Formulation and Evaluation of Polymeric Nanoparticles of an Antiviral Drug for Gastroretention

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ABSTRACT
The aim of present study was to formulate and evaluate nanoparticles of acyclovir by using different hydrophilic polymers. Acyclovir was selected as a suitable drug for gastro-retentive nanoparticles due to its short half life, low bioavailability, high frequency of administration, and narrow absorption window in stomach and upper part of GIT. The nano-precipitation method was used to prepare nanoparticles so as to avoid both chlorinated solvents and surfactants to prevent their toxic effect on the body. Nanoparticles of acyclovir were prepared by using hydrophilic polymers such as bovine serum albumin, chitosan, and gelatin. The prepared formulations were then characterized for particle size, polydispersity index, zeta potential, loading efficiency, encapsulation efficiency and drug-excipient compatibility. The prepared nanoparticulate formulations of acyclovir with different polymers in 1:1 ratio have shown particle size in the range of 250.12-743.07 nm, polydispersity index (PDI) in the range of 0.681-1.0, zeta potential in the range of -14.2 to +33.2 mV, loading efficiency in the range of 8.74-17.54%, and entrapment efficiency in the range of 55.7%-74.2%. Nanoparticulate formulation prepared with chitosan in 1:1 ratio showed satisfactory results i.e. average particle size 312.04 nm, polydispersity index 0.681, zeta potential 33.2 mV, loading efficiency 17.54%, and entrapment efficiency 73.4%. FTIR study concluded that no major interaction occurred between the drug and polymers used in the present study.

KEYWORDS: Nanoparticles; gastro-retentive; nano-precipitation, polydispersity index, zeta potential; entrapment efficiency.

Introduction
The oral route of drug administration is the most convenient and commonly used method of drug delivery due to their considerable therapeutic advantages such as ease of administration, patient compliance, and flexibility in formulation (Garg et al., 2008; Gupta et al., 2007). However, this route has several physiological problems, such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract due to variable gastric emptying and motility. Furthermore, the relatively brief gastric emptying time in humans, which normally means 2-3 hours through the major absorption zone, i.e., stomach and upper part of the intestine, can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose (Rouge et al., 1996). These difficulties have prompted researchers to design a drug delivery system which can stay in the stomach for prolonged and predictable period (Deshpande et al., 1996; Hwang et al., 1998). Several attempts are being made to develop a controlled drug delivery system, which can provide therapeutically effective plasma drug concentration for a longer period, thereby reducing the dosing frequency and minimizing fluctuations in plasma drug concentration at steady-state by delivering the drug in a controlled and reproducible manner (Sood et al., 2003).

Different methodologies have been reported in the literature to increase the gastric retention of drugs, like intra-gastric floating systems, hydro dynamically balanced systems, extendable or expandable, microporous compartment system, microballs, bio/muco-adhesive systems, high-density systems, and super porous biodegradable hydro gel systems (Singh et al., 2000). After oral administration, such a dosage form would be retained in the stomach for several hours and would release the drug there in a controlled and prolonged manner, so that the drug could be supplied continuously to its absorption sites in the upper gastrointestinal tract (Streubel et al., 2006). Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility of drugs that are less soluble in a high pH environment. It is also suitable for local drug delivery to the stomach and proximal small intestine (Rao et al., 2005). Gastroretention helps to provide better availability of new products with suitable therapeutic activity and substantial benefits for patients.

ABBREVIATIONS: Bovine serum albumin (BSA); molar (M); ultraviolet (UV); Fourier transform infra red (FTIR); polydispersity index (PDI).
Acyclovir, a cyclic analogue of the natural nucleoside 2-deoxyguanosine, is clinically used in the treatment of herpes simplex, varicella zoster, cytomegalovirus, and Epstein Barr virus infections (Brien et al., 1989). Absorption of orally administered acyclovir is slow, variable, and incomplete, with a bioavailability of 15%-30% (Shao et al., 1994) and the elimination half-life of acyclovir is approximately 3 hours. It has narrow absorption window and is primarily absorbed from stomach and upper part of the small intestine (Groning et al., 1996); there is a need to develop an effective formulation with enhanced gastric residence time.

