Controlled drug delivery system overcomes a number of challenges associated with conventional delivery approaches. Maximum therapeutic efficacy associated with targeted delivery, sufficient residence time and bioavailability in optimum concentrations with little hazardous events. Various approaches such as liposomes, nanocarriers, microparticles and prodrug were reported for targeted and sustained delivery of therapeutic moieties (1). Biodegradable carriers grabbed attention in development of controlled release systems due to their ability to reabsorb in body having wide pharmaceutical applications in oral drug delivery (2), gene delivery (3), nasal drug delivery (4), buccal drug delivery (5), intra-humoral (6) and local drug delivery (7), ophthalmic drug delivery (8) and colonic drug delivery (9).

Chondroitin sulfate (Chs) is an acidic mucopolysaccharide found in bones, cartilages and connective tissues and belongs to glycosaminoglycan family containing N-acetylgalactosamine and uronic acid linked by β(1→4) and β(1→3) linkages, respectively (10). Chs has exhibited anti-inflammatory, anti-atherogenic and anti-tumor activity in animal models and was extensively used in development of colon targeted drug delivery system (11).

Sodium alginate (NaAlg) is a salt of alginic acid derived from brown seaweeds belonging to family Phaeophyceae, composed of D-mannuronic acid and D-glucuronic acid, a biodegradable and hemocompatible polymer not accumulating in human organs and forming reticulate structure with calcium ions and glutaraldehyde and this characteristic was used in fabrication of sustained release system for variety of therapeutic agents (12).

Fluorouracil (5-FU) an active metabolite of doxifluoridine was synthesized and patented by Charles Heidelberger in 1957. 5-FU is the drug of choice in colon cancer. Due to short biological half-life, acid lability and variant bioavailability it is administered by repeated intravenous route and produced fatal adverse effects. It is the need of time to generate 5-FU target delivery system to meet challenges associated with current therapy. Different polymeric combinations such as chitosan/cho-
dextran sulfate microcapsules, poly (lactide-co-glycolide) microspheres, pectin and sodium alginate microspheres have been presented in the literature for controlled delivery of 5-FU (13, 14).

In present study, efforts were made for successful fabrication of Chs and NaAlg microspheres encapsulating model drug - 5-FU. In addition to SEM, FTIR, PXRD, EXD, DSC and TGA and release studies, zeta size analysis, percent encapsulation efficiency, drug loading and swelling studies were performed to validate objectives of study.

EXPERIMENTAL

Materials

5-Fluorouracil was obtained as a gift sample from Pharmedic Laboratories (Pvt.) Ltd. Pakistan. Chondroitin sulfate and Tween-80 were purchased from Sigma-Aldrich. Sodium alginate was purchased from Sigma, Germany. Magnesium stearate (Chemical Pure) and glutaraldehyde (25% water solution) were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Paraffin oil (36#, food grade) was purchased from Jinan, China. All other chemicals and reagents used were of analytical grade.

Methods

Preparation of chondroitin sulfate-sodium alginate microspheres

Chemical emulsion cross link method was used to fabricate chondroitin sulfate-sodium alginate (Chs-NaAlg) microspheres. In brief, aqueous solution of NaAlg was prepared by dissolving NaAlg in water overnight under constant stirring at 100 rpm at 50°C. To this solution, Chs was added and stirred thoroughly for 30 min at 100 rpm using high-speed stirrer (Stuart series 600, Germany). 5-FU solution was added to above mixture and stirred for 1 h at 60°C. The resultant drug polymer mixture was emulsified into liquid paraffin to form water-in-oil (w/o) emulsion stirring at 600 rpm speed for 45 min in separate beaker containing liquid paraffin oil 100 mL, Tween-80 5% v/v and magnesium stearate 0.3% w/v. To this was added 2% of GA solution containing 0.5 mL of concentrated HCl. Stirring was continued for further 4 h, microspheres formed were filtered and washed repeatedly with acetone to remove oil as well as excess amount of surfactant and unreacted GA. The microspheres were dried under vacuum and stored in desiccator before further analysis. Different formulations and their compositions are given in Table 1.

