Effect of Method of Preparation on Chitosan Microspheres of Mefenamic Acid


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ABSTRACT
Mefenamic acid loaded chitosan microspheres were prepared both by thermal and gluteraldehyde cross linking methods and high level of entrapment of Mefenamic acid was obtained in gluteraldehyde cross linking method. The microspheres exhibited good swelling properties. DTA confirmed that Mefenamic acid was in solid dispersion in gluteraldehyde cross-linked chitosan microspheres. These microspheres exhibited faster release at low loading in comparison to high loading. Fitting the dissolution data of microspheres in Rigter-Peppas equation showed that at low loading the diffusional exponent (n) were high. As the polymer content increased the value of diffusional exponent approached to one, which is indicative of nearly zero order release.

Keywords: Chitosan microspheres, Mefenamic acid, controlled release, gluteraldehyde, diffusional exponent.

INTRODUCTION
Chitosan is biodegradable modified natural carbohydrate polymer (polysaccharide) derived from chitin, which occurs predominantly in animals of arthropoda and marine crustaceans. Chitosan has great pharmaceutical application because of its biocompatibility, high charge density, and nontoxic in nature. [1] Chitosan is used because of its property to improve the solubility of poorly water soluble drugs [2-4] as well as to control the release of drugs by slow erosion from hydrated matrix. [5-7] The gelling property of chitosan offers diverse uses including microencapsulation and controlled release via microparticulate system. [8] Different methods have been used to prepare chitosan particulate system e.g. Cross linking [9], thermal cross linking [10], iontophoretic gelation [11], spray drying [12], and precipitation coacervatin. [13]

Mefenamic acid is selected as a model drug to investigate the use of chitosan to control the release of poorly water soluble drug. The plasma half life of Mefenamic acid is short (2 h) and long term use of Mefenamic acid is ended in rheumatoid arthritis. [14] Mefenamic acid loaded chitosan microspheres were prepared by different cross linking methods using emulsification phase separation technique to study size, shape, drug-polymer interaction, drug state in microspheres and in vitro release profile in different medium with or without surfactant and also compare in vitro release profile with conventional marketed Mefenamic acid tablets.

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MATERIALS AND METHODS
Chitosan was obtained from India Sea Food (Batch no.BX26-20/AN/40/1005), Mefenamic acid from Blue Cross Laboratory Ltd. (Nashik, India). All other reagents were analytical grades.

Determination of molecular weight of chitosan
Chitosan solutions of different concentration i.e., 0.001, 0.003, 0.005, 0.007, 0.009, and 0.01 g/mL in acetate buffer (pH 2.8) were prepared and intensities were measured by Zetasizer Nano ZS (Malvern Instrument, UK). These intensities were plotted (Debye plot) and from the intercept of the plot molecular weight of chitosan was obtained (Fig. 1). The molecular weight of chitosan was necessary because in thermal cross linking method chitosan and cross linking agent citric acid was taken in certain molar ratio. [15-16]

Preparation of microspheres
Thermal cross linking
[17]
Citric acid, the cross linking agent, was added to a 30mL of a 2.5 % (w/v) aqueous acetic acid solution of chitosan maintaining a constant molar ratio between chitosan and citric acid (3.5×10^-3 mol chitosan : mol citric acid ). The chitosan cross linker solution was cooled to 0°C, and then added to 25 mL sesame oil previously maintained at 0°C with stirring for 10 min. This emulsion was added to 175 mL sesame oil maintained at 120°C, and cross linking was carried out in a glass beaker with vigorous stirring (1000 rpm) for 40 min. The microspheres obtained were filtered, then washed with diethyl ether and dried. The drug loaded microspheres were prepared by the method described above, except that the drug was dispersed in the chitosan solution with stirring at different drug: polymer ratio (viz., 1:3, 1:5, 1:10, and 1:15).
Glutaraldehyde crosslinking

A 2.5 % (w/v) chitosan solution in 2 % (v/v) aqueous acetic acid was prepared and the drug was dispersed in it. This dispersed phase was added to 125 ml of continuous phase of light liquid paraffin containing 0.5 % (w/v) span 80 to form a w/o emulsion. Stirring was continued at 2000 rpm using a three blade mechanical stirrer. About 2.5 ml of 25 % (v/v) aqueous glutaraldehyde solution was added drop by drop at each of the following intervals: 15, 30, 45 and 60 min. Stirring was continued for 2.5 h to obtain microspheres, which were separated by vacuum filtration, washed first with petroleum ether (60-80°C), then with distilled water to remove the adhering liquid paraffin and glutaraldehyde, respectively. The microspheres were then dried in room temperature and stored in a desiccator.

