Cyclodextrins in topical gel formulation as photoprotective system for Nabumetone

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Abstract. Photostability studies applied on aqueous solutions of Nabumetone, an anti-inflammatory drug used in the treatment of osteoarthritis and rheumatoid arthritis, have confirmed the sensitivity to light of this compound, revealing the 6-methoxy-naphthalene-aldehyde as the main photoproduct. In this work, the stability of NA-cyclodextrin (CD) complexes was investigated in gel formulation potentially suitable as sustained-release systems. The photodegradation experiments were realized under stressed conditions according to the ICH rules and monitored by spectrophotometry. The spectral data were processed by Multivariate Curve Resolution (MCR), able to estimate spectra and concentration profiles of the components involved in the kinetic process. NA entrapped in cyclodextrin and formulated in solution and gel preparations were exposed to an irradiance power of 350 W/m². Encapsulation percentage of the drug in several cyclodextrins was measured, recording an increase of the water solubility in the order hpβCD>mβCD>βCD. No significant photoprotection of NA was measured in aqueous solution. On the contrary, the gel containing the hpβCD-complex showed relevant stability. The photoprotective ability of this formulation was further increased by adding ascorbic acid 2%, still detecting 90% of the starting concentration after 90 min of light exposure.

1. Introduction
The safety of the drugs, during production, storage, distribution and use by patients, is affected by exposure to several causes of degradation, including temperature and light [1]. In particular, the exposure to light could affect the physicochemical properties of either a pure compound or a pharmaceutical formulation. A great amount of liquid or in topical gel preparations, liquid or in gel, are classified as sensitive to light in the U.S. and European Pharmacopeia [2], consequently, the development of advanced photoprotective systems is still demanding. Furthermore, the use of appropriate protective containers or packaging may also be involved in the photostabilization strategies of the drugs [3]. When the pharmacokinetic behavior of a drug is not favorable and the absorption through the skin take too long, an active compound should be also protected after application, allowing a prolonged exposure to sunlight. In these cases, several approaches of encapsulation into supramolecular systems have been proposed, very often supported by the addition of antioxidants and solar filters [4-7]. In the pharmaceutical field, cyclodextrin (CD) complexes represent the most used encapsulation matrices to realize photoprotective carriers [8]. These cyclic oligosaccharides can incorporate lipophilic drugs into the hydrophobic cavities by non-covalent
complexation [9]. Moreover, the variety of available CDs, both natives and modified, allows the incorporation of drugs with different size.

In this work, the light stability of Nabumetone (4-(6-methoxy-2-naphthyl)butan-2-one) (NA) was investigated in solution and gel formulations. Photoprotection of NA was performed after entrapping the drug into CD matrices. According to the ICH rules, the photodegradation tests were made in an appropriate standard irradiation chamber on drug solutions and CD complexes in solution and gel formulations.

The photodegradation profiles were monitored by spectrophotometry and the spectral data were processed by Multivariate Curve Resolution (MCR). This chemometric procedure was chosen because particularly suitable for following transformation kinetic processes as it is able to estimate spectra and concentration profiles of the components involved [10-13].

2. Materials and methods

2.1. Chemicals, Instruments and software

NA, ascorbic acid (AA), propylene glycol, microcrystalline cellulose, βCD, mβCD, and hpβCD were purchased from Sigma-Aldrich (Milan, Italy). Ethanol and methanol were from J.T. Baker (Holland). UV spectra were recorded by using a Perkin-Elmer Lambda 40P Spectrophotometer by setting the following instrumental conditions: λ range 200–450 nm, scan rate 1 nm/s; time response 1 s; spectral band 1 nm. Spectral acquisition and elaboration were made by using the dedicate software UV WinLab® (Perkin-Elmer, Waltham, MA).

A light cabinet Suntest CPS+ (Heraeus, Milan, Italy) equipped with a Xenon lamp was used to perform the photodegradation experiments. The ID65 standard filter was set to simulate sunlight in a spectral range between 300 and 800 nm.

Multivariate analysis was performed by the software Matlab® computer environment (Mathwork Inc., version 7).

