

Full Paper

Solvent Effects in Electrocoagulation of Selected Plant Pigments and Tannin

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Received: 9 April 2006; in revised form: 27 April 2006 / Accepted: 29 April 2006 /

Published: 29 April 2006

Abstract: Electrocoagulation of a plant extract and certain substances representative of selected classes of plant pigments, viz. chlorophyll, a carotenoid, a phenolic substance and a tannin, was performed in ethanol containing varying amounts of water (15-75%). The results showed that the extent and efficiency of coagulation of these substances tends to vary in a manner directly related to the water content of the solvent, although the tannin and the phenolic substance were less sensitive to the solvent composition and are equally well coagulated in all solvent systems studied. The findings can be applied to the removal of these substances from aqueous alcoholic plant extracts using the electrocoagulation technique.

Keywords: Electrocoagulation, electrolytic decolourisation, plant pigments, phenolic compounds, tannins

Introduction

Electrocoagulation has been a useful alternative technique for clarifying and decolourising certain solutions containing unwanted dissolved substances or suspended matter. However, those solutions were almost always aqueous in nature, for example, potable water [1-2], food wastewater [3], tar-sand and oil-shale wastewater [4], phosphate-containing sewage [5-6], industrial wastes containing cyanide and heavy metals [7-9], and dye-containing textile wastewater [10-11]. Electrocoagulation has also

been used as a purification step in the isolation of a few natural products from crude plant extracts [12-17]. Again, the solvent used in those isolations was also purely aqueous. Trials of natural product isolation using electrocoagulation in alcoholic solutions as a part of the process have been reported in the literature, for example, for the isolation of asiaticoside [18] and the extraction of phenolic compounds from the bark of *Lithocarpus elegans* [19], but a systematic study of electrocoagulation in alcoholic solutions has, to our knowledge, not been undertaken. In view of the fact that a system containing an organic solvent is undoubtedly more useful in isolating natural products in general, we would like to report our study on the effects of solvent in the electrocoagulation of some selected organic substances and plant extracts. For a start, we chose as our model study the system in which ethanol with varying amounts of water is used as solvent.

Results and Discussion

Our previous work carried out with water and alcohol soluble natural dyes (chlorophyllin and crocin) [20] showed that although decreases in the water content of the solvent (ethanol) has some effect on the coagulation efficiency for the dyes, coagulation was more or less complete after 2 hours, especially when iron is used for the electrodes (Figures 1-2).

Figure 1. Plots of residual weight percentage and electrolysis time for each ethanol concentration of chlorophyllin solution at 626 nm; A: with aluminium as electrodes, B: with iron as electrodes.

