Titrimetric and Polarographic Determination of Carminic Acid and its Quantification in Cochineal *(Dactylopius coccus)* Extracts

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Abstract. The purity of synthetic carminic acid was quantified using potentiometric and conductimetric titrations, allowing its use as a secondary standard because there is not a primary one. Polarographic analysis (in ethanol-water 50:50, v/v) of carminic acid showed two reduction signals, each with a different number of electrons. The first reduction had more Nernstian behaviour and can be applied in analyses. Differential pulse polarography (DPP) produced a detection limit of 0.55 µg mL^{-1}. The results indicate that DPP can be used to determine carminic acid content in cochineal extracts.
Keywords: Carminic acid; acid-basic titration; electrochemical determination; cochineal extract; polarography.

Resumen. La pureza del ácido carmínico sintético se cuantificó por valoraciones potenciométricas y conductimétricas, lo que permitió emplearlo como patrón secundario pues no hay patrones primarios para el ácido carmínico. El estudio polarográfico (en etanol-agua 50:50, v/v) mostró que el ácido carmínico se reduce en dos etapas, con diferente número de electrones y que la primera reducción (a E½ = −515 mV / ECS) tiene un comportamiento más nernstiano y puede aplicarse con fines analíticos. El límite de detección obtenido por Polarografía Diferencial de Impulsos (PDI) fue de 0.55 ppm. Estos resultados permitieron determinar el contenido de ácido carmínico por PDI en extractos naturales de grana cochinilla.
Palabras clave: Ácido carmínico, electroquímica, titulación ácido-base, grana cochinilla, polarografía.

Introduction

Carminic acid, or red carmine (CAS: 1260-17-9, CI: 75470) (Fig. 1), has been used for centuries as a colorant and its main source is the female cochineal (*Dactylopius coccus*) insect. Trade in cochineal was a principal income source in Mexico during the Spanish colonial era [1-2].

Development of synthetic colorants (mainly azoic compounds, for reds) led to a substantial drop in natural red carmine production. In recent years, however, cochineal production and its use in foods, cosmetics, pharmaceuticals and textiles has begun to rise because many azoic colorants are now known to be carcinogenic, while carminic acid is non-toxic [3-8]. This increased demand for carminic red highlights the importance of better understanding its chemical behavior and developing trustworthy, simple analytical methods to quantify the amount of colorant in cochineal for quality control purposes.

Several analytical methods have been reported for the determination of carminic acid using spectroscopic and chromatographic methods [9-14]. Few studies have been done, however, on its acid-basic properties [15-19], and still fewer on its redox properties. Only Furman and Stone [20] reported on the electrochemical behavior of carminic acid in a buffered aqueous medium. The present study aim was to determine the purity of synthetic carminic acid through potentiometric and conductimetric titrations and use it as secondary standard because there is not a primary one, and use differential pulse polarography (DPP) to quantify carminic acid extracted from cochineal samples.
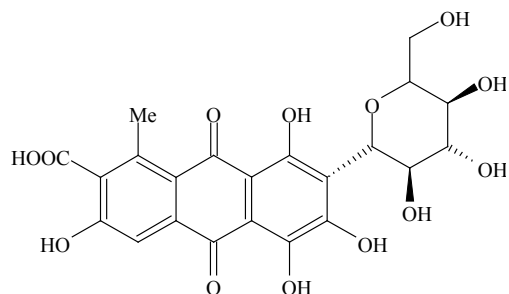
Results and Discussion

Potentiometric titration

Synthetic carminic acid was titrated using 0.091 mol L^{-1} NaOH and colorant concentrations in a 0.01-0.04 mol L^{-1} range; results

Fig. 1. Chemical structure of carminic acid (2-anthracenecarboxylic acid).

were similar for all concentrations. Fig. 2 shows a typical titration curve for a carminic acid solution.

Three equivalence points were identified that indicate the presence of a polyprotic acid. The —COOH carboxyl group and the four —OH phenolic groups in the anthraquinone rings (positions C-3, C-5, C-6 and C-8) are susceptible to proton loss. The first equivalence point can associate with the carboxyl group, which has the most acidic proton. The other two inflections may result from loss of protons from the meta-position phenolic group to the methyl group, and the third may be due to loss from other nearby phenolic group.