2.2 Sample preparation

NA standard solutions were prepared in ethanol at the concentration of 5.0 µg/ml in consideration of the low solubility of the drug in water. CD-complexes were prepared by dissolving an excess of NA (25 mg) in 10 ml of cyclodextrin solution (10mM) and 10.0 ml of Britton-Robinson buffer pH 6.57 (0.04 M phosphoric acid; 0.04 M acetic acid; 0.04 M boric acid; 0.2 M NaOH) under stirring for 20 h at 37° C. Four series of samples (4 x 5) were prepared by using βCD, mβCD, and hpβCD. All the samples were stored for 4 days at 4° C and then filtered through a 0.45 µm membrane. Spectrophotometric measurements were performed after dilution 1:10 of the samples in ethanol. For the solubility tests, the drug content was added to each cyclodextrin solution with concentrations of 1, 5, 7.5, and 10 mM in 10 ml buffer.

Gel formulation (20 g) was prepared according to the European Pharmacopoeia [14]. NA 0.20 g (1% w/w) was emulsified in propylene glycol 2 g under continuous stirring for 15 minutes. 0.60 g of microcrystalline cellulose (gelling agent) and 17.2 g of water were then added and the final emulsion was stirred for 50 min getting a homogeneous white gel.

The gel formulation containing the complex CD-drug was prepared by adding 17.2 g of the CD complex solution to propylene glycol and microcrystalline cellulose.

A last sample was prepared by adding AA to the CD-drug complex before the preparation of the gel, in such a way to obtain a percentage concentration of 2%.

2.3. Photodegradation test

The photodegradation tests were made on all the prepared liquid and gel formulations under the following conditions: irradiation power 450 W/m², corresponding to 27 kJ/m² min, temperature 25 °C. The samples were analysed just after preparation (t= 0 min) and at the several exposure times (10-30-
50-70-100-130-150-210-270-300 min) by UV spectrophotometry. All laboratory experiments were carried out in a dark room to minimize drug photodegradation.

The drug content in gel along the photodegradation experiments was measured by MCR applied to the UV data of the methanol extracts. At this aim, gel 0.5 g was uniformly stratified on a glass plate to form a layer thickness of 0.25 mm and then exposed to forced irradiation. After each irradiation dose, the glass plate was sonicated in acetonitrile 25 mL for 10 min at room temperature. 10 ml of the obtained suspension were centrifuged at 5000 rpm for 10 min and the supernatant was analysed after 1:10 dilution with methanol.

3. Results and discussion

3.1. Photodegradation of NA solutions

An ethanol solution of NA 5.0 µg/ml was subdued to forced photodegradation, under the standard conditions above reported. The spectra, depicted in figure 1, were recorded just after the preparation and at several exposure times up to five hours.

![Figure 1. Absorbance spectra recorded for the ethanol solution of NA at several exposure times.](image)

The spectral data, as an average of five experiments collected during the photodegradation tests, were used to construct the data matrix to be analysed by MCR-ALS. The results reported in figure 2 showed the photodegradation profiles of NA and its photoproducts (A) and the respective absorbance spectra (B).
In accordance with the results reported in the literature, data elaboration confirmed the formation of one major photoproduct (NA-P1) and traces of another by-product (NA-P2). [15] reported the photo-oxidation of the side chain to 6-methoxy-2-naphthaldehyde, as a major product, in butanol solution and the formation of the (4-(6-methoxy-2-naphthyl)-3-buten-2-one) (figure 3).

**Figure 2.** Photodegradation profiles of NA and its photoproducts (A) and their absorbance spectra (B).

**Figure 3.** Chemical Structures of NA and its photoproducts NA-P1 and NA-P2.
This photodegradation process followed the first-order kinetics. In the used solvent, the process seemed to be more efficient than in water [16].

In our experiment, the full degradation of the drug was observed after about 30 min. The degradation process proceeded via first-order kinetics, described by the equation:

\[ \ln(\%\text{NA}) = -k \cdot t + 4.6 \]

where \( \%\text{NA} \) was the percentage of residual drug, \( k \) the photodegradation rate constant, \( t \) the time (min), and 4.6 the logarithm of the starting concentration (100%).

The parameter \( t_{0.1} \) (time to cause 10% degradation) was chosen as a criterion to compare the degradation behaviour of the tested samples. This parameter is conventionally adopted because a drug could no longer be used when its purity falls below 90%. The value of \( k \) was 0.0482 and \( t_{0.1} \) resulted in being 2.08 min, as reported in Table 1.

