

## Comparative study of calix-6-arene and 2-hydroxy propyl $\beta$ -cyclodextrin as ionophores in potentiometric ion-selective electrodes for determination of gallic acid in *Acacia arabica* bark extract

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### ABSTRACT

The present work evaluates the influence of Calix-6-arene and 2-hydroxy propyl  $\beta$ -cyclodextrin as ionophores on the fabrication of gallic acid selective electrodes 1 and 2, respectively. The proposed sensors showed Nernstian slopes of 57.70 and 55.30 mV/concentration decades over concentration range  $10^{-3}$  –  $10^{-5}$  M for sensors 1 and 2, respectively. Fast response and moderate stability time were observed. The selectivity coefficients of the developed sensors indicated excellent selectivity for gallic acid. The proposed electrodes were successfully applied for direct determination of gallic acid in pure form and *Acacia arabica* bark extract without prior separation or pretreatment steps. Thus, they can be used in quality control labs for routine analysis of gallic acid in *Acacia arabica* bark extract.

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### KEYWORDS

Ion selective electrodes;  
Calix-6-arene;  
2-Hydroxy propyl  $\beta$ -  
cyclodextrin;  
Gallic acid;  
*Acacia arabica*.

### INTRODUCTION

Gallic acid (Figure 1) is a major phytoconstituent in *Acacia arabica* which can be used as a bioactive marker for determination of the quality of the crude drug and its formulations. Several reports focused on therapeutic activities of gallic acid as natural polyphenol having hypoglycemic, hypocholesterolemic<sup>[1]</sup>, anti-oxidant<sup>[2]</sup>, anti-tumor<sup>[3]</sup>, anti-inflammatory<sup>[4]</sup>, anti melanogenic<sup>[5]</sup>, anti-bacterial<sup>[6]</sup>, anti-viral<sup>[7]</sup>, neuroprotective<sup>[8]</sup> and cardioprotective<sup>[9]</sup> activities.

Different Methods were reported in literature for determination of gallic acid in *Acacia arabica* bark using HPLC<sup>[10-12]</sup> and HPTLC<sup>[13]</sup>. Although the numerous advantages of ion selective electrodes (ISE), it was not used before for determination of gallic acid in *Acacia*

*arabica* bark extract.

Potentiometric methods using ISE have found wide applications, due to their numerous advantages. The high selectivity of these electrodes imparts a great ad-

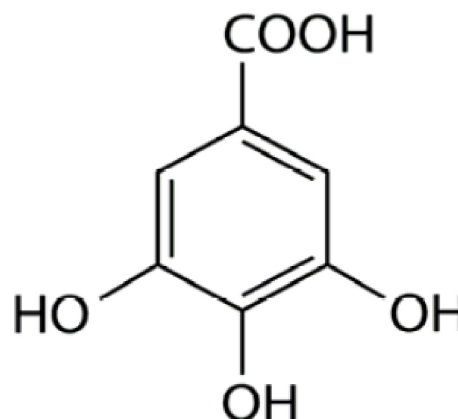


Figure 1 : Chemical structure of gallic acid

vantage over other techniques. No sample pretreatment needed before the analysis. Analytes in colored, turbid and viscous samples can be determined accurately. They show rapid response to changes in the concentration. Furthermore, they may be used for measurement over a wide concentration range. ISE are generally tolerant to small changes in pH. A further advantage is that they are relatively simple and cheap to develop and run. Moreover, the chemical design of the electrodes has been developed to give superior selectivity and response<sup>[14]</sup>.

ISE based on polymeric membranes containing neutral or charged carriers (ionophores) are one of the most successful electrochemical sensors in routine use today<sup>[15]</sup>. The ISE dynamic response is generated by selective complexation of the target ion by ionophores dispersed in a poly (vinylchloride) (PVC) matrix<sup>[16]</sup>.

Calixarenes, cyclic oligomers of phenol-formaldehyde condensates, have drawn much attention as a fascinating class of cyclophanes possessing ionic and molecular-binding properties. Derivatives containing a wide

range of functional groups have been synthesized and shown to exhibit different degrees of receptor ionophoric activity<sup>[17]</sup>. Their ability to complex cations, anions, and neutral molecules has made them a standard among supramolecular host molecules. Their configuration includes a number of selective factors, such as cavity-size, conformation and substituents, which leads to the formation of typical host-guest complexes with numerous compounds and allow for a variety of applications in ion-selective membranes and electrodes<sup>[18-21]</sup>. The structure of calixarenes is shown in (Figure 2A).

