



**IMPROVED CORNEAL ABSORPTION OF TIMOLOL MALEATE BY FORMULATING
AS NANOPARTICULATED MUCOADHESIVE OCULAR DRUG DELIVERY SYSTEM**

Senthil Venkatachalam*, Nirmal Franklin and Merikanapalli V. Harsha

Department of Pharmaceutics, Jagadguru Sri Shivarathreeswara University, Mysore. JSS College of Pharmacy,
Rockland's, Udhagamandalam, Tamil Nadu, India.

***Corresponding Author: Dr. Senthil Venkatachalam**

Department of Pharmaceutics, Jagadguru Sri Shivarathreeswara University, Mysore. JSS College of Pharmacy, Rockland's, Udhagamandalam,
Tamil Nadu, India.

Article Received on 21/12/2016

Article Revised on 11/01/2017

Article Accepted on 01/02/2017

ABSTRACT

Glaucoma is one of the major causes of blindness in the world after cataract. Management of glaucoma through eye drops that reduce IOP (Intra ocular pressure) has major deficiencies including low patient compliance and low bioavailability. Many current anti-glaucoma drugs, available for the treatment of ocular hypertension and open angle glaucoma are associated with rapid and extensive pre-corneal loss caused by drainage and high tear fluid turnover. The objective of this study was design and development of mucoadhesive nanoparticles of Timolol Maleate for increasing the ocular bioavailability from 1-3% to at least 15-20% and to provide sustained release of the drug. The NPs (Nanoparticles) were prepared by using mucoadhesive polymers such as chitosan and poly(acrylic acid). The mean size of NPs was found to be 141.29 nm with PI of 0.339 and zeta potential of the NPs was positive (+23.30 mV). Irritancy Test (HET-CAM Method) no hemorrhage were observed and hence the study certified that the developed formulation is nonirritant. The encapsulation efficiency of the optimized batch showed 69.73% release profile of Timolol Maleate loaded nanoparticles initial burst followed by a very slow drug release and total cumulative release within 24 h ranged from 75 to 90%. *In vitro* release of the drug loaded batch showed that that the formulation undergo anomalous diffusion. In conclusion, the polymerization time extended it showed the maximum entrapment efficiency of 69.75 % and gives a sustained effect in ocular milieu.

KEYWORDS: Ocular drug delivery, Chitosan, Timolol Maleate, Mucoadhesive nanoparticles, Glaucoma.

1. INTRODUCTION

Recently polymeric nanoparticles have been widely investigated as a carrier for drug delivery. Drug delivery in ocular therapeutics is a challenging problem. Various ocular diseases like glaucoma, conjunctivitis, dry eye syndrome, etc., are requiring frequent drug administration. The major problem encountered in drug delivery to the eyes is the attainment of an optimum concentration at the site of action.

Topical ocular therapy is often impaired by natural physiological defense mechanisms like blinking and increase tear turnover after drug application, and also by the barriers of pre-corneal area. As the consequence of these factors the rate of drug loss from the eye can be 500 to 700 times greater than the rate of absorption into the anterior chamber and consequently 1% to 5% or less of the drug applied topically as a solution reaches the inner part of the eye. (Mitan R *et al.*, 2007).

For topical ocular drug delivery system solution and suspensions are frequently used and it will not affect the normal vision. Nevertheless, these formulations not

effective because these are diluted with tear fluids and drained rapidly through nasolacrimal duct. (Sanchez A *et al.*, 2006).

The common sites of application where mucoadhesive polymers have the ability to deliver pharmacologically active agents include oral cavity, eye conjunctiva, vagina, nasal cavity and gastrointestinal tract.

Buccal cavity provides an opportunity to deliver pharmacologically active agents systemically by avoiding hepatic first pass metabolism in addition to the local treatment of the oral lesions (Roy K Pal *et al.*, 2009).

The bioavailability and the duration of therapeutic action of ocular drugs can be divided into two categories, the first is based on the use of drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs. The second involves maximizing corneal absorption and minimizing pre corneal drug loss. To enhance the amount of the active substances reaching the target tissue or exerting a local effect in the cul-de-

sac, the residence time of drug in the tear should be lengthened (sangita D kute *et al.*, 2010).

