C.B.R. NO : 10947

To

SHRI. N. H. ALLOORKAR
PLOT NO. 6, PARIMAL NAGAR, NEAR WATER FILTRATION UNIT, PAWADEWADI ROAD, NANDED, MAHARASHTRA, INDIA.

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For Controller of Patents & Designs
Synthesis, Characterization and Evaluation of Effect of Chemical Modification of Hydroxypropyl Methylcellulose on Drug Release

Nagesh H. Aloorkar*1, Manish S. Bhatia2

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ABSTRACT

A novel polymer, hydroxypropyl methylcellulose (HPMC) chemically modified by reacting with maleic anhydride was synthesized. FTIR and NMR spectroscopy, DSC analysis and determination of physicochemical property like viscosity were used to confirm the formation of novel polymer. Ibuprofen, paracetamol and diclofenac sodium matrix tablets were prepared employing HPMC and novel modified HPMC to evaluate the effect of chemical modification on drug release properties of HPMC. Drug release was found to be increased from modified HPMC matrices and unlike HPMC, release mechanism was found to be due to polymer erosion. Thus, it can be concluded that biocompatible chemical modifications of polymers could make better options accessible for specific formulation objectives.

Keywords: HPMC, chemically modified HPMC, drug release, matrix tablets, polymer erosion.

INTRODUCTION

Hydrophilic matrix systems are widely used for modified drug release because of its simplicity in manufacture. In such systems, the drug release is controlled by a combination of several physical processes which include diffusion, polymer swelling, erosion and dissolution [1-3]. The penetration of water through the tablet and the resultant drug dissolution and diffusion primarily depends on the swellability of the polymer [4]. Polymer swellability, erosion control, and the drug release rate, all depend on the polymer molecular weight, degree of substitution, and the polymer concentration [5-7].

Swelling controlled release systems consist of a drug molecularly dispersed or dissolved in a polymer matrix at low or high concentrations. As water penetrates the polymer, swelling occurs and a thin layer of polymer in the rubbery state is formed. Drug diffusion through this gel layer is
relatively fast. Drug delivery from such systems is governed by the gel layer thickness. During drug delivery as swelling and dissolution compete, the gel layer thickness first increases due to swelling, then remains constant due to synchronization of swelling, drug diffusion, and dissolution, and finally decreases as dissolution takes over [8-14].

The polymer dissolution in a solvent is an important phenomenon in a variety of applications. In controlled release applications of polymers, a solute is dispersed or molecularly dissolved in a polymer phase. The release process can be controlled either by solvent diffusion or by polymer dissolution [15, 16]. Characterization of polymer dissolution has been carried out by various scientists and different mechanisms and mathematical models have been proposed [17-21].

For the formulation of an efficient hydrophilic system, one must select a polymer that will wet, hydrate, and swell to form a gelatinous layer fast enough to prevent the disintegration of the tablet and to protect the interior of the tablet content from dissolving during the initial wetting and hydration phases. For this purpose, various types of cellulosic derivatives and their combinations have been extensively used in the preparation of matrix tablets. Hydroxypropyl methylcellulose is the dominant hydrophilic carrier material used for the preparation of oral controlled drug delivery systems. One of its most important characteristics is the high swellability, which has a significant effect on the release kinetics of a drug. Upon contact with a dissolution medium, water or biological fluid diffuses into the tablet, resulting in polymer chain relaxation with volume expansion. The drug then diffuses out of the device [22, 23].

Modulation of polymer swelling in controlling the drug release is a novel concept [24, 25]. A lot of work has been carried out by various scientists on the modification of cellulosic derivatives and the effect of modification on polymer swelling and drug release has been observed [26-30]. In the present investigation, an attempt has been made to chemically modify hydroxypropyl methylcellulose by substituting some of the hydroxyl groups of it with a biocompatible acid derivative, maleic anhydride for modifying the swelling characteristics. The prime objective for the work was to obtain chemically modified HPMC wherein the mechanism of release is more due to erosion than swelling and diffusion.

**MATERIALS AND METHODS**

**Materials**

Hydroxypropyl methylcellulose (HPMC) K4M was supplied by Lupin Pharmaceuticals, (Goa, India) as the gift sample. Maleic anhydride was procured from Rajesh chemicals, (Mumbai, India). Polyvinyl pyrrolidone K30 (PVP K30) and mannitol were procured from Lobachemie, (Mumbai, India). Ibuprofen and paracetamol were supplied by Mediorals Pvt. Ltd., (Satara, India) as the gift sample, whereas diclofenac sodium was supplied by Sun and Kingly Pharma Pvt. Ltd., (Satara, India) as the gift sample. All the other ingredients used were of analytical grade.

**Methods**

**Modification of hydroxypropyl methylcellulose with maleic anhydride**

Three different ratios of HPMC to maleic anhydride were computed on the basis of the molecular weight and the number of free hydroxyl groups present in each monomer unit of HPMC. Initially required quantity of maleic anhydride was dissolved in water followed by addition of required quantity of HPMC slowly with constant stirring to avoid aggregation. The entire system was heated at 40 °C for 24 h (fig.1). After the completion of reaction, the novel modified polymer was spray dried (Labultima, 2220, Mumbai, India) and passed through a #30
mesh screen. Physicochemical characterization of the modified polymers led to the selection of one modified polymer (1:1 substituted) for evaluation.

![Diagram of chemical modification of HPMC K4M with maleic anhydride]

**Figure 1: Scheme of chemical modification of HPMC K4M with maleic anhydride**

**FTIR spectroscopy**
FTIR spectra of HPMC and modified HPMC were recorded using FTIR spectrophotometer (Jasco 4100, Japan) between wavelengths of 400-4000 cm$^{-1}$.

**NMR spectroscopy**
Nuclear magnetic resonance (NMR) analysis was done to characterize the modified hydroxypropyl methylcellulose using a 300 MHz NMR and $^1$H NMR spectrum was recorded on a Varian Mercury 300 MHz spectrometer using deuterated chloroform as the solvent.

