High molecular weight cross linked chitosan nanoparticles for controlled release of 5-Fluorouracil; Enhances its bioavailability

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Abstract: The aim of study was cross linking of high molecular weight chitosan nanoparticles containing 5-fluorouracil to improve dissolution rate and ultimately enhance its bioavailability by reverse emulsion/micelles method and cross-linking agent i.e. glutaraldehyde (GA 25% aqueous solution in water). The nature and outer morphologies were evaluated by scanning electron microscopy (SEM). Drug release models were functional to support way from cross linked NPs. Cross linking of 5-fluorouracil with glutaraldehyde improved dissolution rate. Mean dissolution time of 5-fluorouracil decreased significantly upon reverse emulsion/cross linking as encapsulated drug is protective and thermally stable within cross linked chitosan NPs. FTIR studies showed formation of intermolecular hydrogen bonding between 5-fluorouracil and GA-co-CHNPs. DSC studies indicated a less crystalline state of 5-fluorouracil in cross linking. SEM showed spherical nanoparticles with somewhat rough surface. 5-FU release followed Korsmeyer-Peppas model which indicate diffusion and dissociation control drug release from GA-co-CH-NPs. 5-FU cross linked chitosan nanoparticles can be safe and useful tool for other chemotherapeutic agents.

Keywords: 5-Fluorouracil, reverse emulsification, nanoparticles, chitosan, drug release mechanism, bioavailability.

INTRODUCTION

Nanotechnology means nanosize drug carriers for remedial and investigative purposes. One nanometer is one billionth ($10^{-9}$) of a meter and a nanoparticle is at least one dimension less than 100 nm. This cut off size showed marked enhanced physical properties than bulk or solution Nanoparticles for drug delivery system (DDS) are applied as nanocarrier to protect encapsulated drug or to deliver it at target site at a control or sustained manner. These NPs must be biocompatible and smaller in size than blood vessel to avoid their blockage Therefore, for biological applications cut-off size for Nanoparticles is not fixed. However, nanoparticles larger 300 nm are quickly in use by reticuloendothelial system (RES) of body and removed from drug delivery system. Relationship of nanotechnology in treatment of cancer has emerged as most intentional area under discussion in research. Nanoparticles range from controlled release nanocarriers to targeting nanovehicles to therapeutically active moieties (Greish, K. (2010).

Fluorouracil (5-FU) is having fluorinated pyrimidine ring in its structure that disrupts DNA synthesis and is second hand to cure various malignant cells of colon, esophageal, stomach, pancreatic, cervical and breast tissues. Toxicity of fluorouracil is above all the way through reserve in pyrimidine nucleotide synthesis by the rate-limiting enzyme of thymidylate synthase (TS), via this rate limiting enzyme (TS), on the whole deoxyuridine monophosphate is converted to deoxythymidine monophosphate (dTMP). Subsequent 5-FU chemotherapy patients with inactivity of dihydropyrimidine dehydrogenase are at elevated neurologic threat. It typically takes as a minimum 3 to 6 weeks, but may take 10 to 12 weeks. About 20% of parent drug is excreted unaffected in urine within 6 hours. Unsympathetic effects and various barriers to efficient administration of 5-FU can be ameliorated through embattled tumor site and confined drug incorporation at the receptor situate (Sarwar et al., 2017).

For DDS, after oral administrations many drug molecules are effective by pH or enzymes of gut so the main rule of polymeric nanoparticles is to encapsulated drug, to improve pharmacokinetics and to control drug release profile. A diversity of biodegradable and biocompatible chitosan is used for advantageous, constant, restricted and targeted site specific delivery within therapeutic range (Li et al., 2010). Chitosan is an innate polymer which is shown to be degraded to some extent after intravenous administration due to its hydrophilic nature; it can be modified with cross linking and functionalization to make it hydrophobic. Therefore, chitosan and derivatives of it are increasingly being used to encapsulate drug molecules of different physical and chemical properties (Riva et al., 2011). After intravenous administration problems like metabolism, immune response or excretion of drugs can be controlled by irreversible cross linking with a cross linker which established a bridge between 5-FU-CH (drug and polymer) Diffusion of drug from CH-NPs can be controlled via this stable linkage with a aldehyde group of

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Pak. J. Pharm. Sci., Vol.32, No.3(Suppl), May 2019, pp.1137-1143 1137
cross linker i.e., glutaraldehyde in our current research work (Patel et al., 2017). In this study cross linking of 5-FU loaded CH-NPs, which will boost up drug delivery, minimize toxicity and furthermore enhance its bioavailability.

