Journal of Applied Pharmaceutical Science Vol. 2 (12), pp. 158-166, December, 2012
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2012.21228
ISSN 2231-3354

Mucoadhesive Polymeric Hydrogels for Nasal Delivery of Penciclovir

Arindam Das[1*], Prof. B. K. Gupta[2], Bhole Nath[1]
[1]Faculty of Pharmacy, Lincoln University College, Mayang Plaza, No. 1, Block A, Jalan SS 26/2, Taman Mayang Jaya, 47301, Petaling Jaya, Selangor, Malaysia.
[2]Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India.

ARTICLE INFO

Article history:
Received on: 11/11/2012
Revised on: 03/12/2012
Accepted on: 15/12/2012
Available online: 30/12/2012

Key words:
penciclovir nasal; nasal mucoadhesion; poly-N-vinyl-2- pyrrolidone hydrogels; chitosan nasal; carbopol nasal

ABSTRACT

The study evaluated different mucoadhesive polymeric hydrogels for nasal delivery of penciclovir . Gels containing poly-*N*-vinyl-2-pyrrolidone (PVP) were prepared with crosslinking achieved by irradiation with a radiation dose of 15 kGy being as efficient as 20 kGy. Gels containing chitosan and carbopol were also evaluated. The mucoadhesive properties of gels were measured by a modification of a classical tensile experiment, employing a tensile tester and using freshly excised sheep nasal mucosa. Considering the mucoadhesive force, chitosan gel and gel prepared with 3% PVP in presence of polyethylene glycol (PEG) 600 were the most efficient. The in vitro drug release depended on the gel composition. Higher release rates were obtained from PVP gels compared to chitosan or carbopol gels. The release rate of drug from PVP gels was increased further in presence of PEG or glycerol. Histopathological investigations proved that the PVP was a safe hydrogel to be used for mucosal delivery. The PEG in gel formulations caused less damages to the nasal mucosal compared to formulation containing glycerol.

INTRODUCTION

Mucoadhesive polymers are synthetic or natural macromolecules that are capable of attaching to mucosal surfaces. The history of pharmaceutical mucoadhesives started more than 40 years ago. Mucoadhesion is widely investigated as a promising strategy for prolonged and enhanced drug delivery through various membranes (Grabovac et al, 2005).

Nasal route of administration has gained great interest. It provides the formulator with a large absorptive surface with high vascularity, ensuring rapid absorption of drugs bypassing the hepatic first-pass elimination. Unfortunately, the nasal mucosa poses a permeation barrier for drugs, especially high- molecular weight therapeutics, such as peptides and proteins. In addition, the mucociliary clearance provides another addition to the barrier nature by reducing the contact time after nasal application (Mattrin et al, 1998). Accordingly, improving nasal drug delivery can be achieved by enhancing the permeation and/or prolonging the contact time. The tight junctions that form this barrier to paracellular drug delivery can be reversibly and safely opened. An absorption-enhancing effect has been recorded for some polymers and was attributed to widening of tight junctions (Guo *et al.*, 2004). Gels can be employed for nasal delivery, because they give a high drug transport often provided by the longer residence time of the formulation at the site of absorption (Ugwoke et al, 1999). Bioadhesive polymers can be used to achieve a more intimate contact with nasal mucosa, which results in higher concentration gradient and subsequently increasing absorption (Critchley et al, 1994). The advantages of a nasal gel include the reduction of postnasal drip due to high viscosity, reduction of anterior leakage of the formulation, reduction of irritation by using soothing/emollient excipients, and target delivery to mucosa for better absorption. Nasal mucoadhesive gel can provide a viable alternative to the conventional nasal formulations as they provide a high drug transport often provided by the longer residence time of the formulation at the site of absorption (Behl *et al.*, 1998). Polymer gels and mucoadhesive polymers have been studied for the mucosal delivery of various compounds ranging from small molecule to macromolecular drugs. Various bioadhesive polymers such as polyacrylic acids (carbopol 971P) and chitosan have been

* *Corresponding Author*
Arindam Das
Email: piku_arin2006@yahoo.co.in

used in gel forms for nasal delivery. Chitosan is a cationic polysaccharide obtained from deacetylation of chitin, a structural polymer abundant in cretaceous animals such as crabs and shrimps. Because of its biocompatibilty, biodegradability, and low toxicity, chitosan represents an attractive biopolymer for a variety of pharmaceutical applications (Paul *et al.,* 2000). Chitosan may be a good option in nasal delivery as it binds to the nasal mucosal membrane with an increased retention time, and it is also a good absorption enhancer (Varshosaz *et al.,* 2006). Successful mucosal delivery of insulin has been recorded after nasal application of mucoadhesive gel comprising 2% medium molecular weight chitosan with ethylenediaminetetraacetic acid (EDTA) to diabetic rats. This formulation enhanced the absorption of insulin and reduced the blood glucose by 46% compared to intravenous route (Varshosaz *et al.,* 2006). Poly-*N*-vinyl-2-pyrrolidone (PVP) is an example of polymer applied for the synthesis of hydrogel to be used in different medical applications. In recent years, PVP hydrogels have emerged as an important class of biomaterials as they possess excellent biocompatibility (Rosiak *et al.,* 1995). PVP hydrogels have a wide range of applications including transdermal systems as wound dressings (Benamer *et al.,* 2006), nasal drug delivery systems (Bertram *et al.,* 2006), and eye preparation (Yanai *et al.,* 2006). Roos, Creton, Novikov, and Feldstein (2002) investigated a series of blends of high molecular weight PVP (K-90) and polyethylene glycol (PEG) 600. PVP/PEG blends were referred to in the literature as the pressuresensitive adhesives (PSAs). In some applications, such as wound dressing and transdermal drug delivery systems, PSAs act as adhesives and reservoirs for the drug to be delivered. Crosslinking transforms a linear polymer into a three- dimensional (3D) molecule, resulting in a significant increase in molecular mass with improved mechanical properties. High energy radiation in particular gamma rays can be used to polymerize unsaturated compounds by converting water soluble polymers into hydrogels (Rosiak *et al.,* 1999). Penciclovir was chosen for intranasal delivery in this study as it does not undergo degradation and metabolism in the nasal cavity. The currently available dosage regimens of penciclovir have a number of limitations including the following: (a) variable bioavailability by oral administration, (b) poor percutaneous absorption, and (c) thrombophlebitis after intravenous bolus injection. Earlier attempts in literature for improving the bioavailability of penciclovir were based on chemical modification (amino acid ester prodrug), very limited work has been reported on modification, which includes transbuccal delivery and ocular delivery. The objectives of this study were to (a) investigate the mucoadhesive properties of different hydrogels (PVP, chitosan, and carbopol), (b) study drug release profile of penciclovir , and (c) evaluate the safety of the prepared hydrogels on nasal mucosa.