Determination of microsphere Size, shape and surface characteristics

The size measurement was made with the help of scanning electron microscopy (JSM, JEOL, Tokyo, Japan). Microsphere suspension in glycerin was spread on a glass slide and gold coating was done by using ion sputtering device. The gold coated samples were vacuum dried and examined under microscope.

Determination of Mefenamic acid content in the microspheres

The amount of Mefenamic acid entrapped in microspheres was determined by digesting 20 mg of microspheres in 100 ml methanol-hydrochloric acid mixture [99:1 (v/v)] and the solution was sonicated for 10 min. Then after filtration and subsequent dilution from this the absorbance was determined spectrophotometrically (Shimadzu UV-Pharmaspec 1700, Japan) at 350.2 nm. Each determination was carried out in triplicate and percent entrapment was calculated as

\[
\text{Percent drug entrapment} = \left( \frac{\text{Measured amount of drug}}{\text{Theoretical amount of drug}} \right) \times 100
\]

IR Spectra

The IR spectrum (FTIR Spectrophotometer, Prestige-21, Shimadzu, Japan) of pure drug, drug loaded microspheres, physical mixture of unloaded microspheres were obtained with the help of potassium bromide pellet to monitor structural changes of microspheres.

Thermal Analysis (DTA and TGA)

Samples (2-4 mg) were placed in platinum crucible and kept in Thermo gravimetric Analyzer (DTG -60, Shimadzu, Japan). The heat flow rate was recorded from 0°C to 320°C at a rate of 10°C/min. Platinum was used as a standard reference material to calibrate the temperature and energy scales of DTG instrument.

Swelling Characteristics of Chitosan Microspheres

The swelling behavior of chitosan microspheres was determined using the previous reported method. One hundred milligrams of the microsphere samples were placed in 12 different 50 ml beakers and were kept for 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8 hour in 25 ml of hydrochloric acid buffer at pH 1.2, phosphate buffer at pH 6.8 and at pH 7.4, respectively. After specified time the microspheres were filtered, blotted with filter paper to remove excess water from surface, and then weighed immediately on an electronic weighing balance (Mettler Toledo, AB104 S). Degree of swelling of microspheres was calculated as-

\[
S_{sw} = \left( \frac{W_e - W_0}{W_0} \right) \times 100
\]

Where, \(S_{sw}\) is the degree of swelling of microspheres at equilibrium, \(W_e\) is the weight of swelling microspheres, and \(W_0\) is the initial weight of microspheres.

In vitro Drug Release Studies

Release of Mefenamic acid from microspheres equivalent to 250 mg of Mefenamic acid, was performed in a dissolution apparatus (Veego Dissolution Apparatus, India). The test material was placed in 900 ml of dissolution media at 37 ± 0.5°C using paddle method with a paddle speed of 50 rpm. Different medium, such as hydrochloric acid buffer pH 1.2 with 1 % CTAB [Cetyl Trimethyl Ammonium Bromide], phosphate buffer pH 6.8, phosphate buffer pH 6.8 with 1 % CTAB, phosphate buffer pH 7.4 and phosphate buffer pH 7.4 with 1 % CTAB were used as dissolution media to see the rate and extent of drug release and evaluate release kinetics in those media for prediction of drug release in different pH region of GI tract. An aliquot of 5 ml medium was withdrawn at predetermined time intervals and an equivalent amount of fresh medium was added. Samples were filtered by Whatmann filter paper and drug concentration was determined with UV-visible spectrophotometer after proper dilution of samples.

For better simulation with in vivo release one more design of dissolution study was performed. Microspheres sample equivalent to 250 mg of Mefenamic acid was taken and introduced in a muslin cloth which was fitted with paddle. The study was performed by using hydrochloric acid buffer pH 1.2 with 1 % CTAB for first 2 h followed by phosphate buffer pH 6.8 with 1 % CTAB for another 2 h and in phosphate buffer 7.4 with 1 % CTAB until the end of the experiment. Samples were taken at predetermined time interval and after filtration were analyzed by UV-visible spectrophotometer at respective absorbance maxima (\(\lambda_{max}\)) which were determined by scanning of mefenamic acid in these solvents.

