International Journal of Medicine and Pharmaceutical Research

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# Preparation and Evaluation of Mucoadhesive Opthalmic Niosomes of Acyclovir Using Chitosan and Carbopol

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**A B S T R A C T**

Niosomes have been reported as a possible approach to deliver the drug to ophthalmic cavity. Niosomes were formed using Span (20, 40, 60, and 80) and cholesterol in different molar ratio using Acyclovir as the model drug; Niosomes were formed using Reverse phase evaporation method. The formed vesicles were characterized for entrapment efficiency, particle size, Zeta potential, Stability etc. results of the study showed that Niosomes formed with Span60 and cholesterol containing 90% span 60 and 10% cholesterol gave maximum entrapment efficiency. The optimized Niosomes were further dispersed in polymeric solution of Chitosan and Carbopol separately; in-vitro release pattern suggested that a sustained release can be obtained, with better sustained release in Niosomes dispersed in polymeric solution of Chitosan. Results of studies suggest that Niosomes dispersed in polymeric solution of Chitosan can be used as promising vesicles for sustained delivery of drug to ophthalmic cavity.

**Keywords:** Acyclovir, Chitosan, Carbopol, Niosomes

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**Article History: Received** 31 May 2016, **Accepted** 10 July 2016, **Available Online** 10 August 2016

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Manuscript ID: IJMPR3083

**PAPER-QR CODE**

**Citation:** Raghvendra, *et al.* Preparation and Evaluation of Mucoadhesive Opthalmic Niosomes of Acyclovir Using Chitosan and Carbopol. *Int. J. Med. Pharm. Res.*, 2016, 4(4): 217-222.

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## 1. Introduction

Non-ionic surfactant vesicles (Niosomes or NSVS) are widely studied as an alternative to hydrated surfactant monomers. Non-ionic surfactants of wide structural types have been found to be useful alternatives to phospholipids in fabrication of vesicular system. They are the microlamellar structures formed on admixing of Non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. The present study was to prepare stable mucoadhesive ophthalmic Niosomes of Acyclovir for ocular use, which has got advantages over conventional dosage forms. Vesicles were prepared with the help of chemically stable surfactants, ie. Sorbitan esters (span) using cholesterol as a stabilizing agent.

The formed Niosomes were characterized for their ability to encapsulate Acyclovir and in-vitro release profile in phosphate buffer, Acyclovir is an antiviral drug used against Herpes and Varicella Zoster virus. It is a deoxyguanosine analogue which is used in the treatment of Herpes simplex keratitis, frequent application of eye ointment, 5 times a day is required since there are chances of drug drainage due to lacrimation, tear dilution of drug etc. to overcome this loss of drug Niosomes of Acyclovir were prepared, which were dispersed in solution of mucoadhesive polymers, due to their ability to form non-covalent bond with mucin present in conjunctiva of eye.

## 2. Materials and Methods

**Drug Profile: Acyclovir**

It is one of the most commonly- used antiviral drugs and is commonly marketed under the trade name zovirax. It is extremely selective and low in cytotoxicity. Pharmacologist Gertrude B.Elion was awarded the 1988 Nobel prize in Medicine partly for the development of acyclovir.

**Systematic (IUPAC) name**

2–amino–9– (2–hydroxyethoxymethyl)–3H–Purin–6–one.

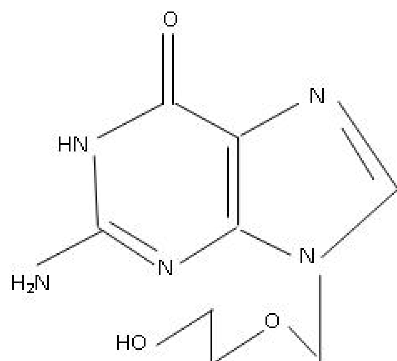


**Figure 1:** Acyclovir

**Formula** : $C_8 H_{11} N_5 0_3$

**Molecular weight:** 225.2 g/mol

**Action and use: Antiviral**

**Character :** A white or almost white, crystalline powder.

**Solubility :** Slightly soluble in water, freely soluble in dimethyl sulfoxide, very slightly soluble in alcohol. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

**Definition:** Acyclovir contains not less than 98.5 per cent and not more than the equivalent of 101.0 percent of 2–amino–9–[(2–hydroxyethoxy) methyl] –1, 9–dihydro–6H purin–6–one, calculated with reference to the anhydrous substance.

