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Short Communication

The characterization of isothiazolinone preservatives in cosmetics

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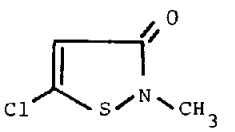
Introduction

The use of new compounds exhibiting antibacterial and antifungal activity employed in the formulation of cosmetic products requires comprehensive studies of their functional and toxicological characteristics. Accurate and sensitive analytical procedures, including microbiological assays, are required to monitor chemical stability, biodegradation caused by micro-organisms and structural modifications due to interaction with other components of the cosmetic formula.

In studies with other antibacterial compounds the authors have successfully employed the techniques of microbiological assays [1, 2], second derivative UV-spectroscopy [3], HPLC [4] and mass spectrometry [5]. This report describes an analytical study of a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one(**I**) and 2-methyl-4-isothiazolin-3-one (**II**) (3:1, m/m) in aqueous solution stabilized with $MgCl_2$ and $Mg(NO_3)_2$, available commercially as Kathon CG.® It is utilized at concentrations ranging from 0.035 to 0.15% m/v [6] in cosmetic products owing to its effectiveness and its lack of irritant effects on cutaneous and mucosal tissues. This investigation was carried out employing standard solutions of Kathon CG that were added to a standard emulsion which in some cases was contaminated with *P. aeruginosa.*



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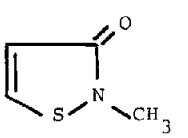

II

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Experimental

Apparatus

Equipment employed included a Perkin–Elmer liquid chromatograph S3-b, an LC-85 UV-detector, and a Sigma 15 data system. The analyses were performed using a 10-μm RP-8 column (Merck), with an eluent of methanol–phosphate buffer 0.004 M, pH 3.5 (20:80, v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 273 nm and the injection volume was 20 μl.

Second derivative spectroscopy was carried out using a Perkin–Elmer model 554 spectrophotometer set at 0.4 a.u.f.s., response factor 5, scan speed 120 nm/min, recorder speed 20 nm/min, and range 200–400 nm.

Mass spectrometry was performed using a VG ZAB-2F double-focussing instrument with reverse geometry, operating at 70 eV (200 mA) and a source temperature of 200°C. Samples were introduced under direct electron impact (DEI) conditions using platinum wire as sample support [7].

Standards

Kathon CG (1.515% m/v in water) was supplied by Rhom & Haas Italia S.p.A. (Milan) and diluted in water or tetrahydrofuran (THF) in the concentration range 0.05–5.0 μg/ml.

Samples

Emulsions containing 10–20% m/v of non-ionic polyoxyethylene derivatives of fatty acids (C_2, C_{18}) (Xalifin 15, from Vevy-Genova) were preserved with 0.01% m/v Kathon CG. Commercial samples of emulsions (1 or 2 g) were diluted to 10 ml with THF.

Results and Discussion

The results were obtained by analysing samples directly after dilution with THF. This solvent dissolves emulsion samples satisfactorily and minimizes sample pre-treatment.

Spectrophotometric studies

The spectra of Kathon CG standard solution diluted with water or THF are reported in Fig. 1A. They show the influence of the two solvents on spectrophotometric resolution; analogous behaviour is observed in the second derivative UV spectra (Fig. 1B). Figure 2 shows a comparison of second derivative spectra of a cosmetic THF-solubilized emulsion with (B) and without (C) the addition of Kathon CG. Spectrum (B) appears identical to that of a Kathon CG standard solution spectrum (A) after eliminating, with this technique, the non-specific background absorbance of the emulsion component, spectrum (C). Consequently, second derivative spectroscopy allows the direct determination of isothiazolinone preservatives in complex cosmetics at the nanomole level, for which normal UV spectroscopy would be ineffective. A calibration of Kathon CG over the range 0.20–1.5 mg % in the presence of cosmetic emulsion preserved with Kathon CG gave a linear response of $y = 0.007x + 0.005$ ($n = 4$) and a correlation coefficient of 0.987. An identical calibration procedure over the range of 0.20–2.0 mg % in the absence of cosmetic emulsion gave a linear response of $y = 0.007x + 0.001$ ($n = 4$) and a correlation coefficient of 0.993. The presence of the cosmetic emulsion is therefore shown to increase the intercept only and not to influence the slope. Moreover, the

