CHAPTER 2

Electrocoagulation of Polyphenolic Compounds

In this study, electrocoagulation (EC) of authentic polyphenolic compounds namely anthraquinone, naphthoquinone, flavonoids and tannin was studied. These compounds containing hydroxyl groups in various numbers and at different positions. The sample solutions were separately operated in the systematic manner to investigate the degree of selectivity of EC process.

2.1 Electrocoagulation of Anthraquionones and a Naphthoquinone

2.1.1 Introduction

Quinones, notably naphthoquinones and anthraquinones, have been known as the most widely distributed natural products. The majority of them exist as coloured phenolic compounds, useful as dyes and pigments. Isolation of these substances from their natural sources normally requires the use of organic solvents before the required compound can be obtained in a reasonably pure state, either to first extract them from the raw material and then partition them between various solvent phases, or as eluents for chromatographic separation. These organic solvents are usually not only costly but also a potential toxic burden to the environment, even if treated in the proper way. [19]

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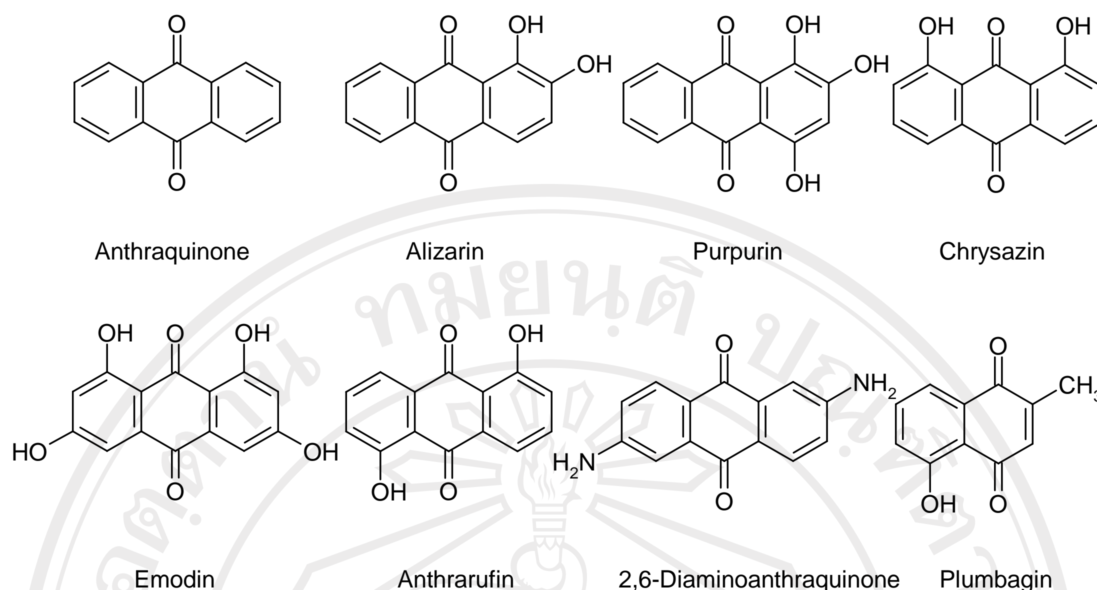

Figure 2.1 Structures of the quinones studied.

Alizarin (1, 2-dihydroxyantharquinone) is found in the roots of the plant (*Rubia tinctorum* L.), and was use as a dye in ancient times in Egypt, Persia and India. It is only rarely used in microtechnique although it has been suggested for demonstrating some metals, with which it forms coloured lakes. Recently, alizarin has been synthesized. Chemical studies on alizarin lead to its synthesis in about 1870. Now, because of its easy synthesis, artists mainly use alizarin in its synthetic form. Alizarin is one of the two main dyes, the other being purpurin, that come from the root of the madder plant. Alizarin tends to be reddish while purpurin tends to be more pinkish.

Purpurin, or 1, 2, 4-trihydroxyanthraquinone, is a naturally occurring red/yellow dye in the roots of the plant madder (with alizarin also). Purpurin is a crystalline compound that is colorless until dissolved in alkalic solutions. It is soluble in ethanol (becomes red) and is soluble in water at boiling and alkaline water (becomes yellow). It

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has the appearance of dark red needles. Purpurin is a fast dye for cotton printing and forms complexes with various metal ions.

Anthrarufin (1,5-Dihydroxyanthraquinone) and chrysazin, a 1,8 isomer, are important starting materials for alizarin and indanthrene dyes. In many cases, the two isomeric dihydroxyanthraquinones give very similar dyes, and a mixture of the two can be used directly. Emodin is found in certain plants, including rhubarb which has shown anti-inflammatory and anticancer effects. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) is a yellow crystalline substance present in the roots of *P. zeylanica*, *P. rosea*, *P. capensis* (syn. *P. auriculata* Thunb.), and *P. europea*, belonging to the family Plumbaginaceae.[20,21]

2.1.2 Experimental

2.1.2.1 Instrumental and apparatus

1) UV/VIS spectrophotometer, model Genesys 10 spectrophotometers, Thermo scientific, USA.
2) FT-IR spectrophotometer, model TENSER27; Bruker, Germany.
3) DC power supplier, model GPR-1810 HD and GPS-3030D; Good Will Instrument Co.Ltd., Taiwan.
4) Vacuum rotary evaporator, model rotavapor-R; Buchi, Switzerland.
5) Vacuum pump, model PC-1; VacUUbrand, Imed, USA.
6) Melting point apparatus, model MEL-TEMP II; Laboratoy devides Inc., USA.
7) pH meter, model pH; Precisa, Merck, Germany.
8) UV-lamp, model CN-6T; Vilber, France.
9) TLC plates (aluminium), silica gel 60 F254; Merck, Germany.
10) Aluminium plates (dimension 15 x 3.5-4 x 0.05cm)

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2.1.2.2 Chemicals

1) Anthraquinone $C_{14}H_8O_2$ (LAB); M&B Ltd., Dagenham, England.
2) Alizarin (1, 2-Dihydroxyanthraquinone) $C_{14}H_8O_4$; Fluka Chemie Sigma-Aldrich, Switzerland.
3) Anthrarufin (1, 5-Dihydroxyanthraquinnone) $C_{14}H_8O_4$; Fluka Chemie Sigma-Aldrich, UK.
4) Chrysazin (1, 8-Dihydroxyanthraquinone) $C_{14}H_8O_4$ (90-95%); ACROS organics, New Jersey, USA.
5) Purpurin (1, 2, 4-Trihydroxy-6-methylanthraquinone) $C_{14}H_8O_5$; Fluka Chemie Sigma-Aldrich, Switzerland.
6) Emodin (1, 3, 8-Trihydroxy-6-methylanthraquinone) $C_{15}H_{10}O_5$ (95%); ACROS organics, New Jersey, USA.
7) 2,6-Diaminoanthraquinone $C_{14}H_{10}N_2O_4$ (97%) ; Aldrich Sigma-Aldrich Chemie, Geramany
8) Plumbagin (5-Hydroxy-2-methyl-1,4-naphthoquinone); Sigma, Sigma-Aldrich Chemie, Germany.
9) Sodium chloride NaCl (99.9%, AR grade); Ajax Chemical Co., Sydney, Australia.
10) Ethanol (Absolute) C_2H_5OH; E. Merck, Darmstadt, Germany.
11) *n*-Butanol $C_4H_{10}O$ Analytical Reagent Grade; Fisher Scientific UK Ltd., UK.
12) Hydrochloric Acid Hydrochloric acid solution (37%); Carlo Erba Reagent Co., Ronando, MI, Italy.

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2.1.2.3 Procedure

(a) Preparation of solutions for electrocoagulation

The 0.01% (w/v) solutions (500 mL) of each of the quinone compounds, viz. anthraquinone, alizarin, chrysazin, purpurin, 2,6-diaminoanthraquinone and plumbagin were prepared in aqueous (85% v/v) ethanol. In the case of anthrarufin, its concentration in this solvent system was somewhat less than 0.01%, due to incomplete dissolution, and for emodin its concentration in the same solvent was reduced to 0.005% due to poor solubility. Sodium chloride 0.5 g (or 0.2% w/v) was added as supporting electrolyte.

The solution was divided into 3 parts; the first 50 mL was scanned for maximum absorption wavelength (λ_{max}), next 250 mL was studied in EC process and the last 200 mL for recovering experiment.

The appropriate wavelength of each sample for ec study are shown in Table 2.1

Table 2.1 The appropriate wavelengths of naphtho- and anthraquinones standard solutions (in 85% aqueous ethanol).

Standard substance	Wavelength (nm)
Anthraquinone	325
Alizarin	435
Anthrarufin	420
Chrysazin	430
Purpurin	485
Emodin	440
2,6-Diaminoanthraquinone	410
Plumbagin	420

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(b) Electrocoagulation procedure

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 in a 400-mL beaker. Direct current (0.3 A, 24-31 V) from the DC power supplier was then passed through the solution. Every 15 min 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.

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. (for calibration curve see appendix)

In the case of anthrarufin, its concentration in this solvent system is somewhat less than 0.01% w/v due to incomplete dissolution. To investigate the EC process, the changing in absorbance at 420 nm was observed.

Table 2.2 and 2.3 show the absorbance and residual weight percentage of quinone solutions during 2 hour of electrolysis.

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Table 2.2 The absorbance of quinone solutions during electrolysis.

Substance	Absorbance/ Time (min)								
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Alizarin	2.21	-0.01	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02
Chrysazin	3.90	1.28	0.43	0.22	0.19	0.18	0.22	0.25	0.34
Purpurin	2.59	0.01	0.00	0.00	0.02	0.01	0.00	0.00	0.00
Anthrarufin	0.56	0.43	0.36	0.32	0.30	0.29	0.29	0.30	0.33
Emodin	1.98	0.61	0.39	0.27	0.22	0.16	0.17	0.23	0.35
2,6-Diaminoanthraquinone	0.73	0.73	0.72	0.72	0.72	0.74	0.73	0.73	0.74
Anthraquinone	2.17	2.16	2.15	2.15	2.16	2.19	2.17	2.18	2.19
Plumbagin	2.18	1.76	1.53	1.42	1.35	1.38	1.39	1.48	1.51

Table 2.3 Residual weight percentage of quinone solutions during electrolysis.

