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COMMUNICATION

Photodegradation Study of Sodium Usnate Solution: Influence of pH

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ABSTRACT

Photodegradation of 2.6×10^{-5} M aqueous solutions of sodium usnate at various pH was studied. Photodegradation appeared to follow first-order kinetics and was found to be pH dependent. The degradation rate constant was calculated to be 9.20×10^{-4} min^{-1}, 5.93×10^{-4} min^{-1}, 9.69×10^{-4} min^{-1}, and 9.88×10^{-4} min^{-1} at pH 6, pH 7, pH 8, and pH 9, respectively.

INTRODUCTION

Sodium usnate is extracted from Iceland Moss or *Cetraria islandica* (1–3). About properties, we can note a highly specific activity toward gram-positive bacteria and a medium activity toward certain gram-negative strains (4–6). Furthermore, usnic acid is known to possess antifungal activity (7). Various lichens contain usnic acid substance. Because of their old medical use, lichens have been the subject of many studies (8–11). For example, usnic acid has a broad antibiotic spectrum (2).

A previous study (12) established that sodium usnate, in the best stability conditions (pH 8), has a 90% shelf life $t_{90\%}$ (time necessary to obtain a decrease of 10% of initial concentration) of 54.6 days and a half-life $t_{50\%}$ (time necessary to obtain a decrease of 50% of initial concentration) of 359.1 days at 20°C. To know better the best storage conditions, we studied the photostability of sodium usnate according to the pH.

MATERIALS

Sodium usnate (Evosina, Varieti and Co. spa, batch 905066) (Fig. 1) is a transparent, yellow-brown liquid soluble in alcohol and in water with opalescence. It is propylene glycol vehicle. All other chemicals used were analytical reagent grade. Water, applied throughout the study, was purified by an Autostill 4000X (Jencons) apparatus. Demineralized deionized water was obtained

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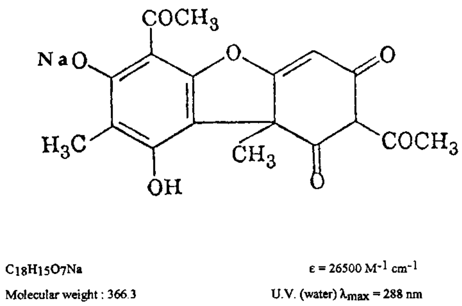

Figure 1. Chemical structure of sodium usnate, wavelength of maximum absorbance λ_{max}, and value of molar absorptivity ε.

from a MilliQ system (Millipore). Methanol (high-performance liquid chromatography [HPLC] grade) was purchased from Merck.

METHODS

Experimental Protocol

Solutions of sodium usnate at a concentration of 2.6×10^{-5} M at various pH were enclosed in spectrophotometer tubes and exposed to the light source in the light-stability cabinet (Original Hanau, 7011, Original Hanau Quarzlampen GmbH, Germany). The experiments were carried out on triplicate samples. The intensity of ultraviolet A (UV-A) and UV-B radiation was measured with an Osram apparatus (Centra-UV-Meßgerät, Germany). The intensity was maintained at 6.45 and 1.47 mW · cm^{-2} for UV-A and UV-B, respectively. All tubes containing sodium usnate solutions were covered with aluminum foil before exposure to eliminate the influence of heat generated by the light within the cabinet. The pH of these solutions was adjusted to the desired values with $Na_2B_4O_7$ 1.3×10^{-4} M, 4.5×10^{-4} M, and 1.5×10^{-2} M. The pH of these solutions were determined with a Metrohm Herisau pH meter (France), model E300B, equipped with a Refill Ingold I 3556 (France) (pH = 0–14 and T = 0°C–80°C) electrode and standardized with Panreac solutions, respectively, at pH 4 and pH 10. These measures were carried out at 20°C.

Table 1

Added Buffer Salt Composition and pH Solutions

Buffer Type	Concentration (M)	pH
None		6
$Na_2B_4O_7$	1.30×10^{-4}	7
$Na_2B_4O_7$	4.50×10^{-4}	8
$Na_2B_4O_7$	1.50×10^{-2}	9

Preliminary Study

The value of the absorption peak of sodium usnate was determined by a spectrophotometric method (Hitachi UV-visible, double-beam spectrophotometer, model U-2000). Slit width was fixed at 2 nm. Solutions were recorded in 1-cm quartz cells over the 200 to 400 nm range (δλ = 2.3 nm). The scan speed was 400 nm · min^{-1}.

