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# Preparation and Characterization of Eudragit Retard Nanosuspensions for the Ocular Delivery of Cloricromene

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## ABSTRACT

The purpose of this study was to improve the stability of cloricromene (AD6) in ophthalmic formulations and its drug availability at the ocular level. To this end, AD6-loaded polymeric nanoparticle suspensions were made using inert polymer resins (Eudragit RS100 and RL100). We modified the quasi-emulsion solvent diffusion technique by varying some formulation parameters (the drug-to-polymer ratio, the total drug and polymer amount, and the stirring speed). The chemical stability of AD6 in the nanosuspensions was assessed by preparing some formulations using (unbuffered) isotonic saline or a pH 7 phosphate buffer solution as the dispersing medium. The formulations were stored at 4°C, and the rate of degradation of AD6 was followed by high performance liquid chromatography (HPLC). The obtained nanosuspensions showed mean sizes and a positive surface charge (ζ-potential) that make them suitable for an ophthalmic application; these properties were maintained upon storage at 4°C for several months. In vitro dissolution tests confirmed a modified release of the drug from the polymer matrixes. Nanosuspensions prepared with saline solution and no or lower amounts of surfactant (Tween 80) showed an enhanced stability of the ester drug for several months, with respect to an AD6 aqueous solution. Based on the technological results, AD6-loaded Eudragit Retard nanoparticle suspensions appear to offer promise as a means to improving the shelf life and bioavailability of this drug after ophthalmic application.

**KEYWORDS:** Cloricromene, AD6, Eudragit Retard, nanosuspensions, quasi-emulsion solvent diffusion, nanoparticles, ophthalmic drug delivery, stability.

## INTRODUCTION

Developing successful drug delivery systems for releasing active molecules to the eye anterior or posterior segments is an exciting challenge for modern pharmaceutical technology. For drugs showing problems of solubility or stability in the eye fluids, or difficulty passing through the corneal barrier, a controlled-release system can help to modulate and/or sustain the amount of drug released, reducing the drainage from the eye surface and the unproductive absorption usually associated with conventional aqueous eyedrop formulations.

Use of polymeric nanoparticles is one of the most interesting approaches to achieving local controlled drug delivery.[1-4] In this field, Eudragit Retard polymer nanoparticle suspensions have been investigated as a carrier system for the ophthalmic release of nonsteroidal antiinflammatory drugs, such as ibuprofen and flurbiprofen.[5-7] Such carriers showed an interesting size distribution and a positive surface charge, along with good stability, that make them potential candidates for use in ocular drug delivery systems. In particular, their positive surface charge (ζ-potential) can allow a longer residence time of nanoparticles on the cornea surface, with a consequent slower drug release and higher drug concentrations in the aqueous humor, compared with classical eyedrops.

Eudragit RS100 (RS) and RL100 (RL) polymers are being used for the enteric coating of tablets and for preparing controlled-release formulations. RS and RL are copolymers of poly(ethylacrylate, methyl-methacrylate and chlorotrimethyl-ammonioethyl methacrylate), containing an amount of quaternary ammonium groups ranging between 4.5% and 6.8%, and 8.8% and 12%, for RS and RL, respectively. These matrixes are insoluble in water at physiological pH values and capable of swelling, so they are good for the dispersion of active compounds.[8-10]

Cloricromene (8-monochloro-3-b-diethylaminoethyl-4-methyl-7-ethoxy-carbonyl-methoxycoumarin hydrochloride; AD6) (Figure 1) is a synthetic coumarin derivative that possesses antithrombotic and antiplatelet actions, inhibits polymorphonuclear cells (PMN) neutrophil function, and causes vasodilatation.[11] AD6 reduces the synthesis of the products of both the cyclooxygenase and the lipooxygenase pathways.[12,13] After its administration, AD6 is hydrolyzed in the blood and tissues to its acid metabolite (cloricromene