RESULTS AND DISCUSSION

Molecular Weight

The absolute molecular weight of chitosan is determined from the intercept point in Y-axis of Debye plot and it was found 159 KDa (Fig. 1).

Microsphere Size, shape, surface characteristics and entrapment efficiency

Table 1 shows the mean particle size, entrapment efficiency and actual drug loading of glutaraldehyde crosslinked chitosan microspheres with drug-polymer ratio of 1:3 (G3), 1:5 (G5), 1:10(G10) (Fig. 2) and 1:15(G15) containing 0.5 % (w/v) span 85. Addition of glutaraldehyde to the dispersion of chitosan in light liquid paraffin caused instantaneous cross linking, but the product obtained did not exhibit good spherical geometry or surface smoothness and moreover, microspheres were found in aggregate. However with the addition of span 85 the microspheres were obtained not in aggregated manner but in separate individual particle. As it is reported in literature that 0.5 % (w/v) of span 85 is satisfactory for producing a stable emulsion, the further batches were prepared using this concentration of span 85. It is also observed a considerable increase in mean diameter of loaded microspheres compared to the unloaded microspheres; this effect may be due to increased viscosity of drug-polymer dispersion constituting the internal phase of emulsion, which leads to large droplets and formulation of...
Table 1. Mean particle size, entrapment efficiency and actual drug loading both unloaded and Mefenamic acid loaded gluteraldehyde cross linked chitosan microspheres

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Theoretical Drug loading (%)</th>
<th>Concentration of Span 85 (% w/v)</th>
<th>Entrapment Efficiency (%)</th>
<th>% Loading</th>
<th>Mean Particle Diameter (µm)</th>
<th>t-test of mean diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>G3</td>
<td>25</td>
<td>0.5 (A)</td>
<td>83.16±0.876</td>
<td>20.79±0.2191</td>
<td>158.96±2.39</td>
<td>A vs. E: P=0.001</td>
</tr>
<tr>
<td>G5</td>
<td>16.67</td>
<td>0.5 (B)</td>
<td>82.21±1.088</td>
<td>13.70±0.1814</td>
<td>132.18±1.67</td>
<td>B vs. E: P=0.001</td>
</tr>
<tr>
<td>G10</td>
<td>9.09</td>
<td>0 (C0)</td>
<td>91.89±1.920</td>
<td>8.36±0.175</td>
<td>162.80±2.73</td>
<td>C vs. C1: P=0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 (C)</td>
<td>89.15±1.915</td>
<td>7.84±0.175</td>
<td>123.11±2.95</td>
<td>C vs. C: P=0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (C1)</td>
<td>80.73±2.922</td>
<td>7.35±0.266</td>
<td>108.36±2.46</td>
<td>C vs. C2: P=0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5 (C2)</td>
<td>71.48±1.993</td>
<td>6.50±0.125</td>
<td>102.72±3.99</td>
<td>C1 vs. C2: P=0.001</td>
</tr>
<tr>
<td>G15</td>
<td>6.25</td>
<td>0.5 (D)</td>
<td>86.18±3.020</td>
<td>5.39±0.2063</td>
<td>120.30±3.55</td>
<td>D vs. E: P=0.02</td>
</tr>
<tr>
<td>G0</td>
<td>0</td>
<td>0.5 (E)</td>
<td>111.01±1.91</td>
<td>111.01±1.91</td>
<td>111.01±1.91</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>25</td>
<td>0.5 (F)</td>
<td>42.63±0.729</td>
<td>10.66±0.1823</td>
<td>324.39±2.93</td>
<td>P vs. S: P=0.001</td>
</tr>
<tr>
<td>T5</td>
<td>16.67</td>
<td>0.5 (G)</td>
<td>45.81±2.11</td>
<td>7.64±0.352</td>
<td>308.05±2.66</td>
<td>O vs. S: P=0.001</td>
</tr>
<tr>
<td>T10</td>
<td>9.09</td>
<td>0.5 (H)</td>
<td>41.80±3.987</td>
<td>3.80±0.363</td>
<td>283.51±2.13</td>
<td>R vs. S: P=0.001</td>
</tr>
<tr>
<td>TB</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>270.47±1.33</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± S.D. (n=3)

