Voltammetric reduction of Ellagic acid nanocomposite Using Carbon nanotube -Modified Glassy Carbon Electrode

*Mohammed Zidan[1], Samer Al-Ali[2], Mohamed Ahwidi [1] , Asmaa Ali [1]

[1]Chemistry Department, Sebha University, Sebha Libya

[2]Faculty of Pharmacy, Isra University, Jordan

*Corresponding Author: moh.zidan1@sebhau.edu.ly

Abstract A new ellagic acid nanocomposite (EAN) based on zinc-layered hydroxide (ZLH) intercalated with an ellagic acid (EA) was synthesized through direct reaction of ellagic acid at 0.01 and 0 .025 Mol/L concentrations with commercially available zinc oxide. The basal spacing of the pure phase nanocomposite was 10.4 Å.""The release of the ellagic acid from the nanocomposite was found to be sustained, and the maximum amount of ellagic acid released is almost 98% of the total at pH 4.0 phosphate buffer saline solution (PBS). A very sensitive electrochemical sensor has been developed by modification of glassy carbon electrode (GCE) with carbon nanotubes (MWCNT). The electrochemical behaviour of ellagic acid was studied at MWCNT/GCE using cyclic voltammetry . A well defined reduction peak was observed in 0.1 M KCl electrolyte solution by solid phase voltammetry. Under the CV techniques, it appears that the catalytic effect of the modification of MWCNT/GCE show higher current response as compared to bare GCE. Peak potential was observed to shift slightly to less positive value by about 220 mV and current was significantly enhanced by about 2.3 folds.

Keywords: ellagic acid nanocomposite (EAN); Modified electrode GC; Cyclic Voltammetry (CV).

تحميل الجهد كهربى الى اقطاب النانوكربون كقطب كاشف لمكشف عن الية سير تفاعل اختزال لحمض الالجيك اسيد

*محمد زيدان1 و سامر حسن الحسين2 و محمد مسعود اىويدى1 و أسماء عمى شحات1

1 قسم الكيمياء ، كمية العموم ، جامعو سبيا

2 كمية الصيدلية ، جامعو اسراء ، عمان االردن

*للمراسلة: moh.zidan1@sebhau.edu.ly

الملخص تم تصنيع مركب نانوكومبوسيت حمض إلالجيك جديد عمى أساس ىيدروكسيد الزنك الطبقات مقحم مع حمض االيالجيك إسيد ومن خالل التفاعل المباشر لمحمض االيالجيك عند 0.01 و 0.025 مول / لتر تركيزات مع أكسيد الزنك المتاحة تجاريا. كان المباعدة القاعدية لممركب النانوي الطور النقي 10.4 Å ". وقد أن إطالق حمض اإللجيك من المركب النانوي يكون مستمرا، وأقصى قدر من حمض اإللجيك الذي تم إصداره يكاد يكون 98٪ من المجموع في ف فوسفات 4.0 العازلة . وقد تم تطوير جياز استشعار الكيروكيميائية حساس جدا عن طريق تعديل القطب الكربون الزجاجي)غمس(مع أنابيب الكربون النانوية. وقد درس السموك الكيروكيميائي لمحمض االيمجيك بالغمس في المركب النانوى باستخدام فولتامتري دوري. لوحظت ذروة تخفيض محددة جيدا في محمول إلكتروليت M 0.1 بوكل بواسطة فولتامتري المرحمة الصمبة. في إطار تقنيات السيرة الذاتية، يبدو أن التأثير التحفيزي لتعديل غمس مركب النانوى تظير استجابة أعمى الحالية بالمقارنة بدون مركب النانوى . وقد لوحظ أن ذروة القدرة عمى التحول قميال إلى قيمة أقل إيجابية بنحو 220 فولت، وتم تعزيز التيار بشكل ممحوظ بنحو 2.3 أضعاف.

الكلمات المفتاحية: قطب الكربون المعالج، حمض الاسكوبيك اسيد، محفز الحديدي المنغنيز .