Micromeritic properties

Flow properties of Chs-NaAlg microspheres were investigated by determining angle of repose, bulk density and tapped density. Fixed base cone method was used to measure angle of repose. Bulk and tapped densities were measured in 10 mL graduated cylinder. The sample contained in cylinder was tapped continuously till no change in volume was observed. Each experiment was performed 3 times (31).

Swelling analysis

Swelling of Chs-NaAlg microsphere was studied at pH 1.2 and 7.4 by the following protocol. Accurately weighed and dried Chs-NaAlg microspheres were immersed in 0.1 M HCl (pH 1.2) and phosphate buffer (pH 7.4) at 37°C. After 24 h, microspheres were withdrawn from solution and an increase in weight of microspheres was measured as a function of time. Swelling ratio (SR) was expressed in following equation (32):

Table 1. Composition and average particle size of Chs-NaAlg microspheres.

<table>
<thead>
<tr>
<th>Code</th>
<th>5-FU (mg)</th>
<th>Polymer load (%)</th>
<th>Chs: NaAlg ratio</th>
<th>Ga (mL)</th>
<th>Average particle size of microsphere (µm) (mean ± S.D.)</th>
<th>Average particle size of 5-FU loaded microsphere (µm) (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-1</td>
<td>200</td>
<td>1</td>
<td>50 : 50</td>
<td>2</td>
<td>112.44 ± 2.499</td>
<td>334.40 ± 1.071</td>
</tr>
<tr>
<td>CS-2</td>
<td>200</td>
<td>1</td>
<td>30 : 70</td>
<td>2</td>
<td>128.00 ± 1.025</td>
<td>375.03 ± 1.811</td>
</tr>
<tr>
<td>CS-3</td>
<td>200</td>
<td>1</td>
<td>20 : 80</td>
<td>2</td>
<td>146.21 ± 1.559</td>
<td>350.57 ± 1.559</td>
</tr>
<tr>
<td>CS-4</td>
<td>200</td>
<td>2</td>
<td>50 : 50</td>
<td>2</td>
<td>155.76 ± 2.055</td>
<td>580.21 ± 2.055</td>
</tr>
<tr>
<td>CS-5</td>
<td>200</td>
<td>2</td>
<td>30 : 70</td>
<td>2</td>
<td>156.77 ± 1.615</td>
<td>565.23 ± 1.615</td>
</tr>
<tr>
<td>CS-6</td>
<td>200</td>
<td>2</td>
<td>20 : 80</td>
<td>2</td>
<td>158.60 ± 1.025</td>
<td>620.99 ± 1.025</td>
</tr>
<tr>
<td>CS-7</td>
<td>200</td>
<td>3</td>
<td>50 : 50</td>
<td>2</td>
<td>160.77 ± 1.115</td>
<td>810.06 ± 1.115</td>
</tr>
<tr>
<td>CS-8</td>
<td>200</td>
<td>3</td>
<td>30 : 70</td>
<td>2</td>
<td>175.01 ± 2.00</td>
<td>858.17 ± 1.46</td>
</tr>
<tr>
<td>CS-9</td>
<td>200</td>
<td>3</td>
<td>80 : 20</td>
<td>2</td>
<td>185.08 ± 2.007</td>
<td>885.29 ± 1.24</td>
</tr>
</tbody>
</table>
Fabrication and in vitro evaluation of 5-fluorouracil loaded chondroitin... 497

where W1 and W2 represent dry and wet weight of microspheres, respectively.