### Table 1. Degradation kinetic parameters calculated for NA in different solutions.

| Samples           | Entrapment efficiency (%) | \( K \times 10^3 \) | \( t_{0.1} \) (min) | \( R^2 \) |
|-------------------|----------------------------|---------------------|---------------------|----------|
| NA-free           | -                          | 48.2                | 2.08                | 0.999    |
| NA-βCD            | 14                         | 23.9                | 4.18                | 0.965    |
| NA-mβCD           | 15                         | 20.6                | 4.85                | 0.970    |
| NA-hpβCD          | 88                         | 4.20                | 23.8                | 0.987    |
| NA-gel            | -                          | 23.4                | 4.27                | 0.977    |
| NA- hpβCD gel     | -                          | 1.99                | 50.25               | 0.999    |
| NA- hpβCD AA gel  | -                          | 1.11                | 90.09               | 0.922    |

In order to minimize light degradation, the stability of NA was investigated by entrapping the drug in cyclodextrin matrices. The CD systems can also enhance the solubility of the drugs in water. In fact, the influence of several cyclodextrins on the solubility of NA has been tested, as described by [17].

A set of NA aqueous solutions was prepared as above described by using βCD, mβCD, and hpβCD, respectively. The most effective cyclodextrin in increasing the solubility of NA resulted in being hpβCD. The incorporation percentage was measured by spectrophotometry as 88, 15 and 14% for hpβCD, mβCD and βCD, respectively. The best performance of hpβCD can be explained by a better fitting of the drug molecule in the cavity of this CD. All the prepared complexes were exposed to light. Figure 4 shows the photodegradation profiles of NA in the different CDs and Table 1 lists the kinetics parameters calculated by MCR.

All the used CDs increased the stability to light of the NA. In particular, the hpβCD-complex showed a \( t_{0.1} \) value of 23.9 min, better than that for the simple solution but still unsatisfactory for the goals we had set.
3.2. Photodegradation of NA in gel

Photodegradation tests were then applied to the above-described formulations prepared in gel. Firstly, gel formulation was made with 1% of the pure drug and exposed to light. The data obtained from the photodegradation experiments were processed by MCR method and the kinetics parameters are listed in Table 1. Also, in this formulation, the drug resulted very sensitive to light showing a \( t_{0.1} \) value of 4.27 min.

A promising result was obtained when the complex \( \beta \text{CD-NA} \) was emulsified in gel. In this case, a clear decrease in NA degradation was measured, with a very successful \( t_{0.1} \) value of 50.25 min. The good performance of this formulation in terms of photostabilization could be attributed to a dual-action of the CD complex: real physical protection of the entrapped drug by means of a molecular shield aided by the increase of the drug solubility in this matrix.

The light-stable formulation was optimized by adding the antioxidant ascorbic acid (2%) to the complex \( \beta \text{CD-NA} \) in gel, showing a very high increase of the stability with a \( t_{0.1} \) value of 90.09 min. The photodegradation profile of this formulation \( \beta \text{CD-NA-AA} \) followed the first-order kinetics and was compared in figure 5 to that of NA 1%, as gel control, and \( \beta \text{CD-NA} \) gel. Table 1 summarizes the degradation rate constants and the values of \( t_{0.1} \) for all the studied matrices.

**Figure 4.** Photodegradation profiles of NA in the different CDs compared with the ethanol solution.
Figure 5. Photodegradation profiles of NA, hpβCD-NA complex and hpβCD-NA-AA formulated in gel.

4. Conclusions
In this work, the anti-inflammatory drug Nabumetone, either in solution or in gel formulations, was demonstrated to undergo photodegradation. The study was performed by adopting photostability tests defined by international rules. In particular, under an irradiance power of 350 W m$^{-2}$, corresponding to 21 kJ m$^{-2}$ min and at a constant temperature of 25$^\circ$ C, the drug resulted degraded of 10% in only 2.08 and 4.27 min, in ethanol and gel, respectively. The design of photoprotective pharmaceutical matrices for topical application is particularly important due to the greater probability of light exposure that can cause a lower bioavailability of the drug and, at the same time, an increase of the risk of formation of toxic photoproducts. The entrapping of the drug into cyclodextrins and the addition of an antioxidant agent was the adopted approach to reduce drug photodegradation. The addition of ascorbic acid to the hpβCD-NA gel complex gave good results with a considerable increase of the drug photostability, reaching a very satisfactory value of 90.09 min for t$_{0.1}$. The proposed system which has been shown to be particularly effective in reducing the photodegradation of the drug could be considered a valuable starting point for the development of innovative pharmaceutical formulations for topical use of Nabumetone.

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