Introduction

Ellagic acid is a dimeric derivative of gallic acid (Scheme 1). It is found in numerous fruits and vegetables including strawberries, raspberries, pomegranates and walnuts. There, it is usually present in ellagitannin as esters of diphenic acid analogue with glucose [1]. The content of ellagic acid is preserved in the food products, such as jams and juices. Its daily intake is estimated to 90 mg [2]. Because of antioxidative and antiviral properties [3] it can be used as a food additive [4]. For its determination in foodstuff the high-performance liquid chromatography with diode array detector [4 - 6] or mass spectrometer [7 9] is used. The separation is achieved by RP C18 column. Limits of quantification are 50 ng/g in fruits [4] and 1.9 µg/L in wines [5]. Voltammetic techniques were used for electrochemical determination of free and total ellagic acid in some fruits and juices [12]. Lately, many articles have described the preparation of ZLH as a starting material followed by intercalation of the anion. However, to the best of our knowledge, little work has been published on the use of ZnO as a starting material to intercalate drug actives.35,36 Therefore, the main objective of this work was to explore the potential use of ZnO

as a starting material for the intercalation of EA for the formation of a new EA–ZLH nanohybrid (EAN). The resulting nanohybrid was then used as a controlled release formulation of drug active EA. A cytotoxicity study of nanohybrids was also carried
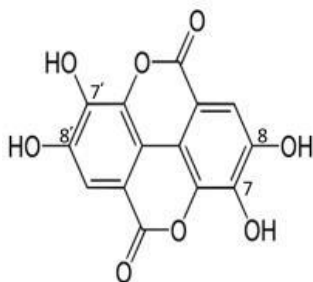
Fig. 1. Ellagic acid structure

2. Experimental
2.1. Instrumentation and electroanalytical analysis methods

Electrochemical workstations of Bioanalytical System Inc. USA: Model BAS 50W with potentiostat driven by electroanalytical measuring softwares were connected to the computer to perform cyclic voltammetry (CV). An Ag/AgCl (3M NaCl) electrode and platinum wire were used as a reference and counter electrodes respectively. The working electrode used in this study was a 3 mm diameter glassy carbon (GC). Unless otherwise stated, the voltammetric experiments were carried out at 25 ± 2oC using 0.1 M KCl as supporting electrolyte. Solutions were degassed with nitrogen for ten minutes prior to recording the voltammogram

2.2. Reagents

Hydrated ellagic acid (97% purity, molecular weight 302.19 g/mol), sodium hydroxide and phosphate-buffered saline solution purchased from Sigma-Aldrich (St Louis, MO) and used as received. The pure commercial ZnO of ACS reagent was used, purchased from Fisher Scientific (Waltham, MA). Dimethyl sulfoxide (DMSO) was purchased from Ajax Finechem (Sydney, Australia) with 0.1% water content used as solvent. Deionized water was used in all the experiments. Unless otherwise specified, the supporting electrolyte was 0.1 M KCl in aqueous media at room temperature. All solutions were deaerated with oxygen-free nitrogen gas for 15 minutes prior to making the measurement.

2.3 Procedures
2.3.1 Preparation of EAN nanocomposite

Ellagic acid nanocomposite was prepared by direct reaction of zinc oxide with a solution of ellagic acid, as reported in our previous preparation[8]. Typically, 0.01 and 0.025 molar of hydrated ellagic acid was dissolved in DMSO followed by 10 minutes stirring and heating for 40°C. The 0.2 g of ZnO was mixed with the solution of ellagic acid and stirred at 70°C for 8 hours. After filtration and washing with deionized water three times, the

sample was dried in an oven at 60°C for 12 hours. The EAN was then powdered and stored in a sample bottle for further use.

