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Determination of photostability of monoammonium glycyrrhizinate

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Abstract

In a previous study, it was shown that monoammonium glycyrrhizinate was thermostable in the pH range 7.0–9.0. To establish the best storage conditions of this sweetener, we have determined the photodegradation of 6×10^{-5} M aqueous solutions of monoammonium glycyrrhizinate (λ_{max}:256 nm, $\epsilon = 10600$ M^{-1} cm^{-1}). The photodegradation appeared to follow first-order kinetics and was found to be pH-dependent. The degradation rate constant was calculated to be 8.85×10^{-4}, 5.38×10^{-4}, 4.33×10^{-4}, 4.66×10^{-4} and 5.12×10^{-4} min^{-1}, respectively, at pH 2.0, 4.5, 6.0, 8.0 and 10.0.

Keywords: Photostability; Monoammonium glycyrrhizinate

1. Introduction

The etymology of *Glycyrrhiza glabra* (Fabaceae) comes from the greek language "glycys" (γλυκυζ) meaning soft and "rhidza" (ριζα) meaning root ; glabra qualifies the appearance of the plant (Garnier, Bezanger-Beauquesne & Debraux, 1961). Glycyrrhiza root is a source of glycyrrhizin, also called glycyrrizic acid (3–5% of this is triterpenic saponin) which is 50–60 times sweeter than sucrose (Edwards, 1991; Fenwick, Lutonsli & Nieman, 1990). Glycyrrhizin is steroid-like, protective of the liver, and has antioxidant, and antivirus properties and can be used in toothpaste for treatment of dental plaque (Davis & Morrice, 1991; Dhepour, Zolfaghari, Samadian, Kobarford, Fazi & Assari, 1995; Dhepour, Zolfaghari, Samadian & Vahedi, 1994; Foussard-Blanpin & Coeurt, 1995). Because of its steroid-like pharmacological activity, there are some side effects, such as sodium retention, with oedema, hypertension (Basso, Dalla, Erle, Bascaro & Armanini, 1994; Kageyama, Suzuki & Saruta, 1994; Van Der Zwan, 1993), and hypokaliemia (Hayashi, Hayashi, Maruyama, & Yanagisawa, 1995; Luchon, Meyrier & Paillard, 1993), so the maximum dose per 24 h is fixed at 125 mg (Bruneton, 1993). Previous studies have established the shelf-life of glycyrrhizin ($t_{50\%} = 22.4$ years at pH = 8, pH where glycyrrhizin is the most stable; Coiffard, Coiffard, Peigné & De Roeck Holtzhauer, 1998). To complete the knowledge about this sweetener, we have studied photostability.

2. Material and methods

2.1. Chemicals

Monoammonium glycyrrhizinate (Ichimaru Pharcos Ltd, Japan) or 20β-carboxy-11-oxo-30-norolean-12-en-3β-yl-2-O-β-glucopyranuronosyl-α-D-glucopyranosiduronic ammonium salt (Fig. 1) is a light yellowish crystalline powder obtained from *G. glabra* (Fabaceae).

All chemicals were of analytical quality. Distilled water was obtained from an Autostill 4000X (Jencons) apparatus. HPLC grade methanol and acetic acid were from Merck.

2.2. Experimental procedure

Solutions of monoammonium glycyrrhizinate (6×10^{-5} M at various pH values were enclosed in spectrophotometer tubes and exposed to the light source in the

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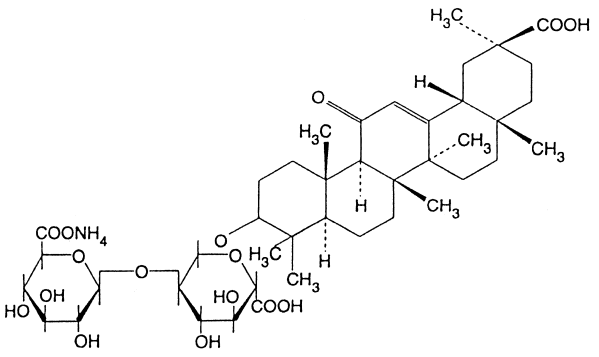

20 β - carboxy - 11 - oxo - 30 - norolean - 12 - en - 3 β - yl - 2 - O - β - glucopyranuronosyl - α - D - glucopyranosiduronic ammonium salt

$C_{44}H_{73}O_{14}N$ $\varepsilon_{max} = 10600\ M^{-1}\ cm^{-1}$

Molecular weight : 839.97 U.V. (water) $\lambda_{max} = 256$ nm

Fig. 1. Chemical structure of monoammonium glycyrrhizinate, wavelength of maximum absorbance (λ_{max}) and molar extinction coefficient (ϵ).

