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Research Article

A Novel Coated Graphite Sensor for Potentiometric Determination of Hydroxychloroquine Sulfate

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Editor:

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Publication Details

Received: Sept 25, 2016 **Accepted:** Nov 05, 2016 **Published:** Nov 19, 2016

Funding: None. **Subject Section:** Analytical Chemistry

Conflict of interest: The authors have read the journal's policy and have the following conflicts:
Construction and analytical application of novel potentiometric sensors for determination of selected pharmaceutical drugs.

Funding: None

Abstract:

A novel coated graphite sensor (CGS) for determination of hydroxychloroquine sulfate (HCQS) has been developed. A polymeric membrane (PVC) was coated on a graphite rod. The optimum membrane contained 3% phosphotungstic acid (PTA) as a counter ion, 48.5% PVC and 48.5% dibutyl phthalate (DBP) as a solvent mediator. The linear range was $9.3x10^{-5}$ -$1.0x10^{-2}$ mol L^{-1} with a Nernstian slope of 28.5 mV/decade and a detection limit of $4.7x10^{-5}$ mol L^{-1}. The constructed sensor was found to be precise and usable within the pH range of 2.0-7.0, and the lifetime of the sensor was 25 days. The sensor showed very good selectivity for HCQ cation with respect to a number of common inorganic and organic species. The drug was determined successfully in pure solutions using the standard addition method.

Keywords: Coated graphite sensor; Potentiometry; Hydroxychloroquine sulfate.

1. Introduction:

Hydroxychloroquine sulfate (HCQS) (Fig. 1), is an antimalarial drug used for treatment of acute attacks of malaria due to *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and susceptible strains of *Plasmodium falciparum* [1-2], where it is reported as being half as toxic as the closely related chloroquine (CQ) yet equally active against *Plasmodium falciparum*[3], and for treatment of a variety of chronic diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), discoid lupus (LD), sarcoidosis, Sjogren's syndrome (SS) and photosensitivity diseases [4-9].

Various analytical techniques have been applied for the determination of HCQS including extraction followed by nonaqueous titrimetric [10] or spectrophotometric methods based on reactions with iodide [11], quinones [12, 13] and cobalt thiocyanate [14]. Because of the nonspecificity of most of these reactions, prior extraction of HCQS is commonly involved in the assay methods. Besides, a number of different HPLC methods have been proposed for determination of HCQ [15] in biological fluids [16] using spectrophotometry [17], fluorescence

[18-20], ultraviolet [21] or tandem mass spectrometry [22] detection. Few electrochemical methods have been used for determination of HCQ was found in the literature, such as differential pulse [23] and squre-wave [24] voltammetry techniques. However; these methods suffer from a variety of drawbacks, i.e. they are costly and unsuitable for large-scale monitoring. Potentiometric sensors based on ion-selective electrodes (ISEs) can overcome these limitations.

Potentiometric sensors have found widespread use for the direct determination of ionic species [25-35]. Coated sensors in which an electroac-tive species is incorporated into a thin polymeric film coated directly on a metallic or graphite conductor have been shown to be very effective for a wide variety of inorganic and organic species [36-40]. Sensors of this sort are simple, inexpensive, durable, capable of reliable response in a wide concentration range for a variety of both organic and inorganic species and suitable for measurements in small volumes of sample or for the desired in vivo applica-tions of ISEs that biomedical researchers have long awaited.

The present work describes construction and investigation of performance characteristics of a novel CGS for the determination of HCQS in pure form.

