DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 21(1), 19-59 (1995)

NEW OPHTHALMIC DRUG DELIVERY SYSTEMS

C.A. Le Bourlais*; L. Treupel-Acar*; C.T. Rhodes**; P.A. Sado*; R. Leverge*

*Laboratoire de Pharmacie Galénique, Biopharmacie et Pharmacie Clinique
Faculté de Pharmacie - Université de Rennes - France
**Department of Pharmaceutics - College of Pharmacy
URI - Kingston - Rhode Island - USA

Abstract

One of the ways to optimize ocular drug delivery is to prolonge precorneal drug residence time. This review focusses on recent findings on the formulation effects in ocular drug bioavailability, employing polymers for the preparation of hydrogels, bioadhesive dosage forms, in situ *gelling systems and colloidal systems including liposomes and nanoparticles. The results observed suggested that mucoadhesion or bioadhesion played a role in the sustained action of drugs more significantly compared to non-mucoadhesive polymers. Encapsulation of drugs in liposomes and nanoparticles was correlated to an increase of the drug concentration in the ocular tissues. However, all the results described suggest that the physico-chemical properties of the encapsulated drug have a significant influence on the effect with the carrier. The results suggest also that the superficial charge, the binding type of the drug onto the nanoparticles and the nature of the polymer were the most important factors regarding the improvement of the therapeutic response of the drug.*

Introduction

Controlled delivery of drugs to the eye is limited by the efficient protective mechanisms that exist in the precorneal area. Absorption of drugs into the eye proper requires good corneal penetration plus prolonged contact time with the corneal tissue. Ideally, the formulation should be able to sustain drug release and to remain in contact with the front of the eye for extended periods of time.

Consequently it is imperative to optimize ocular therapy. One of the ways of optimization is by prolonging precorneal drug retention of a formulation by the addition of soluble polymers, development of viscous gels, development of colloidal suspensions or by the use of erodible and non-erodible inserts (85)(97). A number of systems, soluble and insoluble ocular inserts, are not user-friendly in patients and are often very uncomfortable. From the point of view of patient acceptability, a liquid dosage form is preferable.

This review will focus on recent findings on the mechanisms of formulation effects in ocular drug bioavailability, employing a polymer for the preparation of hydrogels, a bioadhesive dosage form, systems including liposomes and nanoparticles. Bioadhesive systems can be either polymeric solutions (38) or microparticle suspensions (42). Such polymers have demonstrated promising improvements in the ocular bioavailability.

The extent of absorption of an ophthalmic drug is severely limited by physiological contraints such as reflex tearing and reflex blinking that exist in the precorneal area resulting in an important drug loss (52). Tear turnover removes drug and, depending on the delivery system, the dosage form as well.

In most cases, upon instillation into the eye, eye-drops are 30-75 µl in volume and a portion of these drops quickly drain until the eye is back to the normal resident volume of 7 µL.

From the previous description of loss pathways in the front of the eye, it appears that very little drug is available to enter the cornea and the inner tissues of the eye. Actually corneal permeability to drugs is quite low and the reason for an early pulse entry of drug followed by a rapid decline of drug concentration in the tears has to do with the enormous loss of drug from the front of the eye. These processes lead to a typical corneal contact time of about 1 to 2 minutes in humans, for an instilled solution, and an ocular bioavailability that is commonly less than 10% (104) (105) (93).

The cornea is a three layered structure consisting of an hydrophilic sheet (stroma) covered on both surfaces with lipophilic cellular layers epithelium and endothelium. The epithelium facing the tears is a 5-6 cell layer tight junction tissue. This layer is rate limiting for the penetration of most useful ocular drugs. The stroma beneath the epithelium layer containing about 85% water makes up about 90% of the cornea. The last layer endothelium, facing the interior of the eye is a most important layer since it houses the active water pump that accounts for about 75% of the hydration/dehydration activity for the cornea (31) (114).

The mechanism of corneal pilocarpine penetration demonstrates a dual role for the corneal epithelium, both as a barrier to drug penetration and as a reservoir for drug in the intact cornea (106). The pathways for permeation of typical drug molecules varies with the physico-chemical properties of the penetrationg drug.

When a drug with a high oil/water partition coefficient is applied to the cornea, it quickly partitions into the "lipid-like" epithelial layers of the

cornea. However, for such a drug, the aqueous stroma presents a rate-limiting membrane and, therefore, a small concentration gradient across this barrier is expected (29).

The major resistance for small and medium sized peptides is not size but charge; the cornea offers more resistance to negatively charged compounds, as compared to positively charged species.

To optimize ocular drug delivery system, the following characterictics are required (46):

- a good corneal penetration;
- a prolonged contact time with the corneal tissue;
- a simplicity of instillation for the patient;
- a non-irritative and comfortable form (the viscous solution should not provoke lachrymation and reflex blinking);
- appropriate rheological properties and concentration of the viscolyzer

Alternative dosage forms have been tested in the recent past and each has been shown deficient in one or more ways. This review will present the recent dosage forms with their advantages and drawbacks on polymers included in supensions or solutions.

1- POLYMERS - VISCOLIZERS

The objective of this section is to review the effectiveness of the vehicle approach in order to prolonge precorneal residence time of topically applied drugs. A popular issue is to incorporate soluble polymers into an aqueous solution to increase vehicle viscosity, thereby prolonging drug contact with the cornea (13) (51) (61) (87). Typically, these polymers are high

molecular weight molecules (5000-10000 Da) which can not cross biological membranes, including cellulosic components, poly(vinyl alcohol), polyacrylic acid hydrogels, hyaluronic acid, poloxamer and polysaccharidic components. Drugs of various solubilities have been incorporated into these gels, such as pilocarpine, tropicamide, betaxolol, timolol, prednisolone, fluorometholone, progesterone...

