An-Najah Univ. J. Res., Vol. 13 (1999) 57-66

Adsorptive Cathodic Stripping Voltammetric Studies of Emodin

دراسات كهروكيماوية على مركب الايمودين (emodin) بطريقة الانتزاع الكاثودي الامتصاصي

Mohammad A. Hannoun

محمد حنون

Faculty of Pharmacy An- Najah National University- Nablus West Bank (Palestine)

Received: (22/9/1998), Accepted: (31/8/1999)

Abstract

A differential pulse cathodic stripping voltammetric (DP-CSV) method for determination of emodin in Britton- Robinson buffer (pH2) at a hanging mercury drop electrode (HMDE) is described. The method is based on measuring the reductive peak height at –0.25V vs Ag/AgCl reference electrode. The linear relationship between the peak current and emodin concentration allowed the voltammetric determination of emodin over a wide concentration range $1.0 \times 10^{-7} - 2.5 \times 10^{-6}$ M, with a relative standard deviation of 3.3% (10 determinations at 1×10^{-7}M). Adsorption of emodin at HMDE enabled a detection limit of 2×10^{-10}M after 3 min accumulation at 0.0V.

The applicability of the suggested method was found to be suitable for the determination of emodin in roots, stems and leaves of Rumex cyprius plant.

Keyword: Emodin, determination, adsorptive stripping voltammetry.

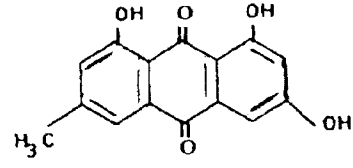
يتضمن هذا البحث وصف لطريقة تعيين مركب الايمودين emodin باستخدام خاصية الانتزاع المهبطي للمركب بعد تجميعه بالامدصاص على سطح قطرة الزئبق المعلقة (HMDE) التي تشكل قطباً سالباً في الخلية الكهروكيماوية. وسط التفاعل المستخدم في هذه الطريقة المحلول المنظم المسمى بريتون وروبنسون عند درجة حموضة تساوي ٢ حيث يتم قياس الجهد مقابل القطب المعروف وهو Ag/AgCl. ولقد وجد ان العلاقة بين قيمة التيار الناتج من عملية الاختزال وتركيز مادة الايمودين هي علاقة خطية حيث تمتد هذه العلاقة ا لمستقيمة ما بين ١ × ١٠$^{-٧}$ وحتى ٢,٥ × ١٠$^{-٦}$ مول/لتر ويكون الانجراف المعياري النسبي ٣,٣%. لقد كان الحد الأدنى من تركيز المادة التي يمكن تقديره هو ٢ × ١٠$^{-١٠}$ مول/لتر وذلك عد تجميعه عن جهد صفر فولت لمدة ٣ دقائق. ان هذه الطريقة قد طبقت بنجاح لتعيين تركيز الايمودين في جذور وسيقان وأوراق النبته الطبية المسماه Rumex Cyprius والتي تسمى في فلسطين (الحميض).

Introduction

Emodin (1,3,8- trihydroxy- 6 – methylanthraquinone), I, is a naturally occuring anthraquinone formed in older Ramnus frangula L., Ceseara sagraela, R. cyprius and other Polygonaceae. It was reported (1-3) that emodin has multivarious effect in pharmacology. Locally in this area (West Bank), R. cyprius grows as a plant and is used in folk medicine for curing some human skin diseases (4, 5).

Emodin was determined in its natural sources by several methods as thin layer chromatography (TLC) (6-8) HPLC (9) , capillary electrophoresis (10,11) and high performance liquid chromatography (HPLC) (12-15). Al-Nuri etal (9) suggested a spectrophorometric method for determination of emodin by measuring the absorbance at 250 nm.

Pal and Jana (16,17) used emodin as a spectrophotometric reagent for



trace determination of Be (II), Ca (II) and Mg (II) using conventional and first derivative spectrophotometry.

To the best of our knowledge, no voltammetric work is yet published on the determination of emodin. The ethanolic extract of emodin from R. cyprius plant was used in our work here to determine emodin concentration in the plant and compared with a reference solution of pure emodin analysed voltammetrically at the same conditions .