**Storage:** Store in a well closed container at room temperature between 15–25 degree C.

**Clinical use:** Acyclovir is indicated for the treatment of herpes simplex virus and Varicella zoster virus infections, including. Genital herpes simplex (treatment and prophylaxis), Labial herpes simplex (cold sores), Herpes zoster (shingles), Acute chickenpox in immune compromised patients, Herpes simplex encephalitis, Acute mucocutaneous HSV infection in immuno compromised patients , Herpes simplex keratitis.

**Mechanism of action**

Acyclovir differs from nucleoside analogues as it contains only a partial nucleoside structure. The sugar ring is replaced by an open- chain structure. It is selectively converted into a monophosphate form by viral thymidine kinase, which is far more effective (3000 times) in phosphorylation than cellular thymidine kinase. Subsequently the monophosphate form is further phosphorylated into the active triphosphate form, aciclo - GTP by cellular kinases. Aciclo- GTP is a very potent inhibitor of viral DNA polymerase; it has approximately 100 times higher affinity to viral than cellular polymerase. Its monophsophate form also incorporates into the viral DNA, resulting in chain termination. It is showed that the viral enzymes cannot remove aciclo- GTP from the chain – which results in inhibition of further activity of DNA polymerase. Aciclo-GTP is fairly rapidly metabolized within the cell, possibly by cellular phosphatases. Therefore the acyclovir can be considered a prodrug. It is administered in an inactive (or less active from) and is metabolised into a more active species after administration.

**Pharmacokinetics**

Acyclovir is poorly water soluble and has poor oral bioavailability (10-20%) hence intravenous administration is necessary if high concentrations are required. When orally administered, peak plasma concentration occurs after 1-2 hours. Acyclovir has a high distribution rate, only 30% is protein – bound in plasma. The elimination half life of acyclovir is approximately 3 hours It is renally excreted partly by glomerular filtration and partly by tubular secretion.

**Toxicity**

Since acyclovir can also incorporate also into the cellular DNA, it is a chromosome mutagen; therefore, its use should be avoided during pregnancy. However it has not been shown to cause any teratogenic or carcinogenic effects. The acute toxicity ($LD_{50}$) of acyclovir when given orally is greater than 1 mg / kg due to the low oral bioavailability.

**Adverse drug reactions**

Common adverse drug reactions associated with systemic acyclovir therapy (oral or IV) include- Nausea, Vomiting, Diarrhea, Headache, Agitation, Vertigo, Confusion,

Dizziness, Oedema, Sore throat, Constipation, Abdominal pain, Rash, Weakness.

**Rare adverse effects include:** Coma, Leucopenia, Fatigue, Seizures, Crystalluria, Hepatitis, Neutropenia, Anorexia, Toxic epidermal necrolysis, Anaphylxis, Stevens Johnson syndrome. Additional common adverse effects, when acyclovir is administered IV, include, Encephalopathy , Injection site reactions, The injection formulation is alkaline (pH 11) and extravasations may cause local tissue pain and irritation. Renal impairment has been reported when acyclovir is given in large, fast doses intravenously, due to the crystallization of acyclovir in the kidneys.

**Chitosan**

**Origin:** Chitin, a polysaccharide of animal origin is obtained from waste material of sea food industries. It occurs in the skeletal material of crustaceans such as crabs, lobsters, hrimps, prawns and crayfish and also from the exo- skeleton and wings of insects' crustacean shells.

**Physical Characters:**

It occurs as odorless, white or creamy-white powder or flakes, fiber formationisquite common during precipitation and the Chitosan may look cotton like.

**Chemical Characters**: Chitosan is a cationic polyamine with a high charge density at pH <6.5 so adheres to negatively charged surface and chelates metal ions. It isalinear polye lectrolyte with reactive hydroxyl and amino groups so available for chemical reaction and salt formation. Numerous studies have demonstrated that the salt form, molecular weight, and degree of deacetylation as well as pH at which the chitosan is used all influence how this polymer is utilized in pharmaceutical application

**Solubility:**

Chitin is insoluble in most of the solvent, acids and bases, Chitosan is insoluble in neutral and alkaline pH values but dissolves in organic and inorganic acids (e.g. acetic acid, formic acid, glutamic acid, lactic acid, hydrochloric acid etc.) to produce viscous polyelectrolyte solutions.