MATERIALS AND METHODS

Materials

Penciclovir was kindly provided by RSTG Chemicals Limited (Hong Kong). Chitosan medium molecular weight (103.200 g/mol) with degree of deacetylation of 76.6% was purchased from Sigma Aldrich Company (St. Louis, MO, USA). Carbopol 974 was obtained from BF Goodrich (Cleveland, OH, USA). PVP known as Luviskol® K-90 was purchased from Aktiengesellschaft (Ludwigshafen, Germany). PEG 600 was purchased from Merck-Schuchardt (Hohenbrunn, Germany). Glycerin BP was purchased from Pacegrove Chemical and Biopharmaceutical Chemicals (Leicestershine, UK). Lactic acid and glutaraldehyde 50% were purchased from BDH Laboratory Supplies (Poole, England). Acetonitrile and triethanolamine were purchased from Sigma Aldrich, Chemie GmbH. All chemicals and organic solvents used were of high-performance liquid chromatography (HPLC) grade and were used as received.

Preparation of Different Hydrogels

Preparation of PVP Gels

PVP gels (3 and 6%, wt/vol) with and without glycerol or PEG 600 were prepared in double distilled water according to the composition presented in Table 1. The polymer was added to the vortex of continuously stirred solvent. Mixing was continued for 3 min before adjusting the pH to 6.4 ± 0.05 using triethanolamine. The mixtures were then filled into glass bottles. Crosslinking was achieved by gamma irradiation. The irradiation of PVP hydrogels was carried out using gamma irradiation with 60Co source at room temperature at radiation doses of 15 and 20 kGy (preliminary study indicated that these doses are appropriate doses for PVP crosslinking). The irradiation time was calculated based on the radiation source dosing rate (51.71 Gy/min). The drug was homogeneously dispersed in the gel after crosslinking in an amount that is sufficient to provide final drug concentration of 20 mg/g.

Preparation of Chitosan Gel

Chitosan solution (2%, wt/vol) was prepared in 1% lactic acid (n = 3). The solution was kept for 4 h for the mixture to equilibrate, and the entrapped air was removed, then two drops of glutaraldehyde have been added and left for 24 h to allow crosslinking process to take place, the pH value of gel was adjusted to 5.6 ± 0.05 using triethanolamine. Lactic acid was used to dissolve chitosan. Lactic acid was found to be biologically safe

Table. 1: Compositions of Various PVP Hydrogels (% wt/vol in water) and Doses of Radiation (kGy)

Gels Code	PVP Concentration	Polyethylene Glycol	Glycerol	Radiation Dose
A	3	—	—	15
B	3	—	—	20
BG1	3	—	1	20
BG2	3	—	2	20
BP1	3	1	—	20
BP2	3	2	—	20
C	6	—	—	15
D	6	—	—	20
DG1	6	—	1	20
DG2	6	—	2	20
DP1	6	1	—	20
DP2	6	2	—	20

(nonirritant) and appeared to be a universal solvent for chitosan (Knapezyk *et al.*, 1993). The drug was incorporated as before.

Preparation of Carbopol Gel

Carbopol gel 2% (wt/vol) was prepared in double distilled water (n = 3). The polymer was added to the vortex of continuously stirred water. The mixture was neutralized using triethanolamine until transparent gel appeared (pH value was 5.6 ± 0.05). The drug was incorporated as before.

Mucoadhesion Performance Evaluation

The mucoadhesive performance of gels was determined by measuring the force required to detach the formulation from nasal mucosal tissue; sheep was selected as an animal model for mucoadhesion performance evaluation. Majithiya, Ghosh, Umrethia, and Murthy (2006) used the sheep mucosa in the evaluation of mucoadhesion of the thermosensitive gelling polymer, Pluronic F127 (PF127) and the mucoadhesive polymer Carbopol 934P. The sheep nasal mucosa was prepared to study mucoadhesion characteristics adopting the procedure described by Gavini, Rassu, Sanna, Cossu, and Giunchedi (2005). Immediately after killing the animal, the nasal mucosa was carefully removed from the nasal cavity of the sheep. Longitudinal incisions were made through the septum wall, and the nose was kept in ice until removing the mucosa. After exposing the nasal cavity on each side of the septum, the mucosal cavity (i.e., the mucous membrane covering the turbinates) was carefully removed using forceps and dissecting scissors. The removal of mucosa was completed within 1 h after killing the animal. The mucosa was cut into small pieces taking into consideration to avoid the areas that were held by forceps.

The mucoadhesive properties of the prepared gels were measured by modification of a classical tensile experiment, using a tensile tester Instron® Texture analyzer model no. 8500 Digital control, with Instron® Series IX automated material tester software, version 8.32.00 (Instron Co., Norwood, MA, USA), equipped with a 5-kg cell. In this test, two equal cylindrical metallic supports with a circular surface of 1.2-cm diameter were constructed; one was connected to the upper movable probe and the other was connected to the lower stationary probe of the apparatus. The mucosa was attached to both upper and lower metal support using cyanoacrylate adhesive (keeping the mucosal side exposed). A 250-mg sample of the gel was placed on the lower mucosa. The upper mucosa was then lowered to the gel surface until contact was achieved between the two surfaces. During the contact phase, the thickness of the gel layer was kept constant (0.5 mm), then the two phases were brought immediately into direct contact with an initial force of 0.5 N for 2 min. The upper mucosa was withdrawn upward at a speed of 5 mm/s until failure occurred between the surfaces. The whole experiment was performed at room temperature and a relative humidity of 50%. This method determines the minimum force needed to separate two surfaces in intimate contact (Bonacucina et al, 2006). During the experiment, the force was recorded as a function of detachment stress (N) until the break point. Data collection and calculation were preformed by the dedicated software, and each measurement was repeated at least five times.