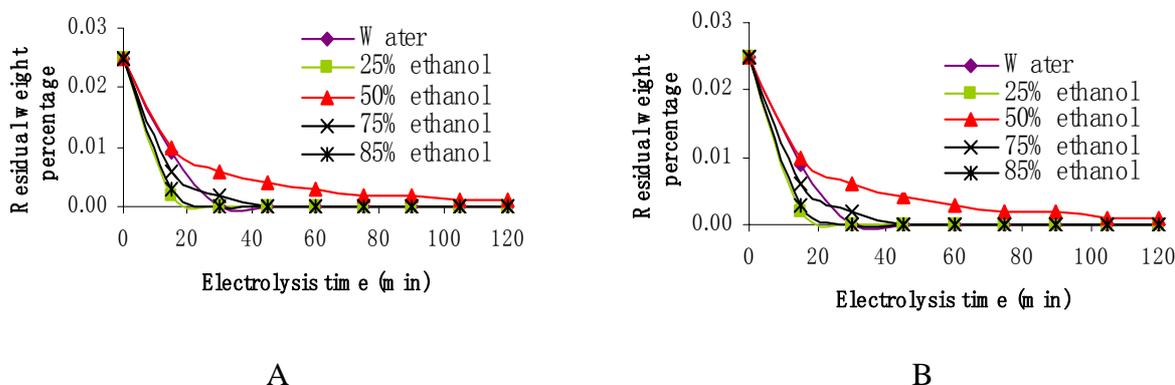
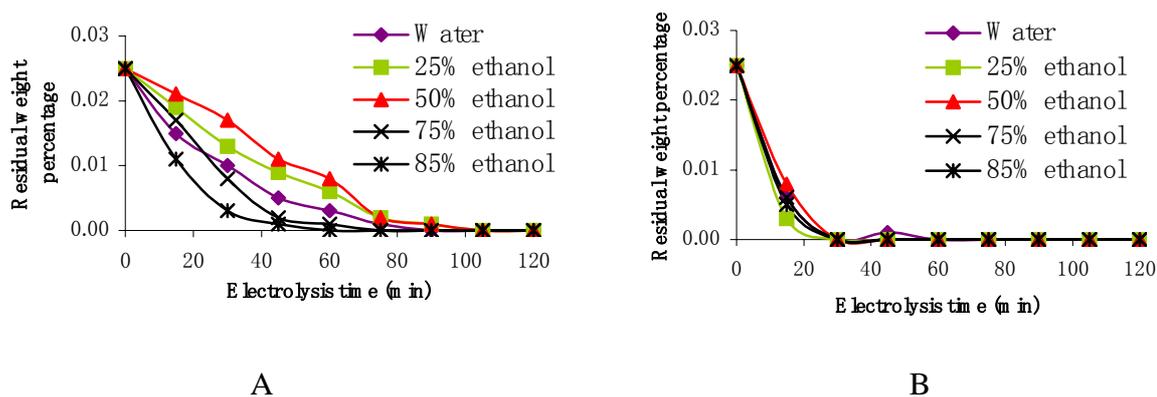
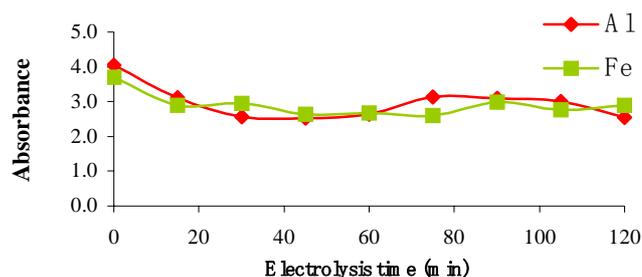


Figure 2. Plots of residual weight percentage and electrolysis time for each ethanol concentration of crocin solution at 440 nm; A: with aluminium as electrodes, B: with iron as electrodes.



However, experiments carried out in this study with water-insoluble dyes (the chlorophylls and the carotenoids) seem to indicate that, contrary to the above result, these pigments seem to be more difficult to coagulate. For example, in Figure 3 it can be seen that the model pigment β -carotene is hardly affected and barely removed from the solution by the electrolytic decolourisation process applied, even when iron electrodes are used (due to the difficulties in solubilizing this pigment, electrocoagulation was only examined in a single mixed solvent system.)

Figure 3. Plots of absorbance at 460 nm and electrolysis time for β -carotene (0.01%) in 27% ethanol; \blacklozenge : with aluminium as electrodes, \blacksquare : with iron as electrodes.



Similarly, for solutions of extracted chlorophyll, the trend is that coagulation seems to be less efficient as the water content in the solvent decreases (Figures 4-5). This may not only be due to the fact that, as the water content in the solvent decreases the process of electrocoagulation itself is naturally retarded, but also to the fact that more pigment is dissolved in the solution as the water content in the solvent decreases and the alcohol content accordingly increases. This trend was repeated in nearly every case of green plant extracts we studied. Thus, for example, when the alcoholic extract of the leaves of *Solanum laciniatum*, which contain solasonine (an important starting compound for steroid synthesis), was subject to decolourisation by electrocoagulation, typical results as shown in Figure 6 (for the yellow pigments) and Figure 7 (for the green pigments) were obtained.