The volumes consumed in the equivalence points were not equal, suggesting that the carminic acid was not pure. The volume increase between the first two equivalence points (ΔV_2) coincided with that between the second and third points (ΔV_3 = 0.96 mL), whereas the first volume was higher (ΔV_1 = 1.29 mL). Given that the three acidic protons of the titrated carminic acid consumed the same quantity of NaOH (0.96 mL), the volume difference (0.33 mL) between the first and second equivalence point implies that the impurity has an acidic character.

Based on these results, carminic acid concentration purity was 87.0% ± 1.5%.

The volumes and pH values at half of each carminic acid equivalence point were used to estimate the approximate pKa values in the ethanol-water (50:50, v/v) medium. The pKa values were obtained through 3 different operators and 3 different carminic acid solutions; the statistical analysis was a double ANOVA:

$$pKa_1 = 3.13 \pm 0.01; \; pKa_2 = 6.20 \pm 0.01;$$
$$pKa_3 = 8.94 \pm 0.05.$$

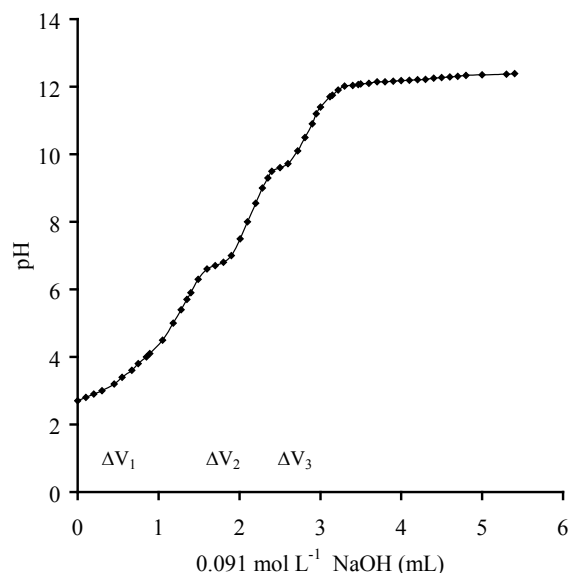These titration results agree with those reported by Schwing-Weill and Wechsler [15] and by Joergensen and Skibsted [16] as far as the existence of three acid hydrogens in the carminic acid molecule, and the pKa values (3.42, 5.55 and 8.25 in an aqueous medium). However, Schwing-Weill and Wechsler [15] mention dimmerization in solution, while in the present study only the acid impurity was detected and no evidence was seen of dimmer formation, no matter the acid concentration employed.

Conductimetric titration

Fig. 3 shows the conductimetric titration results for 10 mL of carminic acid solution with NaOH.

The slope changes between each of the equivalence points are not clearly visible in the conductimetric titration curve, although the overall volume consumed for complete neutralization of carminic acid coincides with that observed in the potentiometric titration. In addition, the first titrated compound was the acid impurity, which is an acid stronger than the carminic acid carboxyl group. The volume consumed for titration of this acid is congruent with the ΔV_2 and ΔV_3 values, that is, the mol percentage of consumed OH⁻ is distributed between carminic acid (87% ± 1.5%) and the acid impurity (13% ± 1.5%).

Polarographic study

The first electrochemical parameter established was the potential range, with an anodic limit of 250 mV and a cathodic limit of −1900 mV.

Two reduction waves with different current intensities (i_d) were observed in the carminic acid (Fig. 4). The second reduction wave (at the most negative potential) had an i_d almost three times greater than the first reduction wave (at a less negative potential).

Logarithmic study of the polarographic waves, E = f [log $(i - i_{ox})/(-i)$], indicated that the first signal had an $E^1_{1/2} = -515$
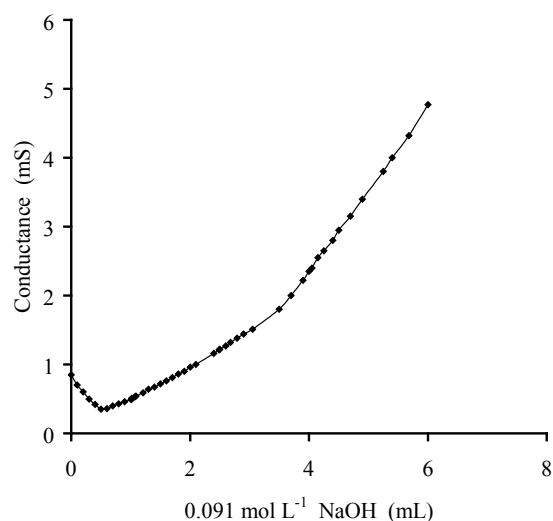