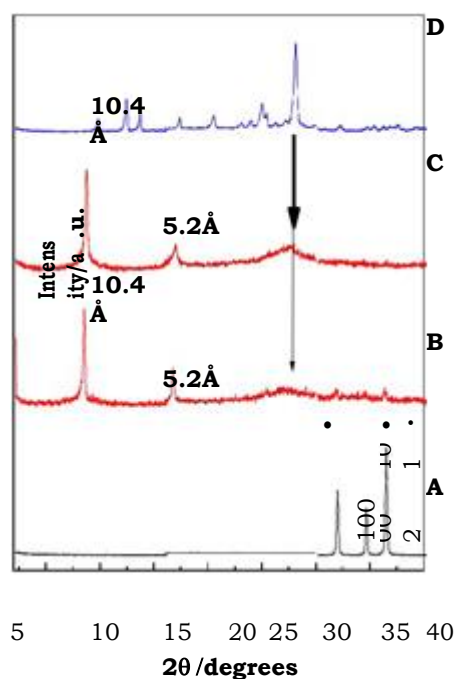## 3. Results and Discussion
3.1.Powder X-ray diffraction

Fig. 2. PXRD patterns for ZnO (A), ellagic acid nanocomposite prepared at two concentrations (0.01 and 0.025 molar for B, and C, respectively) and free ellagic acid (D)

Figures 2A and D show PXRD patterns of the ZnO and free ellagic acid, respectively. In addition, Figures 1B and C show ellagic acid nanocomposite prepared using two concentrations of ellagic acid; 0.01 and 0.025 Mol/L, respectively. As shown in Figure 1A, the ZnO sample shows three intense reflections at 30–40° correspond to diffractions due to 100, 002 and 101 planes (((Reference "Yin M, Gu Y, Kuskovsky IL, Andelman T, Zhu Y, Neumark GF, et al. Zinc oxide quantum rods. Journal of the American Chemical Society. 2004;126(20):6206-7.))) . It was reported previously that the basal spacing for zinc layered hydroxide- nitrate as the interlamella anion is 9.74 Å which appeared at 2θ = 9.12 due to the 200 planes of the monoclinic structure[10]. The PXRD pattern for EAN shown in Figure 2 B and C has a basal spacing of 10.4 Å. Noteworthy, EAN nanocomposites were prepared by both concentrations (0.01 and 0.025 Mol/L) have a broad peak at 2θ= 27.7o, which is due to ellagic acid which adsorbed onto the surface of ZLH. For EAN prepared using 0.01 molar of ellagic acid, ZnO phase was also detected (Figure 1B). Total disappearance of the intense peaks of ZnO phase in Figure 1C indicated that the sample EAN is pure phase and the ZnO was completely converted to ZLH. As a result of a successful intercalation process, EAN prepared using 0.25 Mol/L was subsequently used for further works. The

formation of EAN nanocomposite is assumed through following mechanism (Int J Nanomedicine 2011)
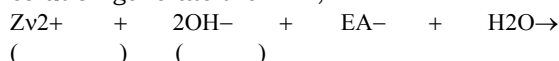
Hydrolysis of zinc oxide in water from hydrated ellagic acid to form zinc hydroxide on the surface of the solid particles
ZnO + H2O   → Zn (OH)2
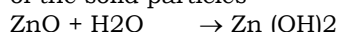
Formation Zn2+ species by dissociation of zinc hydroxide
Zn (OH)2 → Zn2+ + 2OH-
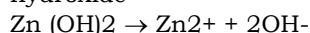
In addition, reaction between Zn2+ ions formed with hydroxyls, H2O and ellagic acid anions in the solution generate the EAN,
Zv2+   +   2OH−   +   EA−   +   H2O→
(   )   (   )

The p reaction condition was 7, this indicating that the ellagic acid have doubly deprotonated hydroxyl group at position 8 and/or 8 (Hasegawa M, Terauchi M, Kikuchi Y, et al. Deprotonation processes of ellagic acid in solution and solid states. Monatsheftef r Chemie/ Chemical Monthly. 2003;134 6 :811– 821. . This means that the ellagic acid has attracted with layers by positions 8 and 8 (Figure 2) and oriented between the interlayers as monolayer with an angle of 22.5˚from z-axis.