**Instrumental analysis**

**Drug content determination**

Known weight of Chs-NaAlg microspheres (equivalent to 20 mg 5-FU) were crushed in an agate mortar with pestle, drug loaded polymeric powder was added in 50 mL distilled water and refluxed for 30 min for complete extraction of 5-FU from microspheres. After refluxation, filtered precipitated solution and 5-FU were analyzed using UV/Vis-spectrophotometer (Perkin Elmer UV/ VIS) at wavelength of 266 nm using calibration curve. Encapsulation efficiency and percent yield was calculated by using the following formulas (30):

\[
\text{Encapsulation efficiency} = \frac{\text{Actual amount of drug in microsphere}}{\text{Theoretical loading}} \times 100
\]

\[
\text{Percent yield} = \frac{\text{Total amount of recovered microcapsule}}{\text{Amount of drug} + \text{Amount of polymer}} \times 100
\]

**Fourier Transform Infrared Spectroscopy (FTIR)**

Drug and polymer interaction was studied by ATR-FTIR spectroscopy (Tensor 27, Bruker, Germany). FTIR spectrum was recorded for 5-FU, Chs, NaAlg, physical mixture of drug and polymers, unloaded microspheres and 5-FU loaded microspheres. Scanning range was kept at 4000-650 cm\(^{-1}\) and resolution was 6 cm\(^{-1}\).

**Thermal analysis (DSC-TGA)**

In order to get phase transition and weight loss preview of 5-FU, Chs, NaAlg, physical mixture of drug and polymers, unloaded and 5-FU loaded microspheres were examined using TA instrument, USA model Q600 series. Before loading sample, both reference pan and sample pan were tarred. After tarring, both sample and reference pans were kept empty. After loading, furnace was heated up to 450°C from ambient temperature with a heat flow rate of 15°C/min in a nitrogen atmosphere having flow rate of 10 mL/min.

**Powder X-ray diffraction analysis (PXRD)**

PXRD was carried out to investigate effect of microencapsulation process on crystallinity of drug using XRD (JDX 3532, Japan). PXRD patterns of 5-FU crystals, Chs, NaAlg, unloaded and 5-FU loaded Chs-NaAlg microspheres were recorded by using Ni-filtered, CuKα radiation, voltage of 60 kV, current of 50 mA and scanning rate was 1°/min over 10° to 40° diffraction angle (2\(\theta\)). The microspheres were triturated to get fine powder before taking scan.

**Scanning electron microscopy (SEM)**

External and internal morphology of microspheres was analyzed by scanning electron microscopy (SEM; JEOL, Japan). Microspheres were fixed on support with carbon-glue and coated with gold using SPI sputter module in high-vacuum evaporator for SEM images at 20 kV. Microspheres internal morphology was studied by embedding them in acrylate adhesive (Trade name: 302, Fushun, China). After drying, blocks were cut with razor blade and the fragments obtained were deposited on SEM stubs.

**Elemental dispersive X-ray (EDX)**

Energy dispersive X-ray analysis was used to identify main components of formulations. This microanalysis technique permits evaluation in rela-

Table 2. Micrometric properties of Chs-NaAlg microspheres.