**Differential Scanning Calorimetric (DSC) Studies**
Thermal analysis of unmodified and chemically modified HPMC was carried out using Mettler Toledo 821° DSC (Switzerland) thermal analyzer. The samples (1-2 mg) were hermetically sealed in an aluminum pan and heated at a constant rate of 10 °C per minute, over a temperature range of 50 °C- 500 °C. Inert atmosphere was maintained by purging nitrogen gas at the flow rate of 20 ml/min.
Viscosity study
The viscosity of varying concentrations (0.1-0.5%) of HPMC and HPMC modified with maleic anhydride solutions was determined at 25 °C using LVDV 2+ Pro viscometer (Brookfield, USA) at a shear rate of 100.

Physical properties of granules and matrix tablet preparation
Granules of paracetamol, ibuprofen and diclofenac sodium were prepared using the wet granulation method. Each formulation contained 100 mg of active drug and 50 mg of HPMC and modified HPMC. Thus the drug to polymer ratio was kept at 2:1 (w/w). The formulae for all the formulations are as given in table 1. Active drug, mannitol, and polymer were passed through a # 12 mesh separately. All the ingredients were weighed accurately and added in a blender in ascending order of their weight and blended for 30 min. The blend was transferred to a glass mortar and granulated with 6% PVP K30 in isopropyl alcohol by trituration with a pestle. The granules were dried in hot air oven (Labhosp, India) at 40 °C for 30 min and passed through a # 60 mesh screen. Magnesium stearate and talc were individually passed through a # 60 mesh screen and added to the granules and blended for 5 min in a blender.

Table 1: Matrix tablets of ibuprofen, diclofenac sodium and paracetamol using HPMC K4M and novel chemically modified HPMC K4M

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of Ingredient</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPM1</td>
<td>HPM2</td>
</tr>
<tr>
<td>1</td>
<td>Ibuprofen</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Diclofenac Sodium</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Paracetamol</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>Chemically modified HPMC</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>HPMC K4M</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>Polyvinyl Pyrrolidone (PVP K30)</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>Mannitol</td>
<td>80</td>
</tr>
<tr>
<td>8</td>
<td>Talc</td>
<td>2.5</td>
</tr>
<tr>
<td>9</td>
<td>Magnesium stearate</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Total weight</strong></td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

Table for one tablet is shown in table. All ingredients are in mg.

Physical properties of granules such as bulk density, tapped density, percent compressibility, Hausner’s ratio, and angle of repose were determined using bulk density test apparatus (Electrolab, India). Percent compressibility and Hausner’s ratio were calculated using the formula

\[
\text{% Compressibility} = \left\{ \frac{(D_t - D_b)}{D_t} \right\} \times 100 \quad \text{-------- Eq. 1}
\]

\[
\text{Hausner’s ratio} = \frac{D_t}{D_b} \quad \text{---------- Eq.2}
\]

Where \(D_t\) and \(D_b\) are tapped density and bulk density respectively.

The tablets were compacted using 10 mm concave punches on a single punch tablet machine (Cadmach, India) to get the tablets of 250 mg weight. A batch of 100 tablets was prepared for all the formulations.
Characterization of the tablets

Physical parameters
Tablets from all the formulations were evaluated for various parameters such as diameter (Vernier caliper), thickness (Micrometer screw gauge), hardness (Pfizer hardness tester), weight variation test and friability (Roche friabilator).

Swelling study
The swelling study was performed as a function of water uptake by tablets prepared using HPMC and modified HPMC. The tablets were placed in separate baskets of dissolution apparatus containing purified water. Tablets were removed and weighed on a digital balance (Adventure, Ohaus Corp., USA) at the regular interval of 1 h. The amount of water uptake was compared from the weight difference.

In vitro dissolution study
In vitro dissolution studies were conducted using USP XXIII type II dissolution apparatus (TDT 08 L, Electrolab, India) in 900 ml of purified water at 37 ± 0.5°C and at a paddle speed of 100 rpm. For ibuprofen the dissolution medium used was phosphate buffer pH 7.2. The study was carried out in triplicate. Aliquots of dissolution medium were withdrawn at specified time intervals, filtered through 0.45 μm filter and were analyzed spectrophotometrically for ibuprofen, diclofenac sodium, and paracetamol at their respective $\lambda_{max}$ of 221, 276, and 243 nm.

RESULTS AND DISCUSSION

Characterization of the novel modified HPMC

FTIR spectroscopy
A relatively characteristic and sharp peak close to 1450 indicates the introduction of ethylene moiety in the chemically modified HPMC and thus confirms the acylation of some free hydroxyl groups in HPMC by maleic anhydride (fig.2)

![FTIR spectra of hydroxypropyl methylcellulose k 4M and novel chemically modified hydroxypropyl methylcellulose K 4M with maleic anhydride.](image)

NMR spectroscopy
The relative increase in the number of protons in the region between $\delta = 3.4-4.8$ and especially in the region close to $\delta = 4.6$ indicates that the 2-butendioic acyl moiety has been substituted in the HPMC (fig. 3a and 3b).
Figure 3a: $^1$H NMR spectrum of HPMC

Figure 3b: $^1$H NMR spectrum of chemically modified HPMC

**Differential Scanning Calorimetric analysis:**
As both hydroxypropyl methylcellulose and modified hydroxypropyl methylcellulose do not have exact melting points and char when heated, no sharp endothermic peaks were observed for both of them indicating no exact melting points. A broad endothermic bend in thermogram 4a from 40-110 $^\circ$C for hydroxypropyl methylcellulose and from 40-110 $^\circ$C in thermogram 4b for modified hydroxypropyl methylcellulose can plausibly be attributable to the vaporization of the moisture present in the samples. A shallow endothermic peak from 130-150 $^\circ$C in the thermogram of modified hydroxypropyl methylcellulose may plausibly be attributable to the glass transition temperature of the polymer.