Table 2. Kinetic constant (K), diffusional exponent (n) and t_{0.5} of different formulation in different medium used in sequential manner by fitting dissolution data in Peppas model.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mean Loading (%)</th>
<th>K</th>
<th>n</th>
<th>r²</th>
<th>t_{0.5} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G3</td>
<td>20.79</td>
<td>0.1283</td>
<td>0.915</td>
<td>0.9938*</td>
<td>4.59</td>
</tr>
<tr>
<td>G5</td>
<td>13.70</td>
<td>0.1262</td>
<td>0.9243</td>
<td>0.9959*</td>
<td>4.43</td>
</tr>
<tr>
<td>G10</td>
<td>7.84</td>
<td>0.1275</td>
<td>0.9576</td>
<td>0.9953*</td>
<td>4.16</td>
</tr>
<tr>
<td>G15</td>
<td>5.39</td>
<td>0.1301</td>
<td>0.9220</td>
<td>0.9957*</td>
<td>4.24</td>
</tr>
<tr>
<td>T3</td>
<td>10.66</td>
<td>0.1136</td>
<td>0.7069</td>
<td>0.9978*</td>
<td>8.13</td>
</tr>
<tr>
<td>T5</td>
<td>7.64</td>
<td>0.1150</td>
<td>0.7229</td>
<td>0.9977*</td>
<td>7.64</td>
</tr>
<tr>
<td>T10</td>
<td>3.80</td>
<td>0.1180</td>
<td>0.7561</td>
<td>0.9969*</td>
<td>7.11</td>
</tr>
</tbody>
</table>

*p<0.001; **p<0.01, t_{0.5}-time required to release drug 50% of total loaded amount.

larger microspheres. However at a particular span-85 concentration the particle size distribution remained unchanged (Table 1).

The t-test was performed for drug loaded microspheres with respect to drug unloaded [GB] microspheres prepared by gluteraldehyde cross linking and there was significant difference (p<0.001) of mean geometric diameter of different drug loaded microspheres for G3 and G5, but for G10 and G15 there was less significant difference (p<0.01 and p<0.02, respectively).

It was seen that the highest entrapment efficiency, when drug-polymer ratio was maintained 1:10 (G10) (Table 1). Beyond this ratio when the polymer concentration was increased the entrapment efficiency decreased.

G10 batch which had optimum characteristics in all respects was further used to study the effect of concentration of surfactant on the drug loaded microspheres. Increasing span 85 concentration beyond 0.5% increased the yield, which might be due to increased number of droplets of w/o emulsion of chitosan and external phase. With increasing span 85 concentration the mean geometric diameter of G10 decreased (Table 1). The t-test was performed every batch of G10 with respect to other batches of G10 prepared with different span 85 concentration. It was seen that there was significant difference (p<0.001) between G10 prepared with different span 85 concentration with respect to G10 prepared without span 85 and less significant difference (p<0.05) between G10 prepared with 1 and 1.5% (w/v) span 85. An increase in Span 85 concentration decreased the total drug content in the chitosan microspheres, that may be due to solubilizing effect of span on mefenamic acid during preparation of microspheres. It was also reported by Dhawan.

Fig. 1. Debye Plot between the intensity of scattered light (KCR/Jo) at various concentrations (C) of chitosan solution

Fig. 2. Scanning electron micrograph of the gluteraldehyde cross linked chitosan microspheres containing Mefenamic acid with theoretical drug:chitosan ratio 1:10 (G10)
Fig. 3. Events recorded during DTA of pure mefenamic acid (I), gluteraldehyde cross linked microspheres blank (II), with theoretical drug-chitosan ratio 1:5 (III), 1:10 (IV), thermally cross linked chitosan microspheres of blank (V), and with theoretical drug-chitosan ratio 1:3 (VI).

Fig. 4. Dissolution profiles of mefenamic acid from gluteraldehyde crosslinking chitosan microspheres G10 (drug:chitosan 1:10) prepared with different span 85 concentration indifferent medias. Each data point represents the mean result ± S.D. of three determinations.
et al. (2003) that with increased concentration of span 85 the microspheres are formed with crack surface which attributes the increased dissolution rate. [15]

For thermally cross linked Mefenamic acid loaded and unloaded chitosan microspheres prepared at 800-1000 rpm (Table 1) shows the mean particle size, entrapment efficiency and actual drug loading. The mean geometric diameter of thermally cross linked chitosan microspheres was greater than glutaraldehyde cross linked chitosan microspheres. With increased drug loading the mean geometric diameter of microspheres were increased; t-test was done of different batches of thermally cross linked chitosan microspheres with respect to blank chitosan microspheres (Table 1) and it was seen that there was significant difference (p<0.001) in mean geometric diameter between drug loaded microspheres with respect to blank microspheres (TB) in all the cases. Microspheres prepared by thermal cross linking resulted in poor drug entrapment efficiency (Table 1). This might be due to increased solubility of Mefenamic acid in the oil medium at high temperature. Table 1 shows that with increasing loading of drug, entrapment efficiency decreases gradually.