light-stability cabinet (Original Hanau, No. 7011, Original Hanau Quartzlampen GmbH). The intensities of UV-A and UV-B were measured with an Osram apparatus (Centra-UV-Meβgerät) This intensity was maintained at 6.45 and 1.47 mW cm^{-2} for UV-A and UV-B, respectively. All tubes containing monoammonium glycyrrhizinate solutions were closed with aluminium foil before exposure in order to eliminate the influence of heat generated by the light within the cabinet. The analysis was carried out on triplicate samples and the difference between the triplicates was less than 1%. The pH of these solutions was adjusted to the desired values with HCl 0.01 M, $Na_2B_4O_7$ 5.90×10^{-5} M, $Na_2B_4O_7$ 2.40×10^{-4} M and NaOH 7.5×10^{-3} M. The 6×10^{-5} M monoammonium glycyrrhizinate solution without any acid or alkali addition had a pH equal to 4.5. The pH values of these solutions were determined with a Metrohm Herisau pH-meter, model E300B, equipped with a Refill Ingold I 3556 (pH = 0–14, T = 0–80°C) electrode and standardized with Panreac solutions, respectively, at pH = 4 and pH = 10. These measurements were carried out at 20°C.

2.3. Assay

Monoammonium glycyrrhizinate concentrations, initially and at various times, were determined by a spectrophotometric method (Hitachi UV-visible, double beam spectophotometer, model U-2000). The possible interference of degradation products with monoammonium glycyrrhizinate was studied using high performance liquid chromatography. The HPLC system consisted of a Merck 655 A-12 pump, a Waters Lambda Max model 481 LC variable wavelength detector and a D-2500 Merck integrator. Chromatography was carried out with a microbondapack C18 phase column, and eluant was methanol-water-acetic acid (60:34:6). The detector was set to the wavelength of maximum absorbance.

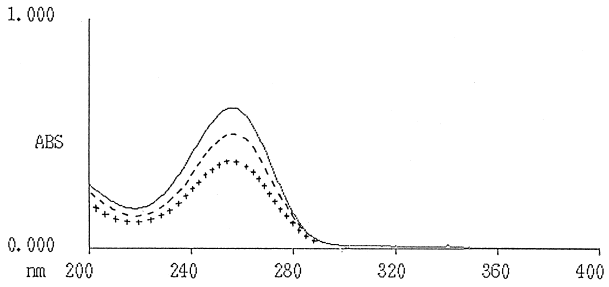

Fig. 2. Set of UV spectra obtained during photolysis of 6×10^{-5} M monoammonium glycyrrhizinate in aqueous solution at pH 4.5 (optical path 1 cm) : (–) initial time, (– –) 180 min, (+ +) 720 min.

3. Results and discussion

3.1. Kinetics of monoammonium glycyrrhizinate degradation at pH 4.5

The optimum response of monoammonium glycyrrhizinate was obtained at 256 nm. Under the experimental conditions, a linear calibration (correlation coefficient, $r > 0.99$), was obtained over monoammonium glycyrrhizinate concentrations ranging from 1.20×10^{-5}–9.50×10^{-5} M. The HPLC protocol shows that the absorbance at 256 nm involves only the intact molecule. The concentration of monoammonium glycyrrhizinate was calculated from a Beer's plot of concentration versus absorbance. The analyses were carried out on triplicate samples and the difference between the triplicates was below 1%.

The order of the photodegradation reaction was determined by the least squares linear adjustment method and by calculation of correlation coefficients, in order to choose between the zero order-kinetics and the first-order kinetics. The photodegradation of monoammonium glycyrrhizinate (pH = 4.5) was expressed as the rate of change of absorbance at 256 nm. A set of UV spectra obtained during photolysis is shown in Fig. 2. This figure demonstrates the gradual decrease in absorbance at 256 nm during photolysis. The degradation rate constant was calculated from the slope of the line of absorbance versus time. The percentage of substance remaining was calculated from a Beer's plot of concentration versus absorbance. The photodegradation of monoammonium glycyrrhizinate in diluted aqueous solution (Fig. 3) follows apparent first order-kinetics and is described by the following equation:

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

Table 1
Photodegradation of aqueous solution of monoammonium glycyrrhizinate at various pH values

Times (min)	C/C_0				
	pH = 2.00	pH = 4.50	pH = 6.00	pH = 8.00	pH = 10.00
0	1.000	1.000	1.000	1.000	1.000
60	0.948	0.874	0.969	0.974	0.969
120	0.899	0.846	0.944	0.947	0.940
180	0.853	0.819	0.920	0.921	0.911
240	0.809	0.793	0.896	0.896	0.884
300	0.767	0.768	0.873	0.871	0.857
360	0.727	0.743	0.851	0.847	0.831
420	0.690	0.720	0.829	0.824	0.806
480	0.654	0.697	0.808	0.801	0.782
540	0.620	0.675	0.787	0.779	0.758
600	0.588	0.653	0.767	0.757	0.735
660	0.558	0.633	0.747	0.736	0.713
720	0.529	0.613	0.728	0.716	0.691
780	0.502	0.593	0.709	0.696	0.670
840	0.476	0.574	0.691	0.677	0.650
900		0.556	0.673	0.658	0.630
960		0.538	0.656	0.640	0.611
1020		0.521	0.639	0.623	0.593
1080		0.505	0.623	0.605	0.575
1140		0.489	0.607	0.589	0.557
1200			0.591	0.573	0.541
1260			0.576	0.557	0.524
1320			0.561	0.541	0.508
1380			0.547	0.526	0.493
1440			0.533	0.512	
1500			0.519	0.498	
1560			0.506		
1620			0.493		