**Preparation of Chitosan from Chitin:**

Chitin and chitosan are one of the most abundant polysaccharides in nature. The principle derivative of chitin, namely chitosan $(C_6H_{11}O_4N)_n$ is a unique polysaccharide and hydrophilic polymer, which is usually obtained by alkaline deacetylation. Heating chitin with 50% potash at 150-160$^0$c for one hour can do deacetylation of chitin. After deacetylation of chitin, the chitosan obtained is dissolved in acid, filtered and precipitate is washed and dried to get amine free chitosan.

**Incompatibilities:** Chitosan is incompatible with strong oxidizing agents.

**Stability and Storage Conditions:** Chitosan powder is a stable material at room temperature although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool, dry place.

**Applications:** Following are some of the applications of chitosan. Used in controlled drug delivery application. Used as a components of mucoadhesive dosage forms, rapid release dosage forms, improved peptide delivery, colonic drug delivery systems, and use for gene delivery. It is processed in to several pharmaceutical forms including gels, films beads, microspheres tablets and coating for liposomes. Used as a flocculating agent to remove impurities from contaminated water. Used in clarification of beverages such as fruit juices and beers. Soil treated with chitin has been found to reduce the severity of disease caused by several soils born fungal pathogen. Used as a fungicide in protection of crops and coating of apples. Chitosan treated papers are resistant to water penetration, a good surface for writing upon with ink. Used in textile industries to improve the appearance of fabrics by adding luster and brightening their colour. Used as thickener and emulsion stabilizer in cosmetic preparations especially in hair care products.

**Carbomer**

**Chemical Name** Carbopol

**Empirical Formula & Molecular weight:**

Carbomers are synthetic high molecular weight polymers of acrylic acid that are cross linked with either ally sucrose or allyl ethers of pentaerythroil. They contains between 56-68% of carboxylic acid (-COOH) groups as calculated as dried basis. The molecular weight of carbomer resins is theoretically estimated at 700 000 to 4 billion. In an effort to measure the molecular weight between crosslink (Mc) researchers have extended the network theory of elasticity to swollen gels & have utilized the inverse relationship between elastic modules and Mc. Generally carbomer resins have low viscosity and lower rigidity will have higher Mc values.

**Structural Formula:**

CHHCCOHH On, Acrylic acid monomer unit in carbomer resins carbomer polymer are formed from repeating units of acrylic acid. The polymer chains are cross linked with allyl sucrose or allylpentaerythritol.

**Solubility:** Soluble in water and after neutralization in ethanol (95%) and glycerin

**Application in pharmaceutical formulation (or) Technology:** Carbopol is mainly used in liquid or semisolid pharmaceutical formulations as suspending, viscosity – increasing agents. Carbomer such as carbopol 934p or 974 p may additionally be used in oral preparations, in suspensions, tablets or sustained release tablet formulations. In tablets Carbomer are used as dry or wet binders and as a rate-controlling Excipients. In wet granulation process water or an alcohol/water blend is used as the granulating fluid. Carbomer resins have been also used in sustained release, matrix beads as enzyme inhibitors of intestinal proteases in peptide containing dosage forms, as a bio adhesive for cervical patch, carbomer are also employed as emulsifier, preparation of oil-in-water emulsions for external use.

**Stability and storage condition:** Carbomers are stable, hygroscopic materials may be heated at temperature below 104$^0$C for up to 2 hrs without affecting their thickening efficiency. However exposure to excessive temperatures can result in discoloration and reduced stability. Complete decomposition occurs with heating for 30 minutes at 2600 C. Exposure to light causes oxidation that is reflected in a decrease in dispersion viscosity.

**Method**

Niosomes of Acyclovir were prepared by Reverse phase evaporation method, accurately weighed amount of surfactant and cholesterol along with dicetyl phosphate were dissolved in Chloroform and Methanol mixture (2:1). The solvent system containing surfactant and cholesterol was placed in a round bottom flask, chloroform methanol mixture was evaporated at $55^{oC}$ under reduced pressure at 150 rpm using Rotary evaporator, after the solvent was evaporated the thin film formed at the walls of the flask was re-dissolved by using ether and drug in 4ml of acetone, 6 ml of phosphate buffer of pH 7.4, the mixture was vortexed for 10 minutes and then swirled by hand again it was vortexed for 15 minutes. The dispersion was allowed to evaporate; hydration was done by using phosphate buffer of pH 7.4 which was followed by Rotary evaporation for 20 minutes. Left for overnight, all the steps were carried under laminar air flow bench. The so formed, Niosomes by reverse phase evaporation method were dispersed in varying concentration of Chitosan and Carbopol separately. Preformed vesicles were incubated in the respective polymeric solution at 37ºc for 10 minutes, the polymeric solution was not removed and allowing the vesicles to remain dispersed in the polymer solution. Finally 0.01% Benzalkonium chloride was added as a preservative, all the steps were carried under laminar air flow bench. The final solution was filtered.