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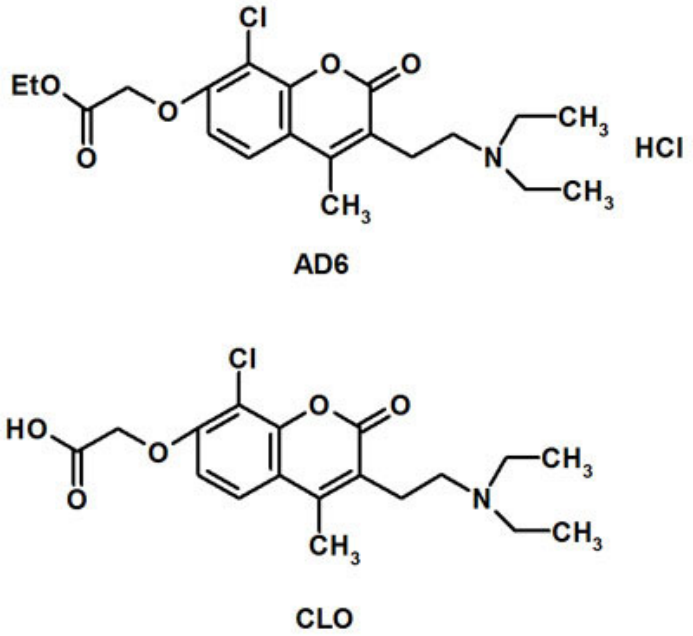


**Figure 1.** Chemical structure of AD6 and its metabolite CLO. AD6 indicates cloricromene; CLO, cloricromene acid.

acid [CLO]; Figure 1). In platelets and leukocytes, AD6 is taken up as an ester and converted to CLO.[14] The latter is ineffective in vitro under conditions where the ester AD6 is active, but it is the active form in the intracellular environment. To enter cells, however, AD6 must be in the ester form.

AD6 has been recently proposed also for ocular application in the treatment of uveitis. Bucolo et al[15] showed that AD6 attenuates the degree of inflammation and tissue damage associated with endotoxin-induced uveitis in the rabbit eye and protects against experimental rat uveitis, reducing the expression of adhesion molecules such as P-selectin and ICAM-1.

Given that the ester form AD6 is freely soluble but unstable in aqueous solutions, where it is rapidly converted into the insoluble acid form, a delivery system able to ensure a slow and prolonged release of this active form at an intraocular level could improve the therapeutic benefits of this pharmacological agent. Therefore, in the present work the feasibility of RS and RL matrices for the preparation of AD6-loaded ophthalmic drug delivery systems was investigated.

RS or RL nanosuspensions were prepared using an adaptation of the quasi-emulsion solvent diffusion (QESD) technique.[6,16] Various operative variables were tested: the drug-to-polymer weight ratio (33% or 50%), the total amount of drug and polymer in the initial ethanol solution (100 or 200 mg), and the agitation speed during the formation of the nanosuspension (20 500 or 24 000 rpm). Such variables could influence the particle size and the drug release.

## Materials and Methods

### *Materials*

RS and RL were a gift from Rofarma Italia Srl (Gaggiano, Milan, Italy). AD6 and CLO were a gift from Bausch & Lomb (Catania, Italy). Tween 80 (Fluka) was purchased from Sigma-Aldrich Chimica Srl (Milan, Italy). Benzalkonium chloride (50%, wt/vol, Ph Eur grade) was purchased from Galeno (Comeana, Italy). All other chemicals were of reagent grade or higher; HPLC-grade water was used for the preparation of all the formulations.

### *Preparation of Nanoparticles*

AD6 and polymer were codissolved at room temperature in 2 mL of ethanol to achieve a 33% or 50% drug-to-polymer weight ratio, and the solution was slowly injected (0.5 mL $min^{-1}$), by a syringe connected to a thin Teflon tube, into 50 mL of isotonic saline containing Tween 80 (0.02%, wt/vol) and benzalkonium chloride (0.05%, wt/vol calculated on the active compound concentration) and kept at low temperature in an iced-water bath. During the injection, the combination was strongly mixed by an Ultra-Turrax T25 (IKA Labortechnik, Staufen, Germany) at the agitation speed reported in Table 1.