In Vitro Release Studies

Vertical jacketed Franz diffusion cell, 15 mm diameter and 12 mL capacity (Crown Glass Co. Inc., Somerville, NJ, USA), was used for in vitro study. Cellophane membrane Spectra Por® dialysis membrane with a molecular weight cut-off of 12.000–14.000 was mounted between the receiver and the donor compartments of the diffusion cells. The receptor compartment was filled with 12 mL of phosphate buffer of pH 5.5 and the system was maintained at 37°C. The receptor compartment was stirred continuously at a controlled speed using a magnetic stirrer. The test formulations (0.5 g) were loaded into the donor compartments before occluding the donor compartments with parafilm. Receptor samples were taken at predetermined time intervals (30, 60, 120, 150, 180, 210, and 240 min). These samples were replaced with equal volumes of buffer to maintain the sink conditions. All in vitro drug release studies were performed in triplicates, and samples were assayed using HPLC (Jain *et al.*, 2005).

The analysis employed an HPLC system consisted of Intelligent Shimadzu two pumping systems LC-10AT VP, equipped with a data processor Shimadzu chromatography software class-VP, version 6.12 SP1, Shimadzu Corporation (Kyoto, Japan). Separation was achieved using a C18 µ-Bondapak column (300 × 3.9 mm i.d.), packed with 5-µm particles (Waters Inc., Bedford, MA, USA). This was maintained at 25°C (column oven). The mobile phase comprised 0.02 M potassium dihydrogen phosphate (pH 3.5) (99%) and acetonitrile (1%), filtered through 0.45-µm Millipore filter and degassed. The mobile phase was freshly prepared before each experiment. The mobile phase was flowing at a rate of 1.5 mL/min with detection being preformed at 254 nm after injecting 20 µL into the HPLC.

Histopathological Estimation of Nasal Mucosa

To evaluate the effect of the prepared hydrogels on the nasal mucosa, histopathological examination of the sheep nasal mucosa incubated in different gels was performed. The sheep's nasal mucosa was pretreated in the same manner as for mucoadhesion measurement. The mucosal tissues were incubated in different gel formulations for 5 h at 25°C. The mucosal tissue was fixed in 10% formalin solution for 48 h and then embedded in paraffin wax. Paraffin sections (7 µm) were cut on glass slides and stained with hematoxylin and eosin (HX–E). Sections were examined for any changes under a light microscope connected to a digital camera. Mucosal tissues incubated in isotonic phosphate buffer saline for 5 h were used as a control for comparison.

Statistical Analysis

Data were expressed as a mean of three experiments ± the *SD*. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS® statistical package (version 13, 2001,

SPSS Inc., Chicago, IL, USA). Statistical differences yielding $p \leq 0.05$ were considered significant. Tukey's multiple comparison post hoc tests were applied.

RESULTS AND DISCUSSION

Mucoadhesion Performance Evaluation

Most in vitro mucoadhesion studies have provided valuable information on the force of adhesion by measuring tensile strength or calculating the detachment force and work of adhesion. Methods using tensile strength usually examine the force necessary for separation of the two surfaces after establishment of mucoadhesion. This force is recorded as a function of the elongation observed at polymer-mucus interfacial (Ponchel et al, 1991). Depending on the direction in which the adhesive is being separated from the substrate (peel), the shear and tensile force necessary to detach a polymer formulation from mucosa can be measured (Hägerström *et al.*, 2003).

Figures 1–3 illustrate the mucoadhesion performance of the prepared gels. Considering the overall adhesion force at different contact time values, chitosan gel and gels prepared with PVP (3%) in presence of PEG showed the greatest force of adhesion.

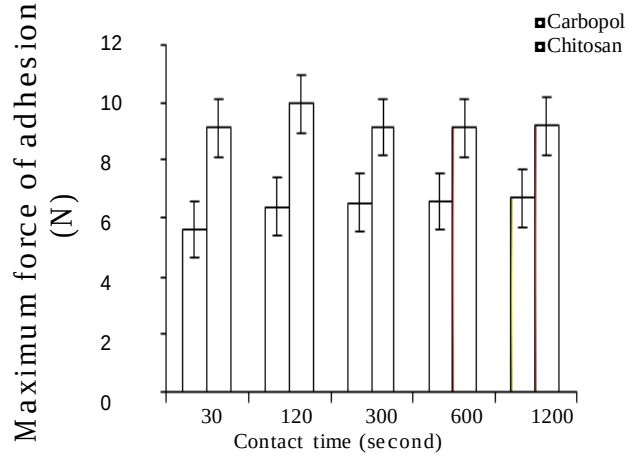

Fig. 1: Force of adhesion of chitosan and carbopol gel.

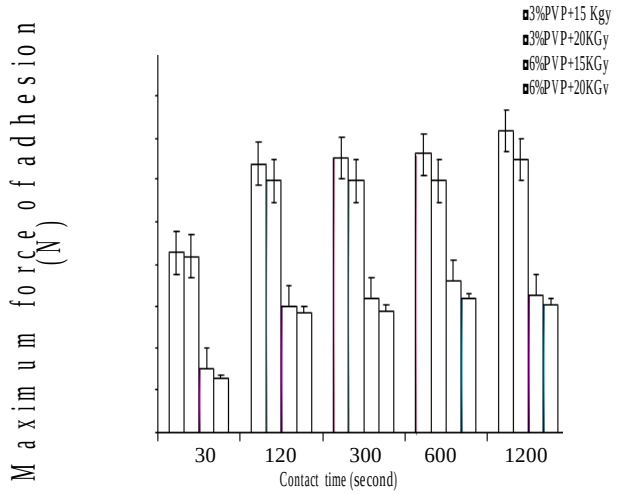

Fig. 2: Effect of radiation dose and PVP concentration on the mucoadhesive effect of PVP gel.