Substance	Residual weight percentage/ Time (min)								
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Alizarin	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chrysazin	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Purpurin	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Emodin (x10^2)	0.50	0.15	0.10	0.07	0.06	0.04	0.04	0.06	0.09
2,6-Diaminoanthraquinone	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Anthraquinone	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Plumbagin	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

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(c) Compound recovery experiments

A solution (200 mL) of each of the four well-coagulated quinone compounds, viz. alizarin, chrysazin, purpurin and emodin, was placed in a 250-mL beaker. Two aluminium plates (15 x 4 cm) were used as electrodes. These were placed 3 cm apart and dipped 6.5 cm into the magnetically-stirred solution. Sodium chloride (0.4g) was added as supporting electrolyte.

Direct current (0.3 A, 22-24 V) was then passed through the solution via the two electrodes for an appropriate time as follows: 40 min for alizarin, 75 min for chrysazin and emodin, and 15 min for purpurin. The resulting mixture was filtered through a buchner funnel. The precipitate was collected and stirred in a sufficient volume of 10% hydrochloric acid solution to completely dissolve it. The acidic solution obtained was extracted with *n*-butanol (50 mL), the alcoholic solution was evaporated to dryness and the residual solid treated again with a small amount of 10% hydrochloric acid solution, then filtered, washed with water and dried to afford the recovered compound. TLC, IR, m.p., and mixed m.p. were used to confirm the integrity of each recovered quinone.

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2.1.3 Results and Discussion

In the first part of this investigation (experiment 2.1.2.3 b), a number of pure samples of representative quinones, viz. alizarin, purpurin, chrysazin, emodin, 2,6-diaminoanthraquinone, anthrarufin and plumbagin (Figure 2.1) were separately electrocoagulated using direct current and aluminium plates as electrodes in an aqueous alcoholic solution containing sodium chloride as the supporting electrolyte. The absorbance of each electrolysed sample solution was measured at an appropriate wavelength at regular intervals during electrolysis and electrocoagulation curves were obtained for each quinone as a plot of percent residual weight of the sample or absorbance versus electrocoagulation time, as shown in Figures 2.2-2.3 respectively.

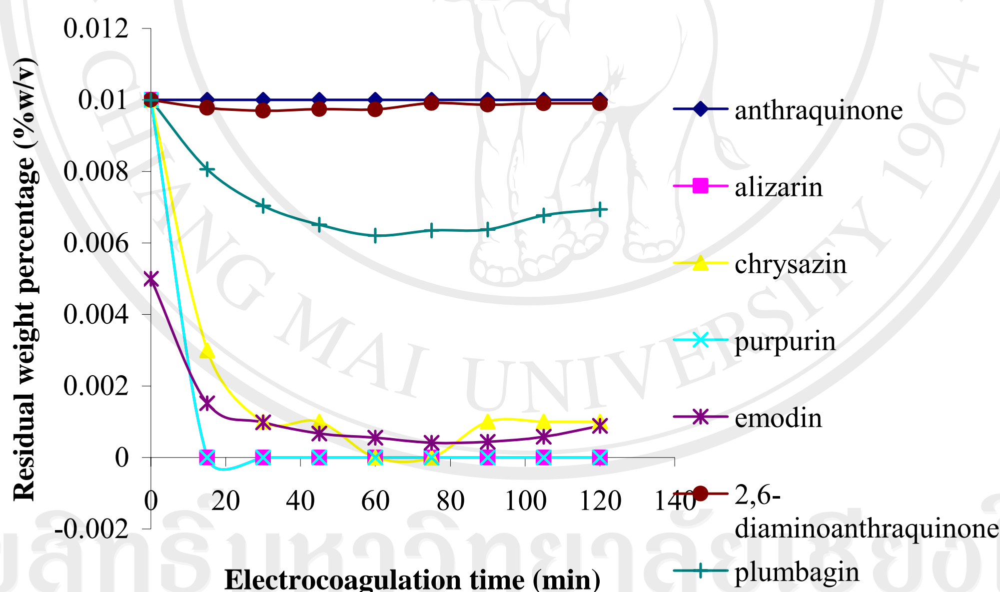

Figure 2.2 Plots of residual weight percentage (%w/v) and electrolysis time of quinone substances in 85% aqueous ethanol; ◆, antraquinone, ■, alizarin, ▲, chrysazin, ×, purpurin, ✱, emodin, ✹, 2,6-diaminoanthraquinone and +, plumbagin.

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When absorbances were plot versus electrocoagulation time the results can be shown in Figure 2.3 (all 8 quinone substances)

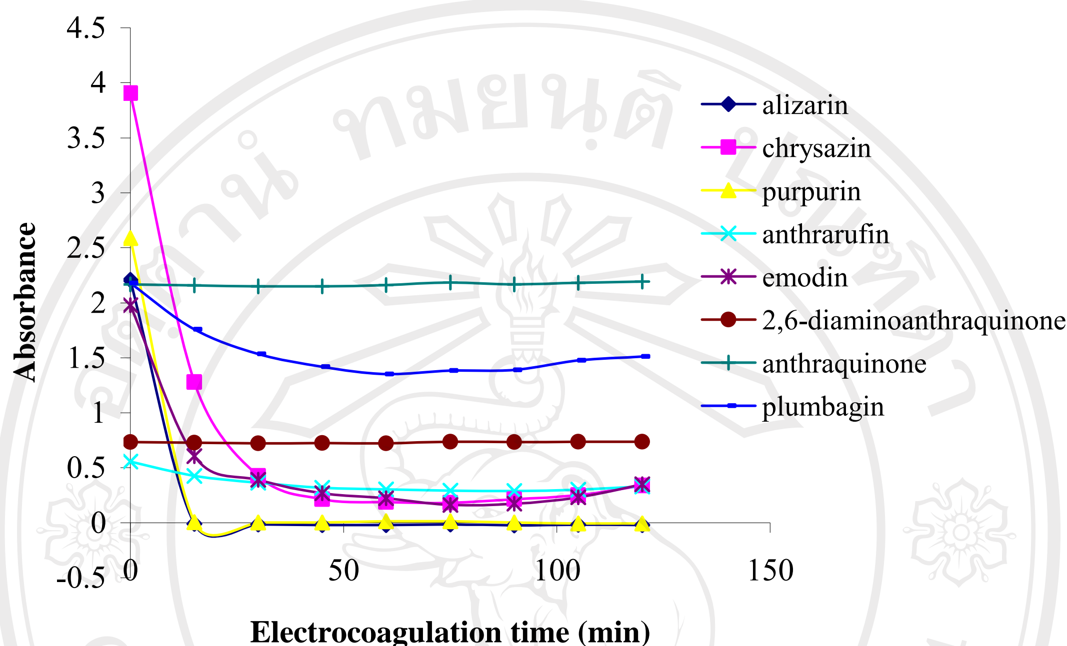

Figure 2.3 Plots of absorbance and electrolysis time of quinone substances in 85% aqueous ethanol ◆, alizarin, ■, chrysazin, ▲, purpurin, ×, anthrarufin, *, emodin, ✸, 2,6-diaminoanthraquinone, +, anthraquinone and, ●, plumbagin.

From the results above it can be seen that the quinones under study may be roughly divided into three categories. The two most rapidly and completely coagulated quinones were alizarin and purpurin, being completely coagulated within 15 min under the experimental conditions used. It can also be noted that these two compounds have adjacent phenolic hydroxyl groups (Figure 2.1). This catechol-like structure thus seems to be particularly prone to coagulation by a metal ion, probably due to intramolecular complexation.

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The second category of quinones comprised chrysazin and emodin, which were coagulated somewhat less completely and more slowly than the first two ones. In this case, it can also be seen from their structures that they have no adjacent hydroxyl groups, although they both possess two phenolic groups flanking a quinone carbonyl group (Figure 2.1). This may then be the next best functional group arrangement of a quinone for complexing with metal ions released by the electrolytic process, although exactly why this should be so remains to be explained. In any case, however, this is better than the arrangement seen in the third category, in which no hydroxyl group for anthraquinone, only one phenolic hydroxyl group flanks a carbonyl group, as seen in anthrarufin and plumbagin, no hydroxyl group but 2 amino group in the 2 and 6 position of 2,6-diaminoanthraquinone which were barely coagulated and give nearly horizontal coagulation cures (Figure 2.2 and 2.3).

Four studied quinone namely alizarin, purpurin, chrysazin and emodin, which can be coagulated by EC process very well, were recovered in experiment 2.1.2.3 (c). The percentage recovery of quinone compounds are shown in Table 2.4.

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Table 2.4 Percentage recovery of quinone compounds

Compounds	% Recovery
Anthraquinone	-
Alizarin (1,2-dihydroxy anthraquinone)	55.0
Purpurin (1,2,4-Trihydroxy-6-methylanthraquinone)	27.7
Anthrarufin (1,5-dihydroxy anthraquinone)	-
Chrysazin (1,8-dihydroxy anthraquinone)	56.0
Emodin (1,3,8-Trihydroxy-6-methylanthraquinone)	64.0
2,6-Diaminoanthraquinone	-
Plumbagin	-

The results show that more than 50% of alizarin, chrysazin and emodin can be recovered by this method. Purpurin shows the lowest percentage yield of recovery because this substance has 3 hydroxyl groups in its structure and is easy to dissolve in aqueous solution. When the solubility of substance in aqueous solution is high, it causes the problem in solvent extraction step and lead to the lower yield at the end of recovery process.

The melting point of recovered compounds were determined and compared with authentic sample (in case of purpurin and emodin the value was obtained from references) and the mixed melting point of the sample were determined. The experiment could not be done with recovered emodin because the compound was obtained in a small amount. The results are shown in Table 2.5.

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Table 2.5 Melting point of authentic sample, recovered compound and mixture of recovered-authentic substance.

Substance		Authentic sample Mp (^oC)	Recovered compound Mp (^oC)	Mix Mp (^oC)
Alizarin	1	289	290	292
	2	290	291	291
	3	289	291	291
	$\overline{X}$	289	291	291
Purpurin	1	253-256*	210-212	216-220
	2		210-212	216-218
	3		212	216-220
	$\overline{X}$		211	216-219
Chrysazin	1	193-197 *	188-192	190-196
	2		190-192	194-196
	3		188-192	190-196
	$\overline{X}$		189-192	191-196

* Melting point obtained from Fluka chemical's handbook.

The recovered compounds were dissolved in 95% ethanol then separated by TLC. The solvent system EtOAc : MeOH : H_2O = 100:17:13 v/v was used, and the sample was developed on silica gel plate. The results are as shown in Table 2.6

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Table 2.6 TLC test of studied quinones.