3.2. Enhancement Study

that ellagic acid exhibit electro-catalytic behavior towards the electrochemical reduction of ellagic acid due to its fast electron transfer capability (see figure 1(a) and (b)). The current was significantly enhanced by about 2.3 folds.
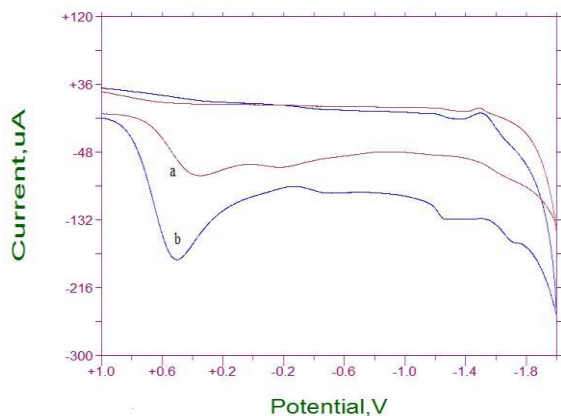
Fig. 3.   Cyclic   voltammograms for reduction of 0.1 mM EAN nanocomposite ellagic acid obtained in 0.1 M KCl (pH 4), with potential scanning in the positive direction from -1000 to +1000 mV vs Ag/AgCl at a scan rate of 100mV/s at 25°C at (a) bare GC electrode with ellagic acid and (b) modified MWCNT/GC.

3.3 Effect of varying pH
The pH was varied from pH 2.0 to 11.0 to determine its effect on the catalytic reduction of 0.1 mM EAN nanocomposite ellagic acid at the

MWCNT/GC modified electrode. Fig. 4 shows that the optimum peak current was achieved at pH 4. With the rise in pH, the peak current decreased due to pH dependency of half wave potential which indicate involvement of protons in the electrode process and finally descanted as we moved towards more alkaline pH due to lower number of available protons. With the increasing pH from 4.2 to 9.0, peak current is shifted to more negative potentials. The linear dependence of peak potential on pH can be expressed by the following relation:

Ep/V (vs.Ag/AgCl) = - 0.0358pH - 0.8203; R2=0.98
Figure 3 shows cyclic voltammograms obtained for the reduction of nanocomposite ellagic acid EAN in 0.1 M KCl supporting electrolyte at pH 4 at modified GC electrode with Ellagic acid (a), and modified electrode with carbon nanotube (CNT) (b). The observation on the reduction potential of Ellagic acid shows an absence of electroactivity on the reverse scan or rereduction, indicating the irreversibility of the oxidation process. The electrocatalytic performance of MWCNT/GCE was advantageous over that observed at the bare GC. The enhancement of reduction peak current at ellagic acid -modified GCE reveals
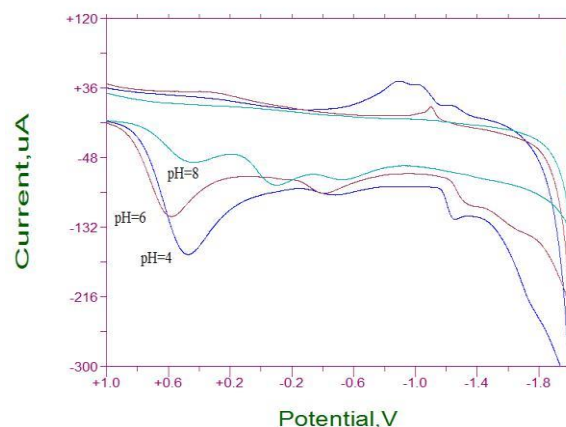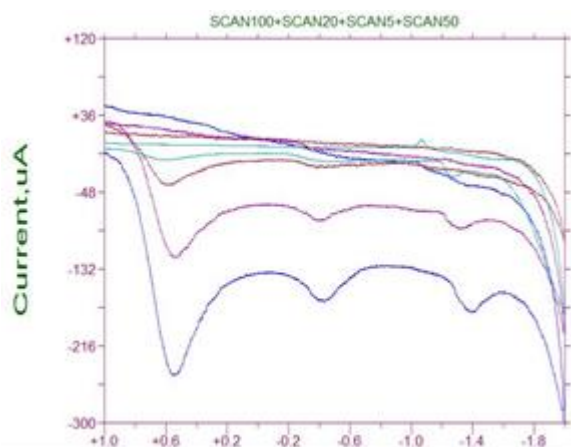