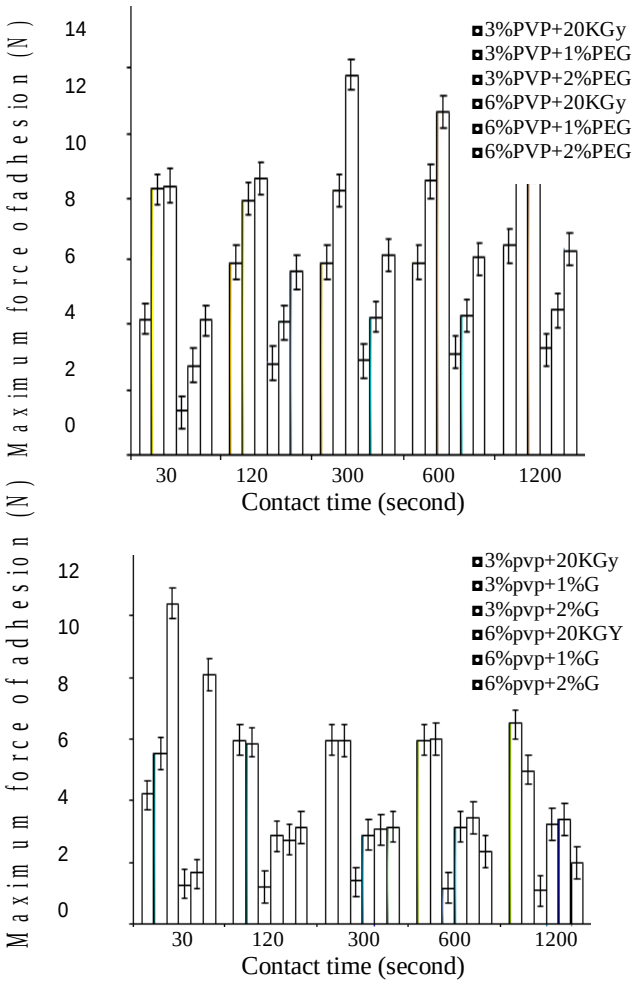

Fig. 3: Effect of incorporation of (A) polyethylene glycol 600 (PEG) or (B) glycerol (G) on the mucoadhesive properties of PVP gel.

Gels can be arranged according to their adhesiveness in descending order as 3% PVP with PEG ≈ chitosan > 3% PVP at 15 kGy > carbopol > 3% PVP at 20 kGy > 3% PVP with glycerol > 6% PVP with PEG > 6% PVP with glycerol > 6% PVP at 15 kGy > 6% PVP at 20 kGy. An electron transfer process has been previously adopted to explain mucoadhesion (Peppas *et al.*, 1996). According to this process, electron transfer occurs on contact between adhering surfaces resulting in the formation of an electrical double layer at the interface, with subsequent adhesion because of attractive forces. Studies have shown that the mucoadhesive properties of polymers containing ionizable groups are affected by the pH of the surrounding media. For cationic polymers like chitosan, the positive charge will favor binding to negatively charged groups (such as carboxyl or sulphate) on the mucin or cell surface (Fiebrig at al, 1995). The results shown here are in good agreement with the work of (Lehr *et al.*, 1992). Thus, at nasal pH (5.5–6.5), the cationic polymer, chitosan, exists in the ionized form. The NH3+ groups on the deacetylated *N*-acetyl groups can thus undergo electrostatic interaction with the negatively charged mucus. It should be noted that the mucoadhesion of chitosan polymers may be weakened by its

solubility in that acidic medium; however, this effect can be neutralized by chemical crosslinking (Lehr *et al.,* 1992).

On the other hand, the adhesion behavior of carbopol gel was significantly lower than that of chitosan gel ($p < 0.05$) (Figure 1). This can be returned to the experimental pH (nasal pH). A previous study showed that carbopol did not adhere when applied as gel to buccal mucosa in organ culture, whereas, chitosan gel showed up to 4 days retention (Needleman *et al.,* 1995).

As poly(acrylic acid) hydrogels contain coiled macromolecules, it is unable to form an elastic polymer network due to the repulsion of negative charges; many of the active groups are shielded inside the coils and do not actively participate in the adhesion process (Tamburic *et al.,* 1997). As carbopol is strongly coiled into a spiral form in dry powder state, when dispersed in water, the molecules become hydrated and slowly unwind, generating an increase in viscosity. To ensure maximum viscous effects, the molecules must unwind completely. The most common method to unwind the molecules involves neutralization of the negative charges along the polymer chains with appropriate base. Repulsion between these charges contributes to unfold the structure while intertwining of chains yields a 3D matrix. The result is the instantaneous formation of a highly viscous gel (Hernández et al, 1998). Triethanolamine was preferred as a neutralizing agent, since relatively higher viscosity could be obtained using organic amines than using inorganic bases. This effect was described previously by Tamburic and Craig (1995) with the cations generated by amines, resulting in a greater expansion of polymer molecules than the smaller sodium cations generated by inorganic bases as sodium hydroxide, hence monovaliant salt could generally lead to lower hydration of poly (acrylic acid).

Figure 2 shows the effect of radiation dose and the PVP concentration on the mucoadhesive efficiency of PVP gels. The data revealed no significant differences between gels containing the same concentration of polymer and radiated with different radiation doses. The results revealed that 3% PVP gels have significantly higher forces of adhesion than 6% PVP gels ($p < 0.05$).

The physical and mechanical characteristics of the PVP gels can depend on the radiation dose as well as the presence of additives in solution. The irradiation causes crosslinking between the PVP chains and consequently results in the formation of a polymer network that influences the mechanical behavior of the resultant product (Güner *et al.,* 1997). The effect of radiation dose on crosslinking density has been studied and showed that increasing of crosslinking density by increasing the radiation dose can result in lower maximum swelling percent (Can et al, 2005). Water uptake is higher when the network is connected by relatively low number of intermolecular bonds, and it decreases as the crosslinking density increases. Therefore, the swelling of crosslinked hydrogels depends on the absorbed irradiation dose and the polymer concentration. Also, the swelling degree depends on the polymer concentration; the higher the polymer concentration, the lower is the equilibrium water uptake (Benamer *et al.,* 2006). In agreement with this, our study revealed reduced mucoadhesion with increasing PVP concentration. However, increasing the radiation dose from 15 to 20 kGy did not produce significant effect on the adhesion force. This can be explained taking into consideration that exposing the system to 15 kGy of radiation could have produced the maximum crosslinking with any further increase in the radiation dose producing no significant effect.