Fig. 2. Potentiometric titration of 0.01 mol L⁻¹ carminic acid in ethanol-water (50:50, v/v) with 0.091 mol L⁻¹ NaOH.
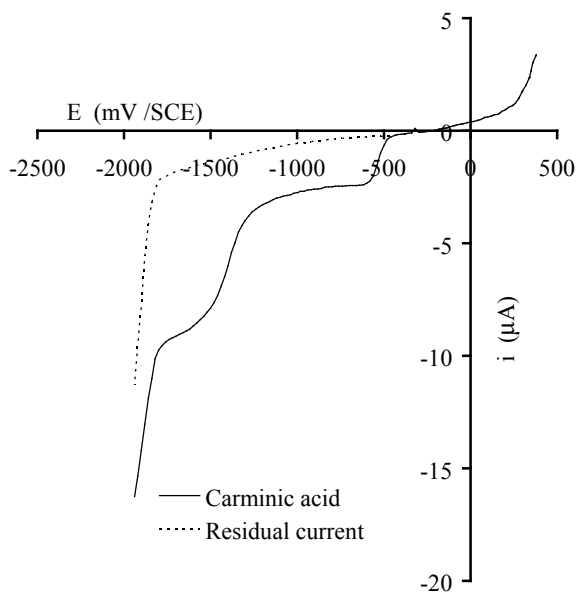
Fig. 3. Conductimetric titration of 0.01 mol L⁻¹ carminic acid in ethanol-water (50:50, v/v) with 0.091 mol L⁻¹ NaOH.

Fig. 4. Typical CP signals of carminic acid (8.6×10^{-4} mol L^{-1}) in 0.1 mol L^{-1} LiClO$_4$ Scan rate: 4 mV s^{-1}.

Fig. 5. Calibration curves of carminic acid for the two reduction waves (Classic Polarography). Supporting electrolyte: 0.1 mol L^{-1} LiClO$_4$.

mV and a slope of 40 mV, which corresponds to a quasi-Nernstian system with an exchange of at least two electrons. The second signal had an $E^2_{1/2} = -1345$ mV and a slope of more than 120 mV, meaning it was a complex system with non-Nernstian behavior. That the i_d value for the second wave was more than double that of the first suggests it involved at least four electrons.

other authors. For example, in cochineal samples from Mexico, Wouters and Verhecken [21] reported a carminic acid percentage of 13%, while Méndez and coworkers [13] established levels of 12.8 ± 0.3%. The carminic acid levels observed here in cochineal from Oaxaca, Mexico, ranged from 12.1 to 16.4%, depending on extraction procedure.

Current response and calibration curves

The first carminic acid Classic Polarography (CP) signal had i_d values that increased with concentration, making it applicable in quantification. The i_d values were measured at -800 mV and at -1700 mV in a linear range of carminic acid from 5×10^{-5} to 3×10^{-4} mol L^{-1} (25-150 µg mL^{-1}) (Fig. 5).

DPP was used to improve the detection limit (LOD). The second DPP reduction signal was badly defined and so only results from the first reduction are discussed. The i_d values were measured in a concentration range of carminic acid from 4×10^{-6} to 3×10^{-4} mol L^{-1} (2-150 µg mL^{-1}). Figure 6 shows a section of the calibration curve corresponding to the lowest concentrations of the studied range.

Table 1 shows the straight line equations, as well as the linear regression coefficient (r) and LOD for CP and DPP.

The DPP detection limit was lower, by a factor of 16, than that observed with CP, as usual. Table 2 is a comparison of quality parameters between the present polarographic procedure and procedures based on chromatographic and spectrophotometric methods.