Patton and Robinson (85) reported that an increase in the corneal penetration of an ophthalmic drug would be maximum at a viscosity of about 15 to 150 cP, any further viscosity increase showing less effect on drainage rate and tear film thickness and often associated with interference with vision and resistance to eyelids movements.

Polymer solutions generally exhibit one of two types of behavior that is newtonian behavior or non-newtonian behavior (6) (85). Solutions exhibiting newtonian behavior where the viscosity is constant at fixed temperature and pressures and independent of the rate of shear are poorly tolerated in the eye because of the transmitted shearing forces associated with blinking and rapid eye movements. Formulations of polymers that display non-newtonian or pseudo-plastic properties in which the viscosity decreases with increasing shear rate, thereby offering significantly less resistance to the movement of the lid over the globe than viscous newtonian formulations, show much greater acceptance (31).

1.1- Non-mucoadhesive polymers

1.1.1- Cellulosic polymers

Cellulosic polymers are widely used as newtonian viscolyzers into ophthalmic preparations:

- methylcellulose;

- hydroxyethylcellulose (HEC);
- hydroxypropylmethylcellulose (HPMC);
- hydroxypropylcellulose (HPC);

Those viscolyzers present common properties:

- they present a wide range of viscosity (400 to 15000 cP);
- they are compatible with many topically applied drugs;
- they can be easily sterilized by heat (120°C);
- they increase the stability of the lachrymal film;
- they are topically well tolerated.

Those polymer were introduced into artificial tear preparations over 40 years ago in order to retard canalicular drainage and improve contact time.

Comparison of cellulosic polymer solution in ophthalmic formulations

Ludwig and Van Ooteghem (62), studied the clearance of a fluorescent tracer through the addition of the viscolyzers HEC, HPC, and HPMC. The instillation of 25 mPa.s HPMC and HPC solutions produced a high fluorescence of the tear film, but also a fast elimination because of discomfort, blurred vision, lachrymation and reflex blinking. The HEC profile showed however, a lower fluorescence intensity compared with HPMC and HPC, but a slower elimination and thus a higher ocular retention. So, the various cellulosic polymers tested seemed to affect the retention of the tracer somewhat differently. HEC was rated more comfortable than HMPC and HPC.

Similar results were demonstrated by Benedetto et al. (6) as they found that HEC seemed to be the most effective to retard the elimination of the tracer from the precorneal area. However, based on the AUC values, HPC was preferred to enhance the amount of sodium fluorescein at the eye surface.

1.1.2- Polyvinyl alcohol (PVA)

PVA was introduced into ophthalmic preparations in the early 1960s and was reported to have a superior corneal contact time as a newtonian polymer solution based on animal studies. PVA can lower the surface tension of water, reduce interfacial tension at an oil/water interface and enhance tear film stability. This film-forming property together with ease of sterilisation, compatibility with a range of ophthalmic drugs and an apparent lack of epithelial toxicity has led to the widespread use of PVA as a drug delivery vehicle and artificial tear preparation (6).

Many studies have compared the precorneal residence of HMPC and PVA ophthalmic solutions with neither polymer solution demonstrating outsdanding precorneal residence over the other. It appeared that vehicles exhibiting newtonian flow properties showed comparable effects as ophthalmic vehicles (6) (85).

Thermes et al. (118) demonstrated that PVA formulation increased transcorneal penetration of timolol. Aqueous solutions of timolol and that containing the non-mucoadhesive polymer PVA generated maximum ocular concentrations at 10 or 30 min post-instillation, but PVA did not radically alter the concentration versus time profile compared to polyacrylic acid (PAA). This was consistent with previous observations using the ß-blocker L-653,328 in a range of viscous solutions of HEC (32), this was consistent also using pilocarpine in different vehicles (18).

1.1.3- Poly (vinylpyrrolidone) (PVP)

Podder et al. (88) demonstrated that isoviscous 0.75% hydroxypropylcellulose (HPC), 3.75% PVA, 6% PVP, and 2.5% hyaluronic acid (HA) all reduced the systemic absorption of timolol, but not

to the same extent. PVP, which was most effective in enhancing ocular timolol absorption, was also most effective in reducing systemic absorption. There was an approximately 2-fold difference between PVP and other three polymers, HA, HPC and PVA. Conceivably, spreading of the drained dose may also be influence by the chemical nature of the polymer independently of viscosity, thus explaining the differences in effectiveness in reducing systemic timolol absorption among the polymers.

Saettone et al. (97) examined the influence of different isoviscous polymers on the activity of pilocarpine in rabbit and man. They found that, using the AUC of the miosis response curve, PVA appeared significantly more active in both species when compared to aqueous, Poly(vinylpyrrolidone) or hydroxypropylcellulose solution.

Newtonian polymers studied for 20 years lead to contradictory results. For a same viscosity, results seem to depend finally on the nature of the polymer. The comparison between the polymers does not reveal always the same rank order.

1.2- Bioadhesive - Mucoadhesive polymers

Bioadhesion is not a new concept, given that cells attach to each other with great strength. Increased attention is given to the possibility of using bioadhesive or mucoadhesive polymers for drug delivery purposes (93). Due to the hydrophobic groups on bioadhesive polymers and the large amount of water associated with mucin, two possible adhesion mechanisms, hydrogen bonding and/or interpenetration of a swollen gel network with hydrated mucin, would seem most likely (110) (23).