Experimental

Apparatus and reagents

The stripping voltammeter EG&G, model 264B coupled with 303A stand was used. Differential pulse cathodic stripping voltammograms (DP- CSV) were obtained using x-y recorder, model RE0150 (Princeton Applied Research). The three- electrode system was composed of a hanging mercury

drop electrode (HMDE), a Ag/AgCl reference electode and a platinum wire auxiliary electrode. A pulse amplitude of 50 mV was selected to obtain DP-CSV at intervals of 0.5 seconds and a scan rate of 10 mVs^{-1}. The pH measurements were obtained with a Hanna HI 1230 PH/ reference elecrode and a Hanna HI 8424 pH meter. Doubly distilled water was used to prepare all the solutions and standards.

All chemicals were of BDH, and emodin, tech 90 +% (molecular weight = 270.24g/mole) was purchased from Aldrich Chemical Company. The R. cyprius plant from which emodin was extracted in ethanol was collected from Nablus local area in the West Bank. The leaves,stems and roots of this plant were dried in the shadow before the extraction procedure after which it was determined voltammetrically. Emodin solution in ethanol seemed to be stable for at least several weeks at room temperature.

Procedure

To obtain an adsorptive CSV for emodin (differential pulse mode), 10 ml of Britton Robinson (BR) buffer of the selected pH (pH2) were placed in the voltammetric cell. The purging with pure (99.999%) nitrogen was initially accomplished for 8 minutes, with stirring. Between measurements, the purging and stirring were done for 0.5 minute only. After the formation at a new HMDE, an accumulation of 60s at 0.0 V was carried on. An automated scanning from 0 to – 1.2 V was usually made at the end of the accumulation, with 15sec- equilibration time selection. A digital micropipet was used to insert sample volumes accurately to obtain successive measurements.

Results and Discussion

Reduction Peaks of Emodin

The effect of pH on the adsorptive differential pulse stripping voltammetric peak of emodin was investigated; the solution exhibited one cathodic peak in the pH range1.0- 10.5. The adsorptive stripping response of emodin is strongly dependent on the pH of the solution. A sharp increase in the peak response was observed at pH range 1.0-2.0. The height of this peak

decreases gradually and becomes broad as the pH increases (Fig. 1), and disappeares at pH > 10.5.

The peak potential shifted towards more negative values as the pH increased. This behaviour clearly shows that protons participate directly in the reaction process. From these data, the optimum pH (2.0) for the determination of emodin was chosen.

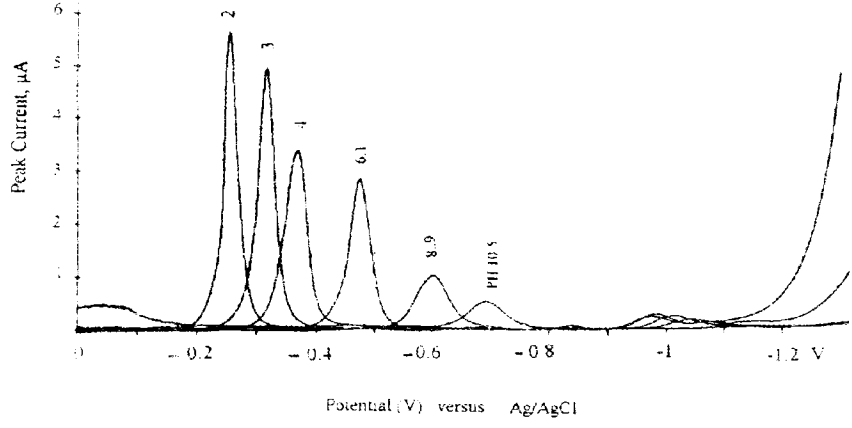


Fig (1) Effect of pH of BR buffers on the DP- CSV peak current of $5x10^{-6}$M emodin
Eacc.= 0V, $t_{acc.}$ = 60 s, scan rate $10mVs^{-1}$ other conditions as mentioned in the procedure.

Effect of accumulation potential

The effect of accumulation potential on the peak current of 2×10^{-6}M emodin is illustrated in Table (1). The scanning was carried always from 0 to -1.0 V, and to achieve this, an equilibration time of 15 sec was utilised to switch any accumulation potential back to 0V. The peak potential ranged from – 0.22 to – 0.24V, and the optimum accumulation potential was selected as 0V which gave 1.6 µA current at -0.24 V.