**Characterization of Vesicles**

**Particle size**

Niosomal suspension was characterized for particle size and particle size distribution and the size of unilamellar vesicles was determined by using Malvern Zetasizer ver. 2.15. Size and size distribution measurements were obtained automatically as the instrument is microprocessor controlled.

**Morphology and structure of vesicles**

The prepared Niosomes were observed by means of an Olympus BH-2 microscope at magnification of 40X. A small drop of Niosomal suspension was placed on a glass slide having a small cavity; prior to it the suspension was diluted with few drops of phosphate buffer and covered with cover slip. The sample was examined for vesicle formation. Transmission electron microscopy (TEM) was performed in order to determine the morphology and structure of Niosomes. TEM was done with the help of Philips Morgani 268D Transmission Electron Microscope. The vesicle formulations were examined by scanning electron microscopy at in order to determine size, shape and lamellarity.

**Entrapment efficiency**

Acyclovir encapsulated Niosomes were separated from un-entrapped drug by dialysis method for 24 hrs. An aliquot of freshly purified Niosomal dispersion (5mg lipid ml-1) was diluted with 10% of TritonX-100 in a ratio of 1:9 vol/vol. the detergent dissolved the Niosomes and yielded a clear solution. Then it was filtered through whatman filter paper and absorbance of resulting solution was measured spectrometrically at 252nm. The percentage of entrapped Acyclovir was determined by applying the following formula

**% Entrapment = ($A_E$ x 100) / AI**

$A_E$ is the amount of drug entrapped

$A_I$ is the initial amount of drug taken

**Ocular irritancy test**

Ocular irritancy test was performed in order to evaluate the tolerability of final formulation formed, this test was based on Drazie Eye Test. Albino Rabbits were used as test species. Animals were evaluated before exposure in order to ensure normal eye. 1 animal was used for each formulation to be evaluated. Right eye was designated as test eye and the left eye served as matched control. Dosage volume of 0.01ml was selected, instillation was made into lower conjuctival sac, normal blinking was allowed. Sterile solution was used to irrigate the ocular surface. Pre and post exposure evaluations were performed by external observation with proper illumination. Observations were made at 1, 24, 48 and 72 hours after exposure. Ocular changes were graded by scoring system that Includes rating any alterations to eyelids, conjunctiva, cornea and Iris.

***In-vitro* release study trough dialysis membrane**

The Niosomal preparation of Acyclovir was placed in a dialysis bag of effective length 10 cm, which acts as a donor compartment. Dialysis bag was placed in a beaker containing 250 ml of phosphate buffer saline of pH 7.4, which acts as a receptor compartment. The temperature of receptor compartment was maintained at 37±2ºc and the media was agitated at a moderate speed using a magnetic stirrer. Aliquots of sample (5ml) were withdrawn periodically at regular interval of time for 10 hours after each withdrawal fresh phosphate buffer was replaced. The collected samples were analyzed at 252 nm by using phosphate buffer saline as blank.

## 3. Results and Discussion

**Particle size**

Results of particle size analysis shows that vesicle size decreases with decrease in HLB value. Vesicle size decreases in the following manner Span20>40>60>80, i.e. higher the HLB value larger are the particles formed.

**Optical microscopy**

Optical microscopic evaluation showed the formation of non-aggregated vesicles, when the sample used was subjected to size reduction prior to observation under microscope. It was observed that Span20 formed clusters of vesicles whereas the vesicles formed with Span60 were most optimum in appearance.

**Electron microscopy**

**a) Scanning electron microscopy**

SEM reports revealed that Niosomes formulated were round in shape, Niosomes obtained from optimized formulations were spherical in shape and were large and unilamellar in nature, since all the formulations have cholesterol aggregation was not observed. Results shown in fig (1)

**b) Transmission electron microscopy**

Further characterization was done with the help of negative stain TEM. TEM reports showed the formation of Niosomes having close spherical shape. It was observed that the vesicle size of Niosomes lie in nanometer range, having large aqueous space. Vesicle size decreases in the

following manner Span20>40>60>80, i.e. higher the HLB value larger are the particles formed.