Attia et al., prepared niosomes of acyclovir by using cholesterol, span 60, and dicetyl phosphate to improve its poor and variable bioavailability. The niosomal dispersion when compared to the free solution has shown more than 2-fold increase in drug bioavailability and increase in the mean residence time of acyclovir reflecting sustained release characteristics (Attia et al., 2007). Palmberger et al., developed a novel oral delivery system for the acyclovir, utilizing thiolated chitosan as excipient, which is capable of inhibiting P-glycoprotein (P-gp). Three chitosan-4-thiobutylamidine (Chito-TBA) conjugates with increasing molecular mass (Chito-9.4 kDa-TBA, Chito-150 kDa-TBA, and Chito-600 kDa-TBA) were synthesized and permeation studies on rat intestinal mucosa and Caco-2 monolayers were performed. They found that Chito-150 kDa-TBA/GSH might be an appropriate sustained release drug delivery system for acyclovir, which is able to enhance acyclovir transport due to efflux pump inhibition (Palmberger et al., 2008). Kharia et al., optimized the floating drug delivery systems of acyclovir using psyllium husk and hydroxypropylmethylcellulose K4M as the polymers by 3² full factorial design to improve the oral bioavailability of acyclovir. The optimized formulations followed Higuchi’s kinetics while the drug release mechanism was found to be anomalous type, controlled by diffusion through the swollen matrix (Kharia et al., 2010). Stulzer et al., prepared microparticles containing acyclovir and chitosan cross-linked with tripolyphosphate using the spray-drying technique. The results obtained indicated that the polymer/acyclovir ratio influenced the final properties of the microparticles, with higher ratios giving the best encapsulation efficiency, dissolution profiles, and stability (Stulzer et al., 2009).

The aim of the present study was to formulate gastroretentive nanoparticles of acyclovir to deliver the drug at a controlled rate to its absorption site so that its oral bioavailability can be enhanced. Mucoadhesive polymers, such as bovine serum albumin, chitosan, and gelatin, were selected to prepare gastroretentive nanoparticles as they intensify the contact between dosage form and the site of absorption, thereby reducing the luminal diffusion pathway of the drug (bioadhesion) and lead to significant improvements in oral drug delivery (Lueben et al., 1994, Park and Robinson, 1984). These mucoadhesive polymeric nanoparticles in the stomach will offer various advantages such as (i) Longer residence time of the dosage form on mucosal tissues in the stomach. This will improve absorption of the drug and increase the drug bioavailability. (ii) Higher drug concentration at the site of adhesion absorption, which will create a driving force for the paracellular passive uptake. (iii) Immediate absorption from the bioadhesive drug delivery system without previous dilution and possible degradation in the luminal fluids (Hejazi and Amiji et al., 2003)

Materials and Method

Acyclovir, bovine serum albumin (BSA), and gelatin were obtained as a gift sample from Modern Laboratories (Indore, India); chitosan was obtained as a gift sample from Indian Sea Foods (Cochin, India). All other chemicals and reagents were of laboratory grade and were used as procured.

Preparation of Nanoparticles

Nanoparticles were prepared according to the nanoprecipitation method with slight modification (Elshafeey et al., 2010). Briefly, 200 mg of polymer (bovine serum albumin, chitosan, and gelatin) was dissolved in 25 ml of acetone separately. The acyclovir 100 mg was dissolved in 2 ml of dimethyl sulfoxide. Both solutions were mixed and then 50 ml of water was added and stirred for a half hour. Acetone was eliminated by evaporation under reduced pressure using rotary flash evaporator and the final volume of the suspension was adjusted to 10 ml. Then this suspension was centrifuged at 15000 rpm at 4°C for half an hour. The supernatant was discarded and precipitate was washed 3 times with distilled water. The nanoparticles thus obtained were dried overnight in oven at 60°C and stored in a desiccator.