The solution immediately turned into a pseudo-emulsion of the drug and a polymer-alcoholic solution in the external aqueous phase. The counterdiffusion of ethanol and water out of and into the emulsion microdroplets, respectively, and the gradual evaporation of the organic solvent, determined the in situ precipitation of the polymer and the drug, with the formation of matrix-type nanoparticles (Figure 2). Ethanol residues were left to evaporate off under a slow magnetic stirring of the nanosuspensions at room temperature for 8 to 12 hours.

### *Scanning Electron Microscopy*

A scanning electron microscope (SEM) (XL-30 Philips, Eindhoven, The Netherlands) was used to evaluate the size and morphology of nanoparticles (Figure 3). The nanosuspensions were deposited on a glass disk applied on a metallic stub and evaporated under a vacuum overnight. Before the SEM analysis the samples were metallized under an argon atmosphere with a 10-nm gold palladium thickness (EMITECH-K550 Sputter Coater, Houston, TX). To determine the size distribution of the samples, at least 300 particles for each preparation were sized from electron microphotographs by an image analysis (IMG VIEW, created by PL Fabbri, CIGS, Modena, Italy).

**Table 1.** Mean Size (nm) With the Relative Abundance (Area %), PI, and ζ-Potential ± SD (Zeta) of AD6-loaded RS or RL Nanosuspensions Obtained at 24 000 rpm (Batches A) or 20 500 rpm (Batches B)*

| | | | T = 0 | | | | T = 4 months | | | | T = 12 months | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Size | | | | Size | | | | Size | | | |
| Sample | % Drug (wt/wt) | Drug + Polymer Total Amount (mg) | nm | Area % | PI | Zeta | nm | Area % | PI | Zeta | nm | Area % | PI | Zeta |
| A/50/1/RL | 50 | 100 | 154.3 | 74.5 | 0.65 | 27.2 ± 8.6 | 102.5 | 94.3 | 1.0 | 22.7 ± 2.8 | 110.2 | 98.7 | 1.0 | +19.3 ± 2.2 |
| A/50/1/RS | 50 | 100 | 233.3 | 89.7 | 0.88 | 30.9 ± 6.9 | 97.6 | 99.1 | 1.0 | 31.8 ± 6.3 | 156.6 | 98.1 | 1.0 | +29.4 ± 1.1 |
| A/33/2/RL | 33 | 200 | 85.4 | 92.9 | 1.0 | 3.1 ± 4.6 | 2305† | 83.5 | 1.0 | 3.1 ± 4.6 | † | — | — | +8.5 ± 3.3 |
| B/33/1/RS | 33 | 100 | 17.5 | 100 | 0.69 | 3.6 ± 5.3 | 163.2 | 100 | 1.0 | 7.7 ± 3.2 | 145.0 | 88.7 | 1.0 | +5.6 ± 0.9 |
| B/33/2/RS | 33 | 200 | 47.6 | 100 | 0.30 | 18.5 ± 4.4 | 77.8<br>502.6 | 44.5<br>52.1 | 1.0 | 1.4 ± 2.0 | 98.1<br>665.0 | 43.9<br>53.1 | 1.0 | +2.6 ± 2.4 |
| B/50/1/RS | 50 | 100 | 48.7 | 99.6 | 1.0 | 8.6 ± 4.1 | 78.3 | 92.4 | 1.0 | 1.7 ± 2.2 | 79.9<br>668.0 | 78.8<br>20.2 | 1.0 | +3.4 ± 0.2 |

*PI indicates polydispersity index; AD6, cloricromene; RS, Eudragit RS100; RL, Eudragit RL100. Data at 4 and 12 months are relative to samples stored at 4 ± 1°C.

†Particle aggregation was visible in these samples.

