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Development of HPLC and UV spectrophotometric methods for the determination of ascorbic acid using hydroxypropyl-β-cyclodextrin and triethanolamine as photostabilizing agents

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ABSTRACT

In this study, the effect of complex formation with triethanolamine (TEA) alone and in combination with hydroxypropyl-β-cyclodextrin (HP-β-CD) on the photostability of ascorbic acid was evaluated for exposure to artificial and diffuse daylight. The first-order rate constants for the photodegradation reactions were determined. The data obtained showed that these complexes strongly reduced the photodegradation process with an 11- and 35-fold increase in the photostability of ascorbic acid, depending of the ligand concentration and the irradiation source. The multicomponent complex gave a significantly better stabilization for exposure to light than TEA alone.

Due to the fact that the complexation extended the exposure of ascorbic acid to light (without molecular changes), UV spectrophotometric and reversed phase high performance liquid chromatographic (HPLC) methods were developed for the quantitative determination of the vitamin in pure form and in pharmaceutical preparations. These methods were statistically validated, all the validation parameters were found to be within the acceptance range. These results demonstrate that the proposed methods are suitable for the quality control of ascorbic acid, providing simple, rapid, precise, accurate and convenient approaches for routine analysis of bulk drug and pharmaceutical formulations.

1. Introduction

The photostability of drugs represents an emerging topic in the pharmaceutical research field, with the number of drugs which have been revealed to be light sensitive noticeably increasing. The photochemical behavior of a drug molecule is strongly dependent on the microenvironment, and its photoreactivity can therefore be altered by interactions with macromolecules [1]. New technology-based pharmaceutical systems have been proposed in order to enhance stability for such drugs. Some of these approaches have used chemical complexes of drugs with appropriate photoprotective carriers [2–4].

A wide area of supramolecular chemistry focuses on the host–guest complexes formed by the binding of a substrate to molecular receptors via non-covalent interactions [5–7]. In the present work, cyclodextrins (CDs) were used, which are cyclic oligosaccharides with hydroxyl groups on the outer surface and a void cavity in the center. Their outer surface is hydrophilic, but the cavity has a lipophilic character. CD encapsulation of a guest molecule affects many of its physicochemical properties. Naturally occurring CDs and their synthetic derivatives improve certain properties of the drugs, such as solubility, stability, and/or bioavailability [8]. Moreover, in our recent works it was shown that the addition of a suitable auxiliary substance, such as triethanolamine (TEA), can enhance the power of hydroxypropyl-β-cyclodextrin (HP-β-CD) as a result of a combined effect of salt formation and inclusion complexation [9,10].

This current study focuses on ascorbic acid, shown in Fig. 1, due to the fact that it is an important water soluble vitamin. It is highly sensitive to heat, alkali, oxygen and light, and also to contact with traces of copper and iron [11,12]. Although ascorbic acid has been extensively studied in different fields, interest in this vitamin has never waned and further aspects are currently being investigated. Previously, we have studied the influence of complexation with HP-β-CD and TEA on the degradation rate of ascorbic acid in solution. The results obtained showed a pronounced enhancement of aqueous stability with the TEA association complex, whereas the HP-β-CD inclusion complex had a minor effect. In addition, the multicomponent complex produced a significantly better stabilization than that of HP-β-CD alone [13].

The degradation of ascorbic acid in aqueous solutions, however, was found to be very sensitive to laboratory fluorescent lighting exposure. This photochemical behavior has important analytical implications and clearly complicates the assays. The aim of the

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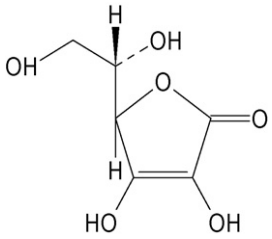

Fig. 1. Chemical structure of ascorbic acid.

present study was to find a suitable photostabilizer complex and to determine its optimum concentration for the analysis of this vitamin. We studied the potential of TEA and the TEA:HP-β-CD combination to reduce the photodecomposition of ascorbic acid, under laboratory fluorescent lighting and simulated sunlight irradiation.

Currently, some difficulties still remain to quantify ascorbic acid due to its extreme instablility in aqueous solutions, especially upon exposure to light. Several analytical methods including chromatography [11,14,15], microcalorimetry [16], fluorimetry [17], electrochemistry [18,19], and spectrophotometry [20–22], have been tried in different pharmaceutical forms, either alone or in combination with other drugs, as well as in foods or biological materials among others. Nevertheless, the majority of these require previous sample preparation steps, such as derivatization or elimination of matrix effects which often makes the method more complicated and laborious. Besides, these methods often do not take special precautions to prevent degradation of the drug.