**Viscosity**
Viscosity of both the polymers was found to be increased as the function of concentration (w/w). Viscosity of HPMC was found to be decreased after its chemical modification (esterification) with maleic anhydride. The reduction in the viscosity of modified HPMC was might be due to its increased solubility and decreased swellability. The data for the viscosity is given in table 2, and fig.5.
Table 2: Viscosity study of HPMC and modified HPMC

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>HPMC K4M</th>
<th>Modified HPMC</th>
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</thead>
<tbody>
<tr>
<td>0.1</td>
<td>126.3</td>
<td>94</td>
</tr>
<tr>
<td>0.2</td>
<td>160</td>
<td>104</td>
</tr>
<tr>
<td>0.3</td>
<td>202</td>
<td>112</td>
</tr>
<tr>
<td>0.4</td>
<td>289</td>
<td>126</td>
</tr>
<tr>
<td>0.5</td>
<td>456</td>
<td>164</td>
</tr>
</tbody>
</table>

Figure 5: Viscosity of HPMC and modified HPMC
Physical properties of the granules

The granules of all the batches exhibited good flow property (angle of repose <30°). Bulk density was found to be between 0.303-0.385 g/cc while the tapped density was found to be in the range of 0.324-0.442 g/cc. From density data percent compressibility (Carr’s index) and Hausner ratio were calculated and were found to be between 8.31-14.28 and 1.10-1.17 respectively. All these properties indicate good flow property of granules, uniform die fill and good compression characteristics. The data for physical properties of the granules is given in table 3.

Table 3: Physical Properties of granules

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Bulk Density (g/cc)</th>
<th>Tapped Density (g/cc)</th>
<th>Carr’s Index</th>
<th>Hausner’s Ratio</th>
<th>Angle of Repose(°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPM1</td>
<td>0.307 ± 0.009</td>
<td>0.332 ± 0.020</td>
<td>9.99 ± 1.45</td>
<td>1.11 ± 0.02</td>
<td>28.17 ± 1.21</td>
</tr>
<tr>
<td>HPM2</td>
<td>0.311 ± 0.0156</td>
<td>0.360 ± 0.031</td>
<td>8.31 ± 1.07</td>
<td>1.16 ± 0.04</td>
<td>25.24 ± 0.52</td>
</tr>
<tr>
<td>HPM3</td>
<td>0.318 ± 0.022</td>
<td>0.388 ± 0.017</td>
<td>14.28 ± 2.40</td>
<td>1.17 ± 0.04</td>
<td>25.48 ± 1.84</td>
</tr>
<tr>
<td>HPM4</td>
<td>0.385 ± 0.015</td>
<td>0.442 ± 0.019</td>
<td>12.79 ± 0.47</td>
<td>1.14 ± 0.01</td>
<td>22.8 ± 0.45</td>
</tr>
<tr>
<td>HPM5</td>
<td>0.330 ± 0.017</td>
<td>0.363 ± 0.021</td>
<td>9.19 ± 1.54</td>
<td>1.10 ± 0.02</td>
<td>27.06 ± 1.19</td>
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<tr>
<td>HPM6</td>
<td>0.303 ± 0.008</td>
<td>0.324 ± 0.010</td>
<td>10.52 ± 1.24</td>
<td>1.12 ± 0.02</td>
<td>24.26 ± 0.63</td>
</tr>
</tbody>
</table>

All Values are expressed as mean± S.D. n =3

Physical parameters of the tablets

Tablets prepared by wet granulation method were evaluated for various official and non official tests. As the granules were free flowing, there was uniform die fill and the tablets of uniform weight with acceptable variation as per I.P. specification (<5%) were obtained. Thickness of the tablets was found to be in the range of 3.33-3.83 mm. diameter was observed in the range of 10.03-10.16 mm. Hardness was observed in the range of 5.83-6.5 kg/cm\(^2\). Percent friability for all the formulations was found to be less than 1%, which is an indication of good mechanical strength of tablets that can withstand the shocks during transportation or shipping. The values obtained for tablet dimensions, hardness and percent friability are as given in table 4. Thus the chemical modification of HPMC has added some advantage with respect to the mechanism of drug release and at the same time the other desirable properties of the HPMC have been retained.

Table 4: Physical parameters of the tablets

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Thickness (mm)</th>
<th>Diameter (mm)</th>
<th>Hardness (Kg/cm(^2))</th>
<th>Friability (%)</th>
<th>Weight Variation Test †</th>
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<tr>
<td>HPM1</td>
<td>3.33 ± 0.29</td>
<td>10.1 ± 0.1</td>
<td>6.17 ± 0.29</td>
<td>0.42 ± 0.17</td>
<td>Passes</td>
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<tr>
<td>HPM2</td>
<td>3.67 ± 0.29</td>
<td>10.16 ± 0.06</td>
<td>6 ± 0.5</td>
<td>0.3 ± 0.22</td>
<td>Passes</td>
</tr>
<tr>
<td>HPM3</td>
<td>3.33 ± 0.58</td>
<td>10.07 ± 0.06</td>
<td>5.83 ± 0.29</td>
<td>0.52 ± 0.09</td>
<td>Passes</td>
</tr>
<tr>
<td>HPM4</td>
<td>3.83 ± 0.29</td>
<td>10.03 ± 0.06</td>
<td>6.5 ± 0.5</td>
<td>0.37 ± 0.10</td>
<td>Passes</td>
</tr>
<tr>
<td>HPM5</td>
<td>3.67 ± 0.58</td>
<td>10.13 ± 0.12</td>
<td>6.33 ± 0.58</td>
<td>0.36 ± 0.07</td>
<td>Passes</td>
</tr>
<tr>
<td>HPM6</td>
<td>3.33 ± 0.29</td>
<td>10.03 ± 0.06</td>
<td>6.33 ± 0.76</td>
<td>.43 ± 0.31</td>
<td>Passes</td>
</tr>
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</table>

Values shown in the figure are the mean of 3 determinations ± SD. † indicates n = 20

Swelling study

It was found that there was swelling initially for 1h, but there onwards erosion dominated in the tablets prepared with modified HPMC (HPM1, HPM3 and HPM5). In formulations containing HPMC, higher swelling was found and the tablets were found to be swelling for 5 h (formulation HPM4, and HPM6) and for 6 h (formulation HPM2) and later erosion of the
polymer matrices started. The earlier but slow erosion of the matrix tablets prepared with modified HPMC as compared to HPMC matrix tablets may be due decrease in the uniformity of the repetitive monomer units of modified polymer due to limited acylation of maleic anhydride. This irregularity in the structure could be responsible for loss of film forming ability and hence swellability while the substitution of hydroxyl group by a relatively non-polar and sterically larger 2-butendioyl moiety may be responsible for slow rate of erosion observed. The data for swelling and erosion of tablets is given in table 5, and fig. 6.