Coating the external surfaces of the eye is a thin film of glycoprotein referred to as tear mucin. Mucin consists of a very large linear peptide chain

to which a large number of oligosaccharide chains are bound and is capable of picking up about 40-80 times its weight in water.

An attractive drug delivery concept is to find suitable natural and synthetic polymers that will attach to this mucin coat and remain in place as long as the mucin is present. Such polymers are refered as mucoadhesives (93).

Many reseachers active in the field of drug delivery have reported on attempts to identify potential bioadhesives. Very accurate methods of investigation of bioadhesion are necessary to be able to class polymers as a function of their bioadhesive power (23). The first method, described by Park and Robinson (82) consisted of a fluorescent technique which could explain the interpenetration of cultured epithelial cells by polymer molecules. Then tensile tests, supplemented by the preparation of stress/deformation curves and stress relaxation tests, were effective methods to characterize bioadhesion (23) (35) (40) (78) (79) (72) (101) (111).

From these numerous studies, a number of polymers possessing mucoadhesive properties have been discovered. Robinson tried to classify bioadhesive polymers concluing that polyanions were better than polycations, for both bioadhesiveness and toxicity reasons. Among polyanions, they showed the important bioadhesion power of carboxymethyl cellulose, and the polyacrylic acids, e.g. polycarbophil and Carbopol 934P (111).

Most of these tests provided the same rank order of adhesiveness for the polymers tested (86).
Dittgen et al. (21) suggested that there was a direct relationship between bioadhesion and the retard effect. The bioadhesion influences both ocular elimination and corneal permeation. The corneal permeation decreases whilst the bioadhesion increases.

1.2.1- Hyaluronic acid

The sodium salt of hyaluronic acid (SH) is a high-molecular-weight biological polymer consisting of linear polysaccharide that is present in extra cellular matrix.

In the eye, SH is present in the vitreous body and in lower concentrations, the aqueous humor. Products based on SH are widely used in intraocular surgery and are mostly used during cataract surgery to maintain the shape of the anterior chamber. SH also showed a topical protective effect for the cornea and conjonctiva during cataract extraction in humans. Some investigations with the use of exogenous SH led to characterize this compound as a typical pseudoplastic polymer with a low viscosity at high shear rates which can be used as a drug delivery system on the eye.

Ludwig and Van Ooteghem (62) found that a 0.25% SH solution increased the precorneal residence time of fluorescein in humans. Snibson et al. (113) used ^{99m}Tc to label SH solutions to quantify the residence time of 0.2% and 0.3% SH solutions in a group of healthy humans and in a group of patients with keratoconjonctivitis sicca (KCS). These authors hypothesized that an alteration of tear mucin in dry eyes might modify the interaction of SH with the ocular surface and explain the effect of SH on the residence time of the tracer only in KCS patients.

Gurny et al. (38), have shown that only solutions containing low concentrations of hyaluronic acid (<0.1%) had the unique capability of prolonging precorneal residence time of pilocarpine in man and rabbit. Similar results have been reported by Camber and Edman (11) showing a significant increase in the miotic response in rabbits using 2% pilocarpine in solutions of SH at different concentrations.

Saettone et al. (100) found that SH solutions and compressed matrices had a bioavailability-enhancing effect for both pilocarpine and tropicamide, this effect being more important for tropicamide.

The capacity of SH to increase the precorneal residence time of gentamicin sulfate (GS) in male volonteers has been under investigation (8). The results suggested that SH increased GS bioavailability on the ocular surface, resulting in higher concentrations for at least 10 min after instillation.

SH is a nonimmunogenic glycosaminoglycan having several uses in ophthalmic therapy, such as protecting corneal endothelial cells during intraocular surgery, replacing vitreous humor, acting as a tear substitute in the treatment of dry eye, and increasing the precorneal residence time of various drugs. This latter use is still the subject of experimentation.

1.2.2- Polyacrylic acid hydrogels

Polyacrylic acids or Carbopol resins are acrylic acids based polymers which are available range of molecular weights and may either be linear, branched or cross-linked. Carbopol 934P is the only member appropriate for use in the pharmaceutical industry, it is lightly cross-linked and has a molecular weight of approximately 3,000,000 Da and readily soluble in aqueous solutions.

Many workers (4) (14) (84) (111) (119) have sugested polyacrylic acids (PAA) as one of the most pseudo-plastic polymers with important bioadhesive properties. Park and Robinson (84) and Ponchel et al. (89) have reported that PAA interact via carboxyl groups and functional groups of the mucus glycoproteins. This polymer had consequently been formulated in produce cross-linked mucoadhesive hydrogels.

Davies and Kellaway (18) evaluated the precorneal clearance of pilocarpine (1%) in Carbopol 934P solution compared to that of an equiviscous non-mucoadhesive PVA solution and buffer (PBS) in the rabbit. By measuring the miotic response intensities, they demonstrated that the precorneal retention of the Carbopol solution was significantly greater than that of the PVA solution, which in turn was significantly greater than that of P.B.S.

Comparable experiments have been carried out by Saettone et al. (97) (100) with pilocarpine in the rabbit eye. The polyacrylic acid gel (5% w/v Carbopol 941) formed a stable precorneal film for up to 2 h post-instillation. A repeat of the study using the less soluble drug tropicamide increased the duration of the effect more significantly compared to a pilocarpine solution.