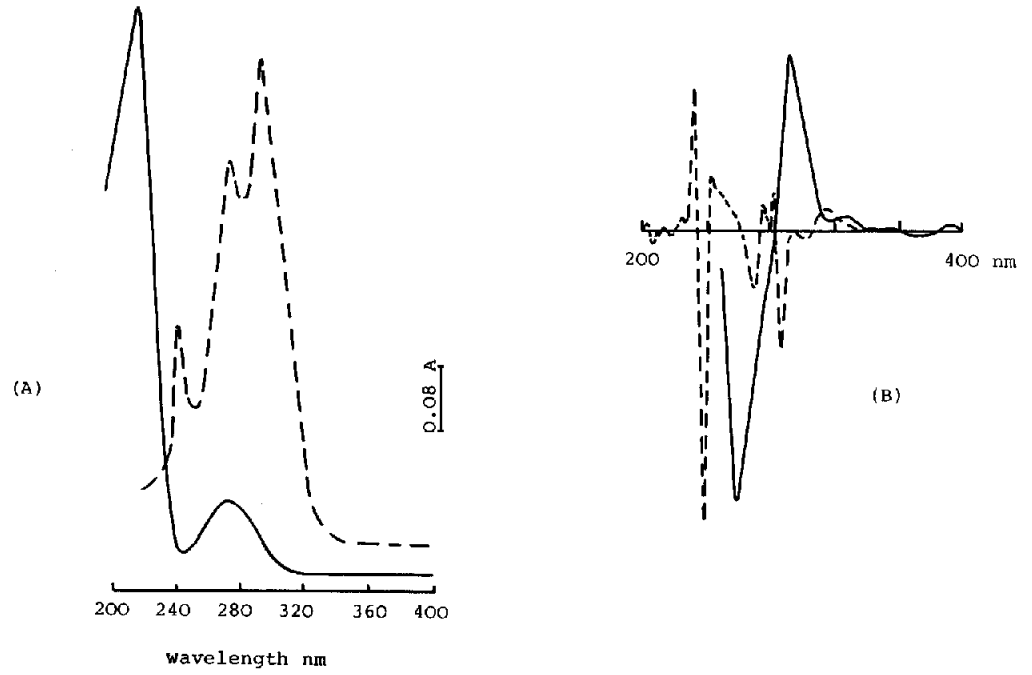

Figure 1
Influence of the solvent on the normal (A) and second derivative (B) spectra of Kathon CG in water (———) and in THF (– – –).

increased intercept introduces a systematic additive error into the quantitative performance of the method.

pH effect

The influence of pH on the stability of Kathon CG standard solution (0.2 ml in 100 ml of water) was studied at pH 4.2, 5.2, 6.2, 7.0 and 8.0 after 1, 7 and 15 days using second derivative spectroscopy. The spectra reported in Fig. 3A demonstrate that the product is stable at room temperature.

UV radiation effect

The second derivative spectra of a standard sample of Kathon CG (0.2 ml in 100 ml of water) before and after 12 h irradiation at 365 nm appear identical (Fig. 3B), thereby indicating the stability of the preservative in UV-A light.

Temperature effect

Figure 3C shows the stability of the standard solution of Kathon CG (0.2 ml of the stock solution diluted with water to 100 ml) towards temperature. The spectra were recorded every 10 min from 20 to 80°C with increases of 1°C/min, each step being 10°C. A significant amount of thermal decomposition appears to take place above 40°C.

High-performance liquid chromatography

Typical chromatograms of Kathon CG in standard solution and added to a cosmetic emulsion are reported in Fig. 4. The capacity factor of the unchlorinated ($k' = 0.98$) and