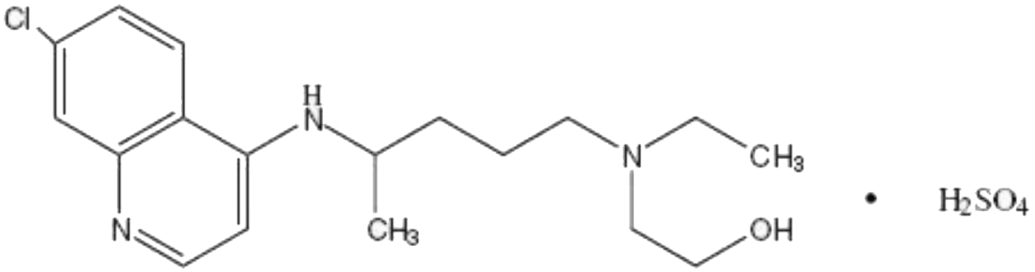

Fig. 1: The chemical structure of hydroxychloroquine sulfate

2. Experimental:

2.1 Reagents and materials:

All chemicals used were of analytical grade and bidistilled water was used throughout all experiments. Dibutyl phthalate (DBP) was purchased from Merck. Phosphotungstic acid (PTA) was obtained from Fluka. Polyvinyl chloride of high molecular weight and tetrahydrofuran (THF) were purchased from Aldrich Chemical Company (USA). The metal salts were provided by BDH Company (UK) as nitrates or chlorides.

Pure-grade hydroxychloroquine sulfate (M. Wt = 433.95 g mol^{-1}) was supplied by MINAPHARM Co., Cairo, Egypt. Standard solution of 1.0×10^{-2} mol L^{-1} hydroxychloroquine sulfate was freshly prepared by dissolving the accurately weighed amount in bidistilled water. Working solutions (1.0×10^{-6} – 1.0×10^{-2} mol L^{-1}) of the drug were prepared. Solutions of NaOH and HCl of concentrations within the range (0.1-1.0) mol L^{-1} were used for adjusting the pH of the medium.

2. 2 Apparatus:

The electrochemical system of the CGS may be represented as follows:

Graphite rod/membrane/test solution/ Ag/AgCl double-junction reference electrode.

An Ag/AgCl double junction reference electrode (Metrohm 6.0222.100) was used as the external reference. Potential and pH-measurements were carried out using 702 titroprocessor equipped with a 665 dosimat made by Metrohm (Switzerland). A circulator thermostat (mLw W20) was used to control the temperature of the test solutions.

2. 3 Preparation of coated graphite sensor:

A pure graphite rod of 1 mm diameter and 12 cm in length was insulated leaving 2 cm at one end for coating and 1 cm at the other end for connection. The coating solution was prepared by dissolving 3.0% PTA, 48.5% PVC, and 48.5% DBP in 5 ml THF. The polished surface of the graphite rod was coated with the active membrane by dipping the exposed end into the coating solution and allowing the film to dry in air for about 1 min [31]. The process was repeated until

a plastic film of approximately 1 mm thickness was formed (9 times). The prepared sensor was preconditioned by soaking for 30 min in 10-2 mol L-1 HCQS solution.

2. 4 Construction of calibration curves:

The constructed sensor was immersed in conjunction with an Ag/AgCl double junc-tion reference electrode in solutions of the drug in the range of 1.0×10^{-6} -1.0×10^{-2} mol L^{-1}. The sensor was allowed to equilibrate whilst stirring and recording the e.m.f. readings within ±1 mV and the mV- concentration profiles were plotted. The regression equations for the linear part of the curves were computed and used for subsequent determination of unknown concentrations of hy-droxychloroquine sulfate.

2. 5 Selectivity:

Bakker protocol [41, 42] is applied to indicate the selectivity behavior of the proposed sensor towards various ionic and nonionic interferying species.