Weinreb (121) evaluated the ocular bioavailability of 0.25% betaxolol suspension based on polyacrylic acid (viscosity 100 to 150 cPs) in comparison with a 0.5% betaxolol solution in rabbits. The results suggested that the suspension provided a more constant release of betaxolol than the solution.

Thermes and Rozier (118) have evaluated ocular bioavailability of 0.5% timoptol® in rabbits compared to 0.5% timolol in isoviscous solutions of PVA, polyacrylic acid and timolol-polyacrylic acid salt (PAA salt). The results indicated that the bioadhesive PAA polymers produced generally lower ocular concentrations than those after PVA, and the concentration versus time profile were flatter. This could be consistent with the slower release of timolol from PAA and the longer retention of the vehicle in the conjonctival sac by mucoadhesion.

Cheeks L. (12) compared several commercial pilocarpine preparations for their efficacy of drug delivery as evaluated by changes in pupils diameter,

and return to base-line pupil size. Adsorbocarpine®, Isoptocarpine®, Pilocar®, all at 2%, Pilopine® HS gel, 4%, were used. In albino rabbits, the order of efficacy as judged by AUC, or maximal pupil diameter change, was Pilopine® HS greater than Isoptocarpine® = Adsobocarpine® greater than Pilocar® = saline.

In a multicenter clinical trial, 13 patients with glaucoma were followed during at least 6 months while self-instilling Pilogel® (once daily). A combination therapy with a ß-blocker showed a greater response on the intraocular pressure in comparison with topical ß-blocker alone. They did not notice any serious side-effects after 6 months of combination therapy (63).

In ocular administration, Carbopol is known as a vehicle for commercial ocular formulation which presents the following advantages:

- Generally more comfortable than insoluble or soluble inserts;
- instilled like ointment;
- less blurred vision as compared to ointment;

but with some disadvantages:

- no rate control on drug instability, e.g. pilocarpine
- matted lids in the morning;
- no true sustaining effect unless dissolution control;
- less toxic than the effect produced with pilocarpine eye drops (Liu, 1989).

Carbopol is also used to stabilize the tear film, and to protect the corneal epithelium with a prolonged action allowing a stable artificial precorneal film.

Polycarbophil

Polycarbophil is a water-insoluble cross-linked polyacrylic acid polymer which swells and incorporates large quantities of water. Gurny (36) has described polycarbophil as very sensitive to pH and electrolyte and hence a number of possible approaches wherein a low viscosity polycarbophil solution, when placed in the eye rapidly thickened to trap drug particles.

Park and Robinson (83) synthesized polymers of polycarbophil crosslinked with divinyl glycol and examined their utility in ocular progesterone delivery in rabbit eyes. The bioadhesive dosage form showed an area under the curve 4.2 times greater than the results obtained for the conventional non-bioadhesive suspension, over the time course of the study.

More recently a slightly different approach was used by Middleton and Robinson (71) to deliver the steroid fluorometholone to the eye using a combination of *in situ* viscosity increase and mucoadhesion. They found that an hypotonic, slightly acid, polycarbophil vehicle could be administered as a drop but would gel in the precorneal pocket. The formulation allowed the normal dose of 0.1% fluorometholone steroid suspension to be decreased by half while maintaining aqueous humor drug levels above the therapeutic minimum for 8 h in rabbits. Administration to a few human volunteers showed the product to be comfortable.

1.2.3- Carboxymethylcellulose

Smart et al. (111) defined sodium carboxymethylcellulose (CMC) as the most mucoadhesive polymer. Woolfson (123) showed the excellent bioadhesive properties of a dry sodium carboxymethylcellulose/sodium carbopol film to cervical tissue with a surface mucus layer compared to HPC and HEC.

In vitro studies were investigated by Pergande (87) comparing ophthalmic drugs formulated with hydrogels based on sodium CMC, PVP and polyacrylic acids. The gels showed diffusion coefficients in the series Carbopol 940 1% > sodium CMC 3% > PVP 23%.

Järvinen et al. (44) evaluated the effects of two polymers (sodium carboxymethyl cellulose and Carbopol 940) on systemic absorption of ophthalmic timolol in rabbits. Statistically significant differences were not observed between the effects of Carbopol 940 and sodium CMC on the systemic values of Cmax, AUC of timolol at 41 cP, suggesting that the systemic absorption of timolol from equiviscous solutions of sodium CMC and Carbopol 940 was not dependent on the polymer. Previously, it has been shown that a 330 cP sodium CMC solution increased timolol concentrations longer (at 30 and 240 min) in the ocular tissues two- to three-fold (50). The 41 cP sodium CMC solution did not prolong corneal contact long enough to increase ocular timolol absorption significantly.

In conclusion, those studies have provided further evidence for the role of mucoadhesive polymers in ocular drug delivery.

1.3- *In situ* gelling systems

There are systems with initially low viscosity and instilled as liquid forms to gelify in contact with the cornea in the cul-de-sac of the eye. This new concept of producing a gel *in situ* was suggested for the first time in the early 1980s (36) .

Several concepts for *in situ* gelling systems have been investigated. Three methods have been employed to cause phase transition on the eye surface: change in pH, change in temperature, and ion activation.

1.3.1- pH-triggered systems: Cellulose acetate hydrogen phtalate latex

A pH-triggered ophthalmic latex is a low-viscosity polymeric dispersion in water which undergoes spontaneous coagulation and gelation after instillation in the conjonctival cul-de-sac. The use of pH-sensitive latex nanoparticles has been described by Gurny (34) (36). By selecting an anionic polymer, CAP (30% w/w, viscosity = 50 mPA.s), the dispersion typically shows a very low viscosity up to pH = 5, and will coacervate in contact with the tear fluid (pH=7.2 to 7.4), forming a gel in few seconds and releasing the active ingredients over a prolonged period of time.