**MATERIALS AND METHODS**

A gift sample of 5-Fluorouracil was received from Pharmedic Laboratories (Pvt.) Ltd. Pakistan. Chitosan (high molecular weight), Glutaraldehyde (25% aqueous solution in water) and Sorbitan monooleate (Span80) was purchased from Sigma-Aldrich (UK). Miglyol oil of medicinal grade and other chemicals like Acetic Acid, potassium di-hydrogen phosphate, sodium hydroxide were also purchased from Merck (Germany). All other solvents and ingredients were of analytical grade.

**Glutaraldehyde cross linked chitosan nanoparticles (GA-co-CH NPs)**

Reverse emulsion/micelles technique is applied for preparation of w/o emulsion consisting of external aqueous phase and internal oil phase and emulsification of innate high molecular weight CH aqueous solution in oil phase, which is composed of miglyol oil and sorbitan monooleate (Span 80) was used as surfactant. Cross-linking of aldehyde group of cross linker, glutaraldehyde (25% aqueous solution in water) with amine group of chitosan (CH) is formed. If W/O emulsion remained stable on standing than filtration, repeatedly washing of nanoparticles were done with ethyl alcohol and then dried. Repeated washing was helpful in minimizing of particles size by controlling of aqueous droplets. However, volume of cross linking agent as well as stirring speed control stability of end product (GA-co-HMWCH-NPs).

**Fig. 1**: Mean particle size of 5-Fluorouracil (3:1) CH-NPs

**5-Fluorouracil loaded chitosan nanoparticles (5-FU/GA-CH NPs)**

Reverse emulsion/micelles technique is applied to stabilized water in oil (W/O) emulsion with surfactant and was provided a dynamic behavior system of important characteristics. Polymerization of nanoparticles were prepared usually large size particles (>200 nm). By using reverse micelle medium ultrafine NPs could be achieved due to Brownian motion of micelle particles, they undergone continuous coalescence followed by re-separation on a time scale and a rapid dynamic equilibrium controlled particles, polydispersity (PDI) and stability of droplets. Under constant vortex mixing reverse micelles were formed, surfactant (Span 80) was dispersed in miglyol oil followed accumulation of high molecular weight CH and 5-FU. Cross-linker glutaraldehyde (25% solution in water) was added with continuous overnight agitation formed a transparent solution. This method was schematically represented (Unsoy et al., 2014).

**Fig. 2**: Zeta potential distributions 5-FU loaded NPs according to the total counts.

**Fig. 3**: FTIR spectrograms of (a) CH (b) 5-FU (c) Physical Mixture (d) 5-FU NPs

**Physicochemical Characterization**

*Effect of glutaraldehyde (GA) on particle size of NPs* Cross linking agent 25% glutaraldehyde solution in water to high molecular weigh chitosan mass ratios, HMW chitosan concentration was remained constant formed average particle size distribution, polydispersity index (PDI) and maximum particle concentration to form cross linked chitosan Nanoparticles.

**Particle size analysis by zeta sizer**

10 mg of 5-Fluorouracil in PBS (25ml Phosphate Buffer Solution) diluted in a volumetric flask at 7.4 pH and for maximum dissolution of drug; resultant solution was stirred with a magnetic stirrer. Then, made up volume of solution up to 100ml with PBS to give 5-FU concentration of 0.01 mg/ml. Average particle diameter was calculated at a fixed angle of 90° by Photon correlation spectroscopy (PCS) (Zetasizer 3000HS, Malvern Instrument, UK). Further dilutions are used to measure particle diameter and PDI (Subedi et al., 2009).

**Scanning electron microscopy (SEM)**

Sample was diluted 10 times and a particle sample was scattered onto an aluminum slab/grid via dual paste and evaluation of particle size and shape were confirmed.
under Scanning Electron Microscope (SEM) (Jeol, JSM-6100 SEM, Japan).

![SEM micrographs of NPs formulations F1 and F5](image)

**Fig. 4:** SEM micrographs of NPs formulations F1 and F5

**Entrapment efficiency (EE %)**
Separation of nanoparticles from encapsulated 5-Fluorouracil were by centrifugation machine 14000rpm, at temp 4 ºC for 30. A uniform solution was obtained from supernatant layer and sonicated it, for 15 min. At a wavelength of 266 nm, the quantity of entrapped 5-Fluorouracil was determined via UV-Spectrophotometer. Drug entrapment efficiency (EE %) was calculated as in Eq 1 (Mitra et al., 2001).

\[
EE\% = \left\{ \frac{(T - S)}{T} \right\} \times 100
\]

Where, T is total amount of drug after centrifugation and S is free amount drug in supernatant.