**Table 5: Swelling study of tablets**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>HPM1</th>
<th>HPM2</th>
<th>HPM3</th>
<th>HPM4</th>
<th>HPM5</th>
<th>HPM6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>248.13±1.14</td>
<td>247.33±1.40</td>
<td>247.47±2.21</td>
<td>247.53±1.68</td>
<td>247.77±1.65</td>
<td>247.87±1.29</td>
</tr>
<tr>
<td>1</td>
<td>456.37±3.98</td>
<td>474.73±3.98</td>
<td>419.37±2.60</td>
<td>435.33±2.91</td>
<td>455.1±2.49</td>
<td>446.17±2.70</td>
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<tr>
<td>2</td>
<td>409.53±3.43</td>
<td>513.83±3.04</td>
<td>388.40±3.54</td>
<td>475.23±2.47</td>
<td>401.23±2.01</td>
<td>484.77±2.50</td>
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<tr>
<td>3</td>
<td>337.4±2.68</td>
<td>527.13±3.52</td>
<td>299.85±2.05</td>
<td>504.9±2.36</td>
<td>316.33±1.55</td>
<td>505.07±2.61</td>
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<tr>
<td>4</td>
<td>271.4±3.25</td>
<td>558.13±3.68</td>
<td>248.53±2.85</td>
<td>524.3±2.36</td>
<td>254.3±2.07</td>
<td>524.87±3.05</td>
</tr>
<tr>
<td>5</td>
<td>202.9±3.86</td>
<td>569.80±2.75</td>
<td>178.17±3.65</td>
<td>587.83±3.46</td>
<td>187.07±2.36</td>
<td>592.03±5.57</td>
</tr>
<tr>
<td>6</td>
<td>148.5±3.15</td>
<td>603.7±2.07</td>
<td>135.8±3.07</td>
<td>502.17±2.32</td>
<td>136.83±2.29</td>
<td>505.87±3.14</td>
</tr>
<tr>
<td>7</td>
<td>58.03 ±3.20</td>
<td>500.73±2.99</td>
<td>23.93±2.93</td>
<td>473.7±2.85</td>
<td>46.4±2.43</td>
<td>486.83±3.12</td>
</tr>
<tr>
<td>8</td>
<td>14.23 ±1.78</td>
<td>307.67±3.16</td>
<td>15.37±2.92</td>
<td>294.1±2.40</td>
<td>9.53±2.80</td>
<td>304.87±2.30</td>
</tr>
</tbody>
</table>

Values shown in the figure are the mean of 3 determinations ± SD.

**Figure 6: Swelling study of tablets**

**In vitro dissolution study**

For all the designed formulations, the drug release at the end of 8 h was found to be varied. The drug release was found to be increased from the formulations prepared with modified HPMC (formulations HPM1, HPM3 and HPM5). An increase of 13-20% in drug release was found from modified HPMC matrix tablets as compared to HPMC matrix tablets. The data for drug release is given in table 6, and fig.7.
This increase in the drug release can be attributed to a marked reduction in viscosity of modified HPMC as well as to the change in the polarity and steric properties of HPMC. The relatively hydrophobic and bulkier group replacing H- of the hydroxyl group of HPMC could be responsible for decrease in the duration of swelling observed. The decrease in the number of free hydroxyl groups as well as physical masking of some adjacent hydroxyl groups due to the hydrophobic bulkier substituent could be responsible for decreased swelling. The disruption of the systematic arrangement of the monomers in HPMC due to a dissimilar substituent could also be a contributing factor. The hydrophobic linear carbon chain along with the terminal carbohydroxyl group may be responsible for retarding erosion rate due to limited access to water molecules. Release of drugs at a consistent rate attributed to erosion could be responsible for a net increase in release at the end of 8 h.

Table 6: In Vitro dissolution profile of tablets

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPM1</td>
</tr>
<tr>
<td>0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>1</td>
<td>32.45±1.14</td>
</tr>
<tr>
<td>2</td>
<td>44.64±1.20</td>
</tr>
<tr>
<td>3</td>
<td>60.85±1.22</td>
</tr>
<tr>
<td>4</td>
<td>68.93±1.07</td>
</tr>
<tr>
<td>5</td>
<td>75.34±0.63</td>
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<td>6</td>
<td>81.13±1.06</td>
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<tr>
<td>7</td>
<td>93.28±1.22</td>
</tr>
<tr>
<td>8</td>
<td>99.06±1.18</td>
</tr>
</tbody>
</table>

Values shown in the figure are the mean of 3 determinations ± SD.

Figure 7: In Vitro dissolution profile of tablets

Acknowledgements
The authors are thankful to the management of Satara College of Pharmacy, Satara for providing the necessary facilities to carry out the work. The authors are also thankful to Mediorals Pvt. Ltd., Satara for the gift sample of Ibuprofen and paracetamol, and Sun and Kingly Pharma Pvt. Ltd. for the gift sample diclofenac sodium.
REFERENCES

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Dear author:

I am glad to inform you that your article 'Synthesis, Characterization and Evaluation of Effect of Chemical Modification of Chitosan on Drug Release' by Nagesh H. ALOORKAR 1* & Manish S. BHATIA 2 has been accepted for publication in Latin American Journal of Pharmacy. In due moment you will receive the page proof consigning the issue where your article will be included.

Many thanks for your interest in our journal.

Yours sincerely,

Prof. Néstor O. Caffini, Editor
Latin American Journal of Pharmacy
E-mail: caffini@biol.unlp.edu.ar
Synthesis, Characterization and Evaluation of Effect of Chemical Modification of Chitosan on Drug Release

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SUMMARY
A novel polymer, chemically modified chitosan was synthesized by reacting with hydrochloride salt of 2-aminoethanoyl chloride. Formation of novel chemically modified chitosan was confirmed by FTIR and NMR spectroscopy and by thermal (DSC) and elemental analysis. Acute oral toxicity study was performed on modified chitosan to check its safety. Ibuprofen, paracetamol and diclofenac sodium tablets were compressed on a single punch tablet machine using the weight granulation method. The drug release was found to be delayed from the formulations containing chemically modified chitosan. Thus, it could be concluded that the change in the hydrophilic balance and the resultant change in the modulation of swelling of the polymer may retard the drug release from polymer matrices.