Cyclodextrins are optically active oligosaccharides that form inclusion complex with organic molecules; because their chemical structure provides well-defined inclusion cavities with a specific receptor function<sup>[22]</sup>. The structure of cyclodextrins is shown in (Figure 2B).

The present work describes the use of calix-6-arene and 2-hydroxy propyl  $\beta$ -cyclodextrin as ionophores for the development of novel sensors for the determination of gallic acid in pure form and *Acacia arabica* bark extract.

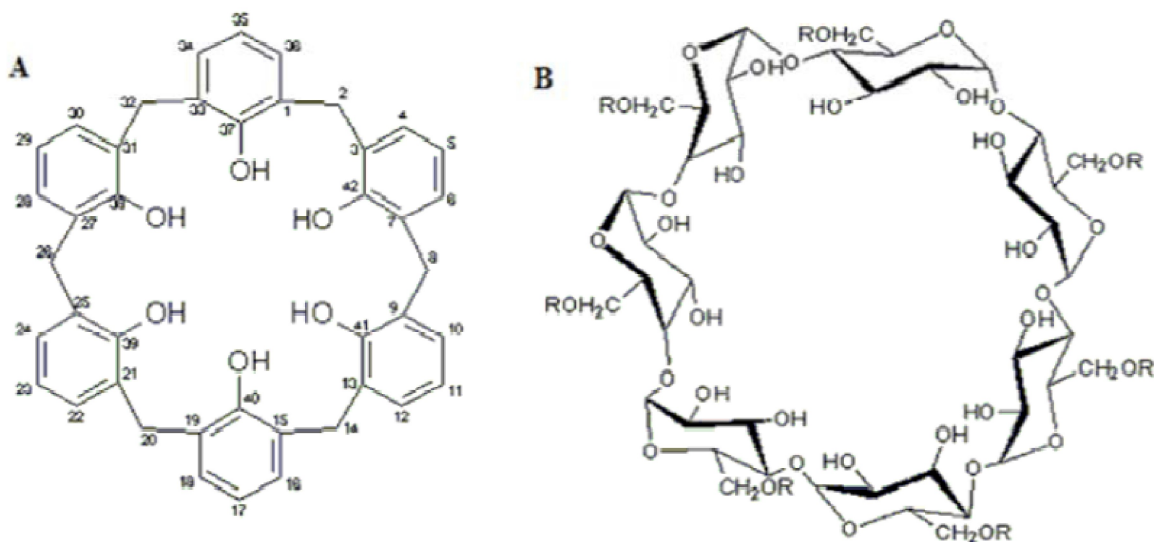


Figure 2 : Chemical structure of: A: calix-6-arene molecule, B: 2-hydroxy propyl  $\beta$ -cyclodextrin molecule

## EXPERIMENTAL

### Apparatus

A Jenway digital ion analyzer; model 3330 (Essex, UK) with Aldrich Ag/AgCl double junction reference electrode no. 113107 (Munich, Germany) was used for potential measurements. A Jenway pH glass elec-

trode no. 924005-BO3-Q11C (Essex, UK) was used for pH adjustments. Solution stirring was done using Bandelin Sonorex magnetic stirrer; model Rx 510 S (Budapest, Hungary).

### Samples

#### Pure sample

Gallic Acid (purity  $\geq 99\%$ ) was purchased from Merck (Hohenbrunn, Germany).

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### Market sample

Acacia arabica bark was collected from (New Valley, Egypt) and authenticated by Phytochemistry Department, Faculty of Pharmacy, Ain Shams University (Cairo, Egypt).

### Chemicals and reagents

All chemicals and reagents used were of analytical grade, and water was bi-distilled. Calix-6-arene and 2-hydroxy propyl  $\beta$ -cyclodextrin were purchased from Alfa Aesar (Ward Hill, Massachusetts, USA). Polyvinyl chloride (PVC) was obtained from Fluka (Steinheim, Germany). 2-Nitrophenyl octyl ether (NPOE) and tetrahydrofuran (THF) were purchased from SigmaAldrich (Steinheim, Germany). Potassium chloride (KCl), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were obtained from Prolabo (Pennsylvania, USA). Toluene, Ethyl acetate and formic acid were purchased from ADWIC (Cairo, Egypt).