2. 6 Potentiometric determination of hydroxychloroquine sulfate:

Hydroxychloroquine sulfate was determined potentiometrically applying the standard addition method [43], in which small increments of the standard solution 1.0×10^{-2} mol L^{-1} of HCQS were added to 50 mL aliquot samples of various concentrations of the pure drug. The following equation was used to calculate the concentration of hydroxychloroquine sulfate sample solution by recording the change in millivolt reading for each increment:

$$C_x = C_s \left(\frac{V_s}{V_x+V_s}\right) \left(10^{n\,(\Delta E/S)} - \frac{V_X}{V_x+V_s}\right)^{-1}$$

Where, Cx and Vx are the concentration and the volume of the unknown, respectively, Cs and Vs the concentration and the volume of the standard solution, respectively, S is the slope of the calibration graph and ΔE is the change in mV due to the addition of the standard solution.

3. Results and Discussion:

3.1 Optimizing the composition of the sensor:

It is well known that the composition of the sensor is mainly responsible for improvement the sensitivity, linear dynamic range and selectivity of a given sensor. Several compositions for the studied sensor were tested along with their other characteristics. Three sensors with different compositions were made and test-ed (sensor 1, 2 and 3). The results (Table 1) show that sensor 2 (3.0% (w/w) PTA) exhibits the best performance (slope 28.5, linear range $9.3\text{x}10^{-5}$-$1.0\text{x}10^{-2}$ mol L^{-1} and detection limit $4.7\text{x}10^{-5}$ mol L^{-1}). However, further addition of the ion-pairing agent (sensor 3), display somewhat sub-Nernstian slope. This behavior may be at-tributed to some inhomogeneities and possible saturation of the membrane [44]. Calibration curve of HCQS coated graphite sensor is shown in Fig. 2.