The prepared formulations were characterized for loading efficiency, entrapment efficiency, particle size, particle size distribution, polydispersity index, zeta potential and drug excipient compatibility studies.

Characterization of Acyclovir Loaded Nanoparticles

Loading Efficiency

Drug content in the preparation was determined by extracting the drug from the nanoparticles with 0.1 M hydrochloric acid. In this method, the nanoparticles (50 mg) were stirred in 50 ml of 0.1 M hydrochloric acid until dissolved; it was filtered through a Millipore filter and the drug content was determined, after suitable dilution, at 254 nm by UV spectrophotometry. The loading efficiency (L) of the nanoparticles was calculated according to Equation 1

\[ L(\%) = \left(\frac{Qn}{Wn}\right) \times 100 \quad \text{...(1)} \]

Where Wn is the weight of the nanoparticles and Qn is the amount of drug present in the nanoparticles (Patel and Patel, 2007).
**Entrapment Efficiency**

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined \( (w) \) by UV spectrophotometer at 254 nm. A standard calibration curve of drug was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation \( (W) \). Effectively, \( (W-w) \) will give the amount of drug entrapped in the particles (Bellare et al., 2005).

Then percentage entrapment of a drug was calculated according to Equation 2
\[
\text{% Drug Entrapment} = \frac{(W-w)}{(W)} \times 100 \quad \text{.....(2)}
\]

**Particle Size, Particle Size Distribution, and Zeta Potential**

The particle size and particle size distribution of the formulation was determined by photo correlation spectroscopy with a zeta master (Malvern Instruments, UK) equipped with the Malvern PCS software. Every sample was diluted with distilled water. The surface charge (Zeta potential) was determined by measuring the electrophoretic mobility of the nanoparticles using a Malvern zeta sizer (Malvern Instruments, UK). Samples were prepared by diluting with distilled water (Pignatello et al., 2006).

**Polydispersity Index**

Polydispersity index is a parameter to define the particle size distribution of nanoparticles obtained from photon correlation spectroscopic analysis. It is a dimensionless number extrapolated from the autocorrelation function and ranges from a value of 0.01 for monodispersed particles and up to values of 0.5-0.7. Samples with very broad size distribution have polydispersity index values > 0.7 (Nidhin et al., 2008).

**Drug-Excipient Compatibility Studies**

The drug excipient compatibility studies was performed by using FT-IR spectrophotometer (Perkin Elmer). The FT-IR spectra of drug, polymers, and formulations were analyzed separately and then correlated for incompatibility.

**Results and Discussion**

The method of nanoprecipitation was used so as to avoid both chlorinated solvents and surfactants to prevent their toxic effect on the body. All the determinations were done in triplicate.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Formulation Code</th>
<th>Polymer</th>
<th>Drug:Polymer ratio</th>
<th>Formulation Code</th>
<th>Loading Efficiency* ± SD %</th>
<th>Entrapment Efficiency* ± SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>NP 1</td>
<td>Bovine Serum Albumin</td>
<td>1:1</td>
<td>NP 1</td>
<td>11.43±2</td>
<td>55.7±6.3</td>
</tr>
<tr>
<td>2.</td>
<td>NP 2</td>
<td>Chitosan</td>
<td>1:1</td>
<td>NP 2</td>
<td>17.54±3</td>
<td>73.4±3.5</td>
</tr>
<tr>
<td>3.</td>
<td>NP 3</td>
<td>Gelatin</td>
<td>1:1</td>
<td>NP 3</td>
<td>8.74±3</td>
<td>74.2±4.9</td>
</tr>
</tbody>
</table>