Although the physicochemical fundamental of polarography is different from that of chromatography and spectrophotometry, the amount of carminic acid found in the analyzed cochineal in the present work is congruent with that described by
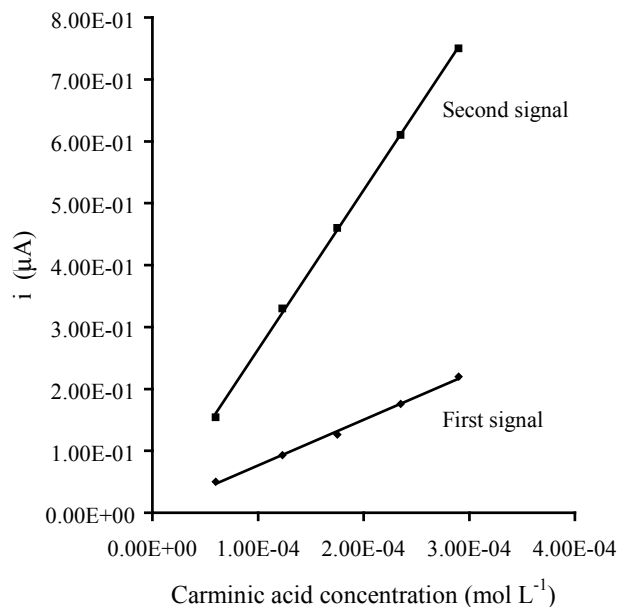
Quantification of carminic acid in cochineal extracts

One of the aims of this study was to propose a simple, precise method for quantifying carminic acid in cochineal based on DPP.
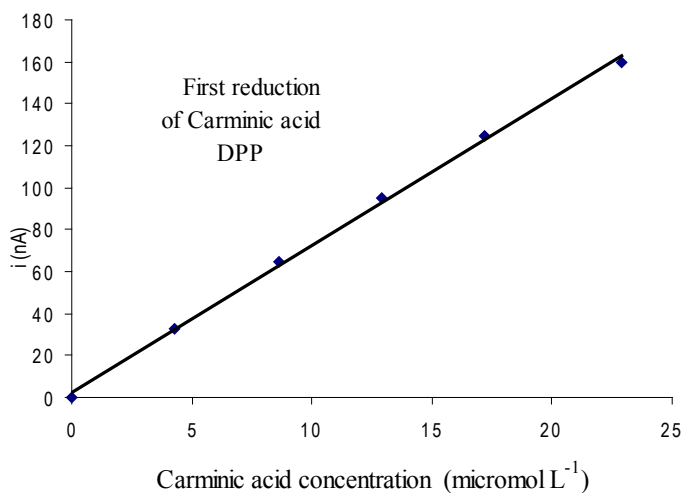

Fig. 6. Calibration curve of carminic acid for the lowest concentrations of the studied range (Differential Pulse Polarography, first reduction signal). Supporting electrolyte: 0.1 mol L^{-1} LiClO$_4$.

Table 1. CP and DPP detection limits for carminic acid (LOD = Xo + 3σ).

Polarography mode	i = f (C*)[a]	r^2	LOD
CP	$i_{d1} = 750.24C* - 0.0014$	0.997	1.8×10^{-5} mol L^{-1} (9 µg mL^{-1})
DPP	$i_p = 7018C* + 0.0025$	0.998	1.1×10^{-6} mol L^{-1} (0.55 µg mL^{-1})

[a] i (µA), C* (mol L^{-1})

Table 2. Quality parameters of procedures for determining carminic acid content in cochineal.

Method	Linear range (µg mL^{-1})	LOD (µg mL^{-1})	Simplicity
Classic Polarography (CP, this study)	25-150	9	Requires previous extraction of carminic acid. Determination by CP is direct and simple in an ethanol-water (50:50, v/v) medium.
Differential Pulse Polarography (DPP, this study)	2-150	0.55	Requires previous extraction of carminic acid. Determination by DPP is direct and simple in an ethanol-water (50:50, v/v) medium.
Chromatography (González *et al.* [11])	5-250	2	Requires previous extraction of carminic acid. Procedure for HPLC is complex, although it includes simultaneous determination of other colorants as well as carminic acid.
Spectrophotometry (Méndez *et al.* [13])	5-75	0.5	Requires previous extraction of carminic acid. Determination by UV-Vis spectrophotometry is direct and simple.