Sample	R_f value		Color	
	authentic	recovered	authentic	recovered
Alizarin	0.90	0.90	Orange-red	Orange-red
Purpurin	0.70	0.70	red	red
Chrysazin	0.90	0.90	yellow	yellow
emodin	0.82	0.82	yellow	yellow

IR spectroscopic data were collected and used to compare between authentic and recovered compounds. All IR spectral data obtained are shown in Figures 2.4- 2.11

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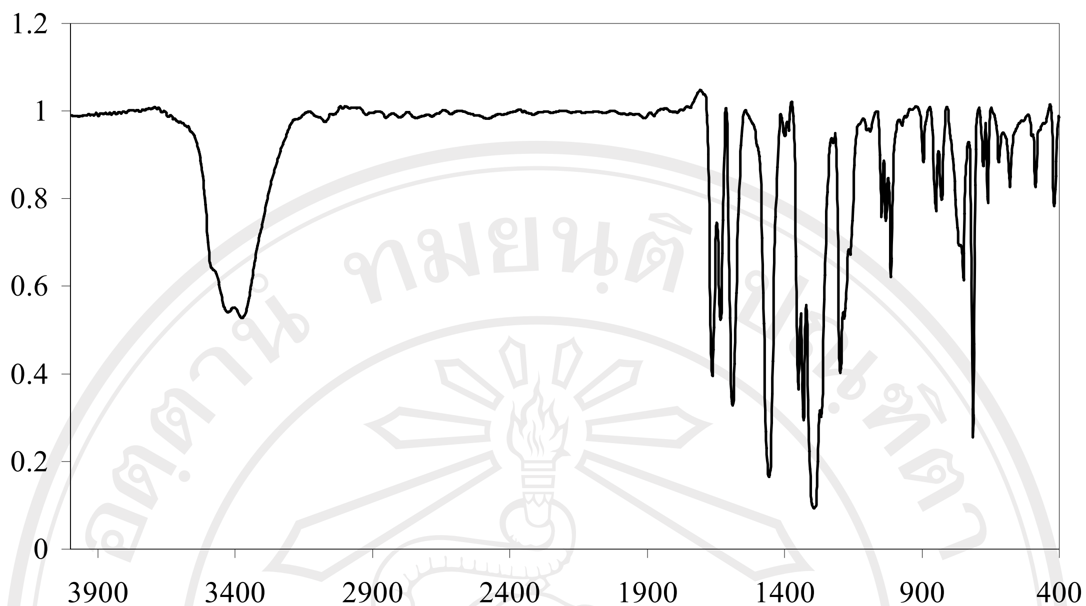

Figure 2.4 IR spectrum of authentic alizarin

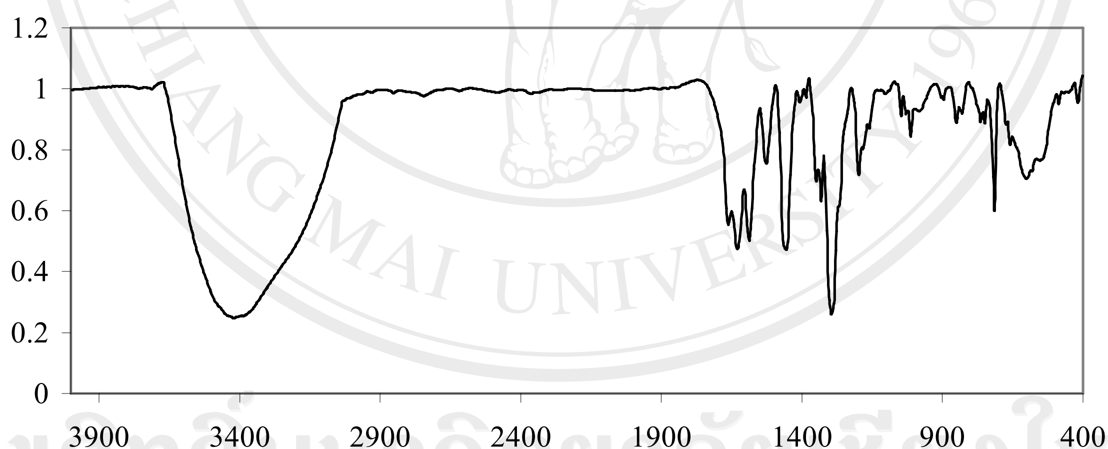

Figure 2.5 IR spectrum of recovered alizarin

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IR (KBr): υ_{max} cm^{-1} 3400 (br, OH stretching), 1700 (s, C=O streching), 1450, 1600 (s, C=C skelton for condensed system), 1675 (v.s, quinone), 770-735 (s, ortho disubstituted)

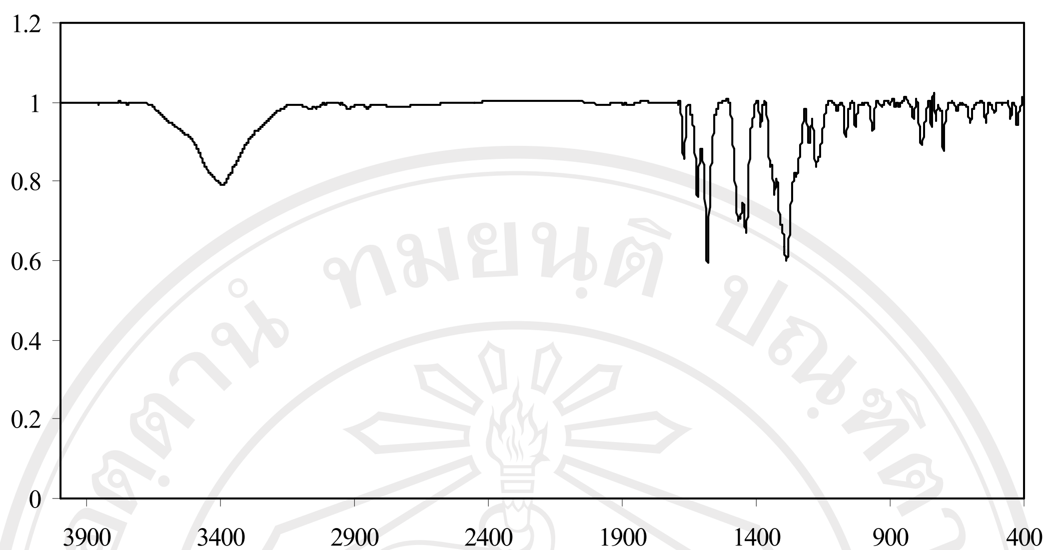

Figure 2.6 IR spectrum of authentic purpurin.

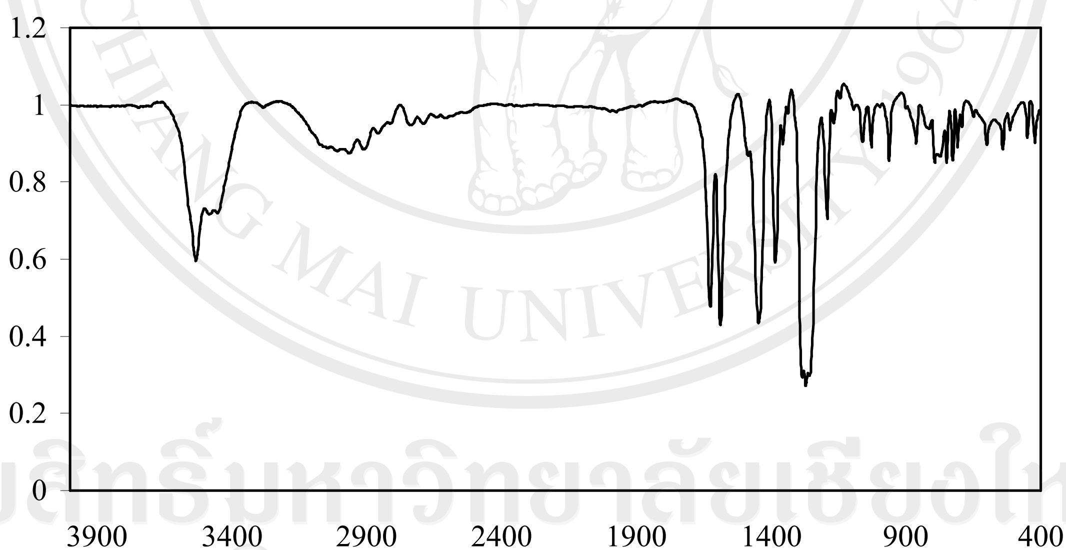

Figure 2.7 IR spectrum of recovered purpurin.

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IR (KBr): υ_{max} cm^{-1} 3400 (br, OH stretching), 1675 (v.s, quinone), 1450, 1600 (s, C=C skelton for condensed system), 885-805 (s, 1,2,4-trisubstituted)

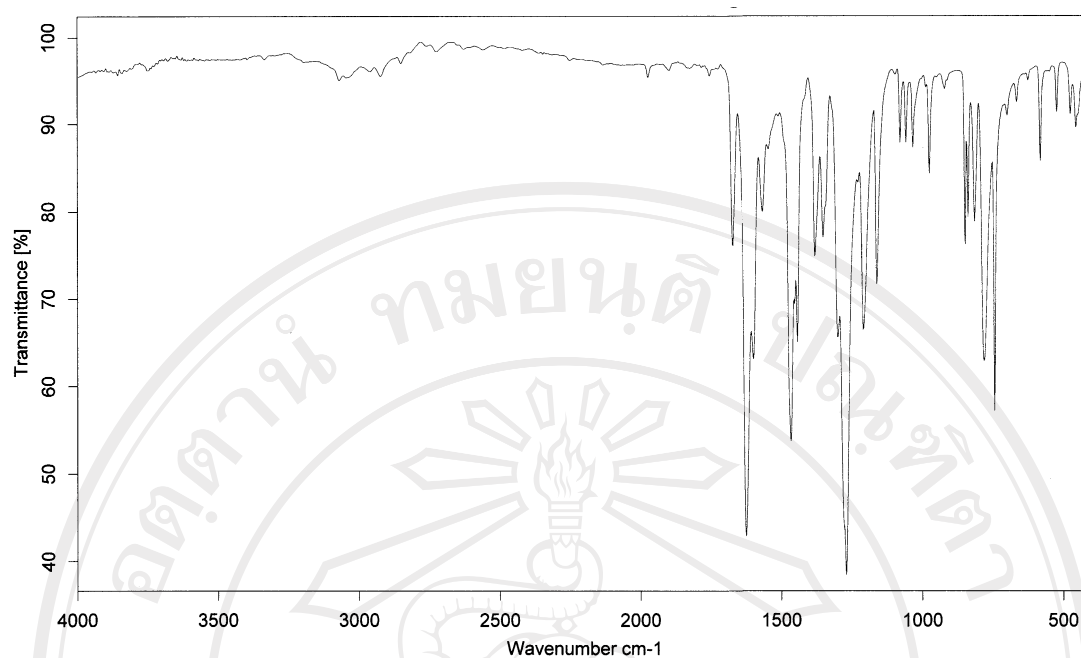

Figure 2.8 IR spectrum of authentic chrysazin.

