td>74±1.4 70±0.5 62±1.2 56±0.8 52±1.8 18±1.2</td>
</tr>
<tr>
<td>F2</td>
<td>78±2.5 74±1.5 70±1.8 64±2.1 58±1.5 25±0.5</td>
</tr>
<tr>
<td>F3</td>
<td>82±1.5 74±1.6 60±0.4 38±1.2 20±0.5 -</td>
</tr>
<tr>
<td>F4</td>
<td>78±0.5 75±0.6 70±0.8 66±1.2 62±1.4 52±1.8</td>
</tr>
<tr>
<td>F5</td>
<td>90±2.5 84±2.4 80±1.5 76±1.2 74±0.6 72±1.8</td>
</tr>
<tr>
<td>F6</td>
<td>82±1.2 71±0.8 66±0.4 38±1.1 28±0.4 18±1.2</td>
</tr>
<tr>
<td>F7</td>
<td>80±2.2 76±1.1 70±1.4 60±2.2 55±1.1 49±0.6</td>
</tr>
<tr>
<td>F8</td>
<td>88±1.8 85±1.2 82±2.4 78±1.5 75±1.0 68±1.2</td>
</tr>
<tr>
<td>F9</td>
<td>75±2.1 62±0.8 56±0.5 42±0.6 35±1.4 28±1.2</td>
</tr>
<tr>
<td>EVA</td>
<td>54±1.6 40±1.8 10±1.2 - - - -</td>
</tr>
</tbody>
</table>

EVA indicates ethylene vinyl acetate (non-mucoadhesive control). Values are given as mean ± S.D.

In-vitro drug release of microspheres

Solubility of RSZ depends on pH. Maximum absorption may be expected with increasing solubility in acid environment. It is known that microspheres constitute multiple-unit dosage forms which have many advantages as compared to tablets. They spread more evenly in the stomach which leads to a decreased risk of high local concentration and of adverse effects. Moreover, these forms are characterized by a high reproducibility of release due to a relatively large surface and a short diffusion way of the drug.

The in-vitro release studies were carried out in 0.1 N HCl (pH 1.2) which indicated that there was a slow and controlled release of drug for all the formulations. The drug release of the microspheres at 1:9 and 9:1 ratio of alginate:
polymers are shown in Figure 2 and 3, respectively. Drug release from the RSZ microspheres was slow, extended and dependent on the type of polymer used. The increasing order of release rate observed with microspheres was Sodium alginate: Sodium CMC < Sodium alginate: Carbopol < Sodium alginate: HPMC. The differences in the drug release characteristics of various microspheres might be due to the differences in the porosity of the coat formed and its solubility in the dissolution fluid. Drug release from alginate-sodium-CMC was slow and extended over a period of 12 hr and these microcapsules were found suitable for oral controlled release formulations.

CONCLUSIONS

Mucoadhesive microspheres offer a unique carrier system owing to its capability to adhere to any mucosal tissue. This ionic orifice method produced spherical and free flowing microspheres. Rosiglitazone maleate release was found to be slow and extended period of time. The release depended on the type of polymer used. The release of drug was prolonged when incorporated within mucoadhesive polymers. The mucoadhesive microspheres prepared by ionotropic gelation technique gave sustained drug release up to 12 h duration. Microspheres containing alginate: sodium CMC gave best reproducible results, would prove to be promising carrier for oral delivery of rosiglitazone maleate and thereby help in the management of type II diabetes mellitus.

REFERENCES

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