Figure 4. Plots of absorbance at 440 nm and electrolysis time for each ethanol concentration of extracted chlorophyll solution; A: with aluminium as electrodes, B: with iron as electrodes; \blacklozenge , 75% ethanol; \blacksquare , 85% ethanol

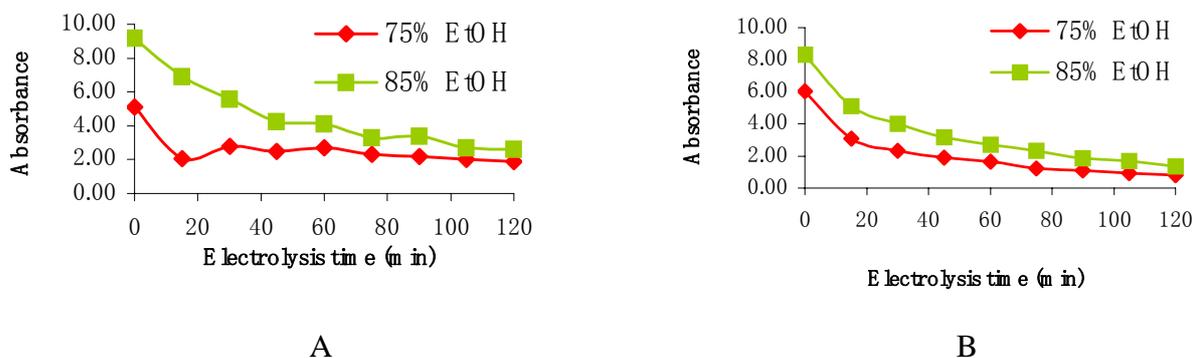
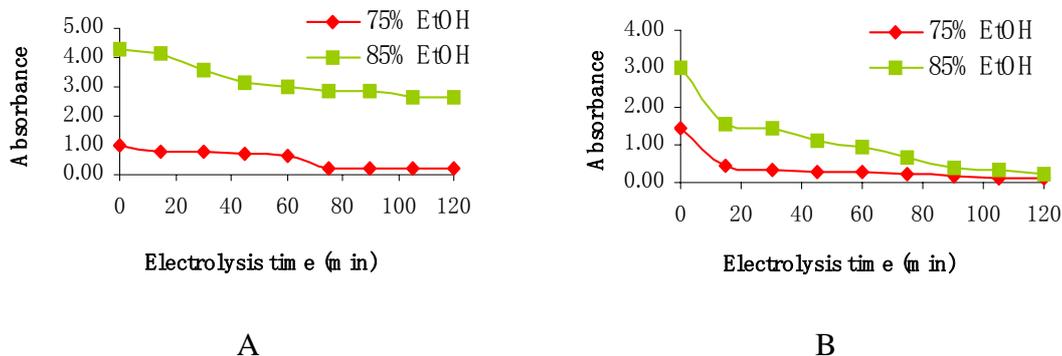


Figure 5. Plots of absorbance at 665 nm and electrolysis time for each ethanol concentration of extracted chlorophyll solution; A: with aluminium as electrodes, B: with iron as electrodes; \blacklozenge , 75% ethanol; \blacksquare , 80% ethanol



Importantly, however, it has been shown by our group that even with this general decreased efficacy of the pigment removal, electrocoagulation is still more efficient than the conventional method of extraction with organic solvents and does not affect the desired natural products, which were subsequently isolated [21].

Figure 6. Plots of absorbance and electrolysis time for each ethanol concentration of *Solanum laciniatum* extract at 408 nm; A: with aluminium as electrodes, B: with iron as electrodes; \blacklozenge , 25% ethanol; \blacksquare , 50% ethanol; \blacktriangle , 75% ethanol; \times , 85% ethanol.