The gamma scintigraphy technique was used to monitor the ocular residence time of a CAP dispersion ophthalmic preparation based on a cellulose acetate phtalate. The gelled sytem constituted an *in situ* microreservoir of high viscosity. The half-life of residence on the rabbit corneal surface was approximately 400 s compared to 40 s for a solution (37). Pilocarpine (2% w/v) formulated with CAP maintained a constant miosis in the rabbit for up to 10 h compared to 4 h with pilocarpine eye drops (34).

This system is however characterized by a high polymer concentration (30% CAP), and the low pH of the instilled solution may be a discomfort (irritative) for the patient.

1.3.2- Change in temperature: Pluronics, poloxamer F127

Sustained drug delivery can also be achieved by use of a polymer that changes from solution to gel at the temperature of the eye (33 to 34°C). An example of this type of polymer is poloxamer F 127, which consists of linked polyoxyethylene and polyoxypropylene units (73). At room

temperature the poloxamer remains a solution, when the solution is instilled onto the eye surface the elevated temperature causes the solution to become a gel, thereby prolonging its contact with the ocular surface.

Mengy and Deshpande (69) studied the effect of flurbiprofen, a non-steroidal anti-inflammatory drug, formulated in Carbopol 940 and Pluronic F127 hydrogels and compared for their effects on ocular hypertension. Unlike the Carbopol 940 gels, the Pluronic F127 gels revealed a rapid onset of action as early as 0.5 h post-administration. Carbopol 940 gels revealed a sustained action at lower polymer concentrations than Pluronic F127 gels. Although viscosities of the two formulations were the same, the difference in the effect on the intra-ocular pressure observed, suggested that mucoadhesion or bioadhesion played a role in the sustained action of Carbopol 940 gels. The sustained action for a period of time of 24 h which was longer than what was obtained with earlier studies, could be due in this case to the lipophilicity of the drug.

Dumortier et al. (25) reported that the plasma concentration profiles resulting from morphine inserts after administration in the conjonctival cul-de-sac in rabbits was prolonged in comparison with that obtained with the poloxamer 407 thermoreversible gel. This result was propably due to the rapid dissolution in the tear fluid, consequently to the acidity of the solution, to the eyelid blinking and tear reflex and to poloxamer surfactive properties.

Regarding the recent results on Poloxamer studies, we can't demonstrate any significant results better than those obtained by Gurny (36) who did not find significant difference between Pluronic and isotonic saline solution containing pilocarpine (4% w/w).

One of the disadvantages of such a system is that it is characterized by a high polymer concentration (25% poloxamer); in addition the surfactive properties of poloxamer may be detrimental to ocular tolerance.

1.3.3- Activation ionique: Gomme "gellan" (Gelrite®)

Gelrite® is a polysaccharide, low-acetyl gellan gum, which forms clear gels in the presence of mono or divalent cations (75). The concentration of sodium in human tears, 2,6 g/l is particularly suitable to cause gelation of the material when topically instilled into the conjonctival sac (96).

Maurice and Srinivas (67) introduced fluorescein as the tracer in Gelrite® solutions and isotonic buffer solutions in humans. The gel afforded a two-fold increase in the penetration of fluorescein compared with buffer solution. Earlier experiments with scintigraphy suggested a considerably greater contact time of fluorescein with the cornea when Gelrite® was used (30). Gamma scintigraphy studies compared isoviscous Gelrite® solution (0.6% w/v) and a hydroxyethylcellulose solution (0.5% w/v), with an isotonic saline solution in man. A significant retention of the Gelrite® formulation compared to the HEC and saline solutions was found, with the mean precorneal residence half-time (± sd) of 1089 ± 1485 s, 891 ± 89 s and 22 ± 19 s respectively.

Gelrite® solutions (0.6% w/v) have shown superior timolol bioavailability over an equiviscous HEC solutions (0.5% w/v) in rabbits (96). The enhanced drug bioavailability at each of the three ocular sites examined was explained by the longer residence time in the conjonctival sac.

Gelrite® has the particularity to be very well tolerated and it can be formulated as an isotonic neutral solution, without the disadvantage to be characterized by a high concentration polymer.

In situ gelling systems seem to be of particular interest especially with Gelrite® system. The lack of significant results with CAP gelling preparations and poloxamer gelling systems would suggest a need for further investigation, including tolerance.

2- COLLOIDAL SYSTEMS

Colloidal carriers (liposomes, nanoparticles) have been widely studied for the past 20 years to increase specificity of action of drugs towards a specific target, to facilitate the bioavailability of drugs through biological membranes, or to protect a drug against enzyme inactivation (91). Those studies have been realized by using different administration routes (systemic, oral, intraperitoneal...). Their use in topical administration and especially the ocular administration has been studied for only 10 years.

As we mentioned in the introduction, the main object in ocular therapy is to increase the contact time between the drug and the corneal epithelium or the conjonctiva, and thus in some cases, to decrease the systemic side-effects (53)(70). Because of their low viscosity, colloidal suspension can be administered as easily as eye-drops with the advantage to present a "reservoir form", that is abble to deliver the drug progressively.

Those suggestions on carriers properties, made the colloidal carriers to be more and more tested in ocular drug delivery. The most important carriers studied are liposomes and nanoparticles.