Table (1): Effect of accumulation Potential on the DP- CSV peak current of 2 x 10^{-6}M emodin in BR buffer, pH2. t_{acc} = 60s and scan rate = 10 mVs^{-1} (HMDE).

Eacc., V	Peak current ,
+0.1	1.4
0.0	1.8
-0.2	1.3
-0.4	1.2
-0.6	0.9
-0.8	0.6
-1.0	0.3

Effect of accumulation time

The accumualtion time effect on the peak current of 5 x 10^{-7} M and 2 x 10^{-6} M emodin is illustrated in Fig (2a). The peak potential for the lower concentration was - 0.22V and for the other one it ranged from – 0.22V (short accumulation times) to – 0.25V (long accumulation times). The linearity of peak current with accumulation time is better for the lower concentration as expected due to lower surface coverage (19). The voltammograms of this concentration are shown in Fig (2b). For longer accumulation times (more than 180sec.), the peak current decreases, perhaps as a result of a desorption effect from multilayers formed at the electrode surface.

Scan rate effect and cyclic voltammetry:

Using DC mode, the scan rate effect on the peak current and peak potential of 5 x 10^{-6}M emodin was studied as shown in Table (2). At slow scan rates, a linearity of peak current with scan rate is observed, indicating adsorption behaviour. Peak currents at faster scan rates are less than expected from linearity, possibly due to slow kinetics of the reduction process. With increasing scan rate, a negative shift of the cathodic peak current is obsorved which indicates a degree of irreversibility of the electrode processs (20).

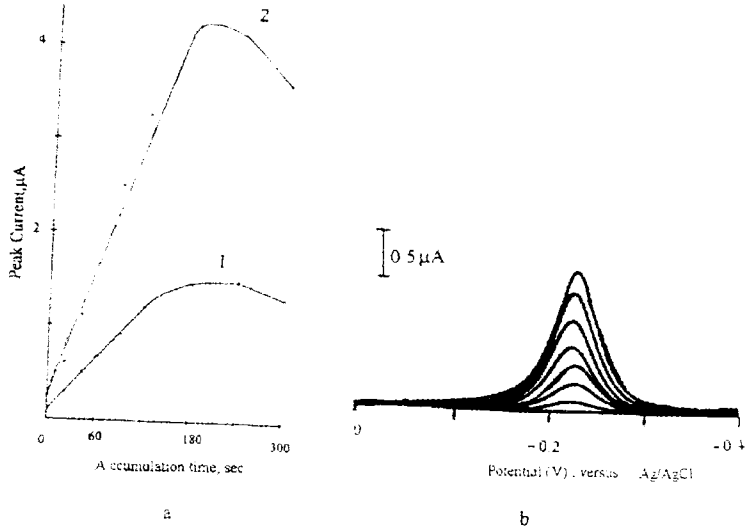


Fig 2a: Effect of accumulation time on the DP- CSV peak current of (1) 5 x 10^{-7}M (2) 2x 10^{-6}M emodin in BR buffer, PH2.

Fig 2b: Voltammograms of 5 x 10^{-7}M emodin accumulated for 0,20, 40, 60, 90, 120 and 180s. in the respective order. The zero current of the blank is also shown. Eacc = 0V.

Table (2): Effect of scan rate on the reductive peak current of emodin (5 x 10^{-6}M) in BR buffer, pH2; t_{acc}= 60s at 0V, scanning from 0 to – 1V.

Scan rate mVs^{-1}	Peak current,
10	0.2
20	0.4
50	0.8
100	1.1
200	1.9
500	2.5
1000	2.2

Three repetitive cyclic voltammograms of 5 x 10^{-6}M emodin at 50 mVs^{-1} are shown in Fig (3a). No anodic peak is observed, which indicates an irreversible behaviour of the reduction of emodin. Fig (3b) shows a small anodic peak at about –0.28V when the scan rate was increaed to 1000 mVs^{-1}. This anodic peak is observed only at such a fast scan rate as time is not enough for the reduced species to escape away from the elecrode surface, so the remaining molecules of it are oxidised in the anodic scan. The anodic shoulder at about 0V is usually obsorved in BR buffers, and is suggested to be due to buffer constituents.