### *Size Analysis and ζ-Potential*

The mean particle size of the formulations was determined by photo-correlation spectroscopy with a Zetamaster (Malvern Instruments Ltd, Worcs, UK) equipped with the Malvern PCS software (version 1.27). Every sample was appropriately diluted with HPLC-grade water, and the reading was performed at a 90° angle with respect to the incident beam.

Electrophoretic mobility was obtained by a laser Doppler anemometer using the same instrument. A suitable amount of the sample (50-100 μL) was diluted with 5 mL of water (HPLC grade) and placed into the electrophoretic cell of the instrument, where a potential of ±150 mV was induced. The ζ-potential value was calculated by the software using Smoluchowski's equation.

### *Drug Loading*

The amount of AD6 associated with polymer matrixes in each formulation was assessed by means of an ultrafiltration procedure. An aliquot of each nanosuspension was loaded into the sample reservoir of a VectaSpin 3 filter device containing a polysulfone ultrafiltration membrane (30K MWCO, Whatman Int Ltd, Maidstone, UK) and submitted to ultracentrifugation at 10 000 rpm (IEC/Centra MP 4R centrifuge equipped with an 851-type rotor [Thermo Electron Co, Waltham, MA]) at 10°C for 20 minutes. Drug concentration in the filtrate was assessed by UV analysis (Shimadzu UV-1601 [Shimadzu Italia, Milan, Italy]), after an appropriate dilution if necessary, at λ = 321 nm and using a calibration curve of AD6 in water. From such a value, the percentage of unencapsulated drug was calculated based on the amount initially added in the preparation.

### *Stability Studies of the Formulations*

The physical stability of the nanosuspensions was evaluated after storage for 12 months at 4 ± 2°C. Exact volumes of each nanosuspension were stored in screw-capped amber-glass bottles and placed in a refrigerator, away from


**Figure 2.** Appearance of 2 AD6-loaded nanosuspensions. Pictures were taken 1 week after the preparation. AD6 indicates cloricromene.

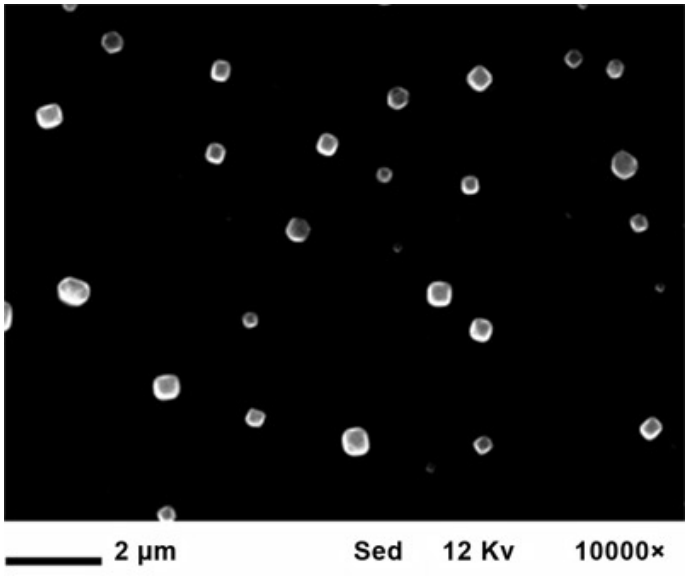


**Figure 3.** Scanning electron microscope analysis of batch A/50/1/RL.

direct light. Aliquots of 500 µL were withdrawn every 2 months to determine particle size and ζ-potential value, as described above.