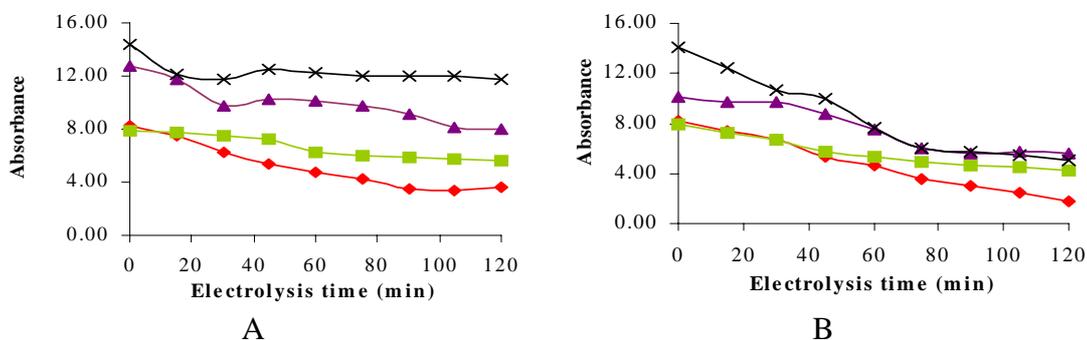
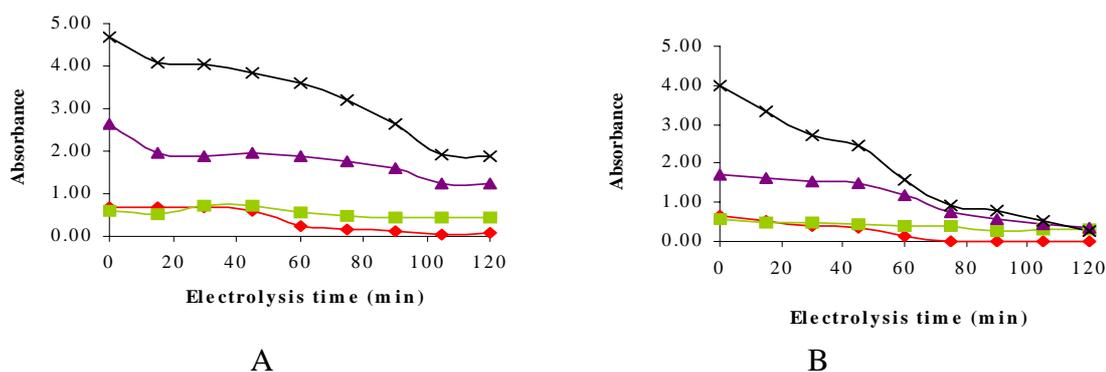


Figure 7. Plots of absorbance and electrolysis time for each ethanol concentration of *Solanum laciniatum* extract at 666 nm; A: with aluminium as electrodes, B: with iron as electrodes; \blacklozenge , 25% ethanol; \blacksquare , 50% ethanol; \blacktriangle , 75% ethanol; \times , 85% ethanol



Another important class of plant pigments is the phenolic substances, which include the tannins, the flavonoids, and the various quinone compounds. Most of these materials (except perhaps the tannins) are poorly soluble in water but more soluble in alcohol or other organic solvents. Many of them are valuable natural products, while others (especially the tannins) are regarded as little more than intractable mixtures with unfavourable biological activities and it is generally preferable to remove them along with the pigments.

In a 100% aqueous medium, it has been shown that tannins can be very efficiently coagulated and removed by electrolysis [22-23]. In this study, we tried to repeat the process in aqueous alcoholic solutions. The hypothesis is that tannins, owing to their polyphenolic nature, should still be easily coagulated by the phenolate salt forming mechanism in addition to the adsorption mechanism [23]. Our experiments showed this to be the case. Thus, for example, at a concentration of 0.1% tannin in up to 85% ethanol, a 250-mL solution was almost completely de-tannized within 15 minutes, using aluminium as electrodes and a current of 0.3 A (Figure 8). At a concentration of 1.0% tannin, the complete detannization time was increased to 80 minutes (Figure 9).

Figure 8. Plot of the residual weight percentage and electrolysis time for 0.1% w/v tannin at 275 nm ; ●, 25% ethanol , ▲, 50% ethanol, ■, 75% ethanol and ◆, 85% ethanolic solution.