KEY WORDS:
Chitosan, acylation, 2-aminoethanoyl chloride, drug release
INTRODUCTION

Chitosan is a biopolymer obtained by deacetylation of chitin, which is the second most abundant polymer next to cellulose available in nature. It is found in the exoskeletons of crustaceans, fungi, etc. It is a polysaccharide formed primarily of repeating units of β (1-4) 2-amino-2-deoxy-D-glucose (D-glucosamine). Generally it is soluble in aqueous medium in the presence of small amount of acids such as acetic acid, lactic acid, hydrochloric acid and so on having pH <6.5 but precipitates above this pH by the addition of alkali solution like aqueous sodium hydroxide [1-4].

Chitosan is widely used in the pharmaceutical field and has been formulated as films, beads, tablets and microparticles. Much attention has recently been paid to chitosan due to its biocompatibility, non toxicity, bactericidal and bacteriostatic activities [5-8]. The primary amino group and hydroxyl groups in the structure of chitosan are highly advantageous for providing unique biological and chemical functions and for conducting chemical modification reactions [9-15]. Various modifications have been carried out in chitosan through chemical modifications and copolymerization methods [16-25].

In the present investigation, an attempt has been made to chemically modify chitosan by using hydrochloride salt of 2-aminoethanoyl chloride having balanced hydrophilic and hydrophobic property and to evaluate the effect of chemical modification of chitosan on drug release form the tablet matrices.

MATERIALS AND METHODS

MATERIALS

Chitosan (DA 88.6%) was supplied by India Sea foods, Cochin, (India) as the gift sample. Hydrochloride salt of 2-aminoethanoic acid was procured from Rajesh chemicals, Mumbai (India). Polyvinyl pyrrolidone K30 (PVP K30) and mannitol were procured from Loba Chemie, Mumbai (India). Ibuprofen and paracetamol were supplied by Mediorals Pvt. Ltd., Satara (India) as the gift sample, whereas diclofenac sodium was supplied by Sun and Kingly Pharma Pvt. Ltd., Satara (India) as the gift sample. All the other ingredients used were of analytical grade.

METHODS

Modification of chitosan with hydrochloride salt of 2-amioethanoyl chloride

Various cyclic anhydrides like succinic anhydride, maleic anhydride, glutalic anhydride, itaconic anhydride, phthalic anhydride, 5,norbornyl-endo-2,3-dicarboxylic anhydride, trimellitic anhydride [2], acetic anhydride [20], and glutaric dialdehyde [26] were used for acylation of chitosan by different researchers. We chose 2-aminoethanol chloride because the hydrolytic product of the chemically modified chitosan using this reagent would be glycine, which is harmless, and biocompatible. Moreover, presence of an amino along with the ester linkage formed would give a definite balance of hydrophilicity and hydrophobicity.

After few trials employing reactant stoichiometry for 5%, 10% and 15% substitution of free hydroxyl and amino groups in chitosan, 10% substitution was found to be appropriate for the targeted release profile of NSAIDs. Therefore a molar ratio for 10% substitution of chitosan with hydrochloride salt of 2-amioethanoyl chloride was computed on the basis of molecular weight, number of monomers and free hydroxyl and amino groups present in each monomer of chitosan. Initially hydrochloride salt of 2-aminoethanoic acid was converted to hydrochloride salt of 2-aminoethanol chloride by reacting with thionyl chloride and distilling off the excessive thionyl chloride used in the reaction. Chitosan (10 g) was dissolved in sufficient quantity of 0.1N acetic acid. Hydrochloride salt of 2-amioethanoyl chloride (3.36 g) was added to chitosan solution and the reaction mixture was heated at 40 °C for 24 h, after which the reaction mixture
N.H.Aloorkar

was treated with 1N sodium hydroxide and a solidified gelatinous mass was obtained. This mass was then freeze dried (Khera Engineering Ltd., India) and passed through #30 mesh screen. The scheme of synthesis is given in fig.1.

**FTIR Spectroscopy**

FTIR spectra of chitosan and chitosan chemically modified with hydrochloride salt of 2- aminoethanoyl chloride were recorded using FTIR spectrometer (Jasco 4100, Japan) between wavelengths of 400-4000 cm⁻¹.

**NMR spectroscopy**

Nuclear magnetic resonance (NMR) analysis was done to characterize the modified chitosan; using a 300 MHz NMR and ¹H NMR spectrum was recorded on a Varian Mercury YH300 MHz spectrometer using deuterated chloroform as the solvent.

**Thermal analysis**

Thermal analysis of chitosan and chemically modified chitosan was carried out using Mettler Toledo 821° DSC (Switzerland) thermal analyzer. The samples (1-2 mg) were hermetically sealed in an aluminum pan and heated at a constant rate of 10 °C per minute, over a temperature range of 40 °C- 300 °C. Inert atmosphere was maintained by purging nitrogen gas at the flow rate of 20 ml/ min.

**Elemental analysis**

Elemental analysis of both chitosan and modified chitosan was carried out using a Thermo Finnigan (Thermo Fischer Scientific Inc.) elemental analyzer (EA 1112).

**Toxicity studies**

The acute oral toxicity studies of chitosan chemically modified with hydrochloride salt of 2- aminoethanoyl chloride were carried out on Swiss Albino Mice according to OECD guidelines (OECD guideline No. 425 (Up and Down Method)) for toxicity studies. The powder of test material was suspended in 0.5% CMC in water. The suspension was freshly prepared before dosing. The concentration of the test material suspensions was adjusted to allow administration of all test doses in a constant volume. All doses were administered orally to the test animals in a constant dosing volume of 0.4 ml /10 g bodyweight of the animal. One group of animals acted as a control group, which received the vehicle alone in a similar manner. The animals were observed for signs of intoxication and mortality, if any, up to the end of 14 days after administration of the dose. The animals were observed during the course of treatment for clinical signs of toxicity and mortality, body weight, ill health, together with any behavioral changes or reaction to treatment. At the end of 14 days after dosing, all surviving mice were sacrificed under pentothal / ether anesthesia. Complete necropsies were carried out on all the animals.

