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Design and monitoring of photostability systems for amlodipine dosage forms

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Abstract

Photostability of amlodipine (AML) has been monitored in several pharmaceutical inclusion systems characterized by plurimolecular aggregation of the drug and excipients with high molecular weight. Several formulations including cyclodextrins, liposomes and microspheres have been prepared and characterized. The photodegradation process has been monitored according to the conditions suggested by the ICH Guideline for photostability testing, by using a light cabinet equipped with a Xenon lamp and monitored by spectrophotometry. The formulations herein tested have been found to be able to considerably increase drug stability, when compared with usual pharmaceutical forms. The residual concentration detected in the inclusion complexes with cyclodextrins and liposomes was 90 and 77%, respectively, while a very good value of 97% was found for microspheres, after a radiant exposure of 11,340 kJ m^{-2}.
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Keywords: Amlodipine; Photodegradation; Supramolecular systems; Liposomes; Cyclodextrins; Microspheres

1. Introduction

Amlodipine (AML) and related compounds, belonging to the 1,4-dihydropyridine class of antihypertensive drugs, are photosensitive since light catalyzes their oxidation to pyridine derivatives (Fig. 1), lacking any therapeutic effect (Yeung et al., 1991; Marinkovic et al., 2000; Ragno et al., 2002).

Nowadays, photostability of drugs represents an emerging topic in the pharmaceutical research field as the number of drugs which are revealed to be light sensitive is noticeably increasing (de Villiers et al., 1992; Tønnesen, 1996; Beijersbergen van Henegouwen, 1997; Albini and Fasani, 1998). Accordingly, many new technology-based pharmaceutical systems are presently proposed in order to enhance stability for such compounds (Tønnesen, 2001). Several of these systems are devoted to the simple use of light-protective wrapping or to the formulation with appropriate excipients. Therefore, photosensitive drugs can be protected from light during preparation or administration (i.e. topical application) by means of photo-absorbent additives directly included into the formulation (Thoma and Klimek, 1991; Béchard et al., 1992; Hasan, 1992; Desai et al., 1994; Ho et al., 1994). Such agents compete with the molecules of the

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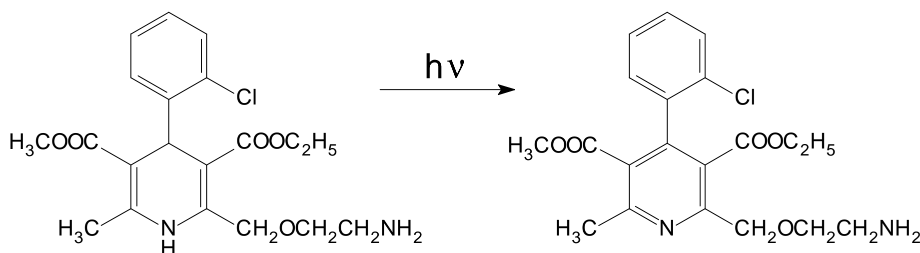

Fig. 1. Chemical structures of AML and its photodegradation product.

drug in absorbing light in the visible and UV spectral regions. Drug stabilization in solid phase by colored additives has been proposed as well (Skowronski et al., 1984; Tønnesen and Karlsen, 1987).

A more recent approach is represented by the possibility of using chemical complexes of drug with appropriate photo-protective carriers. Many studies reporting a reduced amount of degraded drug in supramolecular systems using cyclodextrins (Jiménez et al., 1997; Biloti et al., 1999) or liposomes (Morgan et al., 1995; Bisby et al., 2000; Brisaert et al., 2001), or even a combination of both (Habib and Asker, 1991; Loukas et al., 1995), have been published. In particular, cyclodextrins have been widely utilized in the formation of inclusion complexes with 1,4-dihydropyridines (Mielcarek, 1997; Csabai et al., 1998; Mielcarek and Daczkowska, 1999). The interest excited by the use of liposomes is mainly due to their efficiency as vehicles for a wide number of hydro- and lipo-soluble agents.