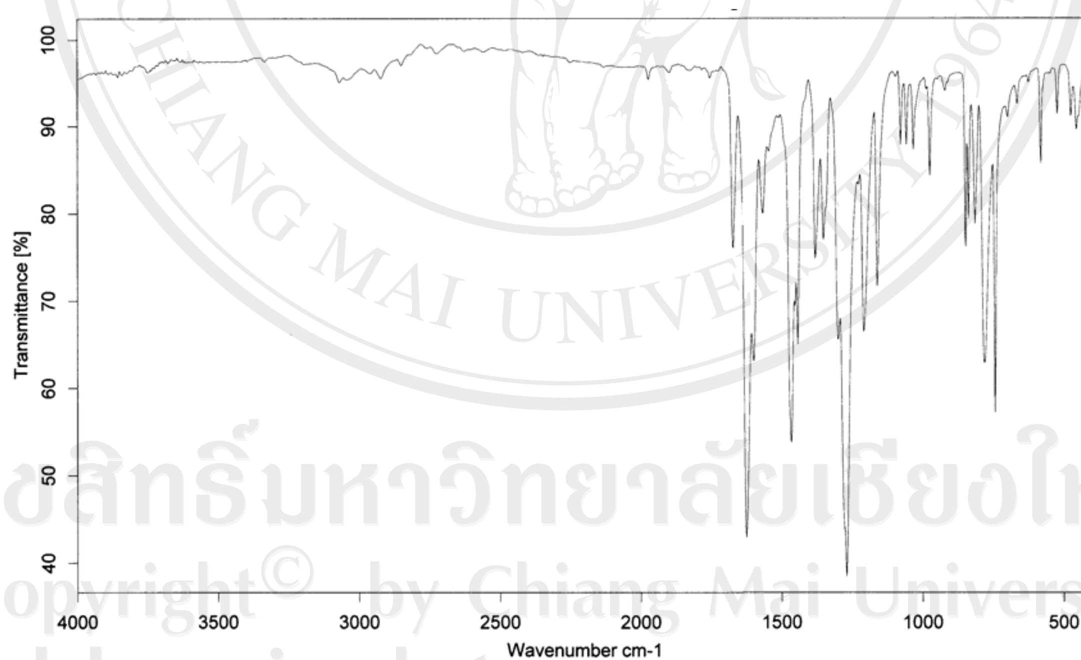

Figure 2.9 IR spectrum of recovered chrysazin.

IR (KBr): υ_{max} cm^{-1} 1675 (v.s, quinone), 1600 (s, C=C skelton for condensed system), 770-730, 710-690 (s, monosubstituted)

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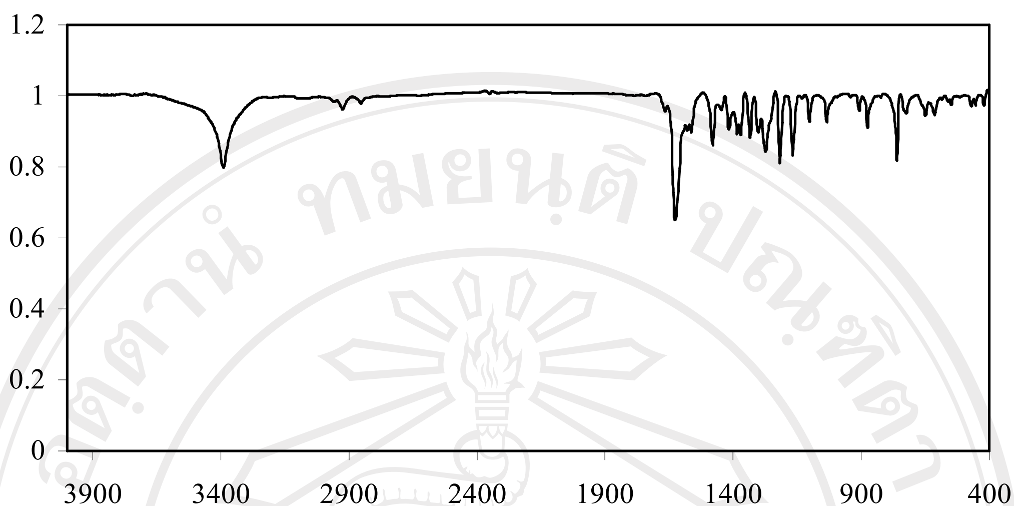

Figure 2.10 IR spectrum of authentic emodin.

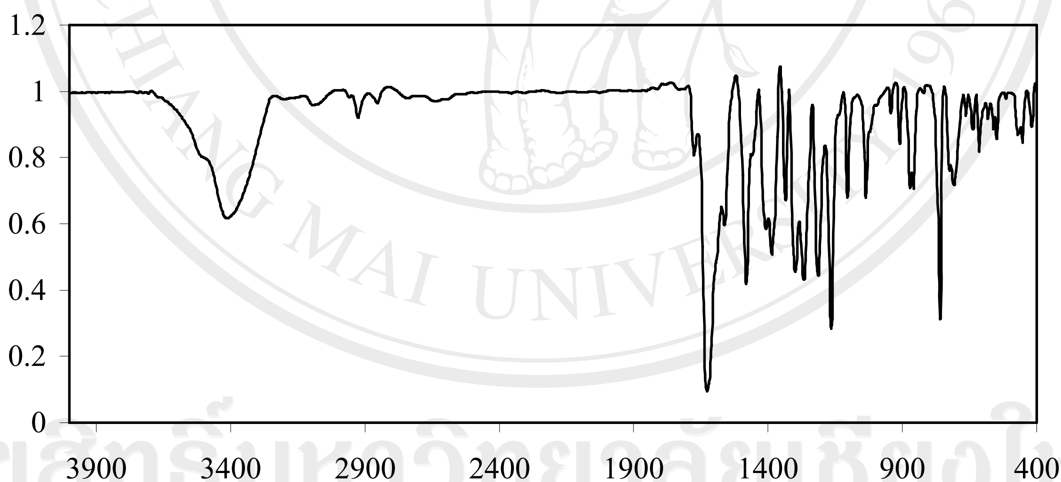

Figure 2.11 IR spectrum of recovered emodin.

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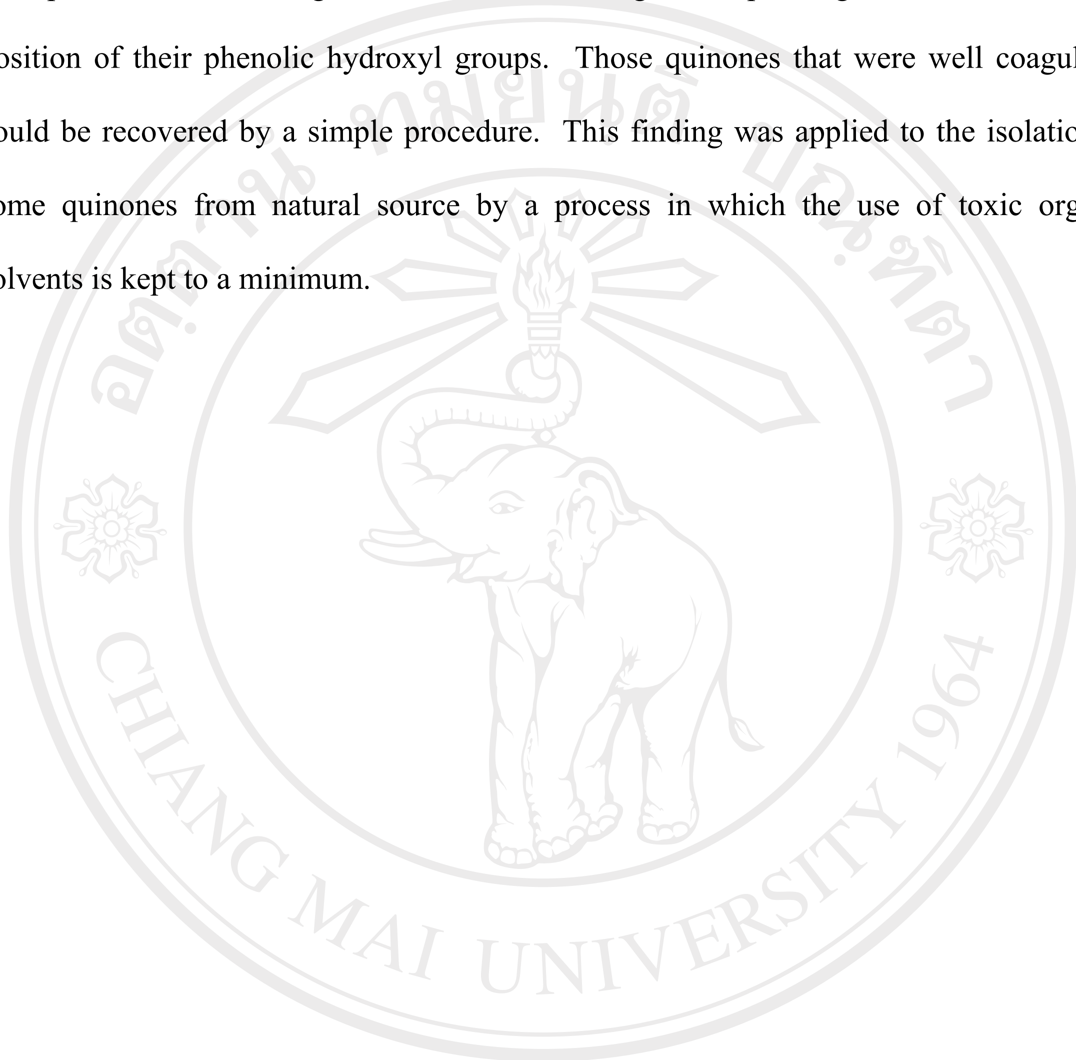
IR (KBr): υ_{max} cm^{-1} 3400 (br, OH stretching), 1675 (v.s, quinone), 1450, 1600 (s, C=C skelton for condensed system), 810-750 (s, meta disubstituted)

The results show that recovered compounds were identical to authentic samples.