**Physical properties of granules and matrix tablet preparation**

Granules of paracetamol, ibuprofen and diclofenac sodium were prepared using the wet granulation method. Each formulation contained 100 mg of active drug and 50 mg of chitosan or modified chitosan. Thus, the drug to polymer ratio was kept as 2:1 (w/w). The formulae for all the formulations are as given in table 1. Active drug, mannitol, and polymer were passed through a # 12 mesh separately. All the ingredients were weighed accurately and added in a blender in ascending order of their weight and blended for 30 min. The blend was transferred to a glass mortar and granulated with 6% PVP K30 in isopropyl alcohol by trituration with a pestle. The granules were dried in hot air oven (Labhosp, India) at 40 °C for 30 min and passed through a # 30 mesh screen. Magnesium stearate and talc were individually passed through a # 60 mesh screen and added to the granules and blended for 5 min in a blender.
Physical properties of granules such as bulk density, tapped density, percent compressibility (Carr’s index), Hausner’s ratio, and angle of repose were determined. Percent compressibility and Hausner’s ratio were calculated using the formula

\[
\% \text{ Compressibility} = \frac{(D_t - D_b)}{D_t} \times 100 \quad \text{----------Eq. 1}
\]

\[
\text{Hausner’s ratio} = \frac{D_t}{D_b} \quad \text{-----------Eq. 2}
\]

Where \( D_t \) and \( D_b \) are tapped density and bulk density respectively.

The tablets were compacted using 10 mm concave punches on a single punch tablet machine (Cadmach, India) to get the tablets of 250 mg weight. A batch of 100 tablets was prepared for all the formulations.

**Evaluation of the tablets**

**Compatibility Studies**

The compatibility of the drug with the excipient was carried out using FTIR spectrophotometer (Jasco, 4100, Japan). FTIR spectra were recorded using potassium bromide (KBR) dispersion method. The base line correction was done using dried potassium bromide. Later on the spectrum of dried mixture of drug and potassium bromide was run followed by drug with excipients.

**Physical parameters**

Tablets from all the formulations were evaluated for various parameters such as diameter (Vernier caliper), thickness (Micrometer screw guage), hardness (Pfizer hardness tester), weight variation test and friability (Roche friabilator).

**In vitro dissolution study**

In vitro dissolution studies were conducted using USP XXIII type II dissolution apparatus (TDT 08 L, Electrolab, India) in 900 ml of purified water at 37±0.5 °C and at a paddle speed of 100 rpm. For ibuprofen the dissolution medium used was phosphate buffer pH 7.2. The study was carried out in triplicate. Aliquots of dissolution medium were withdrawn at specified time intervals, filtered through 0.45 µm filters and were analyzed spectrophotometrically for ibuprofen, diclofenac sodium, and paracetamol at their respective \( \lambda_{max} \) of 221, 276, and 243 nm.

**RESULTS AND DISCUSSION**

**FTIR spectroscopy**

Primary amino group in a definite chemical environment appears in the FTIR spectrum along with the hydroxyl groups in pure chitosan sample in the region 3200-3600 cm\(^{-1}\), while numerous peaks in the same region indicate the presence of amino functional group in more than one distinct chemical environment along with some hydroxyl groups in the modified chitosan (Fig. 2). Though, as reported by other researchers, free amino groups are likely to be preferentially acylated under the reaction conditions and the reaction stoichiometry applied, acylation of free hydroxyl groups is also possible, and from the relative difference in the I. R. spectra, the desired and targeted chemical change in the chitosan structure is evident.

**NMR spectroscopy**

Increase in the variety of protons attached to hetero atoms like oxygen and nitrogen is also clearly indicated by increase in the number of NMR signals observed between 3-5 ppm (Fig.3) and the same has been reported by the other researchers employing other agents for acylation. Thus, the targeted chemical change in chitosan is confirmed.

**Thermal analysis**

As both chitosan and chemically modified chitosan do not have exact melting points and char when heated, no sharp endothermic peaks were observed for both of them indicting no exact melting points. A broad endothermic bend in thermogram from 80-100
°C for chitosan (Fig. 4a) and from 40-100 °C in thermogram for modified chitosan (Fig. 4b) can plausibly be attributable to the glass transition of the polymers and vaporization of the moisture present in the samples.

**Elemental analysis**

The elemental analysis of chitosan and novel chemically modified chitosan is summarized in table 2. It is to be noted that the empirical formula of both polymers can be approximated due to their high polydispersity. The theoretical values of carbon, hydrogen, and nitrogen of chemically modified chitosan were found to be 32, 6.66, and 18.66 (For 100% substitution), whereas the experimental values were found to be 31.51, 6.07, and 1.78 respectively. These values are in good agreement, considering minor probable errors due to the moisture content of the sample, and also it is to be noted that only 10% substitution is attempted and hence difference in the values for nitrogen can plausibly be attributable to it.

**Toxicity studies**

Toxicity Signs like changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity such as lacrimation, piloerection, pupil size, unusual respiratory pattern, circulatory, autonomic and central nervous systems, and somatomotor activity were not observed in any of the animals treated with the test substance. Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy or bizarre behavior were not observed in any of the animals. Other signs like tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were not seen in animals during Study. The data for the survival of the test animals under study is provided in table 3.

The animals that died during study were dissected and observed for changes in the alimentary tract. An observation in the animals which died in the study was severe bloating of and accumulation of air in the alimentary tract. This observation suggests that the dose administered obstructed the gastrointestinal tract and caused severe pressure on vital organs that might had resulted into death. In necropsy, no significant changes were observed in the animals. From observations related to survivals and unaltered histopathological alterations, it can be concluded that modified chitosan has lethal dose above 2000 mg/kg/p.o.

**Physical properties of the granules**

The granules of all the batches showed good flow ability as the angle of repose of granules of all the formulations was found to be below 30°. Bulk density was found to be between 0.383-0.505 g/cc and the tapped density between 0.437- 0.593 g/cc. From density data percent compressibility (Carr’s index) was calculated and was found to be between 10.50- 14.70. Hausner’s ratio was found to be between 0.85-0.9 (Table 4). These all properties indicate the good flow property, uniform die fill, and good compression characteristics.