PVP hydrogel itself is of limited adhesiveness; consequently, blending PVP with other polymers has a significant role in a series of PVP hydrogels as biomedical materials. Figure 3 also shows the effect of PEG on the adhesion performance of PVP hydrogels. It is clear that the forces of adhesion of PVP gels at different time intervals increase as the concentration of PEG increases ($p < 0.05$). PEG is an additive that is typically used in the case of PVP hydrogels for biomedical applications. It also allows changes in the rheological characteristics of PVP hydrogels. The presence of PEG usually increases the elasticity, adhesion, and tacky properties of the hydrogels because of its plasticizing effect. The PEG chains settle among those of PVP avoiding crosslinking and decreasing the physical interactions between PVP chains (Sen *et al.,* 2005). The increase in the force of adhesion of PVP hydrogels containing PEG can be returned to the ability of PEG to form hydrogen bonds with mucosal surfaces. The presence of glycerol decreases the crosslinking density of the PVP network leading to increase in the expected bioadhesive performance owing to the flexibility of polymer chains allowing interpenetration and entanglement of the hydrogel in the substrate (Rosiak *et al.,* 1999). Furthermore, it was reported that glycerol has three folds the hygroscopicity of PEG 600. Consequently, glycerol increases the fluidity of the PVP hydrogel (Rosiak *et al.,* 1999). As a result of the hydration, adhesion might be promoted by tendency to dehydrate and consolidate an intermediate mucin layer. Accordingly, at a contact of 30 s, the order of adhesion was 3% PVP with 2% glycerol > 3% PVP with 1% glycerol > 3% PVP. However, excessive hydration of 3% PVP with 2% glycerol at a contact time of 120 s with mucus membrane resulted in a significant drop in the adhesive strength as compared with 3% PVP with 1% glycerol and 3% PVP without glycerol ($p < 0.05$) (Figure 3). This is clearly an indication of disentanglement at the hydrocolloid-tissue interface due to slippage of the macro-molecular chains of the formulations, leading eventually to the separation of the adhesive polymer and the substrate (Eouani et al, 2001). Ponchel et al. (1991) described a model, whereby the strength of mucoadhesive bond is considered to be a function of both the interaction energy (adhesion work) between the mucoadhesive polymer/ mucosa and the viscoelastic properties at the interfacial layer formed between the two surfaces. The results of force of detachment tests within polymermucin systems are therefore almost certainly a function of viscoelastic moduli of the polymers themselves (Eouani et al., 2001; Tamburic *et al.,* 1995).

The mechanical theory of adhesion assumes that adhesion arises from an interlocking of a liquid adhesive (on

setting) into the irregularities present on the surface. The process by which polymers spread and retain on mucosa will depend principally on the spreadability along with the flowability of the liquid (Peppas *et al.*, 1996). The dispersion will need to be sufficiently mobile to allow spreading and interaction, but not being so mobile to be readily dislodged. Following the mechanical theory of adhesion, only 3% PVP gel with 1% PEG and all 6% PVP gels, which exhibited rheopectic behavior, can be the candidates for mechanical adherence to mucous membranes. As the gel able to thin after being exposed to a shearing force (shear thinning) that facilitates the flow of the formulation (easily spread) over mucosal surface during instillation, followed by a fast tendency to thicken when stress is removed (rheopectic behavior).

In Vitro Release Studies

The amount of drug available at the site of application depends on the release from formulation toward the underlying tissues and on the loss (because of diffusion of the drug and erosion of the formulation) toward the external environment (Bonferoni et al, 1999). There are important factors to be considered in the evaluation of the drug penetration across an artificial membrane: the pH value of the vehicle and drug solubility in vehicle. The release profile of penciclovir from gels through cellulose membrane previously demonstrated that this type of membrane does not exert any effect on release (Diez-Sales *et al.*, 2005). The pH value of the buffer used in release study was chosen to guarantee the solubility of penciclovir as well as to resemble the normal pH range of nasal cavity.

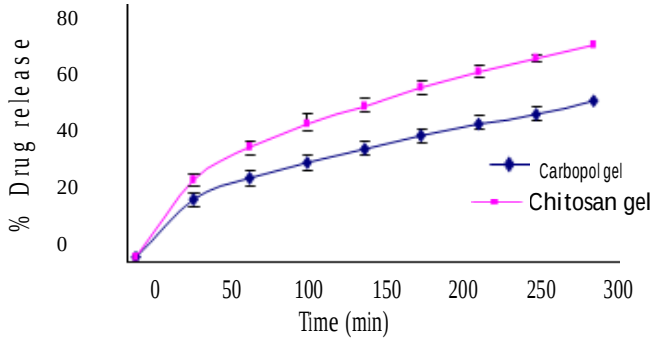

Fig. 4: In vitro release profiles of acyclovir from carbopol and chitosan gel.

Figures 4 and 5 show the in vitro release profiles of penciclovir from different gel formulations. The apparent release kinetic model was determined for each of the promising formulations. The release kinetics was tested for zero-order, first- order, and diffusion kinetic models. For carbopol and chitosan gels, there were a linear relationship between the percent of drug release and the square root of time with the *r*2-values higher than that obtained after fitting the data to other orders, suggesting matrix diffusion system. These results were comparable to that previously reported by Diez-Sales et al. (2005) who studied the release profile of penciclovir from carbopol hydrogels. It has been shown that the Higuchi model is appropriate for describing the release mechanism of penciclovir from carbopol hydrogels. For PVP gels, the obtained results showed that all cases of 6% PVP gels followed diffusion (Higuchi) model of drug release ($r2 > 0.999$). The release of penciclovir from 3% PVP gels without additives was best fitted to diffusion (Higuchi) model. However, the release profiles of 3% PVP gels with different concentrations of glycerol and PEG were best fitted to first-order release kinetic. The alteration in the release kinetic of 3% PVP hydrogels due to the effect of additives can be correlated to the dramatic action of both glycerol as well as PEG on the crosslinking of PVP where the drug release was affected only by the remaining drug concentration in the gel at any time (first-order model).

The data (Figure 4) revealed higher drug release rate from chitosan gel compared to that obtained from carbopol gel. (Bonferoni et al. 1999) have established that the diffusion rate is inversely related to sample consistency, suggesting that the internal structure described by viscoelastic parameters is to some extent relevant to drug release.

The effect of radiation doses and polymer concentration on the release of penciclovir from 3% PVP hydrogels are shown in Figure 5. It is expected that increasing the radiation dose will increase the degree of crosslinking with the result that the release rate is reduced. In our study, increasing the radiation dose provided only a trend of reduced release rate. This results together with the previously discussed mucoadhesion data indicate that radiation dose of 15 kGy is sufficient for the crosslinking of PVP gel.