2.1.4 Conclusions

It has been determined that a variety of structurally different quinones were susceptible to electrocoagulation in different degrees depending on the number and position of their phenolic hydroxyl groups. Those quinones that were well coagulated could be recovered by a simple procedure. This finding was applied to the isolation of some quinones from natural source by a process in which the use of toxic organic solvents is kept to a minimum.



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2.2 Electrocoagulation of Flavonoids

2.2.1 Introduction

Flavonoids, or bioflavonoids, are a ubiquitous group of polyphenolic substances which are present in most plants, concentrating in seeds, fruit skin or peel, bark, and flowers. The structural components common to these molecules include two benzene rings on either side of a 3-carbon ring. Multiple combinations of hydroxyl groups, sugars, oxygens, and methyl groups attached to these structures create the various classes of flavonoids: flavanols, flavanones, flavones, flavan-3-ols (catechins), anthocyanins, and isoflavones.[22]

Of the various class of the naturally occurring compounds based on the flavonoid skelton, the flavones and flavonols are collectively the most abundant groups. They are sometimes known as anthoxanthins (yellow flower pigments). The distinction between flavones and flavonols is an arbitrary one, since flavonols are simply a class of flavone in which the 3-position is substituted by a hydroxyl group.

Apigenin, a common flavone, is used primarily in research as a protein kinase inhibitor that may suppress tumor promotion and that has anti-proliferating effects on human breast cancer cells. Apigenin is also one of several active ingredients in the popular herbal remedy, chamomile. Apigenin is found naturally in many fruits and vegetables, including apples and celery. It is found in several popular spices, including basil, oregano, tarragon, cilantro, and parsley.[23]

Baicalein is a flavonoid originally isolated from the roots of *Scutellaria baicalensis* Georgi. Several different functions of baicalein have been reported.[24] Morin, present in the wood of *Morus tinctoria*., is well known for its dyeing and chelating properties and is use in analytical chemistry as a reagent for metal.

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Quercetin is a member of flavonol class. It is widely distributed in the plant kingdom in rinds and barks. Especially rich sources of quercetin include onions, red wine and green tea. This substance, contains 5 hydroxyl groups at 3, 3',4',5 and 7 position, has been shown to be antioxidant, anti-inflammatory, antiviral, anticancer and gastroprotective activities. [25]

In this experiment polyphenolic compounds based on the flavonoid skeleton were studied. Substances namely apigenin, baicalein, quercetin and morin (Figure 2.12) were prepared in 85% (v/v) ethanolic solution and operated to investigate the degree of selectivity of EC process.

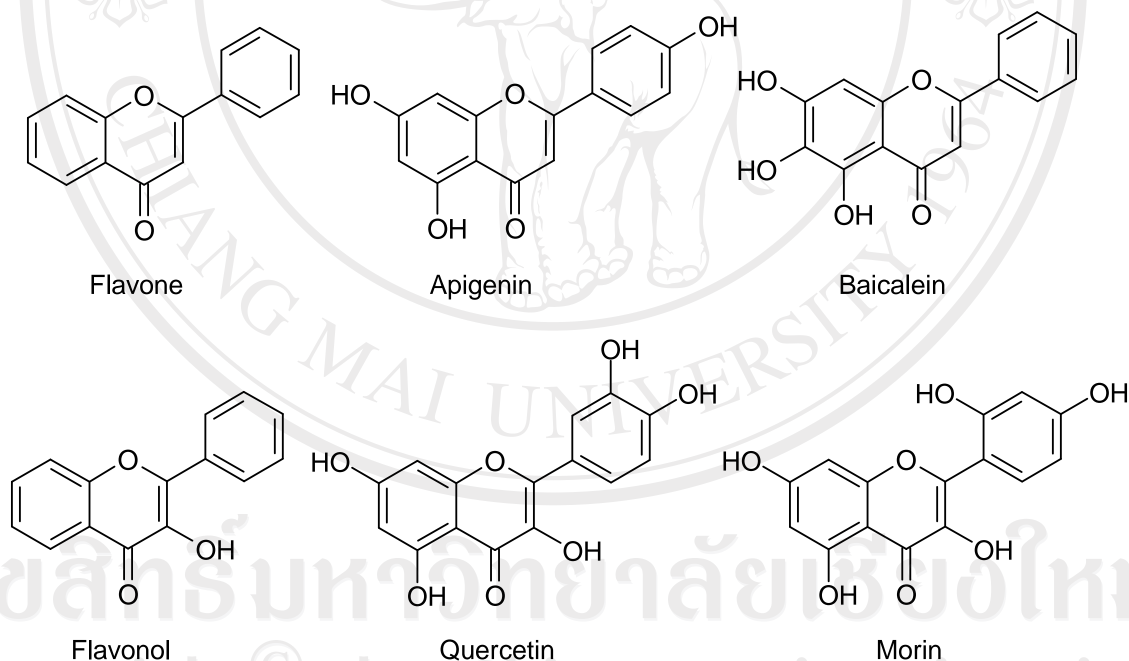

Figure 2.12 Structure of flavonoids studied.

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2.2.2 Experimental

2.2.2.1 Instrumental and apparatus

As indicated in 2.1.2.1

2.2.2.2 Chemicals

1) Morin (3,5,7,2',4'-pentahydroxyflavone) $C_{15}H_{10}O_7$; May&Baker Ltd., Dagenham, England.
2) Quercetin dihydrate (3,3',4',5,7-pentahydroxyflavone dihydrate) $C_{15}H_{10}O_7.2H_2O$; Fluka, Fluka Chemie Sigma-Aldrich, Switzerland.
3) Apigenin (4',5,7-Trihydroxyflavone) $C_{15}H_{10}O_5$; Fluka, Fluka Chemie Sigma-Aldrich, Switzerland.
4) Baicalein (5,6,7-Trihydroxyflavone) $C_{15}H_{10}O_5$; Fluka, Fluka Chemie Sigma-Aldrich, Switzerland.
5) *n*-Butanol $C_4H_{10}O$ (AR grade); Fisher Scientific UK Limited, UK
6) Hydrochloric Acid Hydrochloric acid solution (37%); Carlo Erba Reagent Co.,Ronando, MI, Italy.
7) Sodium chloride NaCl (99.9%, AR grade); Ajax Chemical Co., Sydney, Australia.
8) Ethanol (absolute) C_2H_5OH; E. Merck, Darmstadt, Germany.

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2.2.2.3 Procedure

(a) Preparation of solutions for electrocoagulation

Solutions (500 mL) of each flavonoid compound: 0.01% w/v of morin and quercetin dihydrate, 0.005% w/v of baicalein and 0.004% w/v of apigenin, were prepared in aqueous (85% v/v) ethanol. Sodium chloride 0.5 g (or 0.2% w/v) was added as supporting electrolyte.

The solution was divided into 3 parts; the first 50 mL was scanned for maximum absorption wavelength (λ_{max}), the next 250 mL was studied in EC process and the last 200 mL for recovering experiment.

The appropriate wavelength for absorbance measurement of each authentic lavonoid is as shown in Table 2.7.

Table 2.7 Appropriate wavelength for absorbance measurement of authentic flavonoids (in 85% aqueous ethanol)

Substance	Wavelength (nm)
Morin	360
Quercetin	360
Baicalein	325
Apigenin	340

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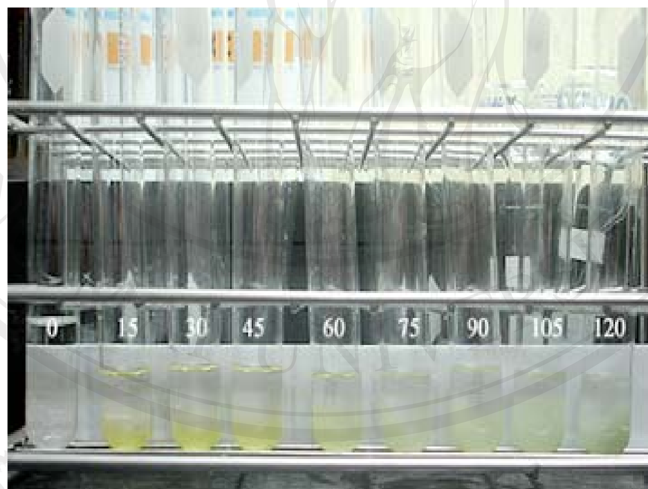
(b) Electrocoagulation procedure

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 in a 400-mL beaker. Direct current (0.3 A) from the DC power supplier was then passed through the solution. Every 15 min 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. The measured absorbance was plot versus the concentration for each compound.

Picture 2.1 shows the fractions collected during 2 hours of electrolysis of apigenin.



Picture 2.1 EC fractions of apigenin during 2 hours of electrolysis.

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Table 2.8 Absorbance of flavonoids solution during electrolysis (120 min).

Substance	Absorbance/ Time (min)								
	0	15	30	45	60	75	90	105	120
Apigenin	3.00	0.45	0.21	0.14	0.12	0.15	0.16	0.13	0.16
Baicalein	2.70	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Quercetin dihydrate	0.79	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.04
Morin	5.05	0.08	0.05	0.05	0.05	0.05	0.05	0.05	0.06

(c) Compound recovery experiments

A solution (200 mL) of each of the four well-coagulated quinone compounds, viz. alizarin, chrysazin, purpurin and emodin, was placed in a 250-mL beaker. Two aluminium plates (15 x 4 cm) were used as electrodes. These were placed 3 cm apart and dipped 6.5 cm into the magnetically-stirred solution. Sodium chloride (0.4 g) was added as supporting electrolyte.

Direct current (0.3 A) was then passed through the solution via the two electrodes for 30 min. The resulting mixture was filtered through a buchner funnel. The precipitate was collected and stirred in a sufficient volume of 10% hydrochloric acid solution to completely dissolve it. The acidic solution obtained was extracted with *n*-butanol (50 mL), the alcoholic solution was evaporated to dryness and the residual solid treated again with a small amount of 10% Hydrochloric acid solution, then filtered, washed with water and dried to afford the recovered compound. TLC, IR, m.p., and mixed m.p. were used to confirm the integrity of each recovered compound.

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2.2.3 Results and discussion

The four flavonoid substances namely morin, quercetin dihydrate, baicalein and apigenin were separately electrocoagulated in an aqueous alcoholic solution containing sodium chloride as supporting electrolyte. The absorbance of each electrolysed sample solution was measured at an appropriate wavelength at regular intervals during electrolysis and electrocoagulation curves were obtained for each flavonoid as a plot of absorbance of the sample versus electrocoagulation time, as shown in Figures 2.13.