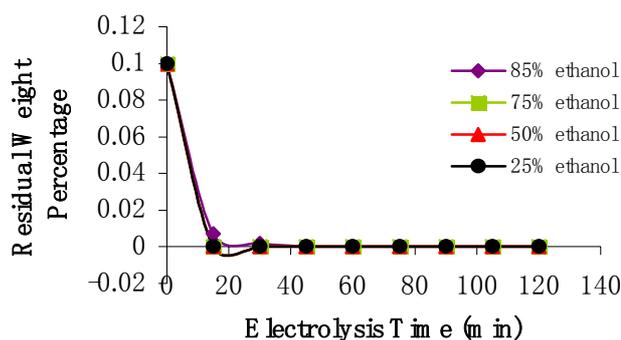
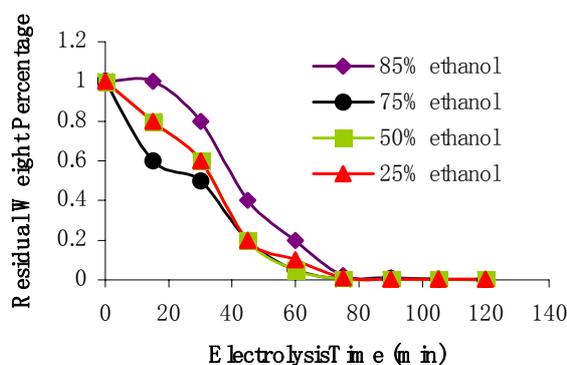


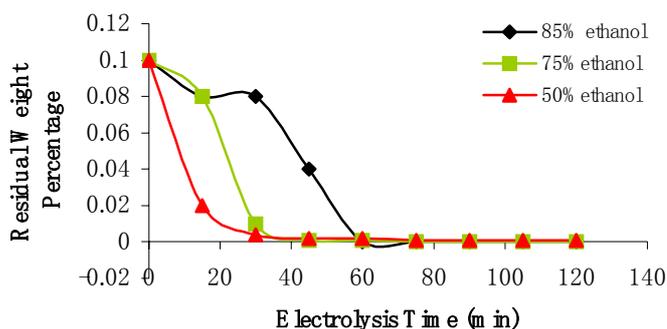
Figure 9. Plot of the residual weight percentage and electrolysis time for 1.0% w/v tannin at 275 nm; ▲, 25% ethanol, ■, 50% ethanol, ●, 75% ethanol, and ◆, 85% ethanolic solution.



Next, to demonstrate the general trend of the effect of water content in the solvent on the coagulation of phenolic compounds other than tannins, the result of electrocoagulation of a known flavonoid, morin, is presented here as a typical example (Figure 10). It can thus be seen that the effect of the decrease of water content in the solvent on the coagulation of this type of substances is similar to

that of the chlorophylls and the carotenoids, i.e., as the water content in the solvent decreases (or the alcohol content increases), coagulating efficacy also seems to decrease proportionally.

Figure 10. Plot of the residual weight percentage and electrolysis time for 0.1% w/v morin at 415, 420 nm ; ▲, 50% ethanol, ■, 75% ethanol and ◆ 85% ethanolic solution (a 0.1% solution of morin in 25% ethanol cannot be prepared.)



For most natural phenolic compounds, however, probably owing to the active polyhydroxy functions of the substances, coagulation which can occur both by reaction and by adsorption tends to be more complete than that of the chlorophylls and the carotenoids, so that after some time (60 minutes in this case), coagulation is virtually complete, even in solvents with a high percentage of alcohol.

Conclusions

It has been demonstrated in a systematic manner that in the electrocoagulation in aqueous alcoholic solutions of some important plant pigments, including tannins, the decrease in the percentage of water in the solvent has some negative effects on the degree and efficiency of their coagulation compared with that observed in 100% aqueous solution. However, the effect is small in the case of tannins and some other phenolic substances. For chlorophylls and carotenoids, this retarding effect is somewhat higher, probably due to only a single mode of coagulation being in operation (viz. adsorption mode). However, even with this unfavourable effect being present, electrocoagulation is still more efficient in removing these organic matrix substances than the conventional method of solvent extraction.