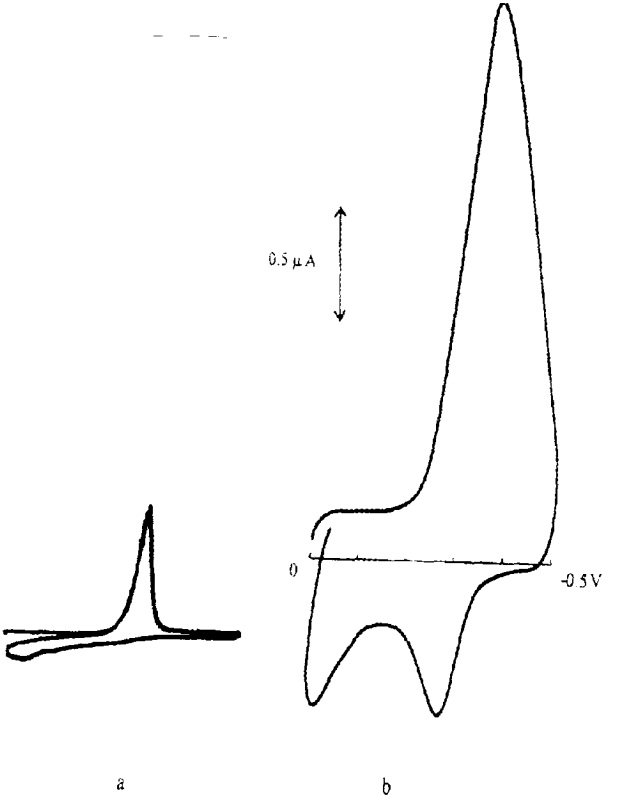


Fig (3): a) repetitive cyclic voltammpgrams (3 cycles) of 5×10^{-6} M emodin after accumulated for 60 sec. At OV. Scan rate = 50mVs^{-1}, b) as in a but scan rate = 1000mVs-1 (1 cycle).

Effect of concentration

The effect of emodin concentration on its DP - CSV Peak current was studied, using the optimum conditions. The detection limit was about 2 x 10^{-10} M after accumulation for 3 minutes at 0V. Longer accumulation times were not attempted as the peak current decreases after 3 minutes accumulation.

The peak current at 1 x 10^{-9} M level (3 minutes accumulation) was equal to 0.25 μA.

As shorter accumulation times gives better linearity for a calibration graph, one minute were repeated 3 times for each point.

At At 8×10^{-7} M level, the average peak current was 0.90 μA and RSD = 3.3% (10 measurements). The linear range extends from 1×10^{-7} to 2.5×10^{-6} M emodin (Fig 4). At higher concentrations (up to 1 x 10^{-5}M) the negative deviation from linearity was observed.

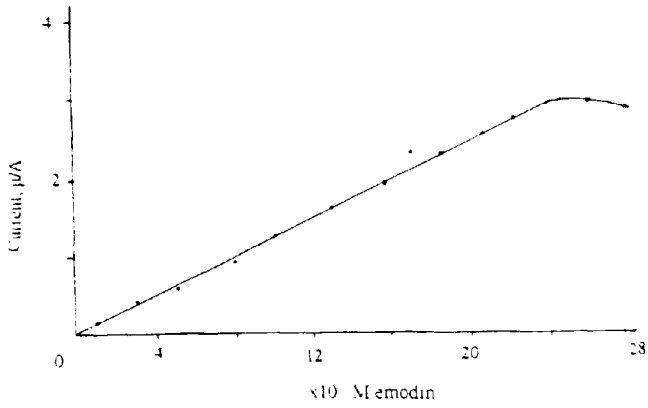


Fig (4) Calibration graph of emodin, after accumulated 60 s. at 0V for other conditions: in the "procedure" part.

Determination of emodin in the plant extracts

To extract emodin from the plant, 25g of each of the dried leaves, stems and roots were extracted in about 250ml ethanol (96%) by heating, and the rotary evaporator was used to decrease the volume of each extract to 100 ml.