* = Average of three determinations
The results of prepared nanoparticulate formulations of acyclovir with different polymers are given in Table 2 and shown in Figure 2. The formulations had very high polydispersity index (PDI) in the range of 0.681-1.0. From the particle size distribution data, it is evident that in case of BSA nanoparticles, mean particle diameter was 250.12 nm and major portion of the particles were in the range of 200-400 nm, for chitosan nanoparticles mean particle diameter was 312.04 nm; and major portion of the particles were in range of 200-525 nm. In case of gelatin nanoparticles mean particle diameter was 743.07 nm and most of the particles were in the range of 480-1200 nm. However, in all the formulations contained a minority population of nanoparticles in much smaller range. For BSA, about 10.1% of the particles were in the range 15-30 nm, for chitosan about 7.1% of the particles were in the range 48-90 nm and for gelatin 14.1% of the particles were in the range 70-160 nm. These minority populations are responsible for larger over all polydispersity indices of the formulations. We are currently exploring the process variables affecting the relative amounts of different populations with an objective to increase the yield of the particles in the smaller range to get much smaller nanoparticles, which have greater degree of monodispersity. Such nanoparticles can be easily separated from the larger sized population by simple methods like filtration.

From the above data it is clear that nanoparticles prepared by using chitosan and BSA exhibited reduction in mean nanoparticulate diameter and narrower granulometric distribution. But the nanoparticles prepared using gelatin as a polymer resulted in nanoparticulate population of large particles. The higher particle size and polydispersity index may be because of absence of emulsifier as the use of emulsifier decreases the surface tension between organic phase acetone and aqueous phase and leads to the formation of smaller solvent droplets, which in turn causes decrease in particle size. It also stabilizes newly generated surfaces and prevents aggregation of the particles as reported by previous researchers (Schubert and Muller, 2003). Therefore results which were obtained in this study may be improved by using increased drug-polymer ratio, using different formulation strategy such as desolvation (for gelatin and albumin) or counter ion induced aggregation (for chitosan and sodium alginate), employing cross linking agent followed by neutralizing residual cross linking agent with cysteine and high speed stirring.

**Zeta Potential**

The measurement of the zeta potential allows predictions about the storage stability of colloidal dispersions. In general, particle aggregation is less likely to occur for charged particles (i.e. high zeta potential) due to electric repulsion. Generally, Zeta potential values above 30 mV (positive or negative values) lead to more stable nanocapsule suspensions because repulsion between the particles prevented their aggregation. A decrease in zeta potential, i.e. electrostatic repulsion, was considered as the cause for the aggregation process (Dalengon et al., 1998). The charge on the surface of the nanospheres will influence their distribution in the body and the extent of uptake into the cells. Because cell membranes are negatively charged, there is greater electrostatic affinity for positively charged nanoparticles. Therefore, the surface of cationic or neutral nanoparticles may be modified to confer a positive charge to enhance efficacy.

The zeta potential values which were in the range of $-14.2 \pm 21.7$ mV, indicates that the colloidal suspension may not be stable and may lead to aggregation. Zeta potential values can be altered by modifying the major components such as surfactants, polymer, and surface composition of the nanoparticles, the presence or the absence of adsorbed compounds, composition of the dispersing phase, mainly the ionic strength, and the pH (Barratt et al., 1999).
Drug-Excipient Compatibility Studies

The results of drug-excipient compatibility studies are shown in Table 3. From the IR data it is clear that functionalities of drug have remained unchanged, including intensities of the peak. This suggests that during the process of formulation polymer has not reacted with the drug to give rise to reactant products. So it is only physical mixture and there is no interaction between them which is in favor to proceed for formulation.