### *In Vitro Drug Release*

The drug release from the nanosuspensions was evaluated in triplicate over 4 hours by a dialysis system consisting of a SpectraPor membrane (cutoff: 3500 Da), mounted on a 5-mL QuixSep microdialyzer (Orange Scientific, M-Medical Srl, Cornaredo, Italy), and soaked in isotonic phosphate buffer solution (PBS, pH 7.4; Sigma). Samples (5 mL) were dialyzed at room temperature and under slow magnetic stirring. At regular time intervals, 1-mL aliquots of the external medium were withdrawn and immediately replaced with the same volume of fresh buffer. The amount of drug released was determined by UV analysis at $\lambda$ = 321 nm (Shimadzu UV-1601). All the tests started 48 hours after the preparation of the nanosuspensions; during those 48 hours, the nanosuspensions were kept at room temperature, in closed bottles, and away from direct light. The possible limiting effect of the dialysis membrane on drug dissolution was checked by running separate experiments in duplicate with a solution in PBS of pure AD6 at the 2 concentrations used in the formulations. Only a limited delay in drug dissolution into the receiving medium was observed (Figure 4).

### *Evaluation of the Chemical Stability of AD6 in RL Nanosuspensions*

The preparation of formulation A/50/1/RL (Table 1) was repeated by changing the amount of Tween 80 in the dispersing aqueous phase (from 0% to 0.4%, wt/vol) and the nature of the latter (isotonic saline or 0.067M PBS, pH 7.0). The systems were characterized immediately after the preparation for pH and osmolality (Osmomat 010, Gonotec Gmbh, Berlin, Germany). The concentrations of AD6 and the metabolite CLO were determined by HPLC in fresh samples and then after 3 and 10 days, 6 weeks, and 3 months of incubation at 4 ± 1°C. The HPLC assay was a modification of the method previously described by Maltese and Bucolo.[17] Briefly, a $C_{18}$ reversed-phase column (Hypersil ODS) with UV detection at $\lambda$ = 318 nm was used. The mobile phase consisted of acetonitrile-water containing 1% triethylamine pH 3.5 adjusted with orthophosphoric acid. An acetonitrile gradient was necessary to achieve a good separation within 10 minutes.

## Results and Discussion

The different formulative variables tested in this study showed no direct effect upon the amount of drug associated with the polymer matrices, drug loading being always higher than 90% of the initial added amount (data not shown). Neither the total amount of drug nor the total amount of polymer in the initial ethanol solution, as well as the drug/polymer ratio, affected the drug loading. It is noteworthy that the ethanol solution produced by co-dissolving the drug and the polymer was perfectly clear. Since AD6 is only partially soluble in ethanol (~10 mg/mL) (C. Bucolo, unpublished data, December, 2004), it can be concluded that the presence of polymers aided the dissolution of the drug.

The average particle size and ζ-potential values were measured immediately after the preparation of the nanosuspensions or after up to 12 months of storage at 4°C (Table 1).

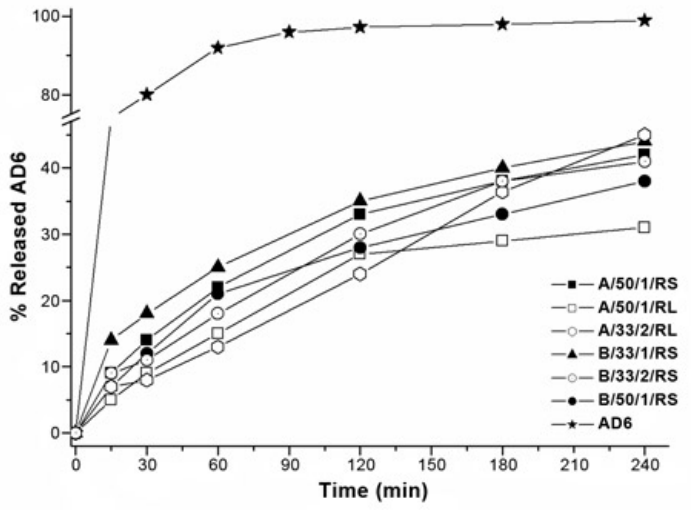


**Figure 4.** In vitro dissolution profile of AD6 from RS and RL nanosuspensions. Values are the mean of 3 replicates; SD bars (always less than 6%) were omitted for clarity. AD6 indicates cloricromene; RS, Eudragit RS100; RL, Eudragit RL100.