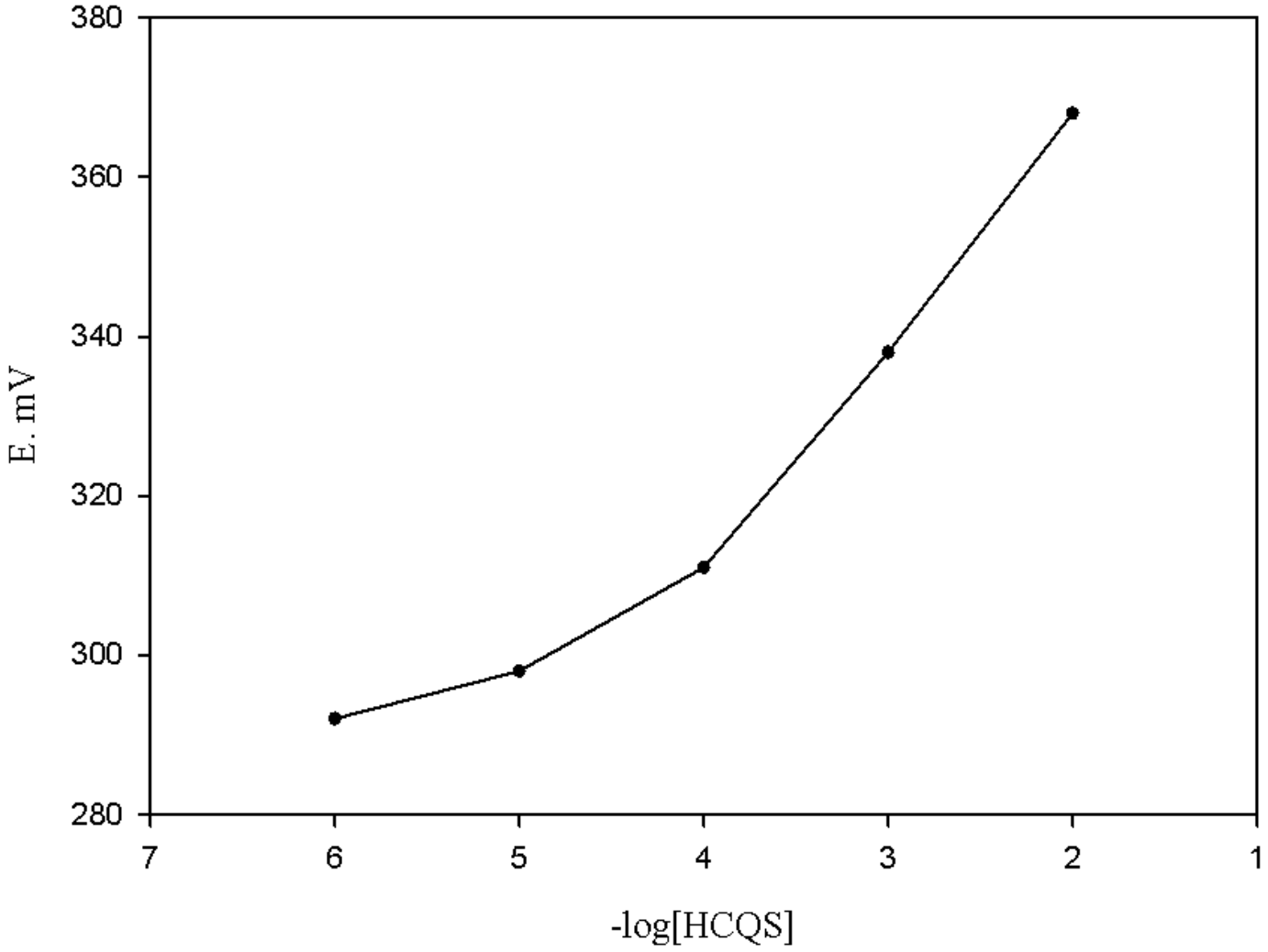

Fig. 2: Calibration curve of HCQS coated graphite sensor

Table 1. Optimization of membrane composition (w/w %) for hydroxychloroquine sulfate sensor

Sensor	Composition % w/w			Slope	Linear range	LOD	r^2
CGS	IPA	PVC	DBP	mV/ decade ± SD	mol L^{-1}	mol L^{-1}	
	PTA						
1	1.0	49.5	49.5	27.0	$8.79x10^{-4}$-$1.0x10^{-2}$	$1.2x10^{-6}$	0.9177
2*	**3.0**	**48.5**	**48.5**	**28.5**	**$9.3x10^{-5}$-$1.0x10^{-2}$**	**$4.7x10^{-5}$**	**0.9997**
3	5.0	47.5	47.5	22.0	$7.39x10^{-5}$-$1.0x10^{-2}$	$1.9x10^{-5}$	0.9999

IPA: Ion-pair agent; LOD: Limit of detection; LR: Linear range.

3. 2. Dynamic response time and reproducibility of the sensor:

The dynamic response time, which representes a significant parameter for a potentiometric sensor, was measured according to IUPAC recommendation [40]. It is defined as the time between addition of the analyte to the sample solution and the time when a limiting potential has been reached [40]. In the present study, the response time of the sensor was measured by varying the HCQS concentration over the range from 1.0×10^{-5} to 1.0×10^{-2}. As shown in Fig. 3, the sensor reached equilibrium in a very short time (~ 10 s).