Soumendra Sahoo *et al.*,(2010) reported that hydrophilic polymers those containing carboxylic group exhibit the best mucoadhesive properties, the mucoadhesive polymers used for the ocular delivery include thiolated (acrylic acid), poloxamer, celluloseacetophthalate, methyl cellulose, hydroxyl ethyl cellulose, poly (amidoamine) dendrimers, poly (dimethyl siloxane) and poly (vinyl pyrrolidone).

Limiting the clearance by increasing the viscosity of a drug formulation and also prolonging the contact time through mucoadhesive interactions will result in an increased bioavailability of the drug. Polymeric enhancers/mucoadhesive polymers would be a promising candidate for these kinds of applications. (A. Anitha *et al.*, 2011).

2. MATERIALS AND METHODS

2.1. Material

Timolol maleate was obtained from Micro labs, Bangalore. Chitosan (medium molecular weight)- (Aldrich), Acrylic acid from Krishna chem industry, Potassium persulfate were supplied by Sigma Aldrich, Calcium chloride dehydrate, Sodium bicarbonate, Sodium chloride and Sodium hydroxide pellets by Sisco,

Potassium chloride by SD fine. chem. ltd. Dialysis bag (MWCO 12KDA) brought from Himedia labs.

2.2. Formulation and optimisation of chitosan-poly acrylic acid (CS-PAA) nanoparticles

Different batches of CS-PAA nanoparticles were prepared with different molar concentration ratios of acrylic acid and chitosan both in atmosphere and inert (Nitrogen) conditions by the template polymerization method. In order to induce polymerisation of acrylic monomers, an initiator potassium persulfate was added to the solution of chitosan dissolved in 50 ml of acrylic acid (AA). The pH value of the system was maintained at about 4. The mixture was maintained at 70°C, under magnetic stirring and nitrogen atmosphere. The reaction was stopped once the mixture became opalescent. The solution was dialyzed against distilled water in a 12-14 KD cut off membrane for three days, to remove the unreacted monomer and other reagents.

When the polymerization of acrylic acid reached a certain level, the inter and intra molecular linkages occurred between carboxyl groups from poly acrylic acid and positively charged amino groups of chitosan. These linkages could make the macromolecular chains of chitosan rolling up, which was responsible for the formation of the gelation of the chitosan solution. (Changzheng Yang *et al.*, 2002).

Table: 1. Varying concentrations of excipients (atmospheric condition)

UNDER ATMOSPHERIC CONDITIONS					
BATCH	ACRYLIC ACID	CHITOSAN	POTASSIUM PERSULFATE	OPALESCENCE	CONSISTENCY
F1	3mM	12mM	0.1mM	Negative	--
F2	6mM	6mM	0.1mM	Negative	--
F3	12mM	3mM	0.1mM	Good	unstable

Table: 2. Varying concentrations of excipients (inert condition)

UNDER INERT CONDITIONS					
BATCH	ACRYLICACID	CHITOSAN	POTASSIUM PERSULFATE	OPALESCENCE	CONSISTENCY
F4	3 mM	3 mM	0.1 mM	Less	stable
F5	6 mM	3 mM	0.1 mM	Moderate	Stable
F6	12 mM	3 mM	0.1 mM	Good	Stable
F7	3 mM	6 mM	0.1 mM	Less	Stable
F8	6 mM	6 mM	0.1 mM	Good	Stable
F9	12 mM	6 mM	0.1 mM	Moderate	Stable
F10	3 mM	12 mM	0.1 mM	Less	stable
F11	6 mM	12 mM	0.1 mM	Less	stable
F12	12 mM	12 mM	0.1 mM	moderate	stable

2.3 Preparation of Drug Loaded Batches

The drug loaded nanoparticles were prepared by dissolving 250mg of Timolol Maleate in 50ml of CS-PAA nanoparticle solution and incubated for 48hrs. Then these nanoparticles were separated from the aqueous phase by ultracentrifugation with 15000rpm for 2hrs. Then the drug loaded nanoparticles were lyophilized by

the freeze dryer system to obtain dried CS-PAA nanoparticles.

2.4 Characterization of Nanoparticles

Selected Nano particles were evaluated by its particle size, zeta potential value and polydispersity index were

measured by photon correlation spectroscopy using a Malvern Zetasizer Nano ZS90,

2.5 *In vitro* drug release study and Release Kinetics

This was studied by using a dialysis bag diffusion technique and the medium as simulated tear fluid of pH 7.4 at 37±0.5°C. In order to elucidate mode and mechanism of drug release, the *in vitro* release data obtained for the formulation was fitted into various kinetic models (A. H. El-Kamel *et al.*, 2002).