For clarity, the intermediate values (collected every 2 months) were not reported. A photograph of 2 sample nanosuspensions appears in Figure 2. SEM pictures (Figure 3) show the presence of definite and regular nanoparticles. No sign of large aggregation was detected during a microscopic examination.

With regard to the preparative variables, when AD6 was loaded in the 2 polymer matrices no significant difference was observed, apart from the agitation speed. All the nanoparticles obtained at 20 500 rpm (batches B) showed lower mean sizes than those obtained at 24 000 rpm (batches A). These findings are quite unusual but could be explained by considering that at the higher agitation speed a greater foaming occurred in the mixture. This could cause an earlier separation of solid nanoparticles from the aqueous medium that limited the size reduction effect induced by stirring. For an alternative explanation of the above results, the role of ethanol during nanoparticle formation must be taken into account. Higher agitation speeds made easier the evaporation of the solvent, with the concomitant rapid precipitation of the polymer upon contact with the aqueous phase[16] and a partial coalescence of particles in larger aggregates. Reducing the stirring speed increased the size of ethanol droplets but slowed down the evaporation rate and rendered more homogeneous the conversion of the nanoemulsion into nanoparticles.

A high polydispersity index was also registered, indicating a high degree of heterogeneity in particle size. Pure RL or RS nanosuspensions usually yielded very small particles (<40 nm) with the QESD technique,[6] forming roughly transparent systems. According to previously described systems loaded with ibuprofen or flurbiprofen,[6,7] in the presence of a carboxylic drug molecule, and irrespective of chemical structure, these polymers formed little and uniform nanoparticles, possibly because of the electrostatic forces occurring between the drug and the ammonium groups in the polymer matrix. The ester form of the drug (AD6) was not able to develop similar interactions and, because of its salted form, led to a wider particle size population.

During storage the nanosuspensions formed a sediment, which could be easily redispersed by manual agitation. No significant change occurred in particle sizes after 12 months of storage in the refrigerator. As previously shown,[7] using benzalkonium chloride in these formulations ensures good microbiological stability over time.

All the formulations exhibited positive ζ-potential values. Such a positive charge is important, since it can facilitate effective adhesion to the cornea epithelial surface, prolonging the drug release time and enhancing the drug's availability in the internal tissues of the eye.[18,19] After 12 months of storage the electrophoretic behavior did not change significantly. In the case of AD6 (Table 1), the hydrochloride salt partially neutralized the polymer positive charges, giving lower ζ-potential values. The relative constancy of the ζ-potential value upon storage indicated that there was no significant drug diffusion out from the nanoparticles into the aqueous phase: in that case, in fact, the free drug molecules in solution could have altered the overall electrophoretic mobility of the suspensions. Previous studies with analogous systems showed that the drug remained on the particle surface after several months of storage, also because of the development of the electrostatic interactions with the polymer.[6,7]

### *In Vitro Drug Release Tests*

In Figure 4 the in vitro dissolution profiles of AD6 from the prepared nanosuspensions are shown. All the formulations linearly released the drug, with ~15% to 25% AD6 dissolved after 1 hour and ~30% to 45% after 4 hours. The tested process variables (drug-to-polymer weight ratio, type of polymer matrix, initial concentration of the ingredients in the ethanol solution, and stirring speed) did not significantly affect the dissolution rate of the active compound, at least in the time interval considered.

Such behavior can be explained by the fact that AD6 release from these systems probably follows a dissolutive mechanism, by which the solubility properties of the drug predominate over any limiting effect that is due to the polymer matrix (diffusion mechanism). It is noteworthy, however, that no burst effect has been observed, indicating that the drug was homogeneously dispersed in the Eudragit matrix and that no significant amount of drug was adsorbed onto the nanoparticle surface.