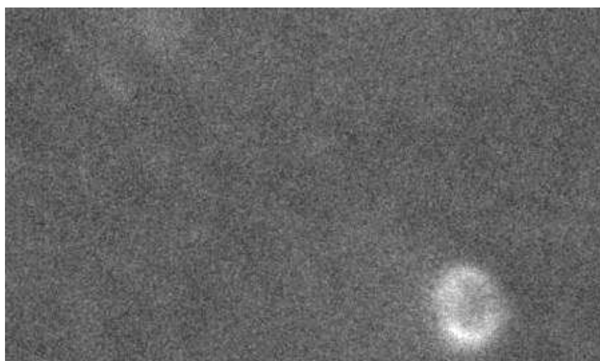

**Figure 2: SEM of NMC2**

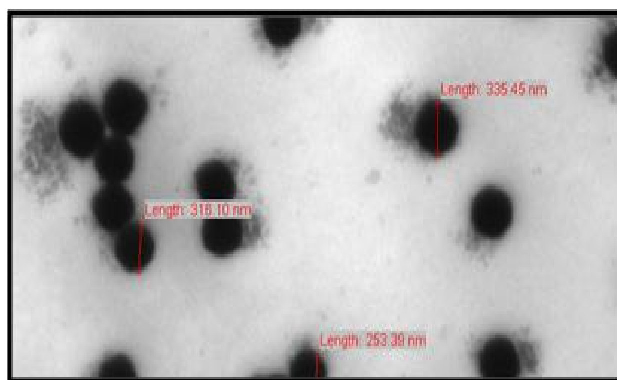


**Figure 3: TEM of NMC2**

Entrapment efficiency of Acyclovir containing formulations are show the entrapment efficiency varies form as low as 20.23% for Span80 vesicles (NMD2) to as high as 56.66% for Span60 vesicles (NMC2), high entrapment efficiency for Span60 formulations can be attributed to its structure, length of side chain, orientation and packaging ability. The presence of $C_{16}$ alkyl side chain is responsible for high entrapment efficiency. In case of vesicles formed with Span80 low entrapment is due to the presence of unsaturated double bond in alkyl side chain which causes the bending of chain as a result of which the membrane formed is less tight in nature that eventually results in increase in permeability of membrane and thus decreases the entrapment. Another cause for high entrapment in vesicles formed with Span 40, 60 may due to their high phase transition temperature whereas for Span80 phase transition temperature is low due to which it undergoes gel to liquid transformation. Thus long saturated alkyl side chain and high phase transition temperature arte the critical factors responsible for high entrapment efficiency

**Ocular irritancy test**

Ocular irritancy test was performed with the aim of determining the potential of Niosomes to be used as delivery system with the ability of being used for ophthalmic delivery of drug. The results of ocular irritancy test showed that Niosomes formed with the help of non-ionic surfactants, dispersed in the polymeric solution of Mucoadhesive polymers do not show any sign of inflammation in cornea, iris and conjunctiva.

***In-vitro* release study**

*In-vitro* release study is performed in order to determine the rate of release of drug from the drug delivery system. It is investigated in order to design a formulation with optimized characters and ability to release the drug in required fashion. In-vitro studies help in predicting the behavior of delivery system that how it may work under ideal situation. It helps in correlating the in-vivo performance. *In-vitro* study for various formulations of Plain Acyclovir Niosomes showed prolonged release character with a Higuchi pattern of drug release which indicates that Niosomes act as reservoir system for continuous delivery. Free drug solution gave a initial high percentage release of 74.56% during the $1^{st}$ hour, whereas the optimized formulation (NMC2) gave release of 7.98%, the release data were analyzed mathematically according to Zero order, first order, Higuchi equation with $R^2$ value of 0.978 for (Plain NMC2),0.865(NMC2+Carbopol), 0.822(NMC2+ Chitosan) optimized formulations. *In-vitro* study was further carried out by dispersing NMC2 in 0.5% chitosan and carbopol 940 solution separately, the results of release rate showed the chitosan gave more sustained release as compared to Niosomes dispersed in carbopol solution, the probable reason behind this may be chitosan gives better adhesion at pH7.4 because of its ability to form ionic bond with negatively charged sialic acid present in mucus layer. Whereas carbopol gives less adhesion because at pH of 7.4 the carboxylic acid group is ionized to greater extent thus reducing the ability to form hydrogen bonding.

## 4. Conclusion

Form the results of various studies performed as a part of this project it can be concluded that Niosomes can be used as an efficient delivery system to deliver drug to eye, there sustained effect can also be further enhanced by dispersing them in polymeric solution of various Mucoadhesive polymers.

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