Experimental Section

General

All of the tested compounds used were of standard reagent grade, and were used as received. Tannin and β -carotene were purchased from Fluka Chemica AG (Buchs, Switzerland); morin

(3,5,7,2',4'-pentahydroxyflavone) was purchased from May & Baker Ltd (Dagenham, England). Crude chlorophyll was obtained from *Spinach oleracea*. Air-dried leaves of *Solanum laciniatum* were obtained from Dr. Jiradej Manosroi, Faculty of Pharmacy, Chiangmai University. Sodium chloride (99.9%, AR grade) was purchased from Ajax Chemical Co. (Sydney, Australia). Absolute ethanol was purchased from E. Merck (Darmstadt, Germany). Acetone and ethanol (95%) were of a commercial grade. Aluminium and iron plates were purchased locally. Direct current was sustained by a GW Instek DC power supply. Absorbance was measured on a Genesys 10 spectrophotometer.

Preparation of solutions for electrocoagulation

A solution of tannin (tannic acid) or morin (0.01, 0.1, or 1.0% w/v) was prepared in aqueous ethanol (25%, 50%, 75% or 85% v/v). Beta-carotene was dissolved in 27% ethanol to give a 0.01% w/v solution. Crude chlorophyll was extracted from *Spinach oleracea* with 75 or 85% ethanol at room temperature by grinding 40 g of the dry plant material with solvent (200 mL). The filtered solution was used directly in the electrocoagulation experiments. For *Solanum laciniatum*, the powdered air-dried leaves (20 g) were refluxed for 3 hours with each aqueous alcoholic solution (25%, 50%, 75%, 85%, 200 mL). After filtration, the deep green solutions obtained were used directly in the electrocoagulation experiments.

Electrocoagulation procedures

1) Morin and tannic acid

Two aluminum plates (dimensions 15 x 4 cm) were used as electrodes. These were spaced 3 cm apart and dipped 5.5 cm deep into a magnetically-stirred aqueous solution (250 mL) of the tested compound (0.01, 0.1 or 1.0% w/v solution) in a 400 mL beaker. Sodium chloride (0.5 g) was added as an electrolyte. Direct current (0.3 A) from the DC power supplier was then passed through the solution. Every 15 minutes during a 2 hour period of electrolysis, a 4 mL sample of the solution was withdrawn, centrifuged and taken for an absorbance measurement at an appropriate wavelength (275 nm for tannin and 415-420 nm for morin). The measured absorbance was then converted into the residual weight percentage of the compound by a calibration curve obtained from a plot of the absorbance versus the concentration for each compound.

*2) Chlorophyll, β -carotene, and *Solanum laciniatum**

Aluminium or iron plates (15.0 × 3.0 cm.) were washed prior to use with acetone to remove surface grease. A pair of aluminium or iron plates 1.5 cm apart was immersed 7.0 cm deep into 200 mL of each solution in a jacketed 250-mL beaker for occasional cooling during electrolysis. The solution was agitated throughout the experiment with a magnetic stirrer (250 r.p.m.). Sodium chloride (0.2 g) was added as supporting electrolyte. Direct current (0.9 A, 16.9-31.6 V) was then passed through the solution *via* the two electrodes. At every 15-minute interval during a 2-hour period of electrolysis, a 4-mL aliquot of the solution was withdrawn and centrifuged for 10 minutes, and the absorbance of the

supernatant solution was measured at an appropriate wavelength of the absorption maximum for each plant solution (440 and 665 nm for chlorophyll, 460 nm for β -carotene and 408 and 666 nm for *Solanum laciniatum*).

Acknowledgements

Dr. Jiradej Manosroi of the Faculty of Pharmacy, Chiangmai University, is thanked for supplying the leaves of *Solanum laciniatum*. This research study is supported financially by TRF (Thailand Research Fund) in the form of Medicinal Chemistry Grant and Royal Golden Jubilee PhD Grant for K. J., and by the Commission on Higher Education PhD Grant for N. C. We also thank the Graduate School, Chiangmai University, for partly supporting K.J and N.C. financially.

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Sample Availability: Available from the authors.