When the release tests were repeated after 6 months of storage at 4°C, no relevant differences were observed in the amount of released AD6 (Figure 5). These findings indicate that the AD6-Eudragit systems have a quite homogeneous structure that did not change much during storage, although dissolution and particle reaggregation phenomena would have occurred in the external aqueous phase. The slight difference in the AD6 release rate observed during the first part of the test for each pair of formulations (freshly prepared or after 6 months of storage) should indicate only that a part of the drug that is initially dissolved in the dispersing phase is gradually readsorbed and kept on the nanoparticle surface, to be released again slowly.

In general, previous studies of RS and RL microparticles showed that the drug release is often more complex, resulting from coexisting dissolutive and diffusion phenomena.[6,10]

### *Stability Evaluation of AD6 in RL Nanosuspensions*

AD6 is a very unstable compound in aqueous solution above pH 5, where it rapidly forms an insoluble precipitate

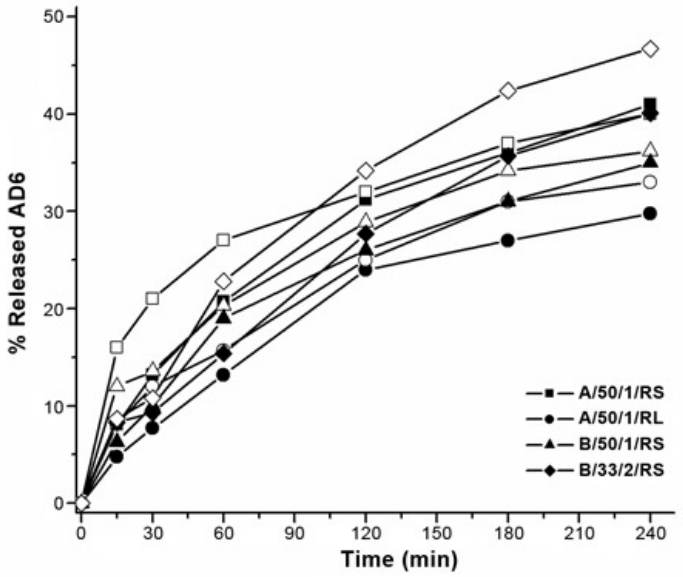


**Figure 5.** Comparison of AD6 release from freshly prepared nanosuspensions (filled symbols) or after 6 months of storage at 4°C (open symbols). Values are the mean of 3 replicates; SD bars (0.4%-4.5%) were omitted for clarity. AD6 indicates cloricromene.

of the acid (CLO). At pH 6.5 ~40% of the drug is hydrolyzed at room temperature and over 80% degraded into CLO after 27 days (C. Bucolo, unpublished data, December, 2004). Such instability strongly limits the formulation of therapeutic eyedrops containing this drug. To verify whether the polymer matrix was able to prevent or reduce the hydrolysis of AD6, we prepared some RL nanosuspensions containing a 50% weight amount of the drug, using saline or a hypotonic pH 7 PBS (0.067M, Italian Pharmacopoeia, X Ed) as the dispersing medium, to have pH values close to neutrality and a tonicity compatible with the ophthalmic application. Furthermore, the preparations were made without any emulsifier or in the presence of increasing percentages of Tween 80 (0.1% to 0.4%, wt/vol). The formulations were tested immediately after the preparation and then after 3 and 10 days, 6 weeks, and 3 months of storage in the refrigerator. Table 2 reports the properties of the tested formulations and the degradation profile of the dispersed AD6 into CLO.

All the systems showed pH values near 7. Such values were maintained for the duration of the test. Only when isotonic saline was used for the dispersion of the polymer were suitable tonicity values (around 330 mOsm/kg) obtained, whereas in the presence of the phosphate buffer the final osmolality was higher (Table 2), thus requiring a finer formulation adjustment before a practical application.