Fig. 4. Cyclic voltammograms pH for 0.1 mM EAN nanocomposite ellagic acid in 0.1 M KCl with varying pH levels for GC electrode modified with modified MWCNT /GC. Potential scanning in the positive direction from -1000 to +1000 mV vs Ag/AgCl at a scan rate of 100mV/s

3.4. Effect of varying scan rate
The effect of varying scan rates on the cyclic

voltammograms of 0.1 mM EAN nanocomposite ellagic acid using MWCNT modified working electrode in 0.1 M KCl supporting electrolyte was studied over scan rate range of 5 – 1000 mV/s. Reduction currents were observed to increase with scan rate due to heterogeneous kinetics [Fig 5]. In the present experiment a linear relationship between log ip and log v1/2 is obtained suggesting the diffusion of ellagic acid at surface of MWCNT/GCE, which can be expressed by the following equation :
Log ip = 0.4 log v – 0.44 ; R2 = 0.98

Fig. 5. Typical cyclic voltammograms of various scan rate 0.1 mM EAN nanocomposite at MWCNT modified GC electrode in .1 M KCl (pH 4), with potential scanning in the positive direction from -1000 to +1000 mV vs Ag/AgCl at a scan rate of 100mV/s.
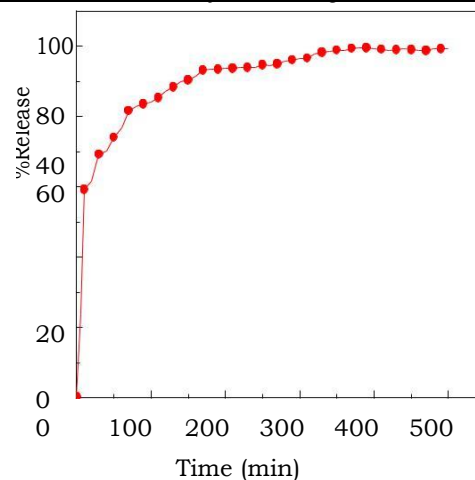
3.5. Release behavior of the EAN

3.5.1 Drug release properties

The ellagic acid release study was performed at room temperature by suspending EAN nanocomposite (200 mg) in phosphate buffer (250 ml) at pH 4.0. The concentration of the phosphate buffer was 0.01 mol/l. The ellagic acid released was measured at a predetermined time by UV-Vis spectrophotometer absorption at λ= 257.5 nm Refrence "Press RE, ardcastle D. Some physico chemical properties of ellagic acid. J

Appl Chem. 1969;19(9):247– 251."Determination of the total amount of intercalated ellagic acid was carried out by sodium carbonate through carbonate ion exchange. The EAN was added to sodium carbonate, and the resulting mixture was heated for 6 hours at 80 °C. After the ellagic acid in the intercalated EAN nanocomposite was completely ion-exchanged by CO_3^{2-} , the concentration of ellagic acid was determined by UV absorption at 257.5 nm.

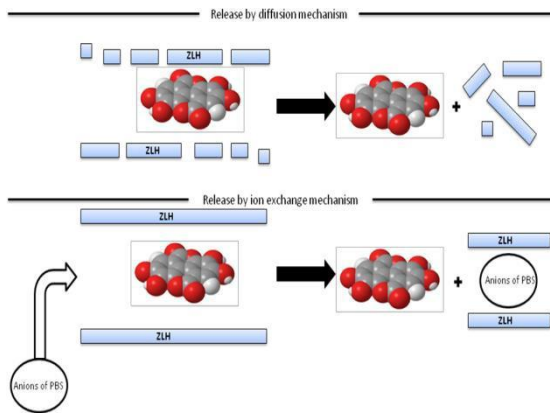### 3.2. Ellagic acid release properties from Enanocomposite

Fig. 6. Release profiles of EA from the EAN in the aqueous solution of phosphate buffer saline at pH 4.0.