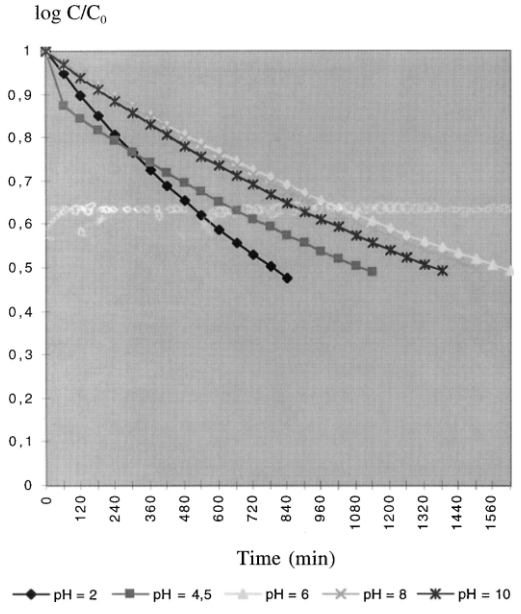

Fig. 3. Kinetic diagram for the photodegradation of monoammonium glycyrrhizinate (6×10^{-5} M) at various pH during irradiation. Data is the average of three determinations.

Table 2
Degradation rate constants of 6×10^{-5} M monoammonium glycyrrhizinate aqueous solutions at various pH values

pH	Degradation rate constants k (min^{-1})±S.E.M.[a]
2.00	$8.85\times10^{-4}\pm0.68\times10^{-4}$
4.50	$5.38\times10^{-4}\pm0.20\times10^{-4}$
6.00	$4.33\times10^{-4}\pm0.15\times10^{-4}$
8.00	$4.66\times10^{-4}\pm0.12\times10^{-4}$
10.00	$5.12\times10^{-4}\pm0.23\times10^{-4}$

[a] S.E.M., standard error of means of three determinations. Significantly different ($P<0.05$) relative to pH.

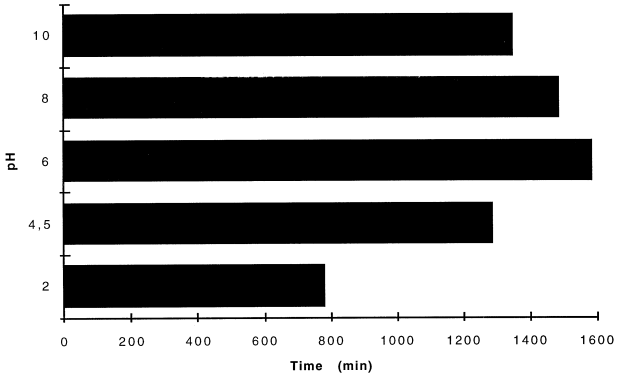

Fig. 4. Half-lives (min) of 6×10^{-5} M monoammonium glycyrrhizinate solution at various pH.

where C and C_0 are the concentrations of monoammonium glycyrrhizinate at time t and initially and k_a is the apparent first order degradation rate constant. Eq. (1) gives the value of the degradation rate constant, which is equal to 5.38×10^{-4} min^{-1}.

3.2. Effect of pH

The photodegradation of 6×10^{-5} M monoammonium glycyrrhizinate in buffer solution, at pH 2, 6, 8 and 10, was studied. The sets of UV spectra obtained during photolysis demonstrate a gradual decrease in absorbance at 256 nm. The degradation rate constants were calculated from the slope of the line of absorbance versus time. The percentage of monoammonium glycyrrhizinate remaining was calculated from a Beer's plot of concentration versus absorbance (Table 1). Whatever the pH, the photodegradation of monoammonium glycyrrhizinate in diluted buffer solution follows apparent first-order kinetics (Fig. 3) and is described by the following equation :

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

where C and C_0 are the concentrations of monoammonium glycyrrhizinate at time t and initially and k_b is the apparent first-order degradation rate constant. At pH 2, 6, 8 and 10 h there are variations of the values of

the rate constant k_b (Table 2). The pH values of the solution have an influence on the photostability of monoammonium glycyrrhizinate. This conclusion has already been reached for many organic molecules (Coiffard, Coiffard & De Roeck-Holtzhauer, 1999; Le, Coiffard, Peigne & De Roeck-Holtzhauer, 1996). The effect of pH on the shelf-life of monoammonium glycyrrhizinate is significant at pH values of 2–6 (Fig. 4). The percentage increase in the stability of monoammonium glycyrrhizinate, by the addition of buffer, was found to be 102% between pH 2 and pH 6.

The present study complements knowledge of monoammonium glycyrrhizinate stability knowledge. This molecule appears to be very photostable and thermostable over a wide pH range. The photostability can justify its incorporation in some formulations. Monoammonium glycyrrhizinate could be used in many domains because of its numerous properties (e.g. UV sunscreen, sweetener and flavour corrector).

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