### Standard solutions

#### Gallic acid standard stock solution ( $1 \times 10^{-1}$ M)

It was freshly prepared by dissolving 1.70 g of pure gallic acid in 100 ml of bi-distilled water.

#### Gallic acid standard working solutions ( $1 \times 10^{-2}$ to $1 \times 10^{-6}$ M)

They were prepared by suitable dilution from stock solution using bi-distilled water.

### Procedures

#### Fabrication of membrane sensors

In a glass Petri dish (5 cm diameter), 0.19 g of PVC and 0.40 g of NPOE were mixed with 0.04 g calix-6-arene or 2-hydroxy propyl  $\beta$ -cyclodextrin for preparation of electrodes 1 and 2, respectively. The mixture was then dissolved in 6 ml THF. The Petri dish was covered by a filter paper and left to stand for 24 h to allow solvent evaporation at room temperature. A master membrane of 0.1 mm thickness was obtained.

From the master membrane, a disk (8 mm diameter) was cut using cork borer and pasted using THF to interchangeable PVC tip that was previously clipped into the end of the electrode glass body. Equal volumes of  $10^{-2}$  M gallic acid and  $10^{-2}$  M KCl were mixed and used as an internal reference solution. Ag/AgCl wire

(1 mm diameter) was immersed in the internal reference solution as internal reference electrode. The electrodes were preconditioned by immersing in  $10^{-2}$  M gallic acid solution for 24 h and stored in the same solution when not in use.

### Sensors calibration

The conditioned electrodes were calibrated by separately transferring 50 ml aliquots of gallic acid solutions covering the concentration range of ( $1 \times 10^{-2}$  to  $10^{-6}$  M) into a series of 100 ml beakers. The sensors and the reference electrode were immersed in each solution with constant stirring using a magnetic stirrer. The potential recordings were recorded. The electrodes were washed with bi-distilled water between measurements. The electrode potential was plotted versus the negative logarithmic concentration of gallic acid. The regression equation for linear part of the curve was computed.

### Study of experimental conditions

#### Identification of slope, response time and operative life of the studied sensor

The electrochemical performance of the studied electrodes was evaluated according to IUPAC recommendation data<sup>[23]</sup>.

The dynamic response time of the electrodes were tested for concentrations of the drug solution from  $1 \times 10^{-6}$  M to  $1 \times 10^{-2}$  M; the sequence of measurement was from low to high concentrations.

The long-term reproducibility and stability of the potentials were evaluated by determining replicate calibration graphs over a period of 5 weeks. The electrodes were stored in  $1 \times 10^{-2}$  M gallic acid standard solution when not in use and washed thoroughly with bi-distilled water between measurements.

### Effect of pH

The electrodes response was tested at different pH values over a pH range 2-11. The pH was adjusted using HCl and NaOH solutions. The concentrations used were  $1 \times 10^{-3}$  M and  $1 \times 10^{-4}$  M. The electrode potential was plotted versus pH.

### Sensors selectivity

The response of the electrodes was examined in presence of a number of possible interfering substances.

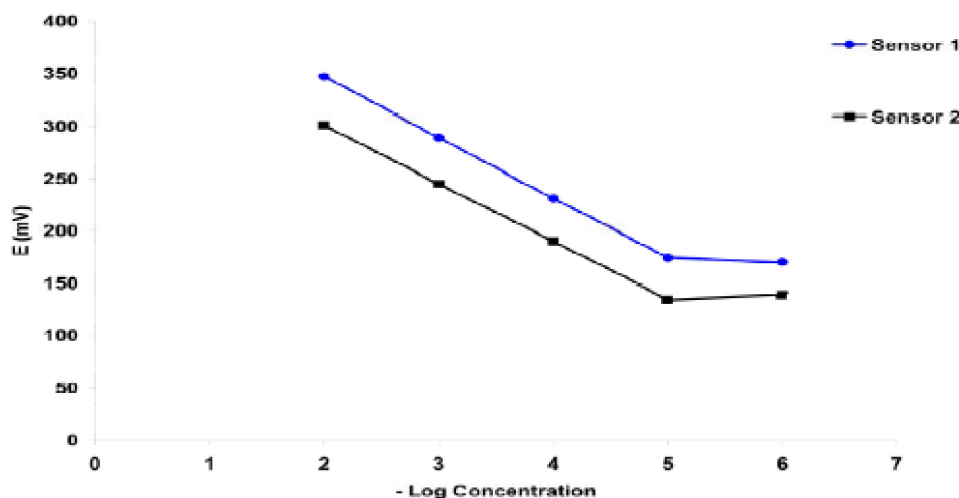


Figure 3 : Profile of the potential in mV vs. -Log concentration of gallic acid for the proposed sensors