2.1- Liposomes

Liposomes are vesicles made of phospholipids layers limiting concentric aqueous cavities. Depending on phospholipids nature and depending on the method of preparation used, it is possible to classify liposomes as MLV (Multilamellar Vesicles), LUV (Large Unilamellar Vesicles), and SUV (Small Unilamellar Vesicles) (91)(90). The vesicles sizes are ranged from 10 nm for SUV to 10 μm for MLV. The external membrane can be positively charged (stearylamine), negatively charged (dicethylphospate, phosphatidic acid...), or neutral (phosphatidylcholin,

dipalmitoylphosphatidylcholin). They usually include cholesterol for its property to maintain the membrane structure of the liposome and so inducing a limiting release of the drug through the membrane out of the vesicle.

Because of the nature of components used for their preparation, liposomes are considered as biocompatible and bioerodible vesicles. They present a particular interest for ophthalmic drug delivery systems (81).

2.2- Nanoparticles

Nanoparticles are polymeric colloidal ranging in size from 10 to 1000 nm. They can be classified into two groups: nanospheres and nanocapsules.

Nanospheres are small solid matricial spheres constituted of a dense solid polymeric network, developing a large specific area (95). The drug can be either incorporated or be adsorbed onto the surface.

Nanocapsules are small capsules formed of a central cavity (oily droplet) surrounded by a polymeric membrane.

Polyacrylamide (9), polymethylmethacrylate (49) (94), albumin (47), gelatin (80), polyalkylcyanoacrylate (33) polylactic-co-glycolic acid and ε-caprolactone (27) can be used. Methods of preparation are numerous but they can be divided into two groups: methods consisting in polymerization of monomers and methods consisting in precipitation of polymers.

Among the former methods, the most commonly used has been developped by Couvreur et al. (15) for nanospheres preparation by polymerization of alkylcyanoacrylate monomers. The mechanism proposed for the formation of nanospheres is an emulsion polymerization process leading to the

formation of submicronic particles. Practically, after addition of the monomer to an aqueous solution, the cyanoacrylic compound let to diffuse through the water phase, polymerizes slowly into micelles by an anionic polymerization mechanism. Three parameters can influence the size of nanospheres: the nature of polymerization medium (presence or absence of surfactant, pH, co-solvent) (15) (103), the nature of the monomer (48) as well as the rate of the mechanical stirring. Molecular weights of polymers constituting the nanospheres were found very low (ranging between 500 to 2000 for isobutylcyanoacrylate). Therefore, these results indicate that nanospheres are most likely constituted by the agglomeration of small oligomeric subunits forming the nanomatricial structure (120). The drug can either be incorporated into nanospheres during polymerization process (hydrophilic drugs) or be adsorbed onto the surface of preformed particles. Factors such as the nature of monomer, the drug concentration in the polymerization medium, the pH of the aqueous medium and the pKa of the drug can influence the sorption capacity of the carrier. Drug in their non ionized form are better entrapped than ionized compounds (41). These alkylcyanoacrylate nanospheres have been widely investigated in cancerology field (16)(17), in intracellular antibiotherapy (124) etc...

Polyalkylcyanoacrylate nanocapsules are prepared by mixing an ethanolic solution of oil and the monomer with an aqueous phase containing non ionic surfactant. The polymeric colloidal suspension occurs immediately. The mechanism proposed for nanocapsules formation is an interfacial polymerization process. This carrier can mainly encapsulate drugs with lipophilic character. The rate of encapsulation is generally related to the solubility of the drug in the oily compartment (2).

Methods requiring the use of preformed polymers have the advantage not to expose a monomer directly to the drug, with the risk to create covalent bounds between polymer and drug.

Several methods of preparation have been developped (47)(80). More recently a nanoagregation method has been developped by Fessi et al. (27). This method consists in the agregation of a polymer solution in a solvent S1 (Acetone) by the addition in a solvent S2 miscible to S1. The agregation leads to the formation of nanospheres with a mean diameter of about 100 to 300 nm. This method presents the advantage to be usable with many polymers (copolymers of lactic acid and glycolic acid, poly-ε-caprolactone, albumin...)

Many drugs have been incorporated with high rate of encapsulation. An alternative use of this method consists to include a small amount of oil which leads to the obtention of nanocaspules by polymer agregation at the interface oil/water. In that case lipophilic drugs can be incorporate in the nanocapsules .

2.3- Colloidal carriers potentialities in ocular drug delivery

2.3.1- Liposomes

The first investigations were realized with liposomes. Smolin et al. (112) studied the effect of idoxuridine encapsulation in liposomes in the treatment of herpes simplex keratitis in rabbits. The administration 3 times a day, of a liposomal suspension of idoxuridine revealed more efficient results compared with idoxuridine solution. Those favorable results have been found with some other authors.

Singh and Mezei (107) revealed a significant increase of triamcinolone concentration in aqueous humor after administration of encapsulated drug in liposomes. Stratford et al. (115) by using inuline as a tracer were able to follow the drug in different compartments of the eye. Encapsulation of inuline in liposomes was correlated to an increase of the drug concentration

in the conjonctiva, the cornea and iris+ciliary body. However, they didn't observe any increase in the aqueous humor.

Comparable results were obtained with Taniguchi et al. (116) who revealed an increase in dexamethasone valerate concentration after administration of the liposomal dosage form.