**Dissolution studies**
Dialysis bag/membrane was engaged through USP type II dissolution equipment by paddle assembly (Pharma Test, Germany) (Lu et al., 2003). Dissolution studies were surrogate indicator of in vivo performance. This bag/membrane was made up of cellulose ester (molecular weight cut-off values 10,000 Daltons), molecular weight of drug and particles size being the base of its selection. 3mg or equivalent NPs containing 5-fluorouracil were packed in a dialysis bag and tied at both ends. The filled dialysis bags were then suspended in dissolution vessels containing 250 ml dissolution medium i.e. phosphate Buffer Saline (PBS) at pH 7.4 (Dave and Patel, 2013). As a function of pH and time all drug release studies were conducted. Temperature of media was adjusted at 37ºC and 39ºC ±1 and speed of paddle was tuned at 100 rpm. 5 mL aliquot were withdrawn from the sample vessels at predetermined intervals. Collected aliquots were diluted rightfully, filtered through Millipore and absorbances were taken at \(\lambda_{max}=260\). Studies were performed in triplicate (n=3). Mean values of cumulative drug release were calculated for plotting the release curve. The percent cumulative release was calculated by following equation:

\[
\text{Cumulative drug release} = \frac{F_t}{F_{\infty}} \times 100
\]

Where \(F_t\) and \(F\) indicate the amount of 5-FU released in time \(t\) and total amount of drug loaded in NPs, respectively.

![DSC thermograms of (a) CH. (b) 5-FU, PM, (c) GA-co-CH NPs](image)

**Fig. 5:** DSC thermograms of (a) CH. (b) 5-FU, PM, (c) GA-co-CH NPs

![PXRD patterns of chitosan, 5-FU, PM and crosslinked NPs](image)

**Fig. 6:** PXRD patterns of chitosan, 5-FU, PM and crosslinked NPs

![Stability studies at 22±5°C and 40±5°C](image)

**Fig. 7:** Stability studies at 22±5°C and 40±5°C

**Pharmacokinetics Modeling of 5-FU Release**
DD Solver software to find out the highest values of regression coefficient was applied in Excel regarding drug release kinetic analysis. In order to investigate mechanism of 5-FU release from chitosan nanoparticles, in vitro release data were analyzed using various kinetic models zero-order, first-order, Higuchi, Hixson Crowell and Korsmeyer-Peppas. Zero-order kinetic model for comparison drug release and to check out the pattern of drug release studied (Barzegar et al., 2008).
High molecular weight cross linked chitosan nanoparticles for controlled release of 5-Fluorouracil

Application of Korsmeyer-Peppas model
A simple relationship which defined drug release from NPs was derived from Korsmeyer-Peppas model equation. This model is usually used to recognized drug release profile from hydrophilic matrix. Log between cumulative percent of drug release versus Log time graph was plotted as shown in Eq 2.

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

Where \( M_t/M_\infty \) is the amount of drug release at time \( t \). \( K \) is a constant which include properties of polymeric system and drug. While \( n \) is kinetic constant related to release mechanism of drug (Brunner, 1904).

STATISTICAL ANALYSIS
SPSS 18 used for statistical analysis. Comparison of formulations was by one-way analysis of variance (ANOVA). \( P<0.05 \) considered significant.

RESULTS

Unloaded/Empty Chitosan NPs
All unloaded/empty formulations not containing drug i.e., 5-fluorouracil, were more than 300nm in particle diameter has been illustrated from table 1.

Loaded/Packed chitosan NPs
Loaded/packed chitosan formulations contain 5-fluorouracil as F3 (3:1) has been illustrated in fig.1 mean particle size was smaller than 192 nm of loaded nanoparticles. In order to make sure its reproducibility as well to minimize any human error, repeated it three times (n=3). Variable concentrations of drug and polymer ratios in Table 2 were interpreted and evaluate results.