Assay Procedure

The sodium usnate concentrations initially and at time t were determined using HPLC. Chromatography was performed using an HPLC system incorporating a Waters model 6000 A pump, a Waters Lambda Max model 481 LC variable-wavelength detector set at 280 nm, and a Merck D-2500 model integrator (Hitachi). The analytical column was a 250 mm × 4 mm i.d. Nucleosil C_{18}, average particle size 5 µm (Merck). Each solution was analyzed under the following conditions. The mobile phase consisted of a mixture of methanol-phosphate buffer (pH 7.4) (70/30 v/v). Chromatography was performed at room temperature, and the flow rate was set at 1.0 ml · min^{-1}. The various solutions (10 µl) were injected into the HPLC system. Each solution was analyzed in

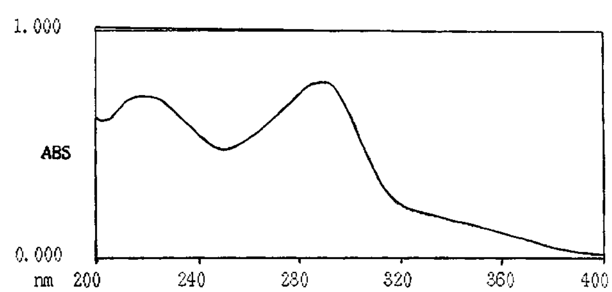

Figure 2. Absorbance spectrum of sodium usnate. Sodium usnate was dissolved in distilled water and analyzed at a concentration of 2.6×10^{-5} M.

triplicate, and the difference among the three samples was less than 1%.

RESULTS AND DISCUSSION

Kinetics of Sodium Usnate Photodegradation

The pH of different solutions are given in Table 1. The absorbance spectrum of sodium usnate showed a minimum at 251 nm and maxima at 217 and 288 nm (Fig. 2). The chromatogram shows that sodium usnate was eluted at 6 min. The order of the photodegradation reaction was determined by the least-squares method linear adjustment and by calculation of correlation coefficients in order to choose between the zero-order and the first-order kinetics.

The degradation rate constant k was calculated from the slope of the line of area of the peak (6 min) versus time. The percentage of substance remaining was calculated. Without any buffer, the photodegradation of sodium usnate in diluted aqueous solution (Fig. 3) follows

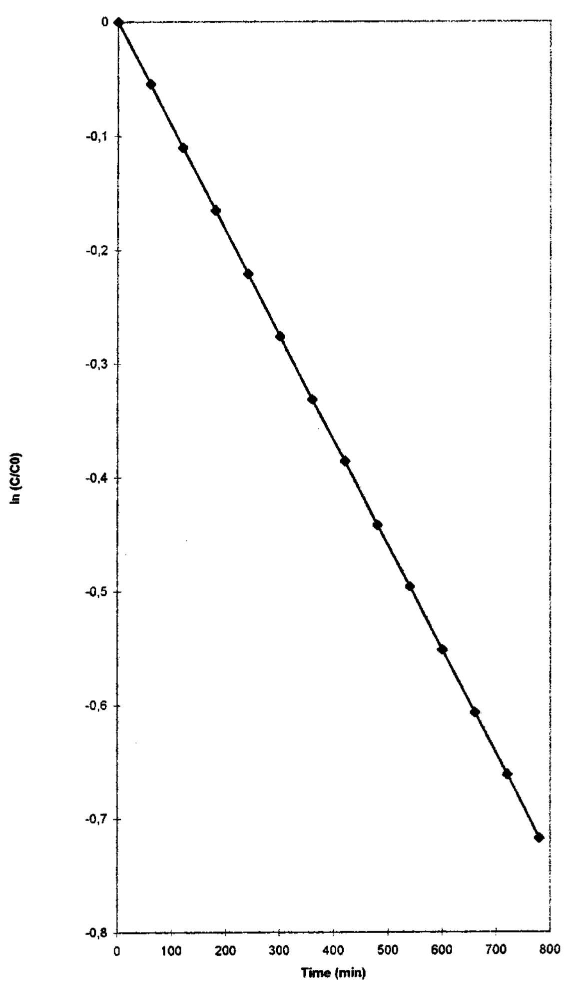

Figure 3. Kinetic diagram for the photodegradation during irradiation of sodium usnate aqueous solution (2.6×10^{-5} M) at pH 6. Data are the average of three determinations.