Ellagic acid release profile for EAN nanocomposite was present in Figure 6. The release profile shows a high initial drug release rate in the initial 10 min, and reaches an almost constant level after 400 min. The first rapid release is due to the "burst effect [7] which can improve it by the adsorbed small amount of ellagic acid on the surface of zinc
layered hydroxide (Figure 1B and C).The maximum amount of ellagic acid released is almost 98% of the total. The release rate of drugs from the nanocomposites obviously depends on the pH; the release rate at pH 7.4 is remarkably lower than that at pH 4.8. This is because, at acidic pH (pH 4.8 and lower), zinc layered hydroxide is unstable Reference, " Khan AI, O' are D. Intercalation chemistry of

layered double hydroxides: recent developments and applications. J Mater Chem. 2002;12:3191– 3198." , and may be easily dissolved and the release of the interlayer anions might occur due to the diffusion and ion exchange processes. On the other hand, at pH 7.4, zinc layered hydroxide is more stable, and as a result, the release would occur through an anion exchange process [13] Therefore, the ellagic acid release from EAN

nanocomposite at pH 4.0 followed the diffusion and ion exchange mechanism (Figure 7).

Fig. 7. Release mechanism of ellagic acid from EAN   nanocomposite

ipc
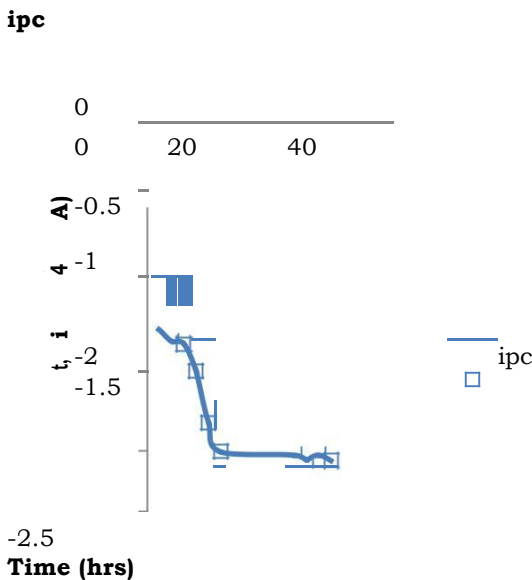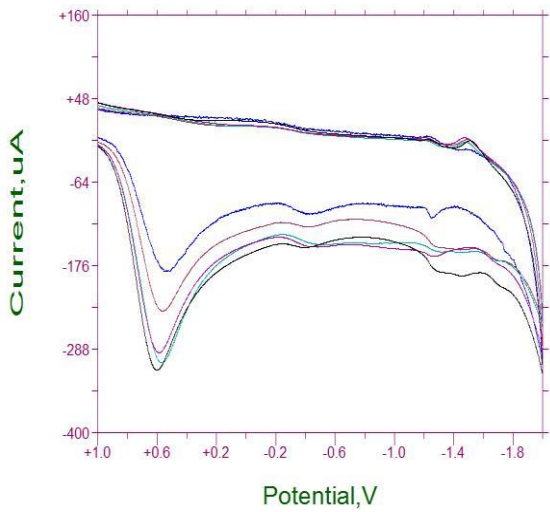
Fig.8. (a,b) . Typical cyclic voltammograms of various release profiles of EA from the EAN in the aqueous solution of phosphate buffer saline at pH 4.0 at MWCNT modified GC electrode, with potential scanning in the positive direction from -1000 to +1000 mV vs Ag/AgCl at a scan rate of 100mV/s.

Ellagic acid release profile for EAN nanocomposite was present in Figure 8. The release profile shows a effect of Cyclic voltammtry with increase time one day which can increase reduction peak by the adsorbed amount of ellagic acid on the surface GC modified electrode after one day the stable adsorbed of reduction EAN ellagic acid.

4. Conclusion

A sensitive and reliable electrochemical method for detection of ellagic acid in real samples using MWCNT as electrode modifier is proposed. Upon modification with ellagic acid, the reduction peak current of ellagic acid was significantly improved due to high catalytic activity and conductivity of ellagic acid. A novel analytical method involving reduction of EAN at the surface of a modified carbon paste

electrode incorporating MWCNT was conducted. The results revealed that EAN reduction was dependent on pH. Owing to the high sensitivity of ellagic acid-modified GCE, this method may be used for the detection of other compounds in food or biological fluids.

5. Acknowledgement

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