Zeta potential analysis
Stability of prepared nanoparticles formulations depends upon system that has a relatively narrow size distribution indicating Polydispersity Index (PDI) values were found to be lesser than 0.2, which further indicates that Zeta potential was found to be within the limit. Stable nanoparticles formulations enhance solubility and bioavailability by being rationale of stable nanoparticles formulations and can be easily dispersed due to stability. Different concentrations of poly:drug as shown in Table 3 and diagrammatically presented in fig. 2 as well were being used for evaluation of stability data.

Percent yield (%yield)
From table 2 Percentage yield of optimized NPs formulation F3 was observed high 92.69% with increased % of polymer and there was a slight difference between NPs formulations F2 and F3 but huge difference was observed between NPs formulations F3 and F5.

Drug Entrapment Efficiency (EE %)
Two NPs formulations were optimized as from Table 2 of ratio 1:3 (181 ± 15.50 nm) and 1:2 (177 ± 11.69 nm) indicating drug entrapment efficiency as per polymer and drug concentration ratio. For further in vitro drug release studies, particle size of optimized ratios 3:1 and 2:1 we selected as optimized NPs formulations F3 and F2.

Fourier Transform Spectroscopy (FTIR)
As exposed in fig. 3 (A), FTIR spectra of CH, 5-Fluorouracil, physical mixture (polymer & drug) and prepared cross linked chitosan nanoparticles containing 5-Fluorouracil taken to authenticate cross-linking between polymer (CH) and 5-FU.

Scanning electron microscopy (SEM)
Nanoparticles formulations F1 and F5, SEM micrographs show in fig. 4. F1 NPs were sphere-shaped and with even surface while F5 NPs is somewhat uneven, inappropriate cross linking between nanoparticles of F5-NPs may impart uneven surface

Differential Scanning Colorimetry (DSC)
DSC thermogram of Cross linked chitosan NPs (GA-co-CH NPs) as showed in fig. 5 endothermic peak at 110°C and a large exothermic-peak at about 290°C.

Powder X-Ray Diffraction (PXRD)
PXRD obtained in fig. 6. Degree of crystalline of chitosan can be determined by XRD analyzer and important for polymer characterization.

Stability studies
As shown in fig. 7 under accelerated conditions of temperature 22±5°C and 40%±5%, samples of prepared 5-FU-CH NPs were drawn after 1, 2 and at end of 3 months.

Dissolution studies
Due to weak acidic nature of 5-Fluorouracil ionize and soluble at high pH solutions in basic medium. From Fig. 8 illustrated dissolution studies of both drug as well as prepared NPs.
**In vitro drug release from NPs**

From Fig. 8 dissolution kinetics of CH-NPs showed that increased concentration of chitosan reduced initial burst release of 5-FU. From Table.4, cumulative release of 5-FU after of first hour was 73.34%, 70.85%, 64.34 %, 42.56 % and 31.78% from F1, F2, F3, F4 and F5, respectively.

**Table 1**: Composition of unloaded CH NPs

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>CH g/mL</th>
<th>GA v/v</th>
<th>Miglyol mL</th>
<th>Span80 mL</th>
<th>Particle size (nm)</th>
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</thead>
<tbody>
<tr>
<td>CH1</td>
<td>50</td>
<td>0.1</td>
<td>120</td>
<td>0.12</td>
<td>300</td>
</tr>
<tr>
<td>CH2</td>
<td>60</td>
<td>0.2</td>
<td>120</td>
<td>0.12</td>
<td>350</td>
</tr>
<tr>
<td>CH3</td>
<td>70</td>
<td>0.25</td>
<td>120</td>
<td>0.12</td>
<td>360</td>
</tr>
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</table>

**Table 2**: Percentage yield, EE% and particle diameter

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>GA v/v</th>
<th>CH: drug ratio</th>
<th>Percent yield</th>
<th>(EE %)</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.1</td>
<td>1:1</td>
<td>87.89</td>
<td>59.55</td>
<td>159 ± 13.39</td>
</tr>
<tr>
<td>F2</td>
<td>0.2</td>
<td>2:1</td>
<td>92.27</td>
<td>67.67</td>
<td>177 ± 11.67</td>
</tr>
<tr>
<td>F3</td>
<td>0.3</td>
<td>3:1</td>
<td>92.69</td>
<td>72.36</td>
<td>181 ± 15.50</td>
</tr>
<tr>
<td>F4</td>
<td>0.25</td>
<td>(2:1):1</td>
<td>85.45</td>
<td>66.86</td>
<td>250 ± 12.45</td>
</tr>
<tr>
<td>F5</td>
<td>0.1</td>
<td>(2:2):1</td>
<td>83.67</td>
<td>66.39</td>
<td>274 ± 14.07</td>
</tr>
</tbody>
</table>