IR Spectra Analysis

In all glutaraldehyde cross linked chitosan microspheres a significant new peak at 1631 cm⁻¹ in the spectra is due to formation of C=N and this is because of imine reaction between amino groups from chitosan and aldehyde groups of glutaraldehyde. C-O stretching and OH in plane bending for C-OH system in COOH group of Mefenamic acid at about 1332.8 cm⁻¹ and 1377.17 cm⁻¹, respectively were also seen in all drug loaded glutaraldehyde cross linked chitosan microspheres. From this it may be concluded that no interaction between drugs with other additives were taken place.

In all thermal cross linked microspheres new peak at about 1165 cm⁻¹ is due to formation of C=N bond between CH (OH) of citric acid and amino group of chitosan. C-O stretching (at about 1240 cm⁻¹) and OH in plane bending (at 1375 cm⁻¹ respectively) for C-OH system in COOH group of Mefenamic acid were also seen in all drug loaded thermally cross linked chitosan microspheres that concludes no interaction between drug and other additives.

Thermal analysis (DTA and TGA)

The physical state of the drug inside the chitosan microsphere was assessed by thermal analysis. Upon incorporation of Mefenamic acid into the chitosan microspheres prepared by glutaraldehyde cross linking, the chitosan and drug gave two endothermic peaks at around 80°C and 230°C for all G3, G5 and G15. For G10 however, there was no peak due to Mefenamic acid but a broad peak was obtained at 105.46°C (Fig. 3IV). When 1:1 physical mixture of unloaded microspheres and Mefenamic acid was subjected to DTA, a peak due to crystalline Mefenamic acid was observed at 232.39°C that indicates presence of crystalline form of drug in all Mefenamic acid loaded microspheres except for G10 where drug was either in solid dispersion or in solid solution.

In thermal analysis result of Mefenamic acid loaded chitosan microspheres prepared by thermal cross-linking method two endothermic peaks were observed for all T3, T5 and T10; which were similar as of 1:1 physical mixture of Mefenamic acid and blank thermally cross linked chitosan microspheres (100.90°C and 229.62°C, respectively for chitosan and Mefenamic acid) (Fig. 3 V & VI). From this it can be concluded that all Mefenamic acid loaded thermally crosslinked chitosan microspheres Mefenamic acid was present in crystalline form as such.

Swelling Characteristics of Chitosan Microspheres

Chitosan microspheres showed good swelling properties, and the percentage equilibrium swelling was dependent upon the chitosan of the microspheres. An increase in the number of the available charged amino groups leads to an increase in porosity of the polymer network. [23] With increased drug loading, the equilibrium swelling was decreased due to dense structure of the membrane at high loadings, and water could not easily diffuse into the matrix of microspheres. The protonation of excess amino groups of poly(acrylic acid) in the stomach pH conditions is responsible for its swelling. [17] It is seen that swelling degree do not change greatly with time in pH 7.4 and pH 6.8; while in pH 1.2 the swelling degree of the microspheres began to decline after the microspheres were swollen for some time, which may indicate the dissolution tendency of microspheres exceeds the ealigumn of imine bond in microspheres due to protonation. [24] The observed swelling rates of cross linked microspheres followed the order G8> G15> G10> G5> G3 and T3> T10> T5> T3. In the present case degree of swelling is very high in solution of pH 1.2 to that of pH 7.4, which is due to inherent hydrophobicity of chitosan microspheres dominating at high pH value, which prevents faster swelling in neutral and alkaline pH. It was seen that after 2.5 h, equilibrium swelling of chitosan microspheres was reached. So the same experiment was done by taking three different batches of each microspheres and equilibrium swelling was seen after 2.5 h in each media.