Microspheres and microcapsules have recently attracted the attention of scientists as inclusions systems for drug controlled release studies and for the potential protection of photosensitive drugs (Sansdrap and Moës, 1993; Filipovic-Grcic et al., 1996; Yüksel et al., 1996; Guyot and Fawaz, 1998).

The photostability of AML when included into cyclodextrins, liposomes and microspheres has been herein investigated. AML-containing matrixes were subjected to stressed degradation studies by comparison of their degradation kinetics to that of the drug, either neat or in solution or in solid dosage form. Analytical determination of AML was performed by measuring the absorbance at 360 nm maximum peak, which was missed in the UV spectrum of the degradation product.

2. Materials and methods

2.1. Apparatus

Absorption spectra were registered on the λ range of 190–500 nm in a 10 mm quartz cell, on a Perkin-Elmer Lambda 40P Spectrophotometer at the following conditions: scan rate $1\,nm\,s^{-1}$; time response 1 s; spectral band 1 nm. The software UV Winlab 2.79.01 (Perkin-Elmer) was used for spectral acquisition and elaboration.

Photodegradation processes were performed in a light cabinet Suntest CPS+ (Heraeus, Milan, Italy), equipped with a Xenon lamp, according to the ICH Guideline for photostability testing (ICH, 1996). The apparatus was fitted up with an electronic device for irradiation and temperature measuring and controlling inside the box. The system was able to closely simulate sunlight and to appropriately select spectral regions by interposition of filters. In the present study, samples were irradiated in a λ range between 300 and 800 nm, by means of a glass filter, according to the ID65 standard of ICH rules; the power was maintained to $350\,W\,m^{-2}$, corresponding to a light dose of $21\,kJ\,min^{-1}\,m^{-2}$, at the constant temperature of 25 °C.

2.2. Chemicals

Amlodipine was a generous gift from Pfizer, Italy. Turkey egg yolk phospholipids, ethylcellulose, polyvinyl alcohol 18-88 (Fluka, Switzerland); Sephadex G-75 (Pharmacia, Sweden); sodium chloride (Carlo Erba, Italy); Pipes [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) sodium salt], β-cyclodextrin hydrate, Britton–Robinson buffer pH 6.57 (85% *ortho*-phosphoric acid, 100% acetic acid, boric acid, natrium hydro-

xide; Sigma–Aldrich, Germany); dichloromethane (Prolabo, Italy); absolute ethanol (J.T. Baker, Holland). All other reagents were of the highest purity commercially available.

2.3. AML ethanol solutions

AML standard solutions in ethanol were used to set up the calibration curves. Solute concentration was within the range 5.0–50.0 $\mu g\,ml^{-1}$.

2.4. AML powder

The drug pure powder was evenly distributed along one of the internal surfaces of a 1 cm quartz cell, before light exposure.

2.5. Preparation of AML–liposomes inclusion complex

Liposomes, with a unilamellar structure, were prepared sonicating 1 g of egg yolk phospholipids (60% of phosphatidylcholine) in 10 ml of water with a Branson sonifier B-15, under a stream of nitrogen for 60 min (20 s sonication, 20 s intermission). The incorporation of AML (12.0 mg in 2 ml of liposomes) was performed under a stream of nitrogen by a 20 min sonication as above described until a clear solution was obtained. AML-free liposomes were prepared in the same way as a control. The liposomal suspension of AML (1 ml) was chromatographed by gel filtration through a column (Sephadex G-75, 0.7 cm × 20 cm) preequilibrated with 50 mM NaCl, 10 mM Pipes, pH 7.0, to remove the external drug. The eluted liposomes (about 2 ml of slightly turbid suspension) were subjected to the controlled photodegradation process at increasing times. Analytical samples for UV analysis were prepared diluting 100 μl liposomal suspension in 20 ml ethanol.