<table>
<thead>
<tr>
<th>Code</th>
<th>Angle of repose (°)</th>
<th>Bulk density (g/mL)</th>
<th>Tapped density (g/mL)</th>
<th>Carr’s index (%)</th>
<th>Haunser’s ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-1</td>
<td>20.21 ± 0.779</td>
<td>0.27 ± 0.005</td>
<td>0.29 ± 0.012</td>
<td>30.76 ± 0.772</td>
<td>1.44 ± 0.012</td>
</tr>
<tr>
<td>CS-2</td>
<td>25.11 ± 0.901</td>
<td>0.28 ± 0.002</td>
<td>0.28 ± 0.011</td>
<td>26.31 ± 0.844</td>
<td>1.35 ± 0.005</td>
</tr>
<tr>
<td>CS-3</td>
<td>29.12 ± 0.821</td>
<td>0.28 ± 0.001</td>
<td>0.25 ± 0.002</td>
<td>34.32 ± 0.922</td>
<td>1.32 ± 0.001</td>
</tr>
<tr>
<td>CS-4</td>
<td>31.44 ± 0.679</td>
<td>0.29 ± 0.005</td>
<td>0.31 ± 0.001</td>
<td>19.40 ± 0.851</td>
<td>1.24 ± 0.001</td>
</tr>
<tr>
<td>CS-5</td>
<td>32.31 ± 0.501</td>
<td>0.31 ± 0.001</td>
<td>0.33 ± 0.004</td>
<td>16.21 ± 0.976</td>
<td>1.19 ± 0.002</td>
</tr>
<tr>
<td>CS-6</td>
<td>32.80 ± 0.744</td>
<td>0.33 ± 0.002</td>
<td>0.35 ± 0.004</td>
<td>14.2 ± 0.916</td>
<td>1.16 ± 0.003</td>
</tr>
<tr>
<td>CS-7</td>
<td>33.40 ± 0.833</td>
<td>0.32 ± 0.004</td>
<td>0.36 ± 0.003</td>
<td>11.11 ± 0.806</td>
<td>1.12 ± 0.002</td>
</tr>
<tr>
<td>CS-8</td>
<td>35.37 ± 0.765</td>
<td>0.35 ± 0.003</td>
<td>0.39 ± 0.001</td>
<td>10.25 ± 0.736</td>
<td>1.11 ± 0.001</td>
</tr>
<tr>
<td>CS-9</td>
<td>38.46 ± 0.803</td>
<td>0.34 ± 0.003</td>
<td>0.38 ± 0.001</td>
<td>10.52 ± 0.716</td>
<td>1.11 ± 0.001</td>
</tr>
</tbody>
</table>
tion to atomic weight and elemental composition of substance under study. Elemental composition analysis of formulation in comparison with pure drug and unloaded microspheres was done by using elemental X-ray spectroscopy.

**Particle size analysis**

All formulations of microspheres were analyzed for their particle sizes and distribution using particle size analyzer (Zetasizer Nano-series ZEN3600, Malvern).

**In vitro drug release studies**

*In vitro* drug release profile of 5-FU loaded microspheres was determined using USP dissolution apparatus-II (paddle method) equipped with autosampler (PharmaTest, Germany). Accurately weighed microspheres (equivalent to 50 mg of 5-FU) were filled in small dialysis bag (dialysis membrane 800 Da), fixed with paddle by silk thread and immersed in dissolution flask with perfect sink conditions in 900 mL dissolution medium. Dissolution studies were performed in phosphate buffer solutions at pH 1.2, 6.8 and 7.4; pH was maintained by using 0.1 M HCl and NaOH solution, separately under rotating speed of 100 rpm at 37 ± 0.5°C. Samples (3 mL) were removed at predetermined time intervals i.e., 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 12 h from center of basket in triplicate. Withdrawn amount was replaced with respective fresh buffer

Figure 1. FTIR spectra (A) 5-FU, (B) Chs, (C) NaAlg, (D) unloaded microspheres, (E) 5-FU loaded microspheres
solutions. All samples were filtered through 0.22 µm membrane filter and analyzed by UV/Vis-spectrophotometer (UV-1601Shimadzu) at 266 nm after appropriate dilution.

**Release kinetic studies**

Data obtained from *in vitro* dissolution was fitted to various kinetic models i.e., zero order, first order and Higuchi to find out the mechanism of 5-FU release from microspheres. To elaborate mechanism of drug release, initial 60% release data were fitted to Korsmeyer–Peppas equation:

\[
\frac{M_t}{M_\infty} = k t^n
\]

where \(M_t/M_\infty\) stands for percentage of drug released at time \(t\), \(k\) is drug release rate constant and \(n\) represents release exponent. The ‘\(n\)’ value is used to characterize drug release mechanisms either diffusion controlled or not as: \(n = 0.45\), Fickian diffusion, \(0.45 < n < 0.89\) anomalous (non-Fickian) diffusion, \(n = 0.89\) Case-II transport; \(n > 0.89\) Super case-II transport. Dissolution data were evaluated using software, DDSolver® (33).
**In vitro drug release**

At acidic pH drug release was below 20% for all formulations. Decreased swelling was observed at acidic pH causing reduced matrix permeability and limiting drug diffusion. An increase in release rate (60-85%) was observed at pH 6.8 and 7.4 in 12 h.