2.6 Sterility studies

According to I.P, sterility tests was carried out by using fluid thioglycolate media (FTM) and soyabean casein digest media (SCDM).

2.7 Irritancy Test (HET-CAM Method)

The chorionic allantoic membrane was treated with the test formulation and checked out for development of endpoints like hemorrhage, hyperemia and coagulation.

2.8 *In vitro* transcorneal permeation study

Goat cornea was used to study the permeation of Timolol Maleate across the corneal membrane. The study was carried out in the Franz diffusion cell. The upper chamber served as a donor compartment in which one drop of drug formulation under study was placed. The lower chamber served as a receiver compartment that was filled with simulated tear fluid. The whole system was maintained at 37±0.5°C. After a predetermined period, 2ml of the medium was removed and the amount of Timolol Maleate was analysed up to 6hrs by UV-Visible spectrometer at wavelength of 294nm. (Himanshu Gupta *et al.*, 2009).

2.9 Release Kinetics

In vitro dissolution has been recognized as an important element in drug development. Under certain conditions, it can be used as a surrogate for the assessment of bioequivalence. Several theories/kinetic models describe drug dissolution from immediate and modified release dosage forms. There are several models to represent the drug dissolution profiles where f_t is the function of t (time) related to the amount of drug dissolved from the pharmaceutical dosage system. To compare dissolution profiles between two drug product model dependent (curve fitting), statistical analysis and model independent methods can be used (Costa P *et al.*, 2001).

In order to elucidate mode and mechanism of drug release, the *in vitro* data was transformed and interpreted at graphical interface constructed using various kinetic models. The zero order release Eq. (1) describes the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of transdermal systems, matrix tablets with low soluble drugs, coated forms, osmotic systems etc., where the drug release is independent of concentration.

$$Q_t = Q_o + K_o t \quad (1)$$

Where, Q_t is the amount of drug released in time t , Q_o is the initial amount of the drug in the solution and K_o is the zero order release constant

The first order Eq. (2) Describes the rules of the system where release is concentration dependent, e.g. pharmaceutical dosage forms containing water soluble drugs in porous matrices.

$$\log Q_t = \log Q_o + K_1 t / 2.303 \quad (2)$$

Where Q_t is the amount of drug released in time t , Q is the initial amount of drug in the solution and K is the first order release constant.

Higuchi described the release of drug from insoluble matrix as a square root of time

$$Q_t = K_H \sqrt{t} \quad (3)$$

Where, Q_t is the amount of drug released in time t , K_H is Higuchi's dissolution constant.

Weibull equation can be applied to almost all kinds of dissolution curves and is given as:

$$\log [-\ln (1 - Q_t)] = b \times \log t - \log a \quad (4)$$

Where, Q_t is the amount of drug released in time t , b is shaping parameter, a is the scale parameter.

The following plots were made: cumulative % drug release vs time (zero order kinetic model); log cumulative of % drug remaining vs time (first order kinetic model); cumulative % drug release vs square root of time (Higuchi model., 1962).

2.10 Mechanism of Drug release

Korsmeyer *et al* (1983) developed a simple, semi empirical model, relating exponentially the drug release to the elapsed time (t)

$$f_t = K t^n$$

K is a constant incorporating structural and geometrical characteristic of the drug dosage form, n is the release exponent; f_t is M_t / M_∞ (fractional release of the drug).

Depending on the relative magnitude of the rate of polymer swelling to the rate of drug diffusion, various release profiles may be possible. The situation where the polymer structural rearrangement takes place rapidly in response to the swelling solvent as compared to the rate of drug diffusion generally leads to Fickian diffusion, or the so-called first order release, characterized by the square root of time dependence in both the amount released and the penetrating diffusion front position in slab geometry.

In case of sorption process is completely governed by the rate of polymer relaxation, the so-called Case II transport, characterized by linear time dependence in both the amount diffused and the penetrating swelling front position, results. In most systems, the intermediate solution, which is often termed Non-Fickian or

anomalous diffusion, will prevail whenever the rates of diffusion and polymer relaxation are comparable.