### Table 2

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Formulation Code</th>
<th>Polymer</th>
<th>Mean Particle Size (nm) ± SD</th>
<th>Size Distribution</th>
<th>PDI ± SD</th>
<th>Zeta Potential (mV) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>NP 1</td>
<td>Bovine Serum Albumin</td>
<td>250.12±18</td>
<td>10.1% (15-30 nm)</td>
<td>89.9 % (200-400 nm)</td>
<td>1.0±0.12</td>
</tr>
<tr>
<td>2.</td>
<td>NP 2</td>
<td>Chitosan</td>
<td>312.04±32</td>
<td>7.8% (48-90 nm)</td>
<td>92.2% (200-525 nm)</td>
<td>0.681±0.15</td>
</tr>
<tr>
<td>3.</td>
<td>NP 3</td>
<td>Gelatin</td>
<td>742.07±45</td>
<td>14.2% (70-160 nm)</td>
<td>85.8% (480-1200 nm)</td>
<td>0.7770±0.14</td>
</tr>
</tbody>
</table>

* = Average of three determination

### Table 3

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Bands</th>
<th>Drug</th>
<th>NP 1</th>
<th>NP 2</th>
<th>NP 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>-OH</td>
<td>3447.8 and 3337.9 cm⁻¹</td>
<td>3442.9 cm⁻¹</td>
<td>3434.5 and 3324.5 cm⁻¹</td>
<td>3441.1 cm⁻¹</td>
</tr>
<tr>
<td>2.</td>
<td>N-H bend, assy.</td>
<td>1632.5 cm⁻¹</td>
<td>1626.4 cm⁻¹</td>
<td>1638.6 cm⁻¹</td>
<td>1635.4 cm⁻¹</td>
</tr>
<tr>
<td>3.</td>
<td>C-C ring str.</td>
<td>1537 and 1483.7 cm⁻¹</td>
<td>1479.2 and 1531.1 cm⁻¹</td>
<td>1456.9 cm⁻¹</td>
<td>1532.1 and 1481.1 cm⁻¹</td>
</tr>
<tr>
<td>4.</td>
<td>Aromatic amines,</td>
<td>1305.9 and 1392.1 cm⁻¹</td>
<td>1387.1 cm⁻¹</td>
<td>1390.4 and 1392.5 cm⁻¹</td>
<td>1393.6 cm⁻¹</td>
</tr>
<tr>
<td>C-N str.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Assy. C-O-C str.</td>
<td>1219 cm⁻¹</td>
<td>1216.8 cm⁻¹</td>
<td>1219.2 cm⁻¹</td>
<td>1216.5 cm⁻¹</td>
</tr>
<tr>
<td>6.</td>
<td>Sym C-O-C str.</td>
<td>1013 cm⁻¹</td>
<td>1011.9 cm⁻¹</td>
<td>1080.3 cm⁻¹</td>
<td>1011.2 cm⁻¹</td>
</tr>
<tr>
<td>7.</td>
<td>N-H wagging</td>
<td>906.5 and 864.2 cm⁻¹</td>
<td>900.1 cm⁻¹</td>
<td>897.3 cm⁻¹</td>
<td>899.5 cm⁻¹</td>
</tr>
<tr>
<td>8.</td>
<td>Mono-substitution</td>
<td>777.1,753.7 and 681.2 cm⁻¹</td>
<td>767.6 and 672 cm⁻¹</td>
<td>771.5 and 675.5 cm⁻¹</td>
<td>769.8 and 670.9 cm⁻¹</td>
</tr>
</tbody>
</table>
in ring                   |                  |                      |                               |                               |                               |

NP1 = Bovine Serum Albumin Nanoparticles, NP2 = Chitosan Nanoparticles, NP3 = Gelatin Nanoparticles

### Conclusion

Among different nanoparticulate formulations prepared by nanoprecipitation method formulation NP 2, with chitosan in 1:1 drug: polymer ratio, showed satisfactory results; i.e. mean particle size of 312.04 nm (majority of the particles were in the range of 200-525 nm), polydispersity index of 0.681, zeta potential of 33.2 and loading efficiency of 17.54%, and entrapment efficiency of 73.4%. FTIR study concluded that no major interaction occurred between the drug and polymers used in the present study.
Acknowledgements

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References