**Evaluation of the tablets**

**Compatibility study**

FTIR spectroscopy revealed that all the important peaks that were present in drugs, ibuprofen, paracetamol, and diclofenac sodium, were found to be present in formulations. Hence, it could be concluded that both chitosan and modified chitosan are compatible with the drugs (Fig.5 a, b, c.).

**Physical parameters of tablets**

Tablets prepared by wet granulation method were evaluated for various official and non official tests. Granules were free flowing, therefore there was uniform die fill and
tablets of uniform weight were obtained with acceptable variation as per I.P. specification (<5%). Thickness of the tablets was found to be in the range of 3.17-3.5 mm whereas diameter was observed in the range of 10.07-10.2 mm. Hardness of the tablets was found to be in the range of 4.83 – 5.67 Kg/cm². Percent friability was found to be below 1% which was an indication of good mechanical strength of the tablets that can withstand the shocks during shipping or transportation. The values obtained for physical parameters are as given in table 5.

**In vitro dissolution study**

For all the designed formulations prepared with chitosan and modified chitosan, the drug release at the end of 8 h was found to be delayed from the formulations containing modified chitosan. A relative decrease of about 10% was found in the release of ibuprofen, diclofenac sodium, and paracetamol from modified chitosan matrix tablets as compared to chitosan tablets. Data for the drug release from chitosan and novel chemically modified chitosan matrix tablets is given in fig.6. Dissolution efficiency of the formulation is provided in table 6.

The decrease in drug release can be attributed to replacement of some free hydroxyl groups as well as free amino groups by ester groups and addition of terminal –NH₂ group to the structure of chitosan. The decrease in the number of free hydroxyl groups in the structure of chitosan might have reduced the water accessible surface area of the polymer and a decrease in the water uptake by the polymer. The reduced uptake of water by the polymer might have contributed to the formation of a permeability barrier controlling the drug release rate. Similarly the loss of uniformity in the repetitive hydroxyl as well as amino groups in the polymer may also have affected the accessibility of the drug for dissolution. It has to be noted that the system has not been investigated in different pH buffers.

**CONCLUSION**

Several researchers have used different types of aldehydes for acylation of chitosan. In the present investigation, we have employed hydrochloride salt of 2-aminoethanoyl chloride, a compound obtained from 2-aminoethanoic acid, which is biocompatible material and has balanced hydrophilicity and hydrophobicity. We have also tried to investigate and quantify the effect of chemical modification of chitosan using this reagent on drug release profile. The formation of modified chitosan was confirmed with FTIR and NMR spectroscopy, and by thermal and elemental analysis. The acute oral toxicity study proved the novel modified chitosan to be non toxic. Chemical modification of chitosan with hydrochloride salt of 2-aminoethanoyl chloride exhibited a retarded drug release from the formulation and 10% modification of chitosan exhibited a near about 10% reduction in the drug release from the formulations. It may be due to addition of ester groups, with higher numbers of terminal –NH₂ groups in place of free hydroxyl and amino groups to the structure of chitosan. Hence, it could be concluded that the resultant change in the hydrophilic balance in the polymer structure may reduce the water uptake capacity of the polymer and may retard the drug release from polymer matrices and these findings can be made use of to synthesize tailor made chemically modified polymers for required type of drug release and thus to fulfill the demands of formulation scientists.

**Acknowledgements**

The authors are thankful to Mediorals Pvt. Ltd., Satara (India) for supplying Ibuprofen and Paracetamol, Sun and Kingly Pharma Pvt. Ltd., Satara (India) for supplying diclofenac sodium and to India Sea Foods, Cochin (India) for supplying chitosan as the gift sample. The authors are also thankful to the management of Satara College of Pharmacy, Satara for providing the necessary facilities to carry out the work.
REFERENCES

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Table 2  Data for the survival of animals in toxicity studies.
Table 3  Data for the survival of animals in toxicity studies.
Table 4  Physical Properties of granules.
Table 5  Physical Properties of Tablets
Table 6  Dissolution efficiency profile of formulations

Figure legends

Figure 1  Scheme of synthesis of chemically modified chitosan
Figure 2  Overlain FTIR spectra of chitosan (A) and chitosan chemically modified with hydrochloride salt of 2-aminoethanoyl chloride (B).
Figure 3  $^1$H NMR spectrum of chitosan chemically modified with hydrochloride salt of 2-aminoethanoyl chloride
Figure 4a  DSC thermogram of chitosan
Figure 4b  DSC thermogram of novel chemically modified chitosan
Figure 5a  Overlain FTIR spectra of paracetamol (A), paracetamol with modified chitosan (B) and paracetamol with chitosan (C).
Figure 5b  Overlain FTIR spectra of ibuprofen (A), ibuprofen with modified chitosan (B) and ibuprofen with chitosan (C).
Figure 5c  Overlain FTIR spectra of diclofenac sodium (A), diclofenac sodium with modified chitosan (B) and diclofenac sodium with chitosan (C).
Figure 6  in vitro dissolution profiles of formulations.
Table 1: Composition of paracetamol, ibuprofen and diclofenac sodium tablets

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of Ingredient</th>
<th>CH1</th>
<th>CH2</th>
<th>CH3</th>
<th>CH4</th>
<th>CH5</th>
<th>CH6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ibuprofen</td>
<td>100</td>
<td>100</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>Diclofenac Sodium</td>
<td>--</td>
<td>--</td>
<td>100</td>
<td>100</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Paracetamol</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>Chitosan modified with hydrochloride salt of 2-aminoethanolyl chloride (10% substitution)</td>
<td>50</td>
<td>--</td>
<td>50</td>
<td>--</td>
<td>50</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>Chitosan</td>
<td>--</td>
<td>50</td>
<td>--</td>
<td>50</td>
<td>--</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>Polyvinyl Pyrrolidone (PVP K30)</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
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<tr>
<td>7</td>
<td>Mannitol</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
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<tr>
<td>8</td>
<td>Talc</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>9</td>
<td>Magnesium stearate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Total weight</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