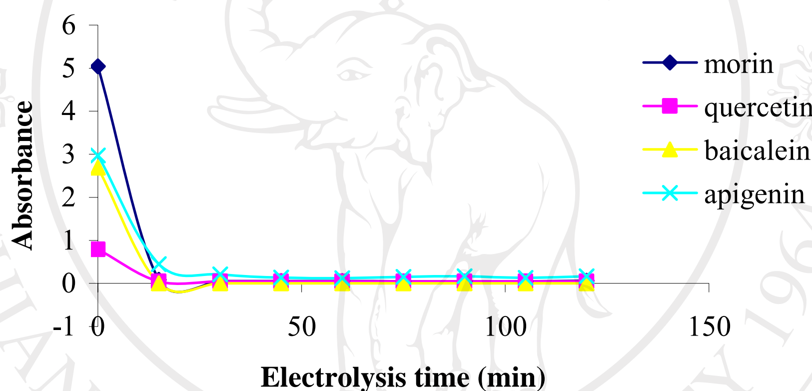

Figure 2.13 Plots of absorbance and electrolysis time of flavonoids in 85% aqueous ethanol. ◆, morin, ■, quercetin, ▲, baicalein, ×, apigenin.

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Based on the previous study (electrolysis of quinones; section 2.1), it was expected that baicalein should be coagulated in the shortest time because of the three adjacent hydroxyl groups on A ring of its structure. Quercetin dihydrate, with two adjacent hydroxyl groups on B ring and two hydroxyl groups located on both sides of carbonyl carbon of C ring, should be the next one which can be coagulated in a short time. Morin with two hydroxyl groups flanking carbonyl carbon on C ring and apigenin which has three separated hydroxyl groups on its structure should be unaffected by

electrolysis. However, the results showed that, all studied flavonoids could be coagulated completely within 15 min or there was no selectivity for electrolysis of flavonoids under the experimental conditions used.

The amounts of the recovery for each substance obtained by extracting the acid solution of the coagulum with *n*-butanol are shown in Table 2.9. In this case, because of the low starting concentration of apigenin, its recovery could not be obtained.

Table 2.9 Percentage recovery of flavonoid compounds

Substance	Percentage recovery
Morin	64
Quercetin dehydrate	61
Baicalein	34
Apigenin	-

The melting point determinations of morin are as show in Table 2.10, while quercetin dihydrate and baicalein were decomposed.

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Table 2.10 Melting point of authentic morin, recovered morin and mix of recovered-authentic substances.

	Authentic sample Mp (^oC)	Recovered compound Mp (^oC)	Mixed Mp (^oC)
1	360	266-270	266-270
2		265-268	266-268
3		264-268	264-268
$\overline{X}$		265-269	265-269

The recovered compounds were dissolved in 95% ethanol then separated by TLC. The solvent system EtOAc : MeOH : H_2O = 100 : 17 : 13 v/v was used; compounds were developed on a silica gel plate with 5 cm of solvent front. The results of TLC test are as shown in Table 2.11

Table 2.11 TLC test of studied flavonoids.

Sample	Rf value		Colour	
	authentic	recovered	authentic	recovered
Morin	0.54	0.56	yellow	yellow
Quercetin dihydrate	0.86	0.86	yellow	yellow
Baicalein	0.72	0.72	yellow	yellow

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The IR spectra of authentic and recovered compounds of morin, quercetin dihydrate and baicalein were studied. All IR spectra obtained are shown in Figures 2.14-2.19

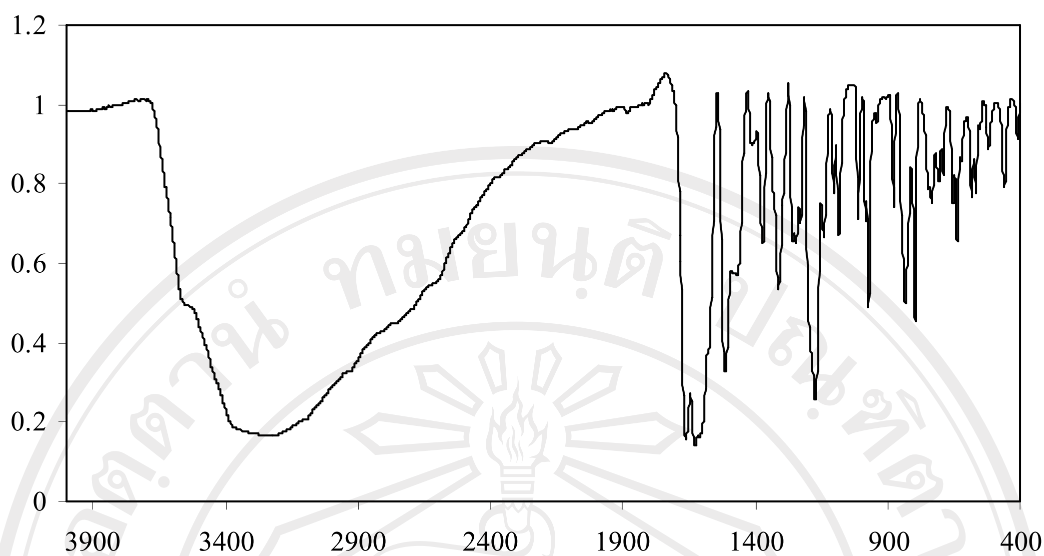

Figure 2.14 IR spectrum of authentic morin.

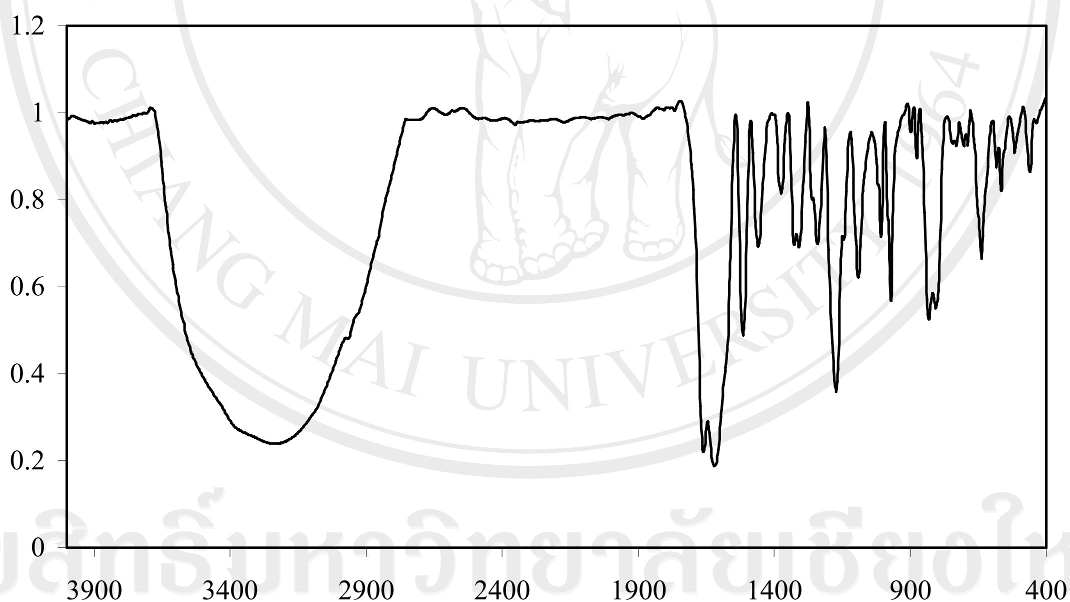

Figure 2.15 IR spectrum of recovered morin.

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IR (KBr): υ_{max} cm^{-1} 3300 (br, OH stretching), 1700 (s, C=O streching), 1160 (s, C-O stretching), 810-750 (v.s, metadisubstituted), 725-680 (m $\rightarrow$ s, metadisubsituted)

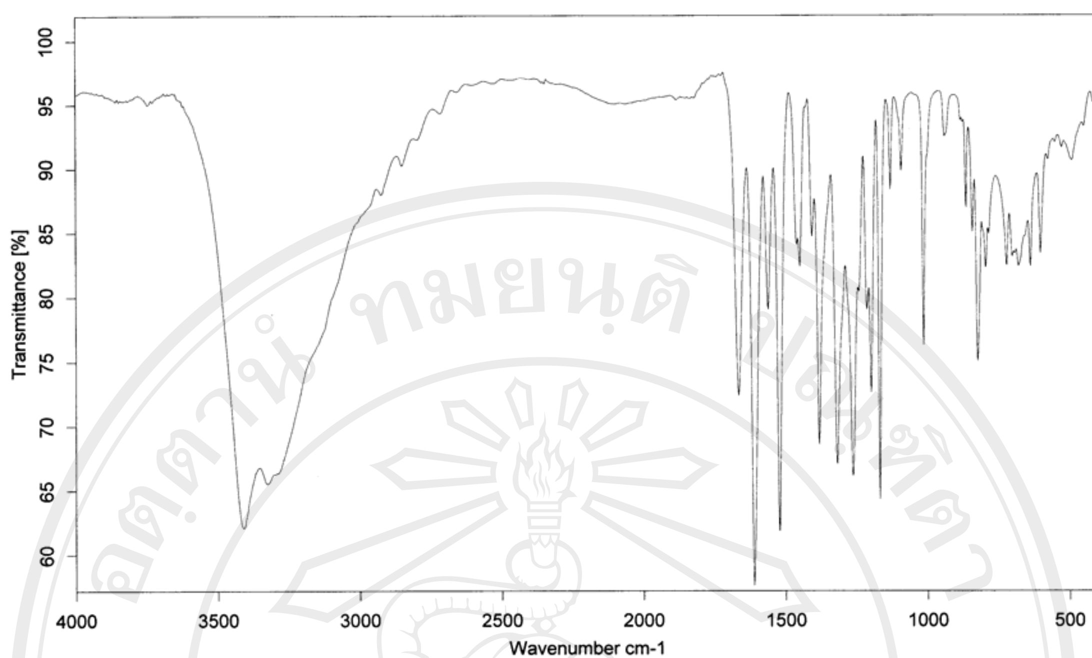

Figure 2.16 IR spectrum of authentic quercetin dihydrate.