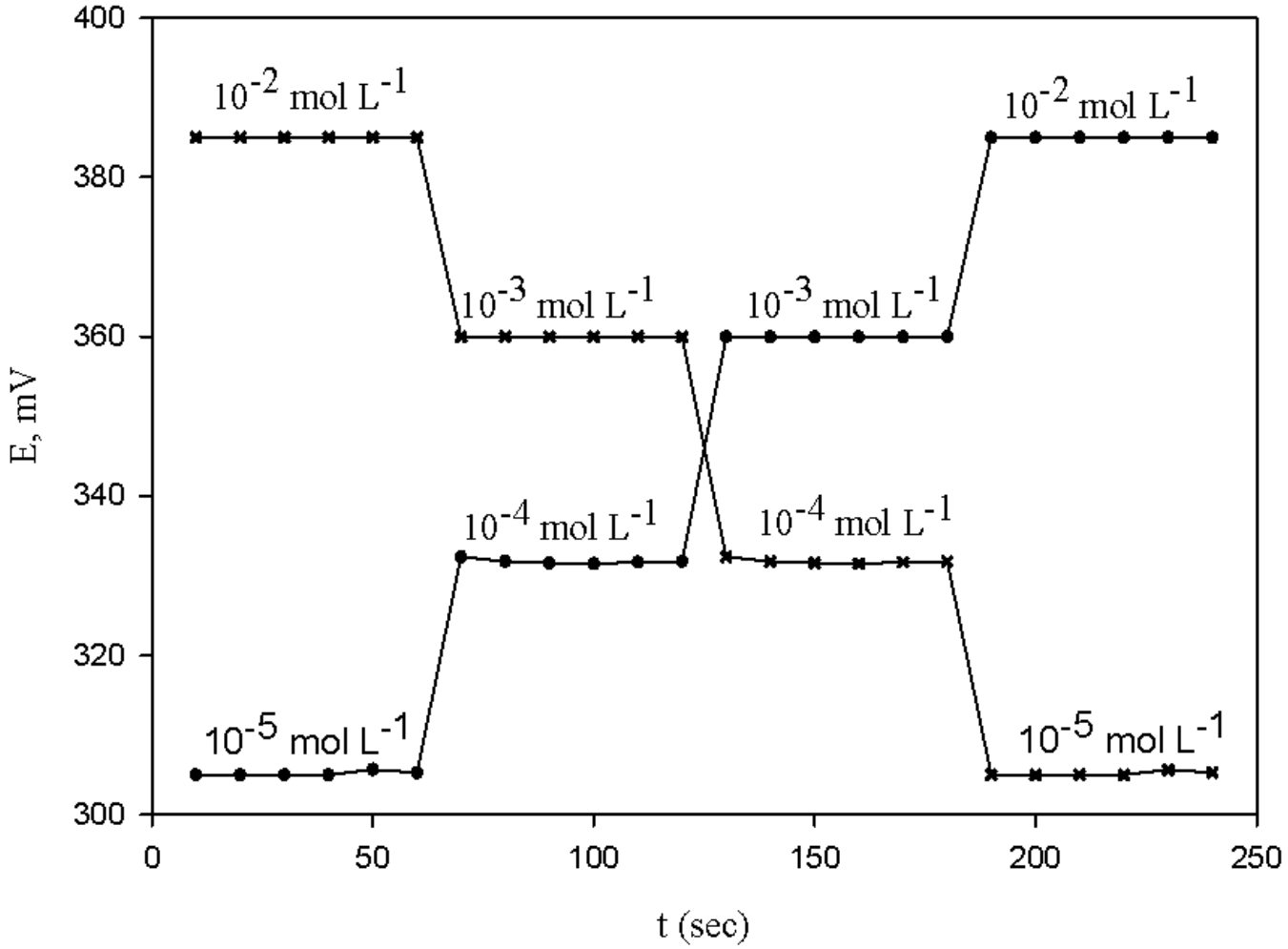

Fig. 3: The dynamic response time of CGS for step changes in concentrations of HCQS from low to high and vice versa

3. 3. Effect of pH:

Since pKa of HCQS is 9.76, therefore at pH 7.90 HCQ is nearly completely ionized, *i.e.* HCQS will be in the cationic form. The concentration distribution diagram for HCQS species is constructed using SPECIES program [41] (Fig. 4).

The influence of pH on the potentiometric response of the fabricated sensor in the pH range of (1–11) at 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1} of HCQS solution was investigated and the results obtained were shown in Fig. 5. The potentials remained constant in the pH range of 2–7. At lower pH values the increase in mV readings was due to the interference of hydronium ion. However, at higher pH values the decrease in the mV readings may be ascribed to the formation of non-protonated drug molecules.