2.6. Characterization of AML–liposomes inclusion complex

A microscopical imagining analysis technique for the determination of AML–liposomal size distribution was applied. The particle size distribution was determined with a computer-controlled Olimpus BH-2 microscope. In all measurements about 10,000 liposomes have been examined, showing a spherical shape with 50–100 μm of inner diameter.

The encapsulation efficiency of AML in the liposomes was determined spectrophotometrically. For this aim, the liposomal suspension was 1:1 diluted with distilled water and subjected to ultracentrifugation at 150,000 × g (4 °C) for 90 min in a Centrikon T-1180 centrifuge (Kontron Instruments, Milano, Italy). The supernatant was removed and 1:50 diluted with ethanol for the determination of unentrapped AML (λ 360 nm). The liposomes containing pellet was suspended in 10 ml of 5% Triton X-100 and sonicated for 20 min; this solution was further 1:10 diluted with the same Triton X-100 solution for the determination of entrapped AML. Recovery of entrapped AML into liposomes was found to be 41.6 ± 2.7% ($n = 3$) of the amount initially added to the samples.

2.7. Preparation of AML–cyclodextrins inclusion complex

A slight AML excess (47 mg) was dissolved in 10 ml of a 0.01 M cyclodextrin solution (1.32 g of methyl-β-cyclodextrin in 100 ml ethanol). 10.0 ml Britton–Robinson buffer pH 6.57 (0.04 M phosphoric acid, 0.04 M acetic acid, 0.04 M boric acid, and 0.2 M NaOH) was added to the sample, under stirring for 20 h at a temperature of 37 °C. A drug-free control solution was prepared under the same condition. The sample was stored for 4 days at a temperature of 4 °C, then filtered through a 0.45 μm membrane and eventually subjected to spectrophotometric analysis.

2.8. Characterization of AML–cyclodextrins inclusion complex

In order to examine the possibility of the formation of inclusion complexes between AML and cyclodextrins, UV absorption spectra of the drug in the presence of α-, β-, methyl-β- and γ-cyclodextrins were recorded. Methyl-β-cyclodextrin was selected because of both a high water-solubility (Cserhati et al., 1996; Loftsson et al., 1996; Connors, 1996) and a marked ability in increasing the drug solubility as well. Moreover, methyl-β-cyclodextrin proved not to cause any appreciable change in the absorption spectrum of an ethanol solution of AML.

The influence of methyl-β-cyclodextrin on the AML solubility was tested according to Higuhi and Connors (1969). For this aim, increasing amounts of

0.01 M cyclodextrin solution and 10 ml of Britton–Robinson buffer pH 6.57 were added to an excess amount (50–200 mg, equivalent to approximately 0.125–0.50 mmol) of AML. Samples were stirred for 12 h, then filtered. The drug concentration was spectrophotometrically determined by UV after appropriate dilution with ethanol of the above solutions. The phase solubility diagram obtained was of type A, according to the classification of Higuhi and Connors, showing a relevant increase of the AML solubility when included into cyclodextrins.

2.9. Preparation of AML-microspheres

Microspheres were prepared by use of 2.0 g ethylcellulose polymer and 0.4 g AML, dissolved in dichloromethane (20 ml). 160 ml of aqueous polyvinyl alcohol (0.45%, w/w) were added to the homogenous solution under continuous stirring at room temperature. The suspension was stirred to room temperature for about 15 min, then warmed to 40 °C in order to completely evaporate dichloromethane (5 h). Microspheres so obtained were washed with about 50 ml of water in turn discharged by decantation for the removal of PVA. Microspheres were collected by filtration through paper and dried under vacuum for 24 h. Spectrophotometric analysis was performed on a sample obtained by appropriate dilution in ethanol of sonicated solution of microspheres.

2.10. Characterization of AML-microspheres

Particles dimensions and outer surface were determined by microscopical imaging analysis (Olympus BX40). A sample of microspheres was distributed on a slide and a mean diameter of 1.6 ± 0.4 mm was sized. The surface was found to be spherical with a well defined shape.