On the other hand, some studies do not lead to an increase in intraocular concentration or an increase in efficacy after incorporation of the tested drug in liposomes. For example, Benita et al. (7) examined the intra-ocular pressure after administration of pilocarpine 0.1% in liposomes in rabbits in comparison with a pilocarpine isotonic buffer solution (1% and 2%). Results obtained with pilocarpine are generally disapointing compared with what was expected and authors conclude to an ineffacy of liposomes to increase pilocarpine bioavailability. Stratford (115) who studied epinephrine bioavailability, found that the liposomal form suspension induced a decrease by half of the concentration in conjonctiva, cornea, iris+ciliary body. Singh and Mezei (108) obtained the same results with dihydrostreptomycine sulphate which decreased in concentration in ocular tissues after administration of the liposomes containing the drugs whatever the type of liposomes it was.

All the results described above suggest that physico-chemical properties of the encapsulated drug have a significant influence on the effect with the liposomes. Actually, it seems that results in favor of the liposomal forms have been obtained essentially with more lipophilic drugs.

It is well-known that hydrophilic drugs are more able to escape rapidly out of the liposomes, than the lipophilic drugs which are incorporated in the liposomal membrane. That's what Stratford et al. (115) reported regarding liposomes of epinephrine with their content releasing rapidly (60% for 3 h) whereas liposomes containing inuline seemed to be more stable (12%

released for 3 h). Comparable results have been reported by Ahmed and Patton (1) with inuline that released only 1% of encapsulated amount from liposomes after 3 h dialysis.

In vivo study confirmed Stratford et al. results. Inuline concentrations in ocular tissues (cornea, and aqueous humor) were always higher with the liposomal drug suspension in comparison with the solution.

Thus, it seems that the association between drugs and liposomes is a predominant factor for ocular drug bioavailability and this association may be in favor with lipophilic drugs. The association drug/ liposome can not only explain the efficacy noticed by some authors. Effectively, if we admit that colloidal carriers can not penetrate the cornea by endocytocis it si necessary for the drug to go out of the vesicle by diffusion or after unstabilization (destructuration) to diffuse then through the cornea and/or ocular tissues.

This necessitates a contact time between the carrier and the corneal epithelium as long as possible. Stratford et al. (115) reported that liposomes containing epinephrine were released from tears as fast as the corresponding solution. However liposomes containing inuline seemed to disapear less rapidly. According to the authors, it would be due to an increase in contact time between liposomes and cornea, resulting in a significant increase in intracorneal concentration of inuline. It is interesting to notice also that the increase in instilled volume leads to an increase in inuline concentration in tears but not associated with an increase in ocular drug tissues concentration (54). Two points are in favor with an increase in contact time between liposomes and cornea to enhance penetration. Effectively, when the cornea has been rinced with a saline solution, the intraocular inuline concentration is decreased. Moreover, administration of empty liposomes just before the administration of liposomes containing inuline revealed a significant decrease in drug concentration. This result

proves that there is a competitive adsorption on the surface area of the cornea among liposomes for a limited number of sites.

Corneal epithelium is covered by mucin negatively charged (114). The same authors reported that adhesion to the mucin can be attributed to an electrostatic interaction or a mucoadhesion. The influence of liposomes charge has been studied by authors. All agree to conclude that positively charged liposomes lead to an increase in drug concentration in ocular tissues.

Singh and Mezei (108) revealed an increase of liposomes - conjonctiva interaction with MLV and SUV positively charged and containing dihydrostreptomycine sulfate.

Meisner et al. (68) compared in an important study, neutral liposomes MLV to positively charged MLV and to negatively charged MLV, containing atropine base and atropine sulphate, in rabbits. The study consisted in the comparison of the mydriatic response of the drug in the different preparations. The results was similar with the both atropine forms base, and sulphate for the same formulation corresponding. However, the response was prolonged with the liposomal suspension compared to the solutions and between liposomes, the positively charged was the one with the greater time profil.

Those results confirmed Fitzgerald et al. (28) studies which revealed a decrease in liposomal suspensions drainage from tears with the positively charged liposomes.

Schaeffer and Krohn (102) also reported in an *in vitro* study, a greater affinity of positively charged liposomes to the cornea. Further investigation lead to the study of coated liposomes with a bioadhesive polymer, Carbopol 1342 (Durrani et al. (26)). Those polymer coated liposomes (REV)

containing pilocarpine were compared to uncoated REV containing pilocarpine. The results revealed a decrease of pilocarpine release from the coated liposomes resulting in a increase of miotic activity. These studies suggest that polymer coated vesicles or similar preparations may improve ocular drug therapy in the future.

2.3.2- Nanoparticles

Investigations conducted on liposomes for ocular drug delivery appeared very encouraging. Some researchers directed their investigations on polymeric carriers. In this part , we won't repeat the results obtained by Gurny who evaluated CAP we talked about in gelling systems.

The first studies concerned Piloplex® systems constituted of pilocarpine-loaded nanospheres of poly(methylmethacrylate-acrylic acid) copolymer. In clinical trials, Piloplex® lowered the intra-ocular pressure. Those systems based on non biodegradable polymers were not investigated further. However, numerous studies have been investigated concerning nanospheres, and for example alkylcyanoacrylate nanocapsules obtained by polymerization of a monomer were under investigation.