The HPLC evaluation of AD6 concentration during storage showed that the stability of the ester drug toward the hydrolysis changed significantly as a function of the dispersing medium used. The systems prepared with saline (S1-S4, Table 2) showed a very high percentage of unmodified drug even 6 weeks after the preparation and still more than 75% of intact AD6 after 3 months. On the other hand, in the phosphate buffered medium (B1-B4) the drug degraded more rapidly, with a 20% hydrolysis after 3 days and 65% after 6 weeks. At the end of the test, only residual amounts of intact AD6 were detected. In the meantime, the formation of CLO gave an abundant white precipitate in the latter formulations, whereas the S1-S4 ones remained almost transparent or lightly cloudy until the end of the experiment.

Given that in the B1-B4 formulations the pH was kept constant by the buffer system, it seems that the polymer matrix was not able to preserve the drug by its aqueous hydrolysis. An effect associated with the phosphate salts may have occurred, but further experiments with different buffer solutions would be needed to prove this. Alternatively, it may be that when the nanoparticles were originated in the buffered medium, the drug was located in the external layers of the particle structure, resulting in a quicker release and contact with the aqueous phase.

**Table 2.** Hydrolysis of AD6 Into CLO (Mean Values ± SD of 3 Replicates) from RL Nanosuspensions Stored at 4 ± 1°C*

| Sample | Tween 80 % wt/vol | pH | mOsm/kg | Percentage Intact AD6 | | | |
|---|---|---|---|---|---|---|---|
| | | | | T = 3 days | T = 10 days | T = 6 weeks | T = 3 months |
| S1 | 0 | 6.64 | 330 | 97.5 | 95.6 | 93.3 | 90.1 |
| S2 | 0.1 | 6.98 | 350 | 100 | 100 | 98.5 | 86.6 |
| S3 | 0.2 | 6.88 | 340 | 91.8 | 88.7 | 86 | 79.2 |
| S4 | 0.4 | 6.82 | 328 | 88.8 | 84.8 | 84.5 | 77.9 |
| B1 | 0 | 7.14 | 360 | 78.7 | 66.3 | 41.6† | — |
| B2 | 0.1 | 7.17 | 410 | 79.1 | 67.6 | 39.8† | — |
| B3 | 0.2 | 7.15 | 380 | 74.5 | 62.1 | 38.9† | — |
| B4 | 0.4 | 7.13 | 390 | 78.8 | 65.8 | 35.8† | — |

*Systems were prepared with saline (batches S1-S4) or a pH 7 phosphate buffer solution (B1-B4) as the dispersing medium. AD6 indicates cloricromene; CLO, cloricromene; RL, Eudragit RL100.
†At the 6th week an abundant precipitate caused by CLO was observed.

The presence of the emulsifier in both systems (S2-S4 and B2-B4) seemed to accelerate the hydrolysis rate of cloricromene. A higher chemical stability of the ester drug was in fact observed when the nanoparticles were formed in saline in the absence of Tween (S1) or with the lowest amount of Tween (S2, Table 2). In this case ~90% of intact drug was present in the nanosuspensions after 3 months of storage. The catalytic effect of Tween on the hydrolysis of AD6 could be explained in terms of facilitating the contact of water with the drug inside the polymer matrix. Moreover, since the presence of the surfactant during the formation of the ethanol/water "quasi-emulsion" aids the mixing of the drug in the Eudragit solution, it is possible that a higher amount of drug remained in the superficial regions of nanoparticles, where the contact with water occurred more quickly.

## CONCLUSIONS

The codispersion of cloricromene hydrochloride (AD6) in RS or RL polymers resulted in nanoparticle suspensions that showed good mean sizes for ophthalmic applications and a positive surface charge. The suspensions allowed for improved corneal adhesion and stability upon storage, particularly at low temperatures.

When preparation occurred in an isotonic saline solution, the dispersion of AD6 in the polymer network protected the ester drug from the hydrolytic cleavage into the inactive and insoluble acid form.

According to preliminary biological evaluation of these systems[20] that showed a higher drug availability in the rabbit aqueous humor after the drug's administration in RL nanosuspensions, AD6-loaded Eudragit Retard nanoparticle suspensions appear to offer a promising means of improving the shelf life and bioavailability of this drug after ophthalmic application.

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