**RESULTS**

Synthesis and size distribution of Chs-NaAlg microspheres

In present investigation, microparticulate systems consisting of Chs and NaAlg were fabricated for colon delivery of 5-FU combining two approaches: timed release and biodegradation. Both are hydrophilic biodegradable and anionic polymers miscible with each other in all proportions to synthesize Chs-NaAlg microspheres by emulsion crosslink method. Different process variables like polymer and crosslinking concentration, stirring speed, stirring time considerably influenced morphology, particle size as well as encapsulation efficiency of formulations.

Encapsulation efficiency, drug loading and micrometric properties of microspheres

Encapsulation efficiency and drug loading of formulations (CS-1 to CS-9) are presented in Table 4.
A linear relationship between 5-FU encapsulation efficiency and polymer concentration was found. Percent drug loading and encapsulation efficiency were increased from 46.31 ± 1.13% to 75.32 ± 1.108% and 31.21 ± 0.143% to 87.11 ± 1.559%, respectively, as polymer concentration was increased from 1 to 3%. Rheological properties i.e., angle of repose, bulk density; tapped density, compressibility (Carr’s index) and Hausner’s ratio of 5-FU loaded microsphere are summarized in Table 2. Bulk and tapped density for all formulations were calculated and used to determine Carr’s index and Hausner’s ratio.

**FT-IR**

Comparison of FTIR spectra of drug loaded and unloaded microspheres with respect to pure drug and polymers is illustrated in Figure 1. In FTIR spectra of Chs and NaAlg peaks appearing at 1320 cm⁻¹, 1130 cm⁻¹, 1090 cm⁻¹ and 1020 cm⁻¹ are attributed to saccharide structure of polymers. IR spectra of NaAlg showed characteristic peak at 3456 cm⁻¹ due to O-H vibrations and at 1620 cm⁻¹ and 1417 cm⁻¹ due to asymmetric and symmetric stretching of carboxylate salt groups of NaAlg, respectively.

**Morphological analysis**

SEM images shown in Figures 2 and 3 illustrate that synthesized Chs-NaAlg microspheres had smooth and spherical surfaces having no agglomeration. Drug loading slightly contributed roughness to microspheres surface. Surface roughness was resulted due to drug entrapment in polymer matrix and caused restricted inward movement of polymers. Microspheres with smooth and non-porous surface were effective in delayed dissolution. The internal scans of blank microspheres indicated porous morphology while compact non porous structure was shown after drug entrapment within polymer matrix.

**Thermal analysis**

DSC-TGA thermograms of pure drug and polymers, loaded and unloaded formulations are shown in Figures 4 and 5, respectively. DSC-TGA curves of Chs and NaAlg showed two weight losses one before 100°C and other before 270°C in polymer mass. The first one was due to elimination of water molecules bound with polymers and the second one exhibited dehydration of saccharide rings, depolymerization, and decomposition of polymers. Around 89.933% of Chs and 75.291% NaAlg disintegrated within 300°C. DSC-TGA thermogram of unloaded microspheres exhibited first thermal event at temperature range 100-110°C accompanied by weight loss ranging from 6 to 8%, indicating loss of residual water present in the sample. Further, this complex remains stable up to 450°C and around 45.63% of sample remained as residue.

**PXRD**

PXRD technique was used to determine nature of drug, either crystalline or amorphous, present in microspheres. PXRD patterns of 5-FU, Chs, NaAlg, physical mixture of drug and polymers, unloaded microspheres and 5-FU loaded microspheres are presented in Figure 6.

**Elemental composition analysis**

EXD surface analysis provides information about elemental and average chemical composition of a material on its surface in 5-10 nm depth by measuring binding energy of electrons associated with atoms. EXD displayed variations in % composition of 5-FU, unloaded and 5-FU loaded Chs-NaAlg microspheres are shown in Table 3 and Figure 7. The quantitative data indicated specific concentrations of carbon, oxygen and sodium.