Kinetic constant incorporates structural and geometrical characters of the drug/polymer system. For non-Fickian release, the n value falls between 0.5 and 1.0 ($0.5 < n <$

1.0), whereas in the case of Fickian diffusion, $n = 0.5$; for zero-order release (case transport), $n = 1$, and for Supercase II transport, $n > 1$. The values of n as estimated by linear regression of $\log (M_t/M_\infty)$ vs $\log (t)$ of different formulations were calculated (table no 3).

Table: 3. Interpretations of Diffusion Mechanisms from Dosage Forms

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
$0.5 < n < 1.0$	Anomalous transport	t^{n-1}
1.0	Case-II transport	Zero order release
Higher than 1.0	Super case-II transport	t^{n-1}

2.11 Stability Studies

For the optimized formulation, stability studies were carried out at $25^\circ\text{C} \pm 2^\circ\text{C}/60\% \text{ RH} \pm 5\% \text{ RH}$ over a period of 90 days (30, 60, 90 days) and evaluated for particle size and drug content.

3. RESULTS AND DISCUSSIONS

Syntheses of Chitosan-poly acrylic acid nanoparticles were prepared by the template polymerization method. As the polymerization time extended, the amount of poly acrylic acid in the solution is increased, and the system changed initially from a clear solution to an opalescent emulsion indicating the formation of CS-PAA nanoparticles. The electrostatic interaction between poly acrylic acid (negative charge) and chitosan (positive charge) promoted the self-assembly of Nanoparticles. The compatibility study between the drug and excipients was carried out to assess the compatibility between Timolol Maleate, chitosan, potassium persulfate, and acrylic acid adopting spectral matching approach and it was checked for any shifting in the functional peaks of drug and non-involvement of functional groups of polymer with the drug. From the FT-IR Spectra, it is clear that there is no interaction between the selected excipients and the drug.

3.1 Zeta Potential

The importance of zeta potential is that its value can be related to the stability of nanoparticles. It has been

reported that positively charged nanoparticles facilitate an effective adhesion to the ocular surface that bears negative charge. (soumendra sahuo *et al.*, 2010).

The zeta potential of F6 and F8 formulation was observed to be +7.42mV and +23.30mV respectively (table 4). The unreacted chitosan molecules carried a positive charge because of their amino groups. So the zeta potential of the nanoparticles was quite high before the reaction started. As the reaction continued, the carboxyl groups of poly (acrylic acid) bonded with the amino groups of chitosan and therefore neutralized the positive charge on the surface and lowered the zeta potential of the nanoparticles.

3.2 Physicochemical Characterisation

Between batches F6 and F8 has shown Nano range particle size. F6 has shown average particle size of 378.1nm with PDI of 0.432 and F8 has shown average particle size of 141.29nm with PDI of 0.339 indicating narrow particle size distribution (table 4).

Since the optimised formulation (Between F1 to F8) F6 and F8 showed appreciable values for the below parameters, they were found ideal for ocular drug delivery. Among all the batches, F8 showed the maximum entrapment efficiency of 69.75%.

Table: 4. Zeta potentials of different batches particle size and poly dispersity index

Batch	AA	CS	$\text{K}_2\text{S}_2\text{O}_8$	Z-Average	Zeta potential	PDI	Drug loading(%)
F6	12mM	3 mM	0.1mM	378.1 nm	+7.42 mV	0.432	18.02±2.5
F8	6 mM	6 mM	0.1mM	141.29 nm	+23.30 mV	0.339	19.18±1.6

AA- Acrylic acid, CS- Chitosan, PDI-Poly Dispersity Index.

3.3 Sterility studies

Sterility of the formulation was the most important. The formulation should not contain any organisms. FTM and SCDM were carried out to determine the presence or absence of fungus and bacteria. In FTM and SCDM the microbial growth was observed by inoculating with positive control. There's no turbidity or growth was observed in inoculated media of the nano formulation (FS8).

3.4 Irritancy Test (HET-CAM Method)

Hen's Egg Test Chorioallantoic Membrane (HET-CAM) - The HET-CAM has been shown to be a qualitative method of assessing the potential irritancy of chemicals. Its well-developed vascularization provides an ideal model for ocular irritation studies. 10 day old, white Leghorn eggs are used in the HET-CAM test. There 5% and 10% drug solution was used and compared with +ve (0.1N NaOH) and -ve (0.9% NaCl) control, there are no

end points like haemorrhage, hyperaemia and coagulation were observed and hence, the study certified

that the developed formulation is non-irritant.