The content of AML in microspheres was calculated by spectrophotometry at 360 nm using a standard regression equation. For this aim 25–30 mg of microspheres was dissolved in 25 ml of ethanol by sonication for about 20 min. This solution was 1:10 diluted with ethanol and analyzed. The amount of drug included was then determined and showed ranging from 28 to 32% (w/w). The release rate of the drug proved to be quite slow, probably affected by the bulkiness of microspheres.

The interference of microspheres excipients was verified by preparing blank samples, in absence of AML. These compounds were demonstrated to have no influence on the reading AML absorbance.

3. Results and discussion

3.1. UV spectrophotometric study of AML photodegradation

The spectral sequence of 20.20 $\mu g\,ml^{-1}$ ethanolic solution of AML is represented in Fig. 2. The light exposure caused a sharp degradation process with the formation of an unique degradation product, namely the pyridine oxidation derivative.

The 360 nm maximum peak minimized (less than 3%) after exhaustive photodegradation, showing that no significant absorption could be detected for the photoproduct at such a wavelength. Measurement of this absorbance peak has been used throughout to directly determine AML concentration. In fact, the photoproduct showed a 269 nm characteristic peak and two isosbestic points at 260 and 306 nm could be clearly singled out.

A calibration graph was obtained by applying a least squares linear regression to the absorbance amplitudes at 360 nm against the increasing concentrations of pure AML. The calibration curve was constructed in the range 5.0–50.0 $\mu g\,ml^{-1}$, obtaining the following equation

$$\mathrm{AML}\ (\mu g\,ml^{-1}) = 103.13\mathrm{Abs}_{360} - 0.238,\quad r^2 = 0.9997$$

A 50% degradation, with respect to the initial value of concentration, occurred after a light dose of only 1400 $kJ\,m^{-2}$, while a dose of 8820 $kJ\,m^{-2}$ was calculated for the complete disappearance of the drug. AML pure powder was analogously subjected to irradiation and a 10% degradation was detected after about 2500 $kJ\,m^{-2}$ of irradiance.

Such results suggested to take into strong consideration the development of new procedures for manufacturing AML-containing pharmaceutical preparations able to completely avoid or, at least, minimize drug degradation. Recent approaches to the problem are based on the formulation of new pharmaceutical

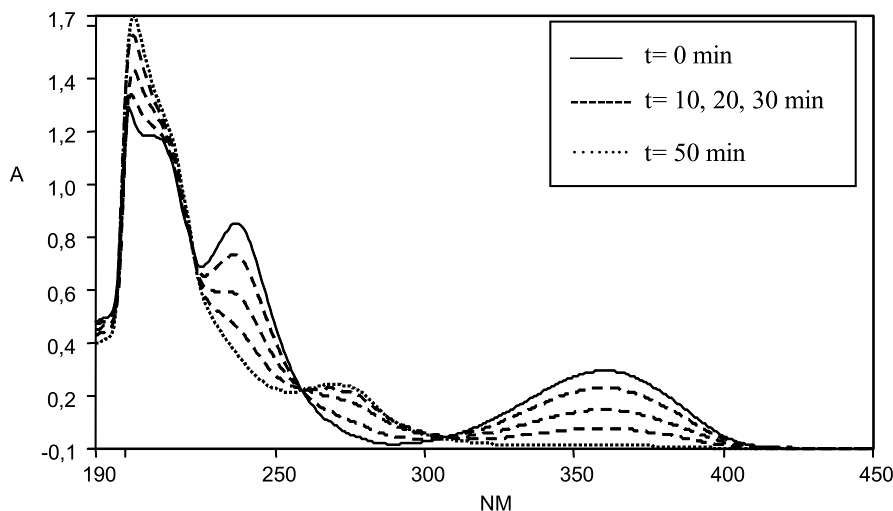

Fig. 2. UV spectra of a 20.20 μg ml^{-1} AML ethanol solution after different times of photodegradation.

carrier systems as an alternative to the use of protective coating materials.

The aim of the present report is the design and monitoring of photo-protected AML-containing systems, namely supramolecular complexes with liposomes and cyclodextrins, and inclusion solid-phase systems based on the use of polymeric cellulose microspheres.