**Swelling studies**

Transport of water through polymeric matrix depends upon rigidity of polymeric network and extent of crosslinking. All formulations had low swelling index at pH 1.2 and high at pH 7.4, as shown in Table 4. These results were due to the presence of carboxylic acid groups of NaAlg which remain com-

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Element</th>
<th>% Weight</th>
<th>% Atomic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unloaded Chs-NaAlg microspheres</td>
<td>C</td>
<td>51.26</td>
<td>59.39</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>40.36</td>
<td>20.34</td>
</tr>
<tr>
<td></td>
<td>Na</td>
<td>10.18</td>
<td>7.10</td>
</tr>
<tr>
<td>5-FU loaded Chs-NaAlg microspheres</td>
<td>C</td>
<td>77.81</td>
<td>82.95</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>26.36</td>
<td>16.77</td>
</tr>
<tr>
<td></td>
<td>Na</td>
<td>0.58</td>
<td>0.35</td>
</tr>
</tbody>
</table>
Figure 6. PXRD spectra (A) 5-FU, (B) unloaded microspheres, (C) 5-FU loaded microspheres
pact in acidic pH and no net charge was developed in polymeric network.

**Factors affecting in vitro drug release**

Drug release from polymeric complex was controlled by a number of parameters including cross linker and polymer concentration. Three different formulations of CS-9 were formulated using 1, 2 and 3% of 25% GA solution. Formulation containing 1% GA showed burst drug release indicating minimum or no crosslinking \( (p < 0.05) \), while formulation containing 3% GA, retarded drug release up to 55% for 8 h \( (p < 0.05) \).

**DISCUSSION**

Synthesis and size distribution of Chs-NaAlg microspheres

Aldehyde groups of GA react with both hydroxyl groups of Chs and NaAlg under acidic conditions and form acetal ring during crosslinking reaction. Acetal ring formation between GA and polymers has been reported in the literature (15). Chs-NaAlg microspheres formed were white, translucent and spherical in appearance. Excellent microspheres were produced when process was carried out with 1-3% polymer load at 600 rpm with
cross linker concentration of 2 mL. Microspheres with large particle size and irregular morphology were produced with polymer load up to 4% and above. It could be due to increased viscosity of sodium alginate. Microspheres with optimum shape and size were produced when agitated at 600 rpm. When agitation speed was kept below 600 rpm, polymer solution did not disperse evenly resulting in agglomerate formation. Previously, sodium alginate-methylcellulose blend microspheres were developed and showed similar results of stirring speed on morphology (16).

Average diameter of formulations, as shown in Table 1, containing 1, 2 and 3% polymer load was in the range of 112.44 ± 2.499 µm, 155.76 ± 2.055 µm and 185.08 ± 2.007 µm, respectively. Microspheres particle size was insignificantly increased after drug loading. This change could be related to entrapment of drug particles within polymeric matrix thereby hindering inward shrinkage of microspheres. Previously, temperature sensitive microspheres of sodium alginate also showed an increase in particle size after drug loading (17).

**Encapsulation efficiency, drug loading and micrometric properties of microspheres**

These results were obtained as maximum amount of drug interacted with polymer resulting in increased percent encapsulation efficiency and drug loading. Formulation CS-9 containing drug: polymer ratio 1 : 3, having NaAlg and Chs in 80 : 20 ratio, showed maximum encapsulation efficiency and drug loading 83.71 ± 71% and 78.21 ± 1.06%, respectively. Increased encapsulation efficiency and