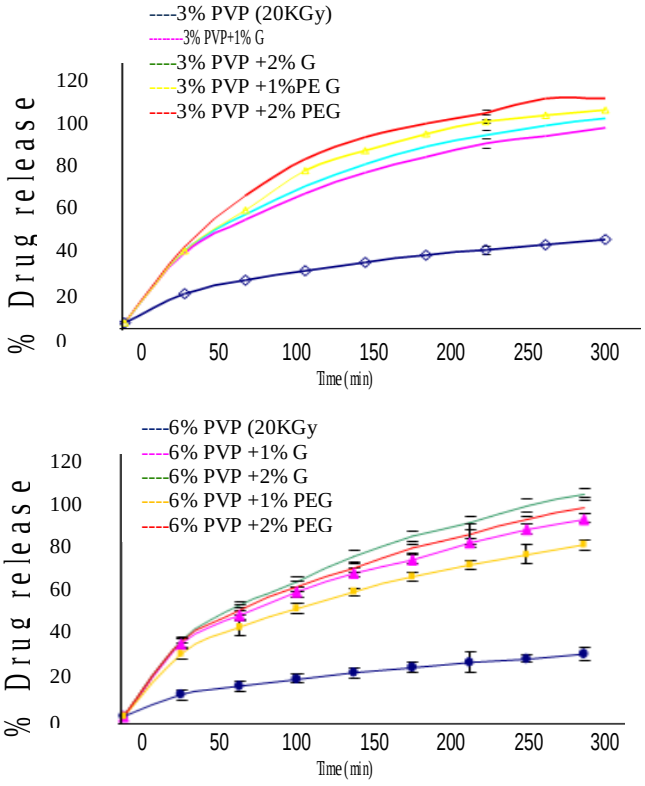

Fig. 5: In vitro release of profiles of acyclovir from (A) 3% PVP gels and (B) 6% PVP gels prepared in water at different radiation doses or prepared in PEG or glycerol solution in water at a radiation dose of 20 kGy.

The presence of PEG and glycerol as cosolvents augmented the release rate of penciclovir from PVP gel (Figure 5). Because of the hygroscopicity of both PEG and glycerol, an increase in the water uptake from the release medium increases the dissolving medium available for the drug in addition to the reduction of the viscosity of the gel. This is expected to increase the drug release rate from the gel. This can explain the increased drug release in the presence of PEG or glycerol in the gel. The higher hygroscopicity of glycerol relative to PEG can also explain the obtained trends of higher release rates in the presence of glycerol compared to gels containing PEG. It was previously pointed out that the viscosity of gel matrix controls the drug release. As the actual pathway for drug diffusion in gels is the fluid phase entrapped in the gel pores, the factors that affect diffusivity in pure liquid phases can control diffusion within gels. Therefore, the gel viscosity is an important factor, which influences the release of drug from hydrogels (Diez-Sales *et al.*, 2005). This estimation can be confirmed through.

Stokes–Einstein equation (Florence *et al.*, 1998):

$$D = \frac{RT}{6phrN},$$

where D is a diffusion coefficient, h is the viscosity, R is a gas constant, T the absolute temperature, r is the radius of the particles, and N is the Avogadro number. From the equation, it is obvious that the diffusion coefficient of drugs depends on the viscosity of the dispersion medium, where the diffusion is inversely proportional to the viscosity of the matrix.

Histopathological Evaluation of Nasal Mucosa

The histological study of the sheep's nasal mucosa showed that the sheep's nasal mucosa is identical to that of human after extensive investigation of all possible models (Illum *et al*,1996; and Shaw *et al*, 2001). Normal respiratory epithelium is a pseudostratified, ciliated, and columnar epithelium. Figure 6 shows a microscopical representation of normal mucosal histology (used as control nasal mucosa). For all gels, no severe damage was found on the integrity of nasal mucosa. The observed changes on nasal mucosa can be summarized as congestion of vessels, complete loss of some parts of the epithelium, and mild inflammation that occurs normally in respiratory epithelium as defense mechanism when particulate matter in inspired air is trapped in a thin layer of surface mucous. Figure 7A illustrated chitosan gel with mild inflammation, loss of ciliary processes, and congestion of vessels, whereas more severe effects were observed with carbopol gel, which showed complete loss of epithelium layer (Figure 7B)

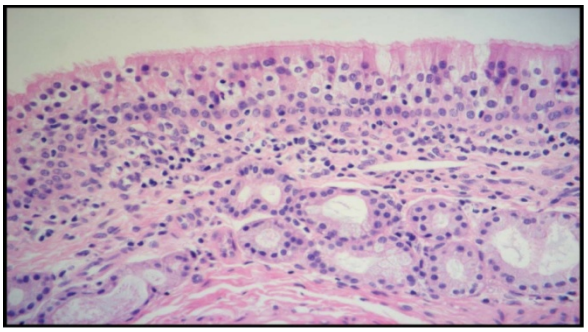

Fig. 6: Photomicrograph of the normal sheep nasal mucosa (control), showing normal respiratory epithelium with preserved cilia (magnification size × 40).

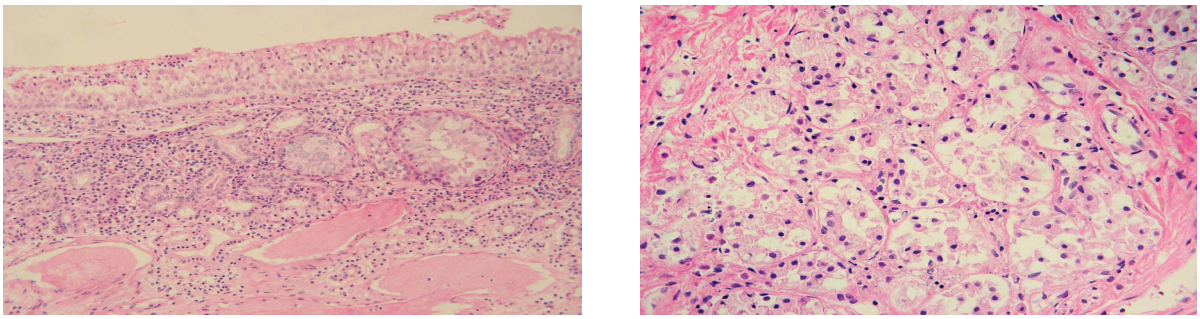

Fig. 7: The histology of the sheep nasal mucosa (A) after application of chitosan gel with the micrograph showing inflammation (a), loss of ciliary processes (b), and congestion of vessels (c) (magnification size, × 20) and (B) after application of carbopol gel with the micrograph showing (a) complete loss of epithelium and (b) mild inflammation (magnification size, × 40).