**Table 3**: Vital features of 5-FU-CH NPs

<table>
<thead>
<tr>
<th>Sample</th>
<th>CH g/100 ml</th>
<th>GA MI</th>
<th>Oil mL</th>
<th>Span 80 mL</th>
<th>NPs</th>
<th>5-FU Mg</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.5</td>
<td>0.1</td>
<td>0.078</td>
<td>0.15</td>
<td>NPs</td>
<td>3</td>
<td>0.098</td>
</tr>
<tr>
<td>F2</td>
<td>0.5</td>
<td>0.15</td>
<td>0.075</td>
<td>0.18</td>
<td>NPs</td>
<td>5</td>
<td>0.09</td>
</tr>
<tr>
<td>F3</td>
<td>0.5</td>
<td>0.2</td>
<td>0.080</td>
<td>0.175</td>
<td>NPs</td>
<td>10</td>
<td>0.08</td>
</tr>
<tr>
<td>F4</td>
<td>0.5</td>
<td>0.25</td>
<td>0.800</td>
<td>0.18</td>
<td>NPs</td>
<td>3</td>
<td>0.1</td>
</tr>
<tr>
<td>F5</td>
<td>0.5</td>
<td>0.3</td>
<td>0.980</td>
<td>0.16</td>
<td>NPs</td>
<td>5</td>
<td>0.11</td>
</tr>
</tbody>
</table>

**DISCUSSION**

**Unloaded/Empty Chitosan NPs**

These nanoparticles cannot perform their planned role and are not appropriate for biomedical applications. Therefore, NPs threshold size for drug delivery applications may be acceptable up to 300 nm (or smaller), as these NPs will stay in blood for longer period of time. Loaded/packed formulations were between 200 and 250 nm in diameter.

**Loaded/Packed chitosan NPs**

It can be analyzed from Fig. 1, particle size intensity for polymer: drug ratios, 1:3 and 1:2, 181±15.50 nm and 177±11.67 nm were respectively. Recently, European Commission is revising definition of NPs to clarify confusions and complexities of interdisciplinary research fields. According to European Commission, a NP is “a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of particles in number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm” (Commission, 2016).

**Zeta potential analysis**

Zeta potential of NPs is robustly correlated to stability data of NPs in an aqueous environment. More stable NPs due to more strong repellent interaction forces between each other ultimately zeta potential will be increased. In addition, due to negative charge on cancerous cell membrane, it was approved binding of positive charge density of NPs plays a vital role. Therefore, in treatment of malignant cells or cancerous cell membrane, positively charged NPs like are model and with increase concentration of 5-fluorouracil zeta potential decreases (Commission, 2017).

**Drug Entrapment Efficiency (EE %)**

As shown in 3:1 polymer and drug ratio drug entrapment efficiency increased up to 72.36%. Hydrophilicity of chitosan polymer increased with increased in drug entrapment efficiency (EE %). Chitosan polymer has to be approved as a promising tumor cells inhibitor (Zhang et al., 2008).
Table 5: Pharmacokinetics modeling of drug release at 1.2 pH and 7.4 pH

<table>
<thead>
<tr>
<th>Pharmacokinetics models</th>
<th>At 1.2 pH</th>
<th></th>
<th>At 7.4 pH</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
<td>F2</td>
<td>F3</td>
<td>F1</td>
</tr>
<tr>
<td>Zero order</td>
<td>R²</td>
<td>0.9806</td>
<td>0.9711</td>
<td>0.9413</td>
</tr>
<tr>
<td></td>
<td>K₀</td>
<td>0.1374</td>
<td>0.1032</td>
<td>0.0405</td>
</tr>
<tr>
<td>First order</td>
<td>R²</td>
<td>0.9352</td>
<td>0.9011</td>
<td>0.7868</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>0.0657</td>
<td>0.0616</td>
<td>0.04</td>
</tr>
<tr>
<td>Higuchi model</td>
<td>R²</td>
<td>0.9638</td>
<td>0.9407</td>
<td>0.9143</td>
</tr>
<tr>
<td></td>
<td>K_H</td>
<td>0.4966</td>
<td>0.5132</td>
<td>0.2930</td>
</tr>
<tr>
<td>Hixon-Crowell</td>
<td>R²</td>
<td>0.9531</td>
<td>0.9744</td>
<td>0.8855</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>0.0018</td>
<td>0.0019</td>
<td>0.0011</td>
</tr>
<tr>
<td>Korsmeyer-Peppas model</td>
<td>R²</td>
<td>0.9765</td>
<td>0.9452</td>
<td>0.9235</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>11.712</td>
<td>3.4542</td>
<td>5.3465</td>
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<tr>
<td></td>
<td>N</td>
<td>0.6541</td>
<td>0.5879</td>
<td>0.4473</td>
</tr>
</tbody>
</table>