## Table 4. Swelling studies, percent drug loading and encapsulation efficiency.

<table>
<thead>
<tr>
<th>Code</th>
<th>Swelling at pH 1.2 (%)</th>
<th>Swelling at pH 7.4 (%)</th>
<th>Drug loading (%)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-1</td>
<td>31</td>
<td>321</td>
<td>46.31 ± 1.13</td>
<td>31.21 ± 0.143</td>
</tr>
<tr>
<td>CS-2</td>
<td>47</td>
<td>343</td>
<td>51.20 ± 0.739</td>
<td>47.08 ± 0.786</td>
</tr>
<tr>
<td>CS-3</td>
<td>50</td>
<td>356</td>
<td>58.92 ± 1.108</td>
<td>50.12 ± 0.610</td>
</tr>
<tr>
<td>CS-4</td>
<td>68</td>
<td>421</td>
<td>65.05 ± 0.652</td>
<td>68.93 ± 1.050</td>
</tr>
<tr>
<td>CS-5</td>
<td>72</td>
<td>458</td>
<td>69.88 ± 0.762</td>
<td>72.12 ± 0.932</td>
</tr>
<tr>
<td>CS-6</td>
<td>77</td>
<td>486</td>
<td>6.83 ± 1.021</td>
<td>75.14 ± 0.996</td>
</tr>
<tr>
<td>CS-7</td>
<td>80</td>
<td>510</td>
<td>71.11 ± 1.137</td>
<td>78.21 ± 1.069</td>
</tr>
<tr>
<td>CS-8</td>
<td>84</td>
<td>530</td>
<td>74.38 ± 0.739</td>
<td>80.01 ± 1.481</td>
</tr>
<tr>
<td>CS-9</td>
<td>88</td>
<td>570</td>
<td>75.32 ± 1.108</td>
<td>87.11 ± 1.559</td>
</tr>
</tbody>
</table>
drug loading with an increase in polymer concentration were also reported in previous studies (18). Compactness of microspheres was increased with an increase in polymer concentration. From the results it was concluded that Chs-NaAlg microspheres possessed good flow properties.

**FT-IR**

FT-IR characteristic peaks of Chs not only include O–H and C–O stretching, but also due to CaO and SO2 at 1640 cm⁻¹ and 1260 cm⁻¹, respectively. Aldehyde groups of GA reacted with hydroxyl groups of NaAlg and Chs under acidic conditions and formed acetal rings. The same was reported in previous literature (19). In 5-FU spectra broad band between 3000 cm⁻¹ and 3500 cm⁻¹ was due to -NH stretching. This band was observed at 3500 cm⁻¹ approximately in spectrum of 5-FU loaded Chs-NaAlg microspheres, could be due to overlapping of -OH band of NaAlg with -NH band of 5-FU. In the spectrum of 5-FU the peak at 1275 cm⁻¹ was due to C-F bond. This peak was observed at 1280 cm⁻¹ in the spectrum of 5-FU loaded Chs-NaAlg microspheres and this shift of peak could be correlated to encapsulation of 5-FU within microspheres. Previous studies illustrated that the presence of drug characteristic peak after encapsulation into polymeric form indicated the presence of drug in its stable form (20).

**Morphological analysis**

NaAlg concentration below 1% w/v resulted in clumping of microspheres, whereas higher NaAlg concentration up to 4% w/v produced discrete microspheres. This was related to an increase in viscosity of polymer solution at higher NaAlg concentration. Moreover, porosity of microspheres depended on polymer composition. High content of Chs exhibited increased porosity. Pieper et al. also reported that porosity of crosslinked collagenous matrices increased as chondroitin sulfate concentration increased and change in surface morphology after drug loading was observed (21). Microspheres with relatively rough and hard surfaces with cracks were produced when process was carried out at higher rotation speed (higher than 700 rpm) and increased GA concentration (Fig. 3C). Due to rapid evaporation of water from dispersed polymer solution in paraffin oil, rigid cross-linked structure resulted. Microspheres with optimum morphology were produced at 600 rpm and with 2% crosslinker concentration (Fig. 2A). When agitation speed was kept below 600 rpm, polymer solution did not disperse evenly resulting in irregular geometry of microspheres (Fig. 3B). Change in microspheres morphology due to rotation speed and GA concentration was also studied previously (22).

**Thermal analysis**

On comparing results of thermograms of microspheres with pure polymers, it was concluded that polymeric complex was more stable; further this was confirmed from the amount of residue remained at the end of experiment and initial decomposition temperatures. 5-FU loaded Chs-NaAlg microspheres exhibited no 5-FU characteristic peak as compared to thermogram of pure drug which exhibited characteristic 5-FU endothermic peak at 287°C due to polymorphism and melting. Further, this complex remained stable up to 450°C with total weight loss 84.31% (23).