Carminic acid was extracted from a dry cochineal sample, from Oaxaca, Mexico, following the procedure shown in Fig. 7.

Water and ethanol were used to extract the colorant from 2 g samples of non-defatted cochineal, as well as from an equal amount defatted with an organic solvent (ethylic ether, methylisobutylketone (MIBK) or chloroform). Only one extraction with 50 mL solvent and 1 h stirring was performed; defatting was made with 25 mL solvent and 1 h stirring. This procedure was repeated three times but no statistical analysis was made because the purpose was only a comparison under similar conditions and the electrochemical determination.

The amount extracted was determined by gravimetric analysis and the extract's carminic acid content quantified with DPP. Table 3 shows the results from the best extractant systems.

Extraction percentage was higher in previously defatted samples. However, the difference in extraction efficacy between use of only boiling water (92°C) and an MIBK + boiling water extraction is not large enough to justify the additional step of defatting and use of an organic solvent. In addition, successive extractions can be done when using only boiling water, which can improve yield.

Conclusions

Acid-basic titration (potentiometric or conductimetric) can be used to determine the purity of synthetic carminic acid, which can be employed as a secondary standard.

The pKa values of carminic acid were estimated in an ethanol-water (50:50, v/v) medium (pKa$_1$ = 3.13 ± 0.01; pKa$_2$ = 6.20 ± 0.01; pKa$_3$ = 8.94 ± 0.05).

Carminic acid exhibited two polarographic reduction signals, each with a different number of electrons. The first signal was the most adequate for quantification purposes.

Very low carminic acid concentrations (LOD = 0.55 µg mL^{-1}) can be detected using DPP.

Extraction of colorant from cochineal is highest when it is defatted (16.4%) compared to non-defatted (12.1%), although extraction using only boiling water is lower cost and non-toxic.
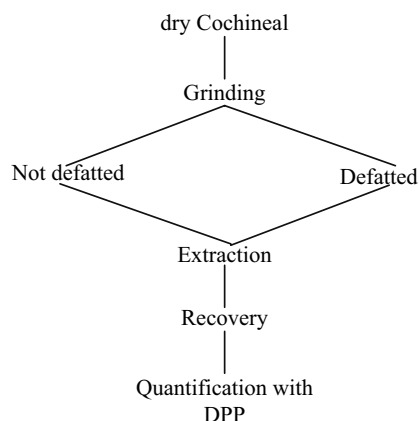
Fig. 7. Procedure for extraction of carminic acid from cochineal.

Table 3. Quantification of carminic acid in cochineal samples (2 g).

Extractant Systems	Extract (g)	Amount of carminic acid in extract (g)	Carminic acid content in extract (%)	Carminic acid content in cochineal (%)
Hot water	0.6040	0.2416	44.0	12.1
Ethanol-water (50:50, v/v)	0.5095	0.2659	52.3	13.3
MIBK + hot water	0.5730	0.3283	57.3	16.4

In addition, when using boiling water successive extractions can be done to increase total carminic acid yield.

Experimental

Reagents

All chemical reagents were analytical grade: Carminic acid (labeled: purity >90%) and $LiClO_4$ were from Aldrich, all other reagents were from Merck. Cochineal samples from the 1998 harvest were obtained from the Centro de Grana Cochinilla in Oaxaca, Mexico.

Supporting electrolyte was a 0.1 mol L^{-1} lithium perchlorate solution in ethanol-water (50:50, v/v). Nitrogen gas (Linde 4.8) was used to eliminate oxygen from solutions.

Equipment

The experimental apparatus was a Tacussel PRG5 (France) potentiostat with a glass electrochemical cell. A dropping mercury electrode (DME) was used as working electrode; a platinum wire was employed as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode.

The acid-base titrations were carried out using a Tacussel S6N (France) potentiometer with a glass combined minielectrode and a Metrohm 654 (Suisse) conductimeter with an electrode (cell constant: $Kc = 0.78$ cm^{-1}).

Procedure

Intensity-potential curves were generated by classic polarography (CP) for different carminic acid solutions at 4 mV s^{-1}. Successive additions of an 8.6×10^{-3} mol L^{-1} carminic acid solution were made in 15 mL of supporting electrolyte. A 50 mV pulse amplitude was used to record the signals with DPP.

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