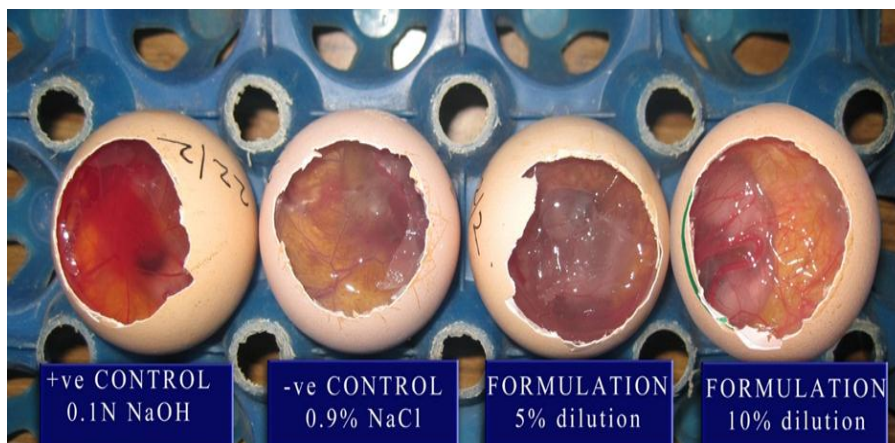


Fig: 3. Hen's Egg Test Chorioallantoic Membrane (HET-CAM)

3.5 *In vitro* drug release study and Release Kinetics

Only about 30% drug was released within 1 hour and then 63% of the drug was released within 12 hours indicating that initially burst effect followed by slow release of drug from the nanoparticle core shell. This

comparison between nanoparticles with marketed formulation demonstrated that the mucoadhesive nanoparticle was better in retention of Timolol Maleate. The transcorneal permeability study has also corroborated this observation.

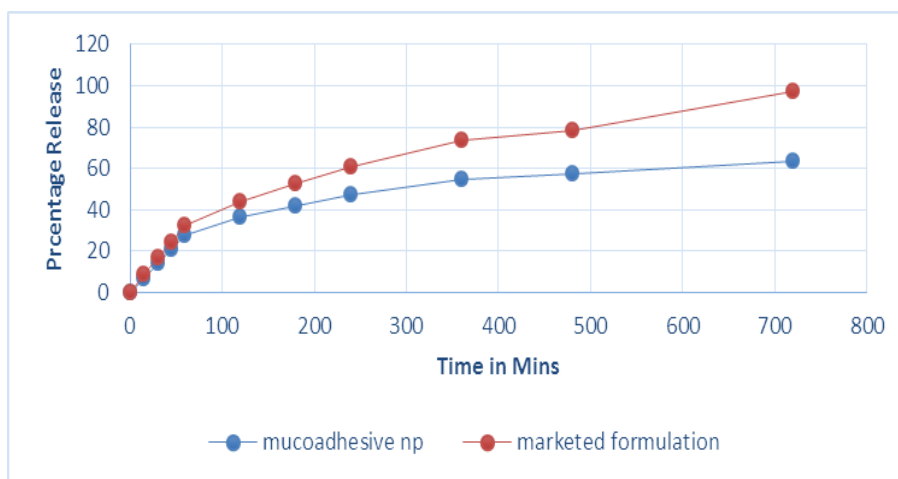


Fig: 4. *In vitro* drug release study

3.6 *In vitro* Permeation Studies

The permeability data for mucoadhesive nanoparticle formulation and marketed formulation shows that the permeation rate of the nanoparticle formulation is slower as compared to same concentration of marketed formulation of timolol maleate. It indicated mucoadhesive nanoparticles presented prolonged period in ocular milieu. Therefore, the mucoadhesive nanoparticle formulation gives a Controlled effect in ocular milieu.

3.7 Release Kinetics

In order to elucidate mode and mechanism of drug release, the *in vitro* data was transformed and interpreted at graphical interface constructed using various kinetic models. The *in vitro* release data obtained for mucoadhesive nanoparticle formulation, in simulated tear fluid pH 7.4, was fitted into various kinetic models. The results are shown.