TABLE 1 : Electrochemical response characteristics of the proposed gallic acid sensors and validation parameters of the assay

Parameter	Sensor 1	Sensor 2
Slope (mV/decade) <sup>a</sup>	57.70	55.30
Intercept	462.45	411.30
R	0.9999	0.9999
LOD (M)	1.02 x 10 <sup>-5</sup>	1.01 10 <sup>-5</sup>
Response Time (sec.)	20	20
Working pH Range	4 - 6	4-6
Concentration Range (M)	10 <sup>-2</sup> – 10 <sup>-5</sup>	10 <sup>-2</sup> – 10 <sup>-5</sup>
Stability (weeks)	5	4
Average Recovery (%) ± SD <sup>a</sup>	100.32 ± 0.32	100.93 ± 0.80
Repeatability (SD <sub>r</sub> )	1.02	0.94
Intermediate Precision (SD <sub>int</sub> )	1.46	1.58
Ruggedness <sup>b</sup>	100.06± 1.31	99.95 ± 1.42

<sup>a</sup> Average of five determinations; <sup>b</sup> Average recovery percent of determining 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> M gallic acid for the studied electrode using Jenway 3310 digital analyzer instead of model 3330

The potentiometric selectivity coefficient ( $K^{Pot.}_{interferent, Primary ion}$ ) was used to evaluate the extent to which a foreign ion would interfere with the response of the electrode to its primary ion.

Selectivity coefficients were calculated by separate solution method where potentials were measured for 1 x 10<sup>-3</sup> M drug solution and 1 x 10<sup>-3</sup> M aqueous interferents solutions, separately. The potentiometric selectivity coefficients were then calculated using the rearranged Nicolsky-Eisenman equation<sup>[23]</sup>.

$$\log K^{Pot.}_{A,B} = [(E_B - E_A) / (2.303 RT / Z_A F)] + [1 - (Z_A / Z_B)] \log [A]$$

Where E<sub>A</sub> is the potential measured in 10<sup>-3</sup> M drug solution, E<sub>B</sub> is the potential measured in 10<sup>-3</sup> M interferent solution, Z<sub>A</sub> and Z<sub>B</sub> are the charges of drug and interferent; respectively, and 2.303 RT / Z<sub>A</sub> F represents the slope of the calibration curve (mV / concentration decade).

### Direct potentiometric determination of gallic acid in *Acacia arabica* bark extract

*Acacia arabica* bark samples were cleaned manually to remove all foreign materials then milled, passed through a stainless steel sieve (20-40 mesh) and stored at 4 °C until use.

An accurately weighed sample (1 g) of the powdered bark was extracted at 88°C for 6 h under reflux with 100 ml of 20% methanol in a round-bottom flask heated in a water bath (optimum conditions for extraction was studied before).

In a 50 ml volumetric flask, 5 ml of the extract was transferred and diluted to the mark with bi-distilled water. The membrane sensors were immersed in conjunction with the reference electrode in the extract. The emf produced was measured by the proposed sensors, and the concentration of gallic acid was determined from the corresponding regression equation.

## RESULTS AND DISCUSSION

### Sensors fabrication

The molecular recognition and inclusion complex-

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ation are of current interest in host-guest and supramolecular chemistry and offer a promising approach to chemical sensing<sup>[24,25]</sup>. The use of selective inclusion complexation and complementary ionic or hydrogen bonding are two main strategies for preparing synthetic host molecules, which recognize the structure of guest molecules<sup>[26]</sup>.

PVC was employed in the fabrication of the membrane matrix as it provides a regular support and reproducible trap for ion association complex in ion selective electrodes<sup>[27]</sup>.

Plasticizer has a great influence on the dielectric constant of the membrane phase, the mobility of the ionophore molecules, and the state of ligands. Plasticizer facilitates the inclusion of organic molecules and ensures stable potentials with long lifetime<sup>[28]</sup>.

### Sensors performance characteristics

The electrochemical performance characteristics of the investigated electrodes were summarized in (TABLE 1).