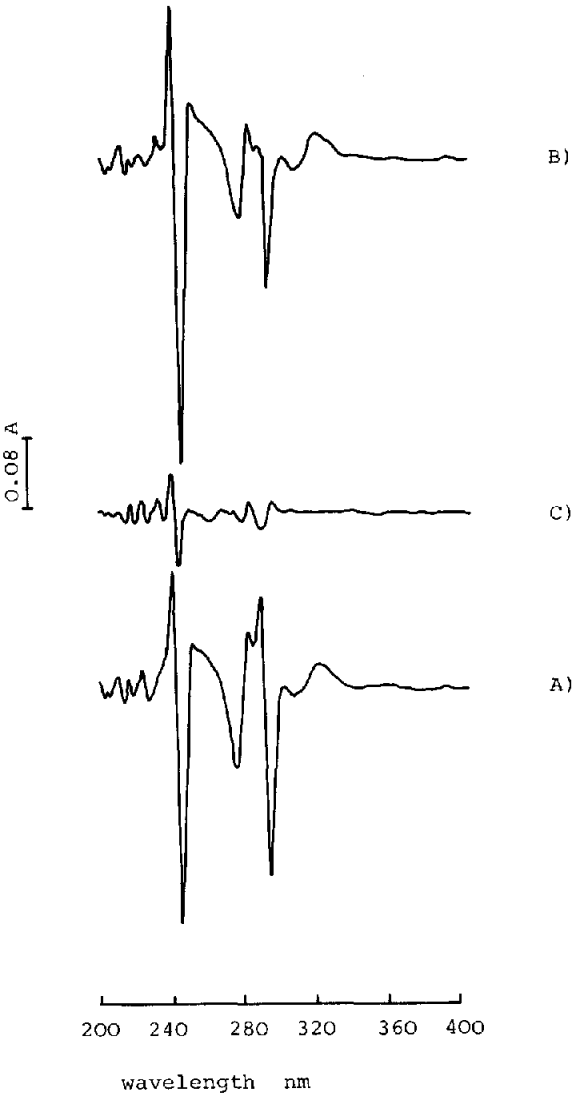

Figure 2
Second derivative spectra of (A) Kathon CG 30.3 μg/ml in THF; (B) cosmetic emulsion dispersed in THF, containing Kathon CG 39.5 μg/ml; (C) cosmetic emulsion preserved with 0.01%, m/v of Kathon CG.

chlorinated ($k' = 4.12$) components demonstrate the high resolution obtained. Peaks were characterized by a stop flow technique and UV scanning. Calibration of 5-chloro-2-methyl-4-isothiazolin-3-one gave a linear response over the range of 0.5–3.0 mg % with $y = 4.718x + 0.23$ ($n = 4$) and a correlation coefficient of 0.998. The other component of Kathon CG, 2-methyl-4-isothiazolin-3-one also gave a linear calibration with $y = 2.132x + 0.115$ ($n = 4$) and a correlation coefficient of 0.999.

Contamination with P. acruginosa

The effect of microbiological pollution has been investigated both in standard aqueous solution and in emulsion containing Kathon CG using second derivative spectroscopy,

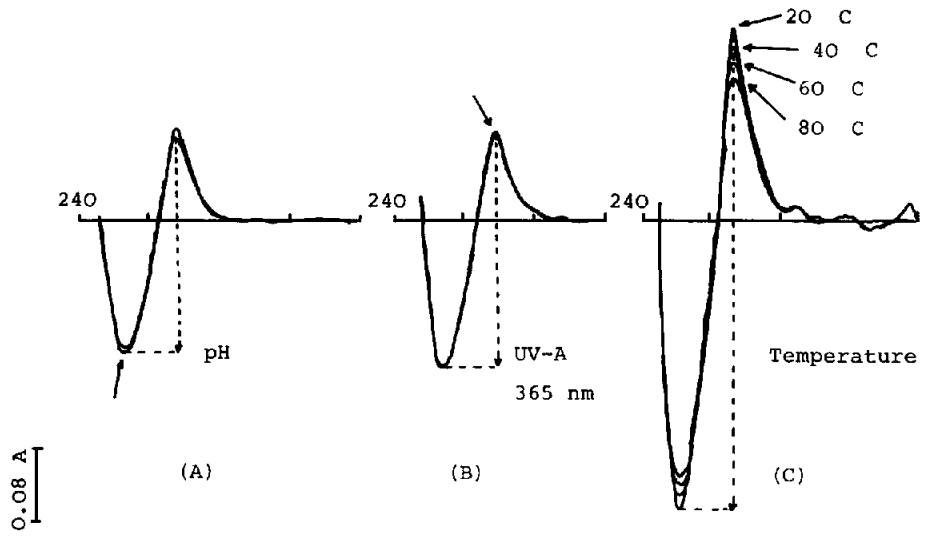

Figure 3
Stability of Kathon CG in water (30.3 μg/ml) towards (A) pH, (B) monochromatic UV-A irradiation (365 nm) and (C) temperature, measured using second derivative spectra.