In-vitro Release Studies

The effect of Mefenamic acid concentration in the microspheres on release rate was investigated. Table 2 indicated the t₀ values for low loadings are less than from that of high loadings. This behavior also has been reported by Bodmer et al. (1989) for sulfadiazine [25] and by Benita et al. (1990) for Nifedipine. [26]

Release Kinetics

Table 2 shows various constants obtained by fitting the solution data to Peppas model, where the values of n (diffusional exponent) varies with the release mechanism and the shape of particle (Fickian transport, n=0.5; zero order release n=1). Clearly, with decreased loading, the value of n increased and approached towards 1. For determination of exponent ‘n’ the portion of the release curve was used up to M/M∞< 0.6. [27] At high loadings (theoretically 25 and 16.67%), the values of t₀ decreased with the decreased Mefenamic acid loading because the entrapment of crystals in the chitosan matrix might have rendered the crystal surface more hydrophilic, thus improving the wetting of crystals by the solvent. The permeation of microspheres at high loading might be low because of low polymer content, low swelling and hydrophobic nature of Mefenamic acid. The t₀ values decreased and the release was fast at low loading (theoretically 9.09 and 6.25 %), because at loadings the hydrophobic nature of drug might have been decreased by high polymer content and polymer swelling. As water penetrates into the polymer, it tends to form a gel layer
that hinders the outward transport of core diffusion, i.e., drug. In swollen condition, the gel layer formed by the polymer contributed substantially to retardation of drug release. Although the gel layer formed by this polymer concentration was thicker than for intermediate loading, there may be a high concentration gradient between the dissolved drug in the microspheres and the solvent that leads to fast release. For G10 the release was faster because of the drug was in solid dispersion as found by thermal analysis.

For in vitro dissolution of Mefenamic acid loaded microspheres hydrochloric acid buffer pH 1.2 with 1% CTAB, phosphate buffer pH 6.8 with 1% CTAB for 2hrs each and phosphate buffer 7.4 with 1% CTAB until the end of the experiment, the exponent for each formulation is greater than that of from the individual medium and approaches toward 1 that is zero order release (Table 2).

**Effect of Span 85 concentration on release of microspheres**

An increase in span 85 concentrations decreased the total drug content in chitosan microspheres (Table 1). Only G10 formulation was prepared with different span 85 concentration to differentiate the effect of span 85 on dissolution behavior of Mefenamic acid in different medium as for G10 the release was faster than other loading at 0.5% (w/v) span 85 and drug is molecularly dispersed. An increase in span 85 concentration decreased the total drug content in chitosan microspheres (Table 1). Release was faster with increased span 85 concentration (Fig. 4), because of cracks and collapsed surface of microspheres. The highest release was achieved with 1.5% (w/v) span 85 in comparison than when it was used 1% (w/v) and 0.5% (w/v). The lowest drug release was achieved from chitosan microspheres prepared without span 85.

By formulating Mefenamic acid in chitosan microspheres in vitro release is sustained and Mefenamic acid solubility was also increased in different media than release from conventional marketed tablet. Surfactant was used in dissolution medium because solubility of Mefenamic acid is very poor in different medium and more over good correlation between bioavailability and in vitro dissolution rate of Mefenamic acid was observed when surfactant was used in vitro dissolution rate. [20] CTAB (Cetrimide) was used because solubility of Mefenamic acid was highest in 1% CTAB containing medium among SLS, tween 80 and CTAB. [20] By delivering Mefenamic acid in chitosan microspheres we can reduce the gastric irritation caused by Mefenamic acid, frequency of dosing in case of patients of rheumatoid arthritis, primary dysmenorrhea, and menorrhagia.

For gluteraldehyde cross linked Mefenamic acid loaded chitosan microspheres, drug is in solid dispersion in G10 where drug-polymer was used in 1:10 and so the rate of release was also faster and uniform for G10 than other formulation. The in-vitro release data was fitted in Peppas model and it was seen that diffusional exponent (n) value was more for G10 than other formulation and for the in vitro release study where different medium were used in sequence for better simulation with in vivo release the value of “n” was very close to 1; that is zero order release (Table 2). The effect of span 85 concentration on in vitro dissolution was also studied and it was seen that 0.5% (w/v) span 85 was optimum for preparation of microspheres with respect to surface characteristics, size, particle size distribution, individuality of particle, entrainment efficiency, and in vitro release profile. There is no evidence of polymer drug interaction by FTIR and DTA analysis. For thermally cross linked chitosan microspheres the drug entrainment was very less and from DTA analysis it was seen that Mefenamic acid is not molecularly dispersed among three formulations of T3, T5 and T10. This present study is holds promise for the further clinical study and screening of various formulations variables through pilot plant scale up.

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