Table: 5. Release Kinetics

FORMULATION	R ²			Peppas's plot
	Zero order	First order	Higuchi's	
Mucoadhesive nanoparticle formulation	0.7791	0.875	0.9502	N=0.534

The best linearity was obtained in Higuchi's plot for mucoadhesive nanoparticle formulation indicating the release from nanoparticles as a square root of time dependent process. The Higuchi's plot for formulation is shown in Fig 5.

The release exponent values 'n' for mucoadhesive nanoparticle formulation was found to be 0.534. Since, the release exponent 'n' values were between 0.5-1, it indicates that the formulation undergo anomalous diffusion.

3.8 Stability Studies

For the optimized formulation Stability studies were carried out at 25°C±2°C/60% RH±5% RH showed a slight change by increase in particle size from 141.29±3.5 to 151.16±2.5 after 90 days storage (Table 6). However the particle size of the formulation would be less than 250 nm would be considered as an ideal formulation for ocular delivery. Hence the developed formulation would be considered to be stable.

Table: 6. Stability Studies of mucoadhesive nanoparticle formulations.

Formulation	Particle Size (nm)	Drug Loading (%)
0 days	141.29±3.5	19.18±1.6
30 days	143.58±4.8	19.08±2.1
60 days	146.44±5.3	19.01%±1.4
90 days	151.16±2.5	18.98%±0.8

CONCLUSION

In vitro release profile of marketed formulation was compared with the optimized mucoadhesive nanoparticle formulation. In this, optimized batch shows an initial burst release of timolol maleate followed by more gradual release phase for the following 12hrs. This comparison between nanoparticles with marketed formulation demonstrated that the mucoadhesive nanoparticle formulation were better able to retain Timolol Maleate. In conclusion, the preparation and optimization of nanoparticulated mucoadhesive ocular drug delivery system of timolol maleate are a viable alternative to conventional eye drops, using natural and cheaper carrier by virtue of its ability to impart mucoadhesiveness and to sustain the drug release.

REFERENCE

- Anitha, A., Deepa, N., Chennazhi, K.P., Nair, S.V., Tamura, H., Jayakumar, R., 2011, Development of mucoadhesive thiolated chitosan nanoparticles for biomedical applications: Carbohydrate Polymers 83(1): 66-73.
- Changzheng Yang *et al.*, Synthesis and characterization of chitosan-poly (acrylic acid) nanoparticles Biomaterials, 2002; 23: 3193-3201.
- Costa, P., Lobo, J.M.S., Modeling and comparison of dissolution profiles. Eur. J. Pharm. Sci., 2001; 13: 123-33.
- El-Kamel, A H., *In vitro* and *in vivo* evaluation of Pluronic F127-based ocular delivery system for Timolol Maleate: Int. J. Pharm., 2002; 241: 47-55.
- Higuchi, W.I., Analysis of data on the medicament release from profiles of drug products. J. Biopharm Stat., 1962; 7: 241-258.
- Himanshu Gupta *et al.*, sustained ocular drug delivery from a temperature and pH triggered in-situ gel system. Drug delivery, 2007; 14: 507-15.
- Korsmeyer, R.W., Gurny, R., Doelker, E, Buri, P., Peppas, N.A., Mechanisms of solute release from porous hydrophilic polymers. Int. J. Pharm., 1983; 15: 25-35.
- Mitan, R., Gokulgandhi, Dharmesh, M., Modi, Jolly, R. Parikh, Drug Delivery Technology, 2007; 7: 30-36.
- Roy S, Anis A, Pramanik K, Prabhakar B. Polymers in Mucoadhesive Drug Delivery System: A Brief Note. 2009.
- Sanchez, A., Alonso, M. J., Nanoparticulate carriers for ocular drug delivery. In: Torchilin V, Nanoparticulates as drug carriers, London, UK: World Scientific-Imperial College Press, 2006; 649-673.
- Sangita D.kute, Somnath C.Sakor, Bhaswat S.Chakraborty. Formulation Approaches in Ocular Drug Delivery System. Int. J. Pharmacy & Technology, 2010; 2(2): 118-145.
- Soumendra Sahoo, Rasmirekha Sahoo, Rajashree Nnada, Tripathy, M.K., Nayak, P.L., Mucoadhesive Nanopolymer – A novel Drug Carrier for Topical Ocular Drug Delivery: Eur. J. Scientific Research, 2010; 46: 3401-409.