The proposed sensors showed Nernstian slopes of 57.70 and 55.30 mV/concentration decades for sensors 1 and 2, respectively over concentration range  $10^{-2}$  -  $10^{-5}$  M as seen in (Figure 3). The Nernstian relation of the electrodes is:

$E = -57.7x + 462.45 \text{ Log [C]}$ ;  $E = -55.3x + 411.3 \text{ Log [C]}$  for sensors 1 and 2, respectively, where [C] is the molar concentration.

The Limits of detection were calculated according to IUPAC recommendations (IUPAC, Analytical Chemistry Division, Commission on Analytical Nomenclature, 2000) from the intersection of the two extrapolated linear portions of the curves and found to be  $1.02 \times 10^{-5}$  M for sensor 1 and  $1.01 \times 10^{-5}$  M for sensor 2.

The electrodes displayed constant potential readings, which did not vary by more than  $\pm 1$  mV on the same day. Linearity range from day to day and calibration slope did not change over a period of 5 weeks for sensor 1 and 4 weeks for sensor 2. This indicates the good stability of the investigated electrodes.

The required time for the electrode to reach values within  $\pm 1$  mV of the final equilibrium potential after increasing the drug concentration 10-folds was 20 seconds for both sensors which indicate the fast response time.

### Effect of pH

The influence of pH on the potential response of the electrode was studied using concentrations  $10^{-3}$  and  $10^{-4}$  M over the pH range 2-11. The proposed sensors showed fairly constant potentials over pH range 4-6. Therefore, this range was assumed to be the working pH range for the proposed sensors.

### Sensors selectivity

(TABLE 2) shows the potentiometric selectivity coefficients of the proposed sensors in presence of some interfering species that may present in *Acacia arabica* bark extract. The results demonstrate an excellent selectivity of the proposed sensors. However, sensor 1 showed relatively higher selectivity and lower response toward the interfering species.

TABLE (2): Potentiometric selectivity coefficients ( $K^{Pot}_{gallic acid, interferent}$ ) for gallic acid selective electrodes

Interferents <sup>a</sup>	Selectivity Coefficient	
	Sensor 1	Sensor 2
Rutin	$6.73 \times 10^{-3}$	$8.68 \times 10^{-3}$
Sucrose	$7.30 \times 10^{-3}$	$8.33 \times 10^{-3}$
Vitamin B <sub>2</sub>	$4.96 \times 10^{-3}$	$5.49 \times 10^{-3}$
Vitamin B <sub>3</sub>	$2.95 \times 10^{-3}$	$4.10 \times 10^{-3}$
Zn <sup>+2</sup>	$1.09 \times 10^{-3}$	$5.97 \times 10^{-3}$
Mg <sup>+2</sup>	$1.83 \times 10^{-3}$	$4.46 \times 10^{-3}$

<sup>a</sup> Aqueous Solutions of  $1 \times 10^{-3}$  M were used

### Potentiometric determination of gallic acid in *Acacia arabica* bark extract

The proposed electrodes were successfully applied for direct determination of gallic acid in *Acacia arabica* bark extract without prior purification or pretreatment steps for the turbid and colored samples. The concentration of gallic acid in the extract prepared under optimized heat reflux extraction conditions are summarized in (TABLE 3).

TABLE 3 : Determination of gallic acid in *Acacia arabica* bark extract by the proposed sensors

Gallic Acid Concentration (mg/g) $\pm$ SD <sup>a</sup>	Sensor 1	Sensor 2	HPTLC Method [13]
	$10.21 \pm 0.73$	$10.03 \pm 0.82$	$10.26 \pm 0.91$

<sup>a</sup> Average of 3 determinations; <sup>b</sup> HPTLC method: using toluene: ethyl acetate: formic acid (6:4:0.8, v/v/v) as mobile phase on TLC aluminum plates precoated with silica gel 60 F<sub>254</sub> at 272 nm

## CONCLUSION

From the obtained results, it was concluded that Calix-6-arene could be considered better ionophore than 2-hydroxy propyl  $\beta$ -cyclodextrin in the preparation of PVC based gallic acid selective electrodes as it showed higher slope, stability and selectivity.

The use of the proposed sensors offer the advantages of fast response, moderate stability time, elimination of drug pretreatment and separation steps and high selectivity in presence of other phyto-constituents in the extract. Thus, the proposed electrodes can be considered for use for routine analysis of gallic acid in *Acacia arabica* bark extract.

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