3.2. AML–liposomes complex photostability

Liposomes were prepared from phospholipids adopting a recommended procedure (Bisaccia et al., 1985; Krämer and Heberger, 1986; Genchi et al., 1999), while AML inclusion was promoted by prolonged sonication in order to get homogeneous liposome families with a small inner volume (~50–100 μm diameter) and a characteristic translucent aspect. The separation of the AML–liposome complex was achieved by gel filtration. Spectrophotometric measurement was performed by appropriately diluting the liposomal complex with ethanol. The amount of drug absorbed into the complex was calculated to vary in a concentration range of 2.3–2.7 mg ml^{-1}.

Light stability studies were carried out by spectrophotometric measurements performed just before exposure and at increasing times (after 0.25, 0.50, 0.75, 1.00, 2.00, 3.00, 6.00, and 9.00 h), by diluting the sample with ethanol every time. Collected results, listed in Table 1, showed that the drug entrapment into liposomes strongly reduced the photodegradation process.

Several species of liposomes would show light sensitivity, so that recent studies deal with photosensitive liposomes which allow a rapid release of incorporated drugs by photolysis (Morgan et al., 1995; Bisby et al., 2000). In order to check light stability of the solution of pure liposomes, a parallel analytical control on the matrix was also performed. For this aim, three samples of liposomal matrix were treated under the same exposure conditions adopted for the AML–liposomes complex. No measurable changes between the spectra recorded before and after light exposure, respectively, were detected, clearly showing that any photo-

Table 1
Rate constants of photodegradation for AML in solid forms, ethanol solution and different inclusion complexes

AML system	k	$t_{0.5}$	$t_{0.1}$	r^2
Ethanol solution	0.267	1.12	0.17	0.9916
Powder	2.481×10^{-2}	12.10	1.85	0.9841
Tablets	9.220×10^{-3}	33.70	5.43	0.9885
Liposome	1.272×10^{-2}	23.62	3.62	0.9903
Cyclodextrin	5.720×10^{-3}	53.63	8.07	0.9764
Microsphere	1.507×10^{-3}	200.00	30.67	0.8433

t is expressed in hours.

degradation occurring during experimental time was negligible.

A complete release of the drug from the liposomes was confirmed by exposure of a control solution, prepared by diluting 100 μl of the AML–liposome complex to 20 ml with ethanol, to the same irradiation conditions. Recorded degradation times and constants were closely similar to those recorded for ethanolic solution obtained by a direct mixing, so suggesting a complete disaggregation of the drug–liposome complex when diluted with a large amount of solvent.

3.3. AML–cyclodextrins complex photostability

Cyclodextrins possess a rigid structure bearing a hydrophobic pocket and an outer hydrophilic surface. Such features suggest their ability in modifying solubility and bioavailability as well as photostability of the host molecule (Loftsson et al., 1996). Moreover, the formation of drug–cyclodextrins inclusion complex often allows a marked stabilizing effect on chemically unstable molecules. Cyclodextrins carry on their inhibitory activity on photo-sensitization side reaction by a potential ability in including the drug through a host–guest type process, so protecting the latter from the diffusion of oxygen which is responsible of unwanted aromatization of the AML dihydropyridine moiety.

All the AML-containing samples were kept to a temperature of 4 °C for 4 days, in order to promote drug inclusion. The spectrophotometric assay was performed by appropriately diluting the cyclodextrin–drug complex in ethanol.

The stability study was carried out by performing spectrophotometric determinations just before light exposure and after the same intervals above indicated for liposomal complex, by diluting the cyclodextrin complex in ethanol each time after irradiation. The results so obtained, and reported in Table 1, showed a drug photo-stabilization even better than the ones recorded for the liposomal complex and the commercial solid formulations of AML.