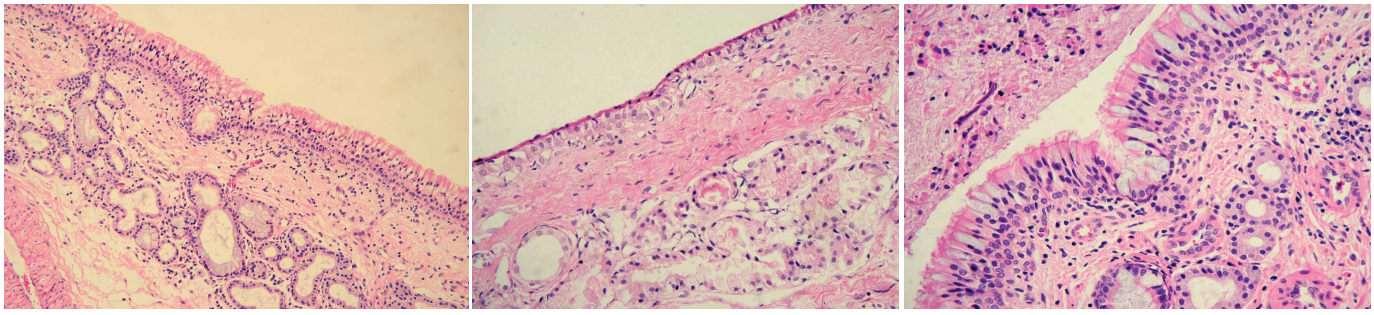

Fig 8. Histological characterization of nasal mucosa after application of (A) PVP without additives with the micrograph showing (a) loss of cilia and (b) mild inflammation (magnification size, × 20); (B) PVP gel containing glycerol with micrograph showing (a) degenerated mucus glands and (b) karyolysis (cells without nuclei) (magnification size, × 40); and (C) PVP gel containing PEG with the micrograph showing preserved cilia (magnification size, × 40).

Practically, loss of the cilia with no sign of inflammation occurred with PVP gels without additives (Figure 8A), whereas PVP gels with glycerol showed congestion of capillaries, degenerated mucous glands with dense mononuclear inflammatory infiltration and karyolysis (cells without nuclei) (Figure 8B). The gel formulation containing PVP with PEG produced no signs of inflammation, and the integrity of cilia was maintained (Figure 8C). These findings indicate that PVP with PEG can be considered as a safe and appropriate nasal gel formula when compared with the other prepared gels. PEGs have frequently been chosen as drug carriers because of their biocompatibility, immunogenicity, minimal toxicity, and good solubility in water or other common solvents (Won et al, 1998). Haslwanter and Rencher (1999) have discovered that incorporation of PVP with PEG mixtures into nasal spray compositions provides enhanced medicinal efficacy and promotes organoleptic acceptance of the preparations. It was found that 0.25% PVP solution would extend nasal mucociliary clearance times from the normal 8–10 to 20–25 min, and the use of PEG in the compositions of this system promoted moisture in the nasal cavity.

CONCLUSION

The study provided a promising mucoadhesive system for nasal delivery of penciclovir . The PVP gels were prepared with the assistance of radiation that imparted the crosslinking with a radiation dose of 15 kGy being as efficient as 20 kGy. Considering the mucoadhesive force, chitosan gel and gel prepared with 3% PVP in presence of PEG 600 showed the mucoadhesive potential. The release characteristics of hydrogels revealed higher release rates from PVP gels with the release rate increasing further in presence of PEG or glycerol. Histopathological investigations proved that the PVP was a safe hydrogel to be used for mucosal delivery. The nasal mucosal damages were less severe with the presence of PEG in gel formulations as compared to that produced by the formulations containing glycerol.

REFERENCES

Behl, C. R., Pimplaskar, H. K., Sileno, J. deMeireles, J., & Romeo, V. D. Effects of physicochemical properties and other factors on systemic nasal drug delivery. Adv. Drug Deli. Rev. 1998; 29, 89–116.

Benamer, S., Mahlous, M., Boukrif, A., Mansouri, B., & Youcef, S. L. Synthesis and characterization of hydrogels based on poly(vinyl-pyrroli- done). Nucl. Instrum. Methods. Phys. Res. B., 2006; 248, 284–290.

Bertram, U., & Bodmeier, R. In situ gelling, bioadhesive nasal inserts for extended drug delivery: In vitro characterization of a new nasal dosage form. Eur. J. Pharm. Sic. 2006; 27, 62–71.

Bonacucina, G., Cespi, M., Misici-Falzi, M., & Palmieri, G. F. Rheological, adhesive and release characterization of semisolid carbopol/tetragly- col systems. Int. J. Pharm. 2006; 307, 129–140.

Bonferoni, M. C., Rossi, S., Ferrari, F., & Caramella, C. A modification Franz diffusion cell for simultaneous assessment of drug release and washability of mucoadhesive gels. Pharm. Devel. Technol. 1999; 4, 45–53.

Can, H. K., Denizli, B., Kavlak, S., & Guner, A. Preparation and swelling studies of biocompatible hydrogel systems by using gamma radiation- induced polymerization. Radiat. Phys. Chem. 2005; 72, 483–488.

Critchley, H., Davis, S. S., Farraj, M. F., & Illum, L. Nasal absorption of desmopressin in rats and sheep. Effect of a bioadhesive microspheres delivery system. J. Pharm. Pharmacol. 1994; 46, 651–656.

Diez-Sales, O., Garrigues, T. M., Herraez, J. V., Belda, R., Martín-Villodre, A., & Herraez, M. In vitro percutaneous penetration of penciclovir from solvent systems and Carbopol 971-P hydrogels: Influence of propylene glycol. J. Pharm. Sci. 2005; 94, 1039–1047.

Eouani, C., Piccerelle, P. H., Prinderre, P., Bourret, E., & Joachim, J. In-vitro comparative study of buccal mucoadhesive performance of different polymeric films. Eur. J. Pharm. Biopharm. 2001; 52, 45–55.

Fiebrig, G., Harding, S. E., Rowe, A. J., Hyman, S. C., & Davis, S. S. Transmission electron microscope studies on pig gastric mucin and its inter- actions with chitosan. Carbohydr. Polym. 1995; 28, 239–244.

Florence, A. T., & Attwood, D. Physicochemical principles of pharmacy. London: Macmillan Press. 3rd ed. (1998).

Gavini, E., Rassu, G., Sanna, V., Cossu, M., & Giunchedi, P. Mucoadhesive microspheres for nasal administration of an antiemetic drug, metaclopramide: In-vivo/ex-vivo studies. J. Pharm. Pharmacol. 2005; 57, 287–294.