Wood et al. (122) evaluated isohexylcyanoacrylate nanospheres labelled with ^{14}C and prepared by the method described above (15). *In vitro* degradation was performed using nanospheres suspensions and freshly collected albino rabbit tears. Degradation of the nanospheres in tears occured at a relatively rapid rate for the first hour with approximatively 19% degradation. At 6 h only 30% approximatively were degradated. It would be caused as it has been demonstrated using I.V. route, by lysosomal enzyme biodegradation (34) (114); lysosomal enzymes being especially numerous in the precorneal area. Similar study (122) showed that after administration of a 25 µL suspension, only 0.1% of initial amount has been

found in the cornea and the conjonctiva. It is interesting to notice that this concentration is relatively constant for 6 h after instillation; this result suggests that the adhesion of the nanospheres to the mucin epithelial surface of the cornea and conjonctiva occured.

Further work has been conducted by Harmia et al. (39), testing polybutylcyanoacrylate nanospheress containing pilocarpine nitrate. Two formulations were tested: the first one containing pilocarpine inside the matrix of the particle, the other one with pilocarpine adsorded onto the surface area of the particle. The miotic response has been evaluated after administration of 50 μL suspension in comparison with the solution. The response intensity was the same with both formulations. However, the formulation containing the drug on the surface of the particle maintained the effect for a longer time. This results were not concluant with pilocarpine included inside the nanospheres, these results were correlated to the results on biodegradation of the particles described above, which show a slow and incomplete degradation of the polymer in tears.

Those results have been confirmed in a study on polybutylcyanoacrylate nanospheres containing progesterone (57). The lipophilic nature of the drug resulted in an encapsulating rate around 100% in the carrier but the strong affininty of the drug for the polymer and the slow degradation of the polymer does not induce a sufficient release of the drug. This resulted in an intraocular concentration lower than after administration of the solution. So it seems that the major problem with the use of alkylcyanoacrylate nanospheres is a very slow drug release out of the matrix, compared to the residence time of the particles in the precorneal area. Some authors, Losa et al. (59) evaluated amikacin sulphate suspensions associated to polybutylcyanoacrylate and reported that all drug molecules which are desorbed from the nanospheres quickly enough diffuse across the cornea more easily than free molecules wich remain in the lacrymal fluid. These results agree with the conclusion of Marchal-Heussler et al. (65) who have

demonstrated that the superficial charge and the binding type of the drug onto nanospheres were the most important factors regarding the improvement of the therapeutic response of Betaxolol chlorhydrate. In other studies, Marchal-Heussler et al. (64) in order to increase the ocular absorption of betaxolol, used nanospheres (NS) or nanocapsules (NC). Three polymers were tested namely polyisobutylcyanoacrylate, a copolymer of lactic and glycolic acid and poly-ε-caprolactone. The decrease in intra-ocular pressure were much more pronounced with the colloidal carriers made of poly-ε-caprolactone (NS or NC) than with the carriers prepared with the other polymers as well as the commercial eye-drops. It seems that the more hydrophobic the carrier, the higher the ocular activity of the drug. It was observed that poly-ε-caprolactone nanospheres and nanocapsules undergo a process of agglomeration after instillation and increase in residence time of the drug in the precorneal area. Furthermore, poly-ε-caprolactone nanocapsules display a better effect than poly-ε-caprolactone nanospheres probably because the drug entrapped is under the non-ionized forme in the oily core of the carrier and can diffuse at a greater rate into the cornea. Similar results were obtained by Marchal-Heussler et al. (66) with carteolol (ß-blocker). Poly-ε-caprolactone nanocapsules induced a better penetration of the drug than nanospheres prepared with the same polymer. Diffusion of the drug from the oily phase toward the epithelium may be more effective than diffusion from the internal matrix of particles. This new dosage form produces very interesting therapeutic effect with much lower drug concentrations. Thus systemic effects could be minimized. Complementary results were proposed by Losa et al. (60). They investigated the potential of polymeric nanocapsules obtained by interfacial polymerization of PIBCA (NC-PIBCA) and interfacial deposition of poly-ε-caprolactone (NC-PC) containing metipranolol, a β-blocker used in glaucoma. Experiments revealed that the type of oil is the most important factor influencing loading efficiency. From *in vitro* release studies, the

authors concluded that the release profiles were not influenced by the thin polymeric coating. This suggests that NC-PIBCA and NC-PC are not able to control release of metipranolol base but the polymer envelop can contribute to the stabilization of the suspension.

Conclusion

This review lead us to conclude that those systems present some interests in ocular drug delivery increasing ocular drug residence time; improving ocular drug bioavailability; diminishing side effects due to systemic absorption; and diminishing the necessary amount of drug for a therapeutic response in the anterior chamber.

As mentioned, numerous studies have been conducted on polymers viscolyzers, bioadhesive delivery systems and colloidal systems, all in rabbits and in humans. Bioadhesive properties of polymers illustrated in this review, seemed to be related to precorneal retention of the drug more significantly in comparison with other isoviscous and non-bioadhesive polymers. Encapsulation of drugs in liposomes and nanoparticles was correlated to an increase of the drug concentration in the ocular tissues. However the various polymers tested seemed to affect the retention of the tracers somewhat differently depending on the nature of the polymer but also the nature of the drug and depending on the choice of the test to reveal the polymer properties.

There is a need for a polymer pattern in which drug could be trapped physically to prolong drug residence time on the corneal surface and preserve visual acuity. Such systems should propably be more hydrophobic than the materials currently employed, and would have to exhibit pseudoplastic behavior to minimize interference with blinking.

Aknowledgments:

The authors thank CIEP (Club international d'échanges pharmaceutiques) for their contribution to the realization to the exchange between the two universities: University of Rhode Island and University of Rennes (France)

CIEP (Club international d'échanges pharmaceutiques)
5, rue Charles Martigny
94701 MAISON ALFORT - France

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