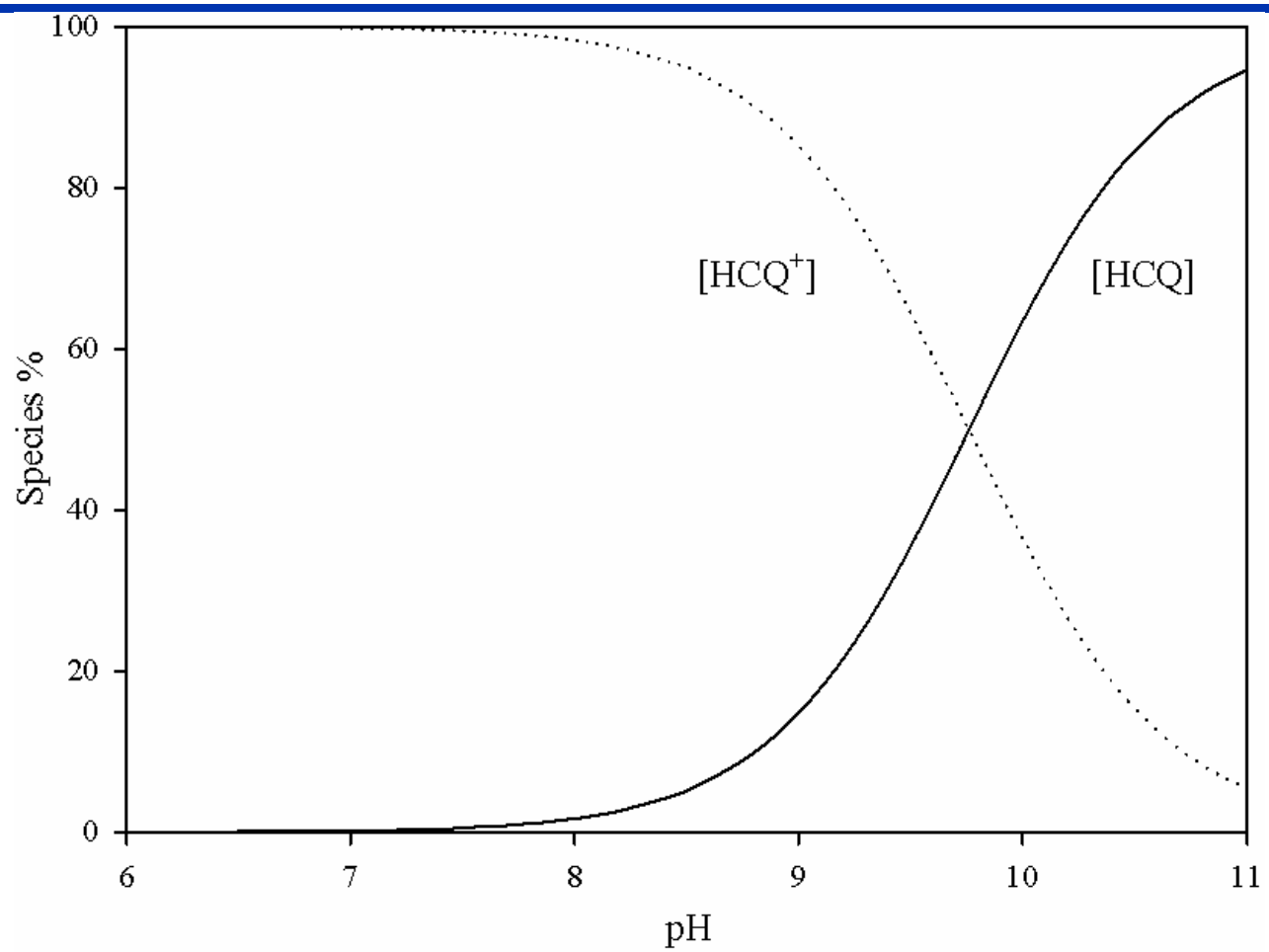

Fig. 4: Representative concentration distribution diagram for HCQ species