The calibration graph of emodin was utilised to determine it in the exracts of leaves, stems and roots of R. cyprius plant. Fig (5) shows three peak currents for emodin in leaves, stems and roots of the analysed plant. Refering to the calibration graph which was made at the same conditions, this plant was concluded to have emodin in its parts as follows:

leaves	0.05 %
stems	0.02%
roots	0.25%

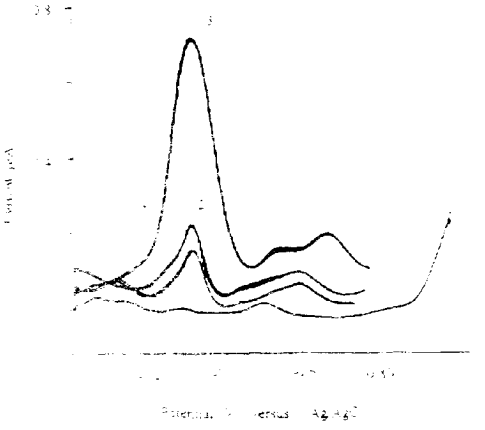


Fig (5): DP- CSV voltammograms of emodin in the ethanolic plant extract (1) 0.005g leaves (2) 0.005g stems (3) 0.005g roots, added consequently to a 10 ml BR buffer pH2 voltammetric cell. Conditions : same as the calibration graph. Measurements were made in duplicates.

References

1. Renee. J.G. and Jeffery. B.H. *Phytochemistry* **37** (1989). 19.
2. Evans. W.C. " Trease and Evan's Pharmacagnosy". Bailliere Trindall. London. (1989).
3. Vestal. P.A. " Ethnobotany of the Ramah Navajo". The Museum. Cambridge. (1952).
4. shtayeh M.S.A. " Medicinal Plants of the West Bank". Department of Biological Sciences. An- Najah National University. Nablus. west Bank (unpublishes work).
5. Palevitch. P.D. and Yaniv. z. "Medicinal Plants of the Hollyland". Tammuz Ltd.. Tel-aviv. (1991).
6. Xiao. S.X.; Peng. J.F. and Huang. N.J. . *Yaown- Fenxi- zazhi* **16(2),** (1996). 125(Ch)
7. kiridena. w.. Poole. S.k.; Miller. k.G. and Pool. C.F.J. *Planar chromatogr. mod- TLC.*, Nov- Dec. **8(6)** (1995). 416.
8. Jiao. Q. and Yu. R. *Yaowu - Fenxi- Zazhi.,* **11(6)** (1991). 326(Ch)
9. Al- Nuri. M. Nidal Za'tar; Abu- Eid. M.. Hannoun. M.; Al Jondi. w.. Hussein. A. and shtayeh. M. *Spectroscopy letter.*, **29(8)** (1996). 1539-1543.
10. Sheu. S.J. and Lu. C.F.J.. *High- Resolut - Chromatogr.,* Apr. **18(4)** (1995) . 269 .
11. Shen. S.J. and Chen. H.R. *Anal- Chim.- Acta.,* 20 Jun **309 (1-3) (1995), 361.**
12. Djozan. D. and Assadi.Y. *Talanta,* Jun.**42(6),** (1995). 861.
13. Sheu. S.J. and Lu. C.F.J. - *Chromatogr., - A,* 9 Jun **704(2),** (1995). 518
14. Toth. Z.A.; Raetikainen. O.; Naaranlahti. T. and Auriola. S.J.; *Chromatogr.*, 5 Feb., **630 (1-2)** (1993). 423 (Ch).
15. Lou. W. and Jiang. P., *Yaowu- Fenxi- Zazhi,* Sep. **9(5)** (1989) . 259 (Ch) .
16. Pal. T. and Jana. N.R. *Analyst (London), **118(10)7,*** (1993). 1337
17. Pal. T. and Jana. N.R. *Talanta,* **40(10),** (1993) , 1519 .
18. Moreia. J.C.; Miller. R.D. and Fogg. A.G.. *Anal. Proc.*, **28,** (1991).
19. Wanag. J. and Bard. A.J. *Electroanal. Chem.*, **16**. (1989) . 1
20. Baizer. M.M. and Lund. H. "Organic Electrochemistry". Marcel Dekker. Inc.. 2nd edn.. New York (1993). p 83.