Formula for one tablet is shown in table. All quantities are in mg

Table 2: Elemental analysis of chitosan and novel chemically modified chitosan

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Empirical Formula</th>
<th>Theoretical (%)</th>
<th>Experimental (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>H</td>
<td>N</td>
</tr>
<tr>
<td>Chitosan (DA 88.6 %)</td>
<td>C₆H₁₁O₃N₁</td>
<td>40.03</td>
<td>5.5</td>
</tr>
<tr>
<td>Modified Chitosan</td>
<td>C₈H₂₀O₈N₄</td>
<td>32</td>
<td>6.66</td>
</tr>
</tbody>
</table>

Table 3: Data for the survival of animals in toxicity studies

<table>
<thead>
<tr>
<th>Test Material</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemically modified chitosan with hydrochloride salt of 2-aminoethanolyl chloride</td>
<td>OXOOX</td>
</tr>
</tbody>
</table>

(Survive =O, Death=X)

Table 4: Physical properties of granules

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Bulk density (g/cc)</th>
<th>Tapped density (g/cc)</th>
<th>Carr’s Index</th>
<th>Hausner’s Ratio</th>
<th>Angle of Repose (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH1</td>
<td>0.403 ± 0.013</td>
<td>0.461 ± 0.009</td>
<td>12.38 ± 1.627</td>
<td>0.89 ± 0.015</td>
<td>27.46 ± 1.53</td>
</tr>
<tr>
<td>CH2</td>
<td>0.383 ± 0.018</td>
<td>0.437 ± 0.009</td>
<td>12.31 ± 2.513</td>
<td>0.88 ± 0.025</td>
<td>27.81 ± 1.09</td>
</tr>
<tr>
<td>CH3</td>
<td>0.446 ± 0</td>
<td>0.516 ± 0.006</td>
<td>13.57 ± 1.004</td>
<td>0.86 ± 0.012</td>
<td>27.11 ± 1.04</td>
</tr>
<tr>
<td>CH4</td>
<td>0.505 ± 0.013</td>
<td>0.593 ± 0.018</td>
<td>14.70 ± 2.380</td>
<td>0.85 ± 0.021</td>
<td>28.11 ± 0.91</td>
</tr>
<tr>
<td>CH5</td>
<td>0.397 ± 0.013</td>
<td>0.456 ± 0.017</td>
<td>12.87 ± 0.408</td>
<td>0.87 ± 0.006</td>
<td>26.98 ± 0.05</td>
</tr>
<tr>
<td>CH6</td>
<td>0.496 ± 0.023</td>
<td>0.554 ± 0.014</td>
<td>10.50 ± 1.807</td>
<td>0.9 ± 0.017</td>
<td>26.98 ± 0.05</td>
</tr>
</tbody>
</table>

All Values are expressed as mean ± S.D. n =3
Table 5: Physical properties of tablets

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Thickness (mm)</th>
<th>Diameter (mm)</th>
<th>Hardness (Kg/cm²)</th>
<th>Friability (%)</th>
<th>Weight Variation Test †</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH1</td>
<td>3.17 ± 0.29</td>
<td>10.17 ± 0.15</td>
<td>5.17 ± 0.29</td>
<td>0.38 ± 0.015</td>
<td>Passes</td>
</tr>
<tr>
<td>CH2</td>
<td>3.33 ± 0.29</td>
<td>10.17 ± 0.12</td>
<td>4.83 ± 0.58</td>
<td>0.16 ± 0.012</td>
<td>Passes</td>
</tr>
<tr>
<td>CH3</td>
<td>3 ± 0</td>
<td>10.2 ± 0.1</td>
<td>5.5 ± 0.5</td>
<td>0.87 ± 0.020</td>
<td>Passes</td>
</tr>
<tr>
<td>CH4</td>
<td>3.17 ± 0.29</td>
<td>10.07 ± 0.12</td>
<td>5.67 ± 0.29</td>
<td>0.30 ± 0.015</td>
<td>Passes</td>
</tr>
<tr>
<td>CH5</td>
<td>3.33 ± 0.29</td>
<td>10.13 ± 0.06</td>
<td>5.33 ± 0.58</td>
<td>0.87 ± 0.026</td>
<td>Passes</td>
</tr>
<tr>
<td>CH6</td>
<td>3.5 ± 0</td>
<td>10.17 ± 0.06</td>
<td>5 ± 0.5</td>
<td>0.48 ± 0.020</td>
<td>Passes</td>
</tr>
</tbody>
</table>

Values shown in the figure are the mean of 3 determinations ± SD. † indicates n = 20

Table 6: Dissolution efficiency profile of formulations

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>CH1</th>
<th>CH2</th>
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<th>CH4</th>
<th>CH5</th>
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<td>0</td>
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<td>0.00</td>
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<tr>
<td>1</td>
<td>9.46</td>
<td>10.10</td>
<td>12.09</td>
<td>11.89</td>
<td>8.42</td>
<td>20.81</td>
</tr>
<tr>
<td>2</td>
<td>16.42</td>
<td>19.00</td>
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Fig. 1

Chitosan + \( \text{HCl} \cdot \text{CH}_2\text{N} = \text{CH}\cdot\text{C}\text{Cl} \)

Hydrochloric salt of 2-aminoethanoyl chloride

Dil. acetic acid

Where, \( R \) may be \(-H, \ OR\cdot \text{COCH}_2\text{NH}_3^+\cdot\text{Cl}^-\)

1N NaOH

Chemically modified chitosan

Where, \( R' \) may be \(-H, \ OR\cdot \text{COCH}_2\text{NH}_2\)
Fig. 2
Fig. 4b
Fig. 5a
Fig. 5b
Fig. 6
DATE: 23-5-08

CERTIFICATE

This is certify that, the research project of M. Pharm / Ph.D. entitled

Acute Oral Toxicity Study of Modified Cellulose, Modified Chitosan and Modified HPMC in mice.

Submitted by Mr. / Miss Nagesh H. Aloorkar

Under guidance of Dr. M. S. Bhatia

has been approved by Institutional animal Ethical Committee of R C Patel College of pharmacy during the committee meeting convened on 20-11-2007

resolution number RCPCP/IAEC/2007-08/23

Chairman IAEC

Principal