Grabovac, V., Guggi, D., & Bernkop-Schnurch, A. Mucoadhesive polymers: Strategies, achievements and future challenges. Adv. Drug Deliv. Rev. 2005; 57, 1713–1723.

Güner, A., & Ataman, M. Effects of inorganic salts on the properties of aqueous PVP solutions. Colloid. Polym. Sci., 1997; 272, 175–180.

Guo, J., Ping, Q., Jiang, G., Dong, J. Q. S., Feng, L., Li, Z., & Li, C. Transport of leuprolide across rat intestine, rabbit intestine and Caco-2 cell monolayer. Int. J. Pharm., 2004; 278, 415–422.

Hägerström, H., & Edsman, K. Limitations of the rheological mucoadhesion method: The effect of the choice of conditions and the rheological synergism parameter. Eur. J. Pharm. Sci., 2003; 18, 349–357.

Haslwanter, J. A., & Rencher, W. F. Nasal spray compositions exhibiting increased retention in the nasal cavity, U.S. Patent No. 5897858, April 27, 1999.

Hernández, M. J., Pellicer, J., Delegido, I., & Dolz, M. Rheological characterization of easy to disperse (ETD) carbopol hydrogels. J. Disper. Sci. Technol., 1998; 19, 31–42.

Illum, L. Nasal delivery. The use of animal models to predict perfor- mance in man. J. Drug Target., 1996; 3, 427–442.

Jain, S. K., Jain, R. K., Chourasia, M. K, Jain, A. K., Chalasani, K. B., Soni, V., & Jain, A. Design and development of multivesicular liposomal depot delivery system for controlled systemic delivery of penciclovir sodium. AAPS PharmSciTech., 2005; 6, 35–41.

Knapezyk, J. Chitosan hydrogel as a base for semisolid drug forms. Int. J. Pharm., 1993; 93, 233–237.

Lehr, C. M., Bouwstra, J. A., Schacht, E. H., & Junginger, H. E. In-vitro evaluation of mucoadhesive properties of chitosan and some other nat- ural polymers. Int. J. Pharm. 1992; 78, 43–48.

Majithiya, R. J., Ghosh, P. K., Umrethia, M. L., & Murthy, R. S. Thermoreversible-mucoaadhesive gel for nasal delivery of sumatriptan. AAPS. PharmSciTech. 2006; 7, 1–7.

Mattrin, E., Schipper, N. G. M., Verhoef, J. C., & Merkus, F. W. Nasal mucociliary clearance as a factor for nasal drug delivery. Adv. Drug Deliv. Rev. 1998; 29, 13–38.

Needleman, I. G., & Smales, F. C. In vitro assessment of bioadhesion for periodontal and buccal drug delivery. Biomaterials. 1995; 16, 617–624.

Paul, W., & Sharma, C. P. Chitosan, a drug carrier for the 21st century: A review. S.T.P. Pharm. Sci. 2000; 10, 5–22.

Peppas, N. A., & Sahlin, J. J. Hydrogels as mucoadhesive and bioadhe- sive materials: a review. Biomaterials. 1996; 17, 1553–1561.

Ponchel, G., Lejoyeux, F., & DucheÃne, D. Bioadhesion of poly (acrylic acid) containing systems. Thermodynamics and rheological aspects. Proceedings of International Symposium on Control Release Bioactive Materials. 1991; 18, 111–112.

Roos, A., Creton, C., Novikov, M. B., & Feldstein, M. M. Viscoelasticity and tack of poly(vinyl pyrrolidone)–poly(ethylene glycol) blends. J. Polym. Sci. Part. B Polym. Phys. 2002; 40, 2395–2409.

Rosiak, J. M., Ulanski, P., Pajewnski, L. A., Yoshii, F., & Makuuchi, K. Radiation formation of hydrogels for biomedical purpose. Some remarks and comments. Radiat. Phys. Chem. 1995; 46, 161–168.

Rosiak, J. M., & Yoshii, F. Hydrogels and their medical applications. Nucl. Instr. Methods Phys. Res. B. 1999; 151, 56–64.

Sen, M., & Avc, E. N. Radiation synthesis of poly(N-vinyl-2-pyr- rolidone)–k-carrageenan hydrogels and their use in wound dressing applications. I. Preliminary laboratory tests. J. Biomed. Mater. Res. A. 2005; 1, 187–196.

Shaw, C. K. L., Cowin, A., & Wormald, P. J. Standardization of the sheep as a suitable animal model for studying endoscopic sinus surgery. Aust. J. Otolaryngol. 2001; 4, 23–26.

Tamburic, S., & Craig, D. Q. M. An investigation into the rheological, dielectric and mucoadhesive properties of poly (acrylic acid) gel system.J. Control Release. 1995; 37, 59–68.

Tamburic, S., & Craig, D. Q. M. Rheological evaluation of poly- acrylic acid hydrogels. Pharm. Sci. 1995; 1, 107–109.

Tamburic, S., & Craig, D. Q. M. A comparison of different in vitro method for measuring mucoadhesive performance. Eur. J. Pharm. Biop- harm. 1997; 44, 159–167.

Ugwoke, M. I., Van den Mooter, G., Verbeke, N., & Kinget, R. Nasal mucoadhesion delivery systems of the antiparkinsonain drug, apomorphine; influence of drug loading on in-vitro and in-vivo release in rabbits. Int. J. Pharm. 1999; 181, 125–138.

Varshosaz, J., Sadrai, H., & Heidar, A. Nasal delivery of insulin using bioadhesive chitosan gels. Drug Deliv. 2006; 13, 31–38.

Won, C. Y., Chu, C. C., & Lee, J. D. Novel biodegradable copolymers containing pendant amine functional groups based on aspartic acid and poly(ethyleneglycol). Polymer. 1998; 25, 6677–6681.

Yanai, R., Yamada, N., Ueda, K., Tajiri, M., Matsumoto, T., Kido, K., Nakamura, S., Saito, F., & Nishida, T. Evaluation of povidone–iodine as a disinfectant solution for contact lenses: Antimicrobial activity and cytotoxicity for corneal epithelial cells. Cont Lens. Anterior. Eye. 2006; 29, 85–91.

How to cite this article:

Arindam Das, Prof. B. K. Gupta, Bhole Nath., Mucoadhesive Polymeric Hydrogels for Nasal Delivery of Penciclovir . J App Pharm Sci. 2012; 2 (12):158-166. .