3.4. AML-microspheres photostability

AML-microspheres preparation was obtained by a well known method (Guyot and Fawaz, 1998), by dissolving variable amounts of drug and a polymer mixture (ethylcellulose and polyvinyl alcohol) in dichloromethane. The stability study was carried out by irradiating microspheres and spectrophotometrically analyzing the appropriate amount of sample immediately before light exposure and after the same

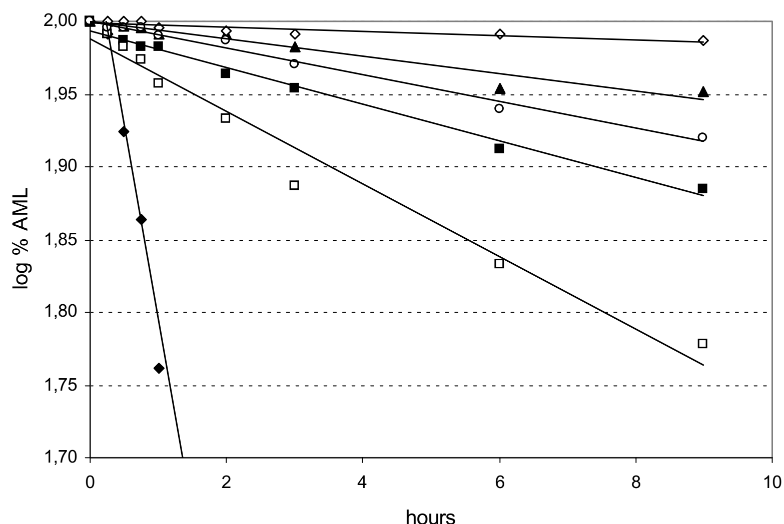

Fig. 3. Photodegradation curves of AML in ethanol solution (◆), powder (□), tablets (○) and its inclusion complexes with liposomes (■), cyclodextrins (▲), microspheres (◇).

intervals selected for above complexes. Spectrophotometric reading was performed by appropriately dissolving microspheres in ethanol.

3.5. Kinetic parameters determination

The results so obtained soon appeared very promising, as even after a prolonged light exposure clearly confirmed a higher stability when compared to other investigated matrixes or drug solid commercial formulations.

The quantitative evaluation of photochemical degradation was described by the equation

$$\log(\%c) = -kt + 2$$

where $\%c$ was the value of %residual drug concentration, k was the photodegradation rate constant, t was the time (h), and 2 was the logarithm of initial percent drug concentration.

The degradation was evaluated on the basis of kinetic photodegradation constant k, half-life time ($t_{0.5}$) and degradation time of 10% ($t_{0.1}$), with respect to the initial drug concentration.

AML degradation curves are plotted in Fig. 3, while Table 1 reports the mentioned degradation kinetic parameters.

Liposomes complex showed a $t_{0.1}$ equal to 220 min, with respect to a value of only 10 min for the ethanolic solution and 110 min for the powder, as shown in Table 1. Cyclodextrin complex showed an even better performance, with a $t_{0.1}$ value of 480 min, better than 325 min shown by AML commercial tablets.

Best results were obtained from stressing study on microspheres, which showed an excellent photostability with a measured residual AML concentration of 97% after 11,340 kJ m^{-2} light dose, corresponding to an extrapolated $t_{0.1}$ value of over 200 h.

4. Conclusions

AML can definitely be protected from light by its incorporation in supramolecular systems, namely liposomes, cyclodextrins and microspheres. Such matrixes prevent drug oxidation to the aromatic derivative through a number of chemical and physical barriers. All the studied systems have shown a high degree of protection, with a degradation rate always lower than in the usual solid drug formulations. Whereas liposomes systems may double the drug half-life, which may be even quadrupled with the use of cyclodextrins, inclusion complexes of the drug into solid systems like microspheres have shown an almost absolute stability to photodegradation. In this case, the rate of drug inactivation was only a little 3% after a 9 h continuous light exposure.

The systems herein studied appear of paramount interest for the development of new pharmaceutical formulations of AML, or other photosensitive drugs, even when their effectiveness is compared to that of customary protective wrapping.

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