Fourier Transform Spectroscopy (FTIR)
Spectrum peaks of Chitosan due to –OH stretching at 3377 cm⁻¹, indicates –C–H stretching vibrations at 2920 cm⁻¹, while suggests >C=O at 1645 cm⁻¹, at 1645 cm⁻¹ and 1025 cm⁻¹ keep up a correspondence of C=C and –CH₂ stretching vibrations.

As revealed in fig. 3 (b), characteristic FTIR spectrum of 5-FU at 3377 cm⁻¹ due to O-H stretching, at 2927 cm⁻¹ is due to C-H stretching, at 1667 cm⁻¹ indicates C-O stretching vibration, at 1030 cm⁻¹ C-O-C stretch shoulders. 5-FU spectrum there is only a slight shifting in some characteristic peaks.

As shown in fig. 3 (c), there was no potential interaction in spectra between 5-FU and CH; therefore new bands were not detected due to formation of compatible and stable 5-FU loaded CH-NPs formulations (Aydin & Pulat, 2012).

Scanning electron microscopy (SEM)
F1-NPs were observed appropriate cross linking of Nanoparticles, with smooth surface, due to which Nanoparticles are stable and the release of drug from them will be in a controlled manner, when compared to F5-NPs having rough surface (Rao et al., 2010).

Differential Scanning Colorimetry (DSC)
DSC confirmed glutaraldehyde (GA) cross linking with polymer (CH) it authenticated that (GA-co-CH NPs) confirmed more thermal stability than individual components. It has been confirmed such thermally stable cross linked chitosan NPs are provide the path for PXRD studies. Our results have been reported similar by (Prasad et al. 2012).

Powder X-Ray Diffraction (PXRD)
Confirmation of formation of new materials via various PXRD pattern of pure chitosan and cross linked chitosan NPs. CH-co-GA NPs indicates less crystalline structure than pure CH in PXRD pattern studies (Pal., 2009).

Stability studies
Dissolution studies and in vitro drug release studies were indicating drug contents stable at the end of 3 months. So it is confirmed that under accelerated conditions of temperature and humidity our prepared nanoparticles formulations were remain stable for 3 months.

Dissolution studies
A significant increase in the solubility of drug in water (5.86 fold), pH 1.2 (3.32 fold) and pH 7.4 (8.54 fold) were observed. Cross linking of CH-NPs increased solubility of drug in the prepared NPs. Due to hydrophilic nature of chitosan it forms a network with lipophilic drug moieties and makes it soluble. Solubility of prepared nanoparticles was increased by several folds than pure drug at low and high pH media (Shilpi et al., 2015).

In vitro drug release from NPs
F1 and F2 nanoparticles formulations show initial burst release due water solubility. Drug release is maintained up to 12 hrs due to cross linking of chitosan NPs. Burst effect in F5 is significantly less than that of F1. This phenomenon can be explained on the fact that less tight cross linking of NPs is devoid of drug and water has to pass the outer layer first to dissolve and diffuse 5-FU out in dissolution media. Drug release data was fitted to various kinetic models. Highest linearity was found for Higuchi model followed by First order kinetics. These results may also be due small particles size will increase surface area of particles that improve dissolution and solubility of NPs ultimately enhance bioavailability of drug (Fahmy et al. 2008).

CONCLUSION
Controlled release nanoparticles of 5-Fluorouracil have been successfully prepared by reverse emulsification/chemical cross linking method. Entrapment efficiency is high in chitosan nanoparticles, up to 72.36%. The nanoparticles displayed anticancer properties in targeted area by controlled manner, reduce toxicity and enhanced bioavailability, thus indicating its potential for chemotherapeutic application.
ACKNOWLEDGEMENTS

The authors are grateful to Faculty of Pharmacy and Alternative Medicines, the Islamia University of Bahawalpur, Pakistan, during this study. Authors are thankful to National Institute of Biotechnology and Genetic Engineering, Faisalabad, Pakistan.

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