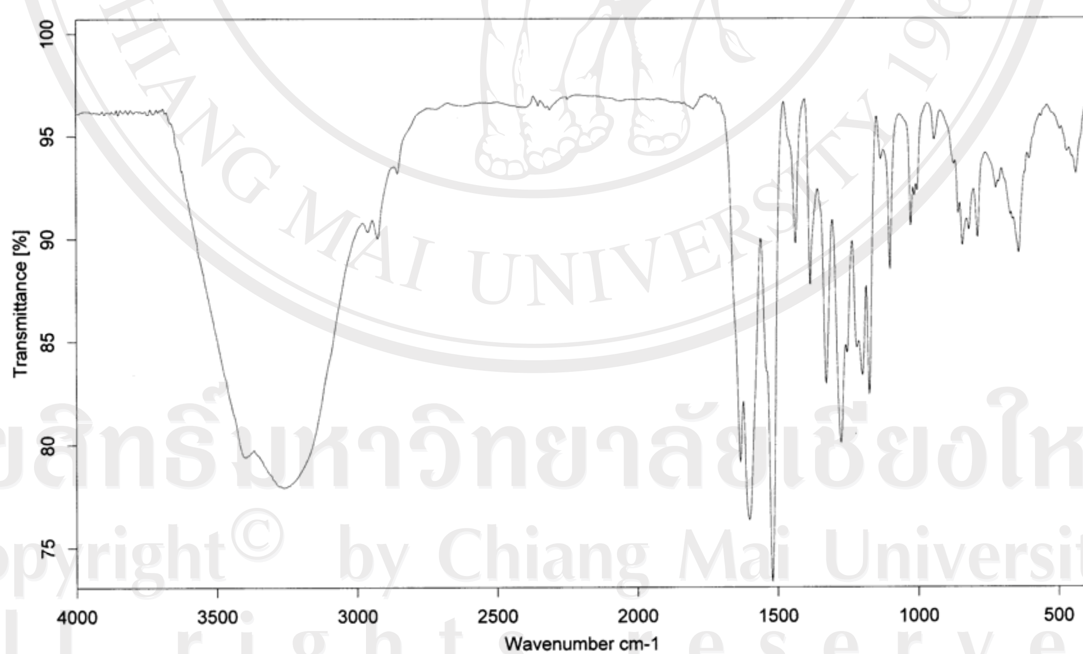

Figure 2.17 IR spectrum of recovered quercetin dihydrate.

IR (KBr): υ_{max} cm^{-1} 3300 (br, OH stretching), 1600 (s, C=O streching), 1160 (s, C-O stretching), 770-735 (v.s, orthodisubstituted)

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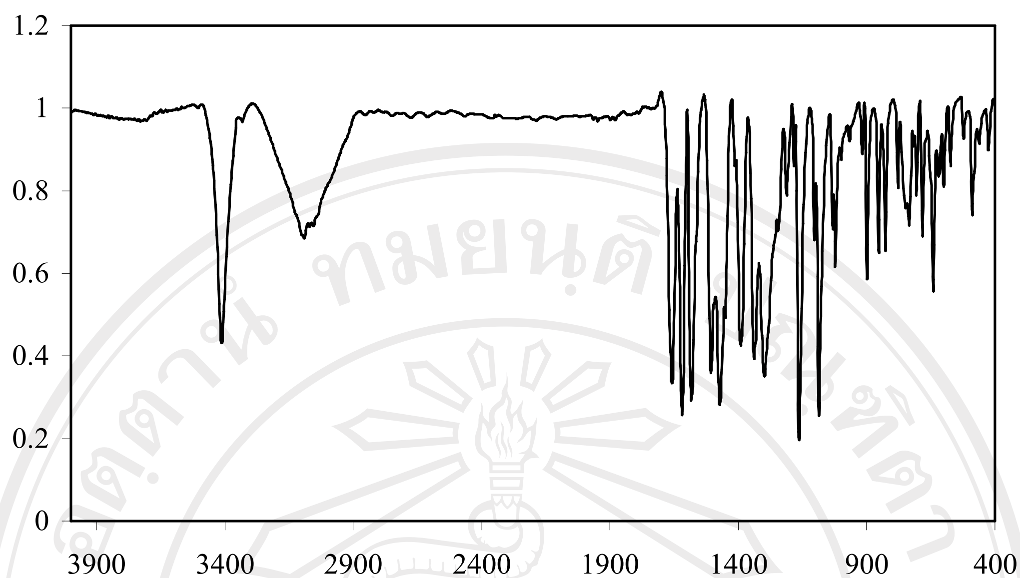

Figure 2.18 IR spectrum of authentic baicalein.

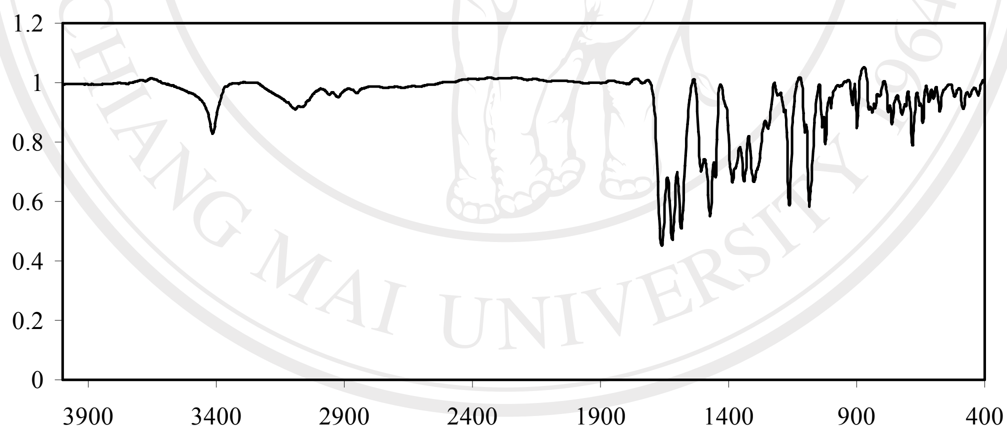

Figure 2.19 IR spectrum of recovered baicalein.

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IR (KBr): υ_{max} cm^{-1} 1600 (s, C=O streching), 1160 (s, C-O stretching), 780-760, 745-705 (s, 1,2,3-trisubstituted)

The results show that the recovered compounds were somewhat identical to the authentic samples.

2.2.4 Conclusion

There are no selectivity of electrolysis of four flavonoids; apigenin, baicalein, quercetin and morin under the studied condition.

2.3 Electrocoagulation of tannin and morin; the solvent effects.

2.3.1 Introduction

Electrocoagulation has also been used as a purification step in the isolation of a few natural products from crude plant extracts. 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 and the extraction of phenolic compounds from the bark of *Lithocarpus elegans.*, 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, a study on the effects of solvent in the electrocoagulation of some selected organic substances and plant extracts was undertaken. In this section, a model study of the system in which ethanol with varying amounts of water is used as solvent was carried out.

Tannin is one of an important class of plant pigments. Normally, tannins are regarded as little more than intractable mixtures with unfavorable biological activities and it is generally preferable to remove them along with the pigments.

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2.3.2 Experimental

2.3.2.1 Instrumental and apparatus

As indicated in 2.1.2.1

2.3.2.2 Chemicals

1) Tannic acid; Fluka Chemica AG, Buchs, Switzerland.
2) Morin (3,5,7,2',4'-pentahydroxyflavone); May & Baker Ltd., Dagenham, England.

2.3.2.3 Procedure

a) 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).

b) Electrocoagulation procedure

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 min 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.

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2.3.3 Results and discussion

The absorbance of tannin and morin (0.01%, 0.1% and 1% w/v) at defined times during electrolysis was measured as shown in Tables 2.12-2.14. The residual weight percentage of compound after calibrating with standard calibration curve is shown in Tables 2.15-2.17 and Figures 2.27-2.32 respectively.

Table 2.12 The absorbance of tannin (at 275 nm) and morin (at 360 nm) solutions (0.01% w/v) during electrolysis.

Substance	Solvent	Absorbance/Time (min)								
		0	15	30	45	60	75	90	105	120
Tannin	25% EtOH	1.99[a]	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.01
	50% EtOH	2.03 [a]	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
	75% EtOH	2.10 [a]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Morin	25% EtOH	1.56 [a]	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01
	50% EtOH	2.42 [a]	0.08	0.03	0.01	0.01	0.02	0.01	0.02	0.02
	75% EtOH	2.61 [a]	0.01	0.01	-0.02	-0.01	-0.02	-0.02	-0.02	-0.02

Note: The solution was appropriately diluted for calibrating with standard calibration curve before UV measuring, a = dilution factor 2, b = dilution factor 8.3, c = dilution factor 10, d = dilution factor 20, e = dilution factor 50, f = dilution factor 100, g = dilution factor 200

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Table 2.13 The absorbance of tannin (at 275 nm) and morin (at 415-420 nm) solutions (0.1% w/v) during electrolysis.

Substance	Solvent	Absorbance/Time (min)								
		0	15	30	45	60	75	90	105	120
Tannin	25% EtOH	1.99 [d]	0.09	0.09	0.09	0.07	0.11	0.10	0.10	0.07
	50% EtOH	2.03 [d]	0.12	0.10	0.10	0.13	0.13	0.12	0.20	0.10
	75% EtOH	2.41 [d]	0.74	0.05	0.05	0.06	0.05	0.07	0.05	0.06
	85% EtOH	2.17 [d]	2.93	0.81	0.08	0.05	0.04	0.04	0.05	0.06
Morin	25% EtOH	1.19 [d]	0.35 [d]	1.30	0.65	0.45	0.35	0.29	0.30	0.26
	50% EtOH	2.33 [d]	0.35 [d]	1.30	0.65	0.45	0.35	0.29	0.30	0.26
	75% EtOH	2.86 [d]	2.36 [d]	0.58 [c]	0.76	0.48	0.28	0.12	0.14	0.21
	85% EtOH	2.76 [d]	2.73 [d]	2.57 [d]	1.13 [d]	0.16	0.07	0.08	0.07	0.03

Note: The solution was appropriately diluted for calibrating with standard calibration curve before UV measuring, a = dilution factor 2, b = dilution factor 8.3, c = dilution factor 10, d = dilution factor 20, e = dilution factor 50, f = dilution factor 100, g = dilution factor 200

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Table 2.14 The absorbance of tannin (1% w/v, at 275 nm) during electrolysis.