**PXRD**

Diffraction patterns of 5-FU exhibited intense and sharp peaks at 2θ of 15.9°, 16.2°, 18.9°, 20.6°,

<table>
<thead>
<tr>
<th>Code</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi</th>
<th>Korsmeyer Peppas</th>
<th>Mechanism of drug release</th>
</tr>
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<td>r²</td>
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Table 5. Results of regression coefficients of model fitting of release data.
28.5° and 32.1° indicating its crystalline nature. The diffraction peak of NaAlg at around 13.47° was a typical characteristic peak of the hydrated crystalline structure, but its intensity was weakened drastically after forming microspheres. Diffraction pattern of unloaded microspheres revealed a broad hump in region of 2θ of 10-25° indicating complex formation between polymers and decreased crystalline behavior of NaAlg. PXRD pattern of 5-FU loaded microspheres presented absence of 5-FU characteristic peak indicating change in crystalline nature of drug after encapsulation. These results are in good agreement with the previous studies (24).

Elemental composition analysis
The carbon and oxygen concentration of 5-FU loaded microspheres slightly decreased due to drug entrapment in microspheres as compared to unloaded microspheres. This change was due to the presence of free carboxyl ion and hydroxyl ion present in unloaded microspheres which decreased after 5-FU loading in microspheres. Change in electronic configuration of microspheres after drug entrapment was also reported in previous study (25).

Swelling studies
At pH 7.4, carboxylic acid group dissociate into carboxylate ions resulting in electrostatic repulsion in polymer chains causing swelling of polymer network. Increased swelling was observed in formulations containing increased concentration of NaAlg. This may be attributed to increased number of carboxyl groups of alginate. Such pH-dependent swelling modulated the release of drug from carrier system. Previous studies also reported the same findings in sodium alginate based microspheres (26).

In vitro drug release
Release behavior was explained as at acidic pH interpolymeric complex exists in gel form, also crosslinking bridge retard drug release in dissolution media while at basic pH microspheres swelling occurred and acetal linkage disintegrate to release drug. In the literature it was reported that at basic pH deprotonation of sodium alginate occurred causing disintegration of polymeric systems and release of drug as soluble ions (27). Results of in vitro studies for best formulation CS-9 are shown in Figure 8.

In the present study, it was found that drug release followed non-Fickian release pattern, as n value ranged between 0.965-0.981 (data given in Table 5). The release of drug from crosslinked Chs-NaAlg microspheres was supposed to take place after swelling, which resulted in degradation of Chs and formation of gel by NaAlg followed by dissolution and diffusion of 5-FU through gel.

Factors affecting in vitro drug release
In the literature it was reported that GA developed rigid crosslinking bridge and reduced free spaces within polymeric complex causing controlled diffusion of drug. Formulations having high sodium alginate content controlled diffusion of 5-FU molecules more effectively and enhanced sustained effect of formulation (28). Increased concentration of Chs enhanced hydrophilic character of formulation resulting in rapid penetration of dissolution media causing augmented drug release (29).

CONCLUSION
Combined approach of pH-dependent and microbial triggered drug delivery system was fabricated by using Chs and NaAlg using GA as crosslinking agent. Zeta size analysis revealed that all formulations were in micrometric range. FTIR and EDX studies of microspheres indicated formation of polymeric network and successful 5-FU entrapment while XRD studies elucidate reduction in crystallinity of 5-FU. TGA and DSC studies revealed that the developed microspheres were found highly thermally stable. In addition, swelling studies and in vitro drug release showed that these microspheres have an ability to protect the therapeutics from acidic environment of stomach and can preferentially deliver the drug at pH 7.4. This type of work could encourage the synthesis of new polymeric complexes to deliver therapeutic agents at target site. However, the study has potential of extension through an in vivo evaluation.

REFERENCES
Fabrication and in vitro evaluation of 5-fluorouracil loaded chondroitin... 507


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