In the present study, UV spectrophotometric and HPLC methods were developed and validated for ascorbic acid that were based on the significant enhancement of its stability in aqueous solutions and of its photostability due to the formation of complexes. These methods were successfully used to assay the total content of ascorbic acid in different pharmaceutical formulations.

2. Experimental

2.1. Chemicals and equipment

Ascorbic acid was obtained from Anedra® (99%) (Buenos Aires, Argentina) and TEA was purchased from Aldrich® (98%) (Milwaukee, WI, USA). HP-β-CD (MW = 1326–1400, degree of molar substitution 7.0) was kindly supplied by the Ferromet (Buenos Aires, Argentina) subsidiaria of Roquette (Lestrem, France). HPLC grade methanol was procured from Sintorgan (Buenos Aires, Argentina). All experiments were performed using water purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other materials and solvents were of analytical reagent grade.

A Shimadzu UV-160A spectrometer (VA Howe, UK) with 10 mm quartz cells was used for all spectral measurements.

The HPLC system consisted of an Agilent 1100 series pump, an autosampler, a multiple-wavelength ultraviolet–visible (UV–vis) detector and a Chemstation software version A.10.02 (Agilent, Waldbronn, Germany). The column used was a Phenomenex Gemini C18 250 mm × 4.6 mm i.d. filled with 5 μm particles, with a precolumn (guard cartridge SecurityGuard C18 4 mm × 3.0 mm i.d.) supplied by Phenomenex (Torrance, CA, USA).

2.2. Photostability studies

The kinetics of photodegradation of ascorbic acid were studied in solution in the presence or absence of TEA alone and in the combination of TEA and HP-β-CD. The effect of different concentrations of TEA was evaluated, while for the combination TEA:HP-β-CD, the concentration of HP-β-CD was kept constant.

In addition, the influence of both pH and light on the photodegradation was studied at different pH values. The studies were performed using 50 mM $NaHCO_3$ adjusted with NaOH or phosphoric acid to different pH values, and McIlvaine buffers prepared from 0.2 M Na_2HPO_4 and 0.1 M citric acid.

Stock solutions of ascorbic acid (0.5 mg mL^{-1}) were prepared in water and protected from light before irradiation. Freshly prepared solutions were used for each experiment in order to avoid any chemical or photochemical effects. Test solutions were prepared by diluting the stock solutions to a final concentration of 1.8×10^{-2} mg mL^{-1} in water and buffer solutions. The aqueous solutions containing increasing concentrations of TEA (0.9454–3.5450 mM) or their combination with HP-β-CD (0.9454–1.7365 mM and 1.00% (w/v), respectively). The samples were exposed to light sources in cylindrical tubes of transparent glass (13 mm i.d. × 100 mm) under continuous stirring, and maintained in a water bath at constant temperature of 25.0 ± 0.1 °C [Haake DC10 thermostat (Haake, Paramus, NJ, USA)] to minimize the degradation produced by effect of thermal reactions. One set of these solutions was irradiated with a Philips mercury arc lamp (emission in the range of 312–577 nm) fixed horizontally at a distance of 50 cm which transmitted light corresponding to exposure behind a glass window. The other set was positioned 160 cm away from daylight fluorescent tubes (Philips, TLT 40W/54), fixed horizontally (emission in the range of 400–600 nm). At specified time intervals, samples were withdrawn and immediately analyzed for remaining ascorbic acid by spectrophotometrically monitoring the decrease in absorbance at 266 nm. Each experiment was performed in triplicate.

2.3. Preparation of stock and standard solutions for proposed methods

Stock solutions of ascorbic acid (0.29 mg mL^{-1}) were prepared in water and protected from light. The standard solutions were prepared by dilution of appropriate aliquots of these stock solutions with TEA 0.9749 mM solution and TEA 0.9749 mM:HP-β-CD at 1.00% (w/v) solution, respectively.

2.4. Conditions of proposed methods

Spectroscopic determinations were carried out at room temperature. The absorbance was measured for each system at 266 nm against a reagent blank prepared under identical conditions without addition of the examined drug.

HPLC experiments were done using isocratic conditions. The mobile phase was filtered through a 0.45 μm Millipore membrane and degassed prior to use. The column temperature was 25 °C, and the injection volume was 50 μL. The assay procedure was performed using the external standard method.

2.5. Validation of proposed methods

The developed methods were validated according to standard procedures (ICH Guidelines, 2005 [23]).

Linearity and range were studied by preparing the calibration curves, which were constructed in triplicates at seven concentration levels. Additionally, linearity of the calibration graphs and conformity with the Lambert–Beer Law for the systems were evaluated by the *F*-test.

The detection (LOD) and the quantification (LOQ) limits were calculated based on the standard deviation (SD) and the slope of the calibration graphs.