Table 2

Photodegradation of Aqueous Solution of Sodium Usnate

	C/C_0			
Time (min)	pH 6	pH 7	pH 8	pH 9
0	1.000	1.000	1.000	1.000
60	0.947	0.965	0.944	0.943
120	0.896	0.932	0.890	0.889
180	0.848	0.899	0.840	0.838
240	0.802	0.868	0.793	0.790
300	0.759	0.837	0.748	0.744
360	0.718	0.808	0.706	0.701
420	0.680	0.780	0.666	0.611
480	0.643	0.752	0.628	0.623
540	0.609	0.726	0.593	0.587
600	0.576	0.701	0.559	0.553
660	0.545	0.676	0.528	0.522
720	0.516	0.653	0.498	0.492
780	0.488	0.630		
840		0.608		
900		0.586		
960		0.566		
1020		0.546		
1080		0.527		
1140		0.509		
1200		0.491		

Table 3

Degradation Rate Constants of 2.6×10^{-5} M Sodium Usnate Solutions at Various pH

pH	Degradation Rate Constants k(min^{-1}) $\pm$ SEM
6	$9.20 \times 10^{-4} \pm 0.62 \times 10^{-4a}$
7	$5.93 \times 10^{-4} \pm 0.33 \times 10^{-4b}$
8	$9.69 \times 10^{-4} \pm 0.60 \times 10^{-4c}$
9	$9.88 \times 10^{-4} \pm 0.65 \times 10^{-4d}$

SEM = standard error of means of three determinations.
[a,c,d] Values are not significantly different ($p < .05$) from each other.
[b] Value is significantly different ($p < .05$) from the [a,c,d] values.

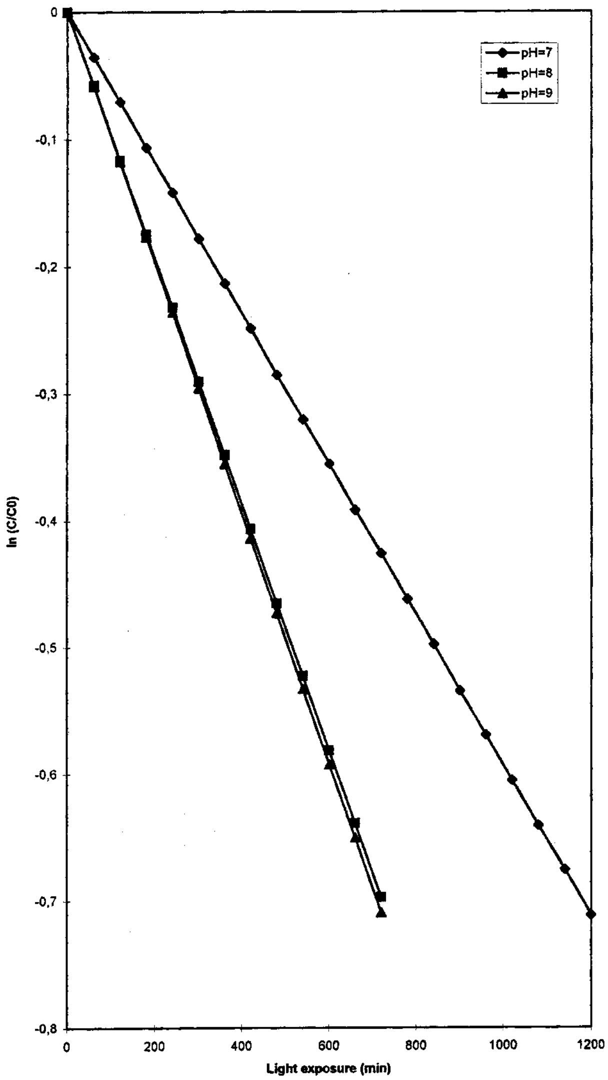

Figure 4. Kinetic diagram for the photodegradation of sodium usnate (2.6×10^{-5} M) at various pH during irradiation. Data are the average of three determinations.

apparent first-order kinetics and is described by the following equation:

$$C/C_0 = e^{-k}a^t \quad (1)$$

where C and C_0 are the concentrations of sodium usnate at time t and initially, respectively, and k_a is the apparent first-order degradation rate constant. Equation 1 gives us the value of the degradation rate constant, which is equal to 9.20×10^{-4} min^{-1}.

Table 4

90% Shelf Lives $t_{90\%}$ *and Half-Lives* $t_{50\%}$ *According to pH*

pH	$t_{90\%}$ (min)	$t_{50\%}$ (min)
6	114.9	754.0
7	178.2	1168.9
8	108.8	715.5
9	107.5	702.5

Effect of pH

The photodegradation of sodium usnate 2.6×10^{-5} M in buffer solution at pH 6, 7, 8, and 9 was studied (Table 2). The degradation rate constant was calculated from the slope of the area of the peak (6 min) versus time. The percentage of sodium usnate remaining was calculated at various pH (Table 3). Whatever pH, the photodegradation of sodium usnate in diluted buffer solution follows apparent first-order kinetics (Fig. 4) and is described by the following equation:

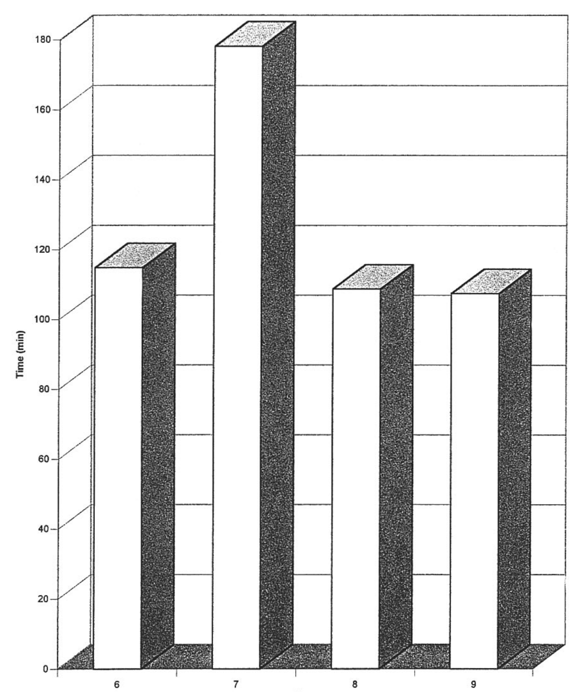

Figure 5. The 90% shelf lives (min) of 2.6×10^{-5} M sodium usnate solutions at various pH.

$$C/C_0 = e^{-k}b^t \quad (2)$$

where C and C_0 are the concentrations of sodium usnate at time t and initially, respectively, and k_b is the apparent first-order degradation rate constant.

At pH 6, 7, 8 and 9, we noted a variation of the values of rate constant k_b (Table 4). The pH of the solution had an influence on the photostability of sodium usnate. This conclusion has already been reached for many organic molecules (13). The effect of pH on the 90% shelf life of sodium usnate is significant at pH values of 6 and 7 (Fig. 5). The percentage increase in the stability of sodium usnate by the addition of buffer was found to be 55% and 64% between pH 6 and pH 7 and between pH 8 and pH 7, respectively. The present study completed the sodium usnate stability knowledge. Because the most stable molecules have an energy of activation E_a between 18 and 25 kcal · mol^{-1} (14), this molecule appears to be relatively thermostable (E_a = 15 kcal · mol^{-1} at pH 8) (12) and photostable.

REFERENCES

1. A. G. Gonzales, J. Bermejo Barrera, P. M. Rodriguez Perez, and C. E. Hernandez Padron, Planta Med., 58, 214 (1992).
2. D. Dubrescu, M. Tanasescu, A. Mezdrea, C. Ivan, E. Ordosch, F. Neagoe, A. Rizeanu, L. Trifu, and V. Enescu, Rom. J. Physiol., 30, 101 (1993).
3. B. Ribard, A. Kapor, G. Argay, P. Engel, Z. Djarmati, and R. M. Jankov, J. Cryst. Spectrosc. Res., 23, 107 (1993).
4. L. Grosso, P. E. Ghirardi, and M. Ghione, Curr. Ther. Res. Clin. E, 45, 1067 (1989).
5. C. Liberman, E. P. Tavares de Oliveira, H. Ilzuka, H. G. Higashi, and V. M. De Goday, Ann. Pharm. Fr., 47, 89 (1989).
6. M. Lauterwein, M. Oethiger, K. Belsner, T. Peters, and R. Marre, Antimicrob. Agents Chemother., 39, 2541 (1995).
7. B. Brocksa, M. Sturdikova, N. Pronayova, and T. Liptaj, Pharmazie, 51, 195 (1996).
8. T. Inone and M. Iwaida, J. Soc. Cosmet. Chem., 14, 57 (1980).
9. A. M. Al Bekairi, S. Qureshi, M. A. Chaudhry, D. R. Krishna, and A. H. Shah, J. Ethopharmacol., 33, 217 (1991).
10. P. Seifert and C. Bertram, Seifen, Ole, Fette, Wachse, 121, 123 (1995).
11. A. Fournel, M. E. Ferreira, A. Plojas, S. Torres, A. Inshausti, G. Yaluff, W. Quilhot, E. Fernandez, and M. E. Hidalgo, Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol., 116, 51 (1997).
12. C. Coiffard, L. J. M. Coiffard, F. Peigné, and Y. De Roeck-Holtzhauer, Arch. Pharm., 331, 128 (1998).
13. M. T. Le, L. J. M. Coiffard, F. Peigné, and Y. De Roeck-Holtzhauer, STP Pharma Sci., 6, 455 (1996).
14. L. Gonzalez Tavares, P. Sanz Saiz, M. J. Perez de la Cruz, M. A. Camacho, and J. L. Martin, STP Pharma Sci., 1, 195 (1991).