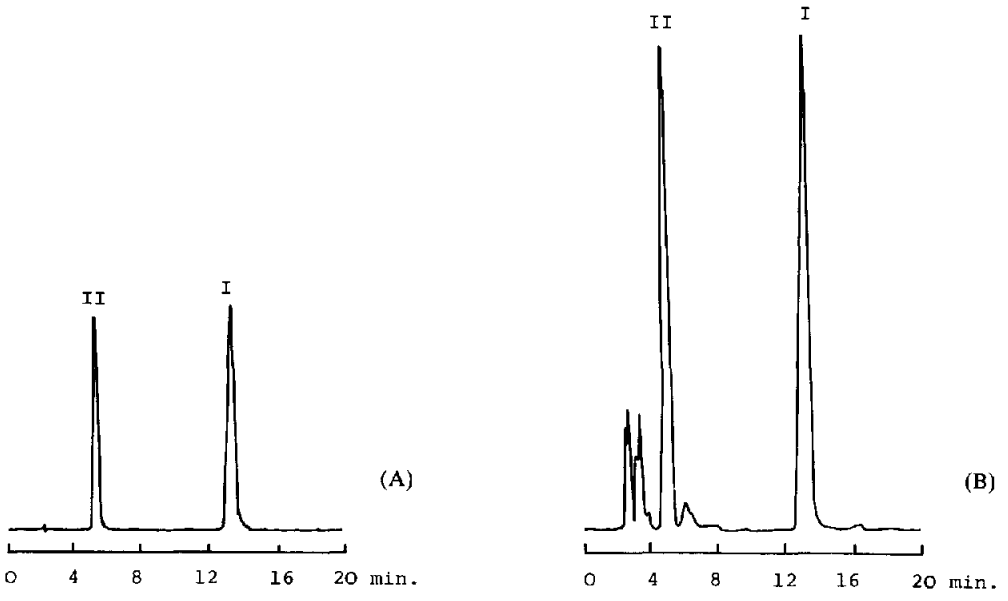

Figure 4
Typical HPLC chromatogram of Kathon CG in a standard solution (A) and added to an emulsion sample (B) measured at 273 nm. Chlorinated (I) and unchlorinated components (II) are well resolved.

HPLC and mass spectrometry. The spectra of the second derivative from a standard solution (0.2 ml % in water) before and after contamination with *P. aeruginosa* is reported in Fig. 5. Whilst the second derivative spectra (Fig. 5A) can be used to measure the loss of Kathon CG, the HPLC chromatogram (Fig. 5B) shows interference by several products of degradation. The chlorinated derivative of the isothiazolinone (**I**) mixture was particularly affected by *P. aeruginosa* enzyme activity. With the aim of identifying the products of Kathon CG metabolism, several fractions were collected by liquid chromatography and each was analysed by mass spectrometry under direct electron impact conditions. The mass spectrometry evaluation was carried out by using a cold cathode filament to control the ion source operating conditions. In this way, the electron emitter has a low temperature and is chemically inert and produces a stable emission current up to mA level. Furthermore, the low sample evaporation induces a "fractional distillation" of the mixture products in the ion source. The peaks at m/z = 87 and m/z = 121/123 in the DEI mass spectra (Fig. 6) derive from CO loss from m/z = 115 and m/z = 149–151 in the molecular peaks of the two components present in the commercial Kathon CG. In the mixture containing *P. aeruginosa* the relative abundance of the m/z = 87 and m/z = 121–123 peaks increase proportionally with the *P. aeruginosa* concentration. The data can be explained by the presence in the mixture of molecules from unchlorinated and chlorinated molecules that have lost the ketone functional group.

Conclusion

The procedures reported permit the determination of Kathon CG in cosmetic material and the study of its metabolism by microbiological activity. The aim was to demonstrate

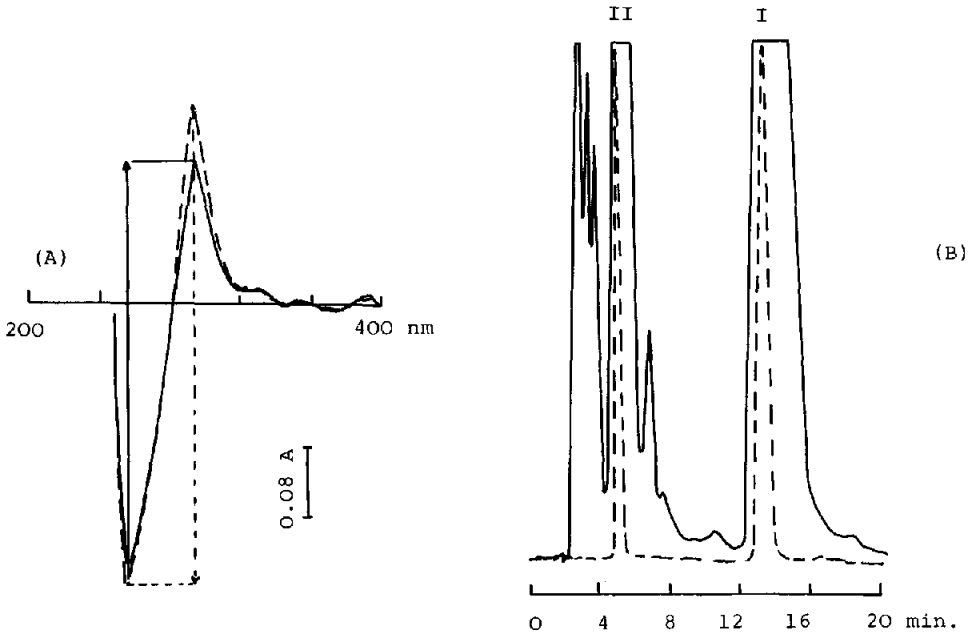

Figure 5
(A) Second derivative UV spectra and (B) HPLC chromatogram of a standard Kathon CG sample (30.3 μg/ml) before (– – –) and after (———) contamination with *P. aeruginosa*.

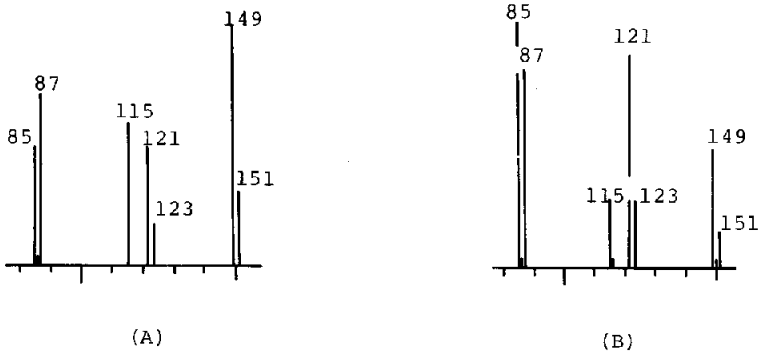

Figure 6
Mass spectra of chromatographic fraction of a standard Kathon CG sample before (A) and after contamination (B) with *P. aeruginosa*. The spectra were obtained by direct electron impact (DEI) technique.

that total isothiazolinone content can be determined by second derivative spectroscopy, not only on standard solutions of the isothiazolinone mixture, but also directly on emulsions after their solubilization with THF. The stability of the preservative was examined at different pH values and at different temperatures and with respect to UV-A irradiation. A pH greater than 8.0 and a temperature above 40°C appeared to decrease its stability.

In addition to the spectroscopic aspects, it has been established that HPLC allows the selective quantitative evaluation of the two isothiazolinone components with good reproducibility at levels of 0.5 μg/ml (twice the signal to noise ratio) in less than 15 min. Kathon CG appears partially unstable towards the microorganism *P. aeruginosa* which was chosen as the contaminating agent for cosmetic emulsions. It has been demonstrated that the chlorinated isothiazolinone derivative undergoes greater biodegradation. These results were qualitatively confirmed through DEI mass spectrometry. As Kathon CG is now widely used, it is, in our opinion, important to have a better understanding of its stability and to develop accurate and rapid methods to evaluate Kathon CG itself as well as its degradation products.

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References

[1] D. S. Orth, *J. Soc. Cosmet. Chem.* **30**, 321–331 (1979).
[2] F. Galiano, A. Bettero and V. Marin, *Relata Technica* **37**, 53–57 (1983).
[3] A. Schmitt, *Applied UV Spectroscopy*. Bodenseewerk Perkin Elmer, 1E, 2–10 (1977).
[4] A. Bettero and C. A. Benassi, *J. Chromatogr.* **280**, 167–171 (1983).
[5] A. Bettero, F. Galiano, V. Marin and C. A. Benassi, Proc. "La Spettrometria di massa nello studio del metabolismo intermedio e della biotrasformazione dei farmaci", Padova (Italy) (1983).
[6] Speciality Chemicals, Kathon CG, Rohm & Hass Company, SC-105-980-E, Philadelphia, USA (1980).
[7] P. Traldi, U. Vettori and F. Dragoni, *Org. Mass Spectrom.* **17**, 587–591 (1982).

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