Substance	Solvent	Absorbance/Time (min)								
		0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Tannin	25% EtOH	2.05^{g}	1.83^{g}	1.15^{g}	0.96^{f}	0.60^{f}	0.49^{c}	1.30	0.87	0.94
	50% EtOH	2.26^{g}	2.05^{g}	1.34^{g}	0.99^{f}	0.49^{e}	2.02	1.38	0.96	0.83
	75% EtOH	2.95^{g}	1.88^{g}	2.60^{f}	1.01^{f}	0.38^{e}	0.67^{b}	0.34^{b}	0.95	0.78
	85% EtOH	2.16^{g}	1.98^{g}	1.55^{g}	1.76^{f}	0.66^{f}	0.83^{c}	0.37^{c}	2.18	1.18

Note: The solution was appropriately diluted for calibrating with standard calibration curve before UV measuring, a = dilution factor 2, b = dilution factor 8.3, c = dilution factor 10, d = dilution factor 20, e = dilution factor 50, f = dilution factor 100, g = dilution factor 200

Table 2.15 Residual weight percentage of tannin and morin solutions (0.01% w/v) during electrolysis.

Substance	Solvent	Residual weight percentage/Time (min)								
		0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Tannin	25% EtOH	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	50% EtOH	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	75% EtOH	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Morin	25% EtOH	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	50% EtOH	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	75% EtOH	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

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Table 2.16 Residual weight percentage of tannin and morin solutions (0.1% w/v) during electrolysis.

Substance	Solvent	Residual weight percentage/Time (min)								
		0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Tannin	25% EtOH	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	50% EtOH	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	75% EtOH	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	85% EtOH	0.10	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Morin	25% EtOH	0.08	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	50% EtOH	0.10	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	75% EtOH	0.10	0.08	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	85% EtOH	0.10	0.08	0.08	0.04	0.00	0.00	0.00	0.00	0.00

Table 2.17 Residual weight percentage of tannin solutions (1% w/v) during electrolysis.

Substance	Solvent	Residual weight percentage/Time (min)								
		0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Tannin	25% EtOH	1.00	0.80	0.60	0.20	0.10	0.01	0.00	0.00	0.00
	50% EtOH	1.00	0.80	0.60	0.20	0.05	0.00	0.00	0.00	0.00
	75% EtOH	1.00	0.60	0.50	0.20	0.05	0.01	0.01	0.00	0.00
	85% EtOH	1.00	1.00	0.80	0.40	0.20	0.02	0.01	0.01	0.00

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In a 100% aqueous medium, it has been shown that tannins can be very efficiently coagulated and removed by electrolysis. In this study, an attempt was made 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. The experiments showed this to be the case. Thus, for example, at a concentration of 0.01% tannin in up to 75% and 0.1% tannin in up to 85% ethanol, a 250-mL solution was almost completely de-tannized within 15 min, using aluminium as electrodes and a current of 0.3 A (Figures 2.20 and 2.21). At a concentration of 1.0% tannin, the complete detannization time was increased to 80 min (Figure 2.22).

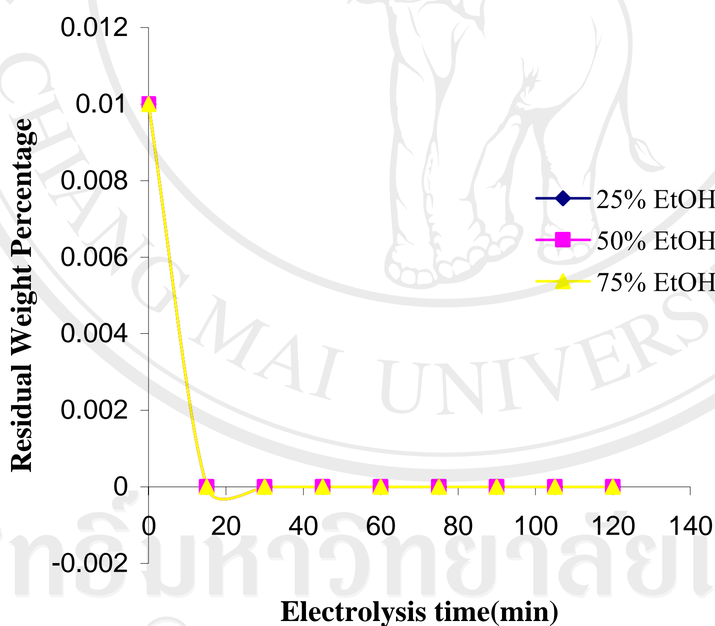

Figure 2.20 Plots of the residual weight percentage and electrolysis time for 0.01% w/v tannin at 275 nm; ◆, 25% ethanol, ■, 50% ethanol and ▲, 75% ethanol

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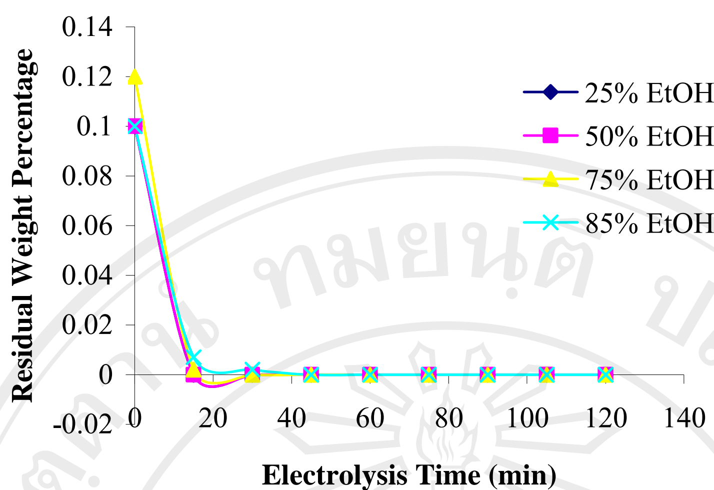

Figure 2.21 Plots of the residual weight percentage and electrolysis time for 0.1% w/v tannin at 275 nm; ◆, 25% ethanol, ■, 50% ethanol, ▲, 75% ethanol and ×, 85% ethanol.

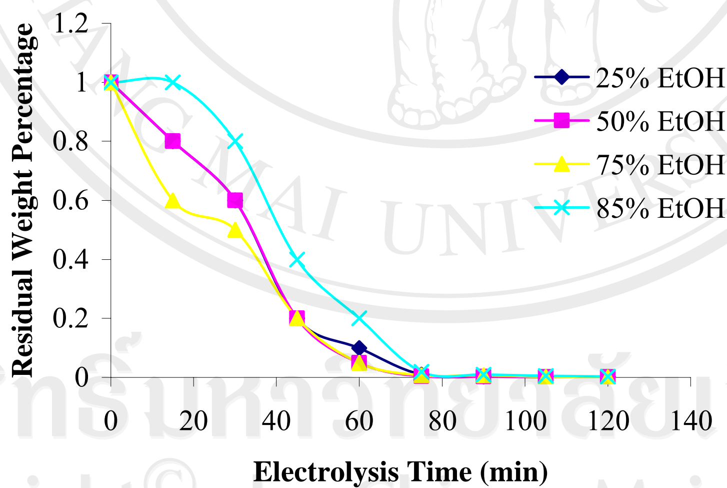

Figure 2.22 Plots of the residual weight percentage and electrolysis time for 1% w/v tannin at 275 nm; ◆, 25% ethanol, ■, 50% ethanol, ▲, 75% ethanol and ×, 85% ethanol.

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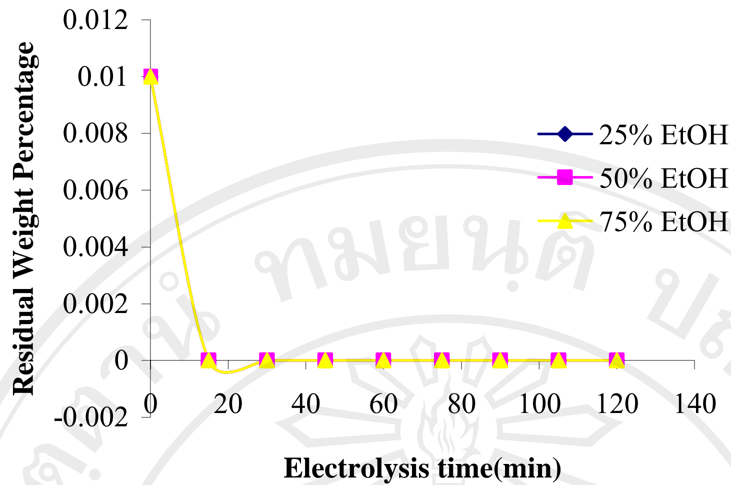

Figure 2.23 Plots of the residual weight percentage and electrolysis time for 0.01% w/v morin at 360 nm; ◆, 25% ethanol, ■, 50% ethanol and ▲, 75% ethanol

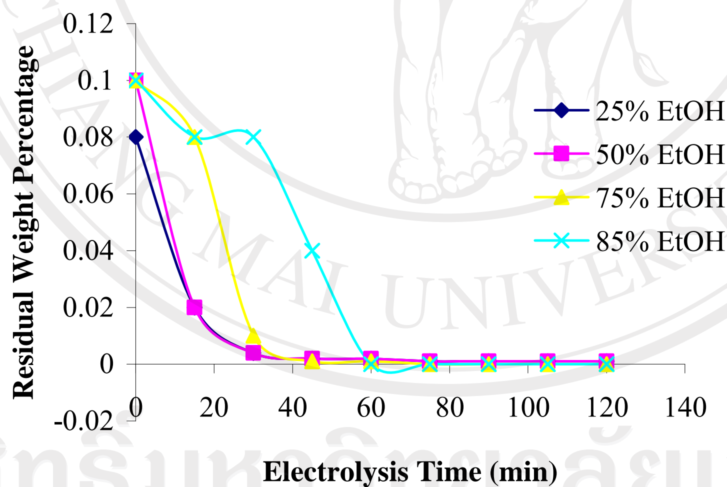

Figure 2.24 Plots of the residual weight percentage and electrolysis time for 0.1% w/v morin at 360, 415 nm; ◆, 25% ethanol, ■, 50% ethanol, ▲, 75% ethanol and ×, 85% ethanol.

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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 2.23 and 2.24). It can thus be seen that as the water content in the solvent decreases (or the alcohol content increases), coagulating efficacy also seems to decrease proportionally.

2.3.4 Conclusions

It has been demonstrated in a systematic manner that in the electrocoagulation in aqueous alcoholic solutions of some important plant pigments, tannins and morin, the decrease in the percentage of water in the solvent has some small negative effects on the degree and efficiency of their coagulation compared with that observed in 100% aqueous solution. However, even with this unfavorable effect being present, electrocoagulation is still more efficient in removing these organic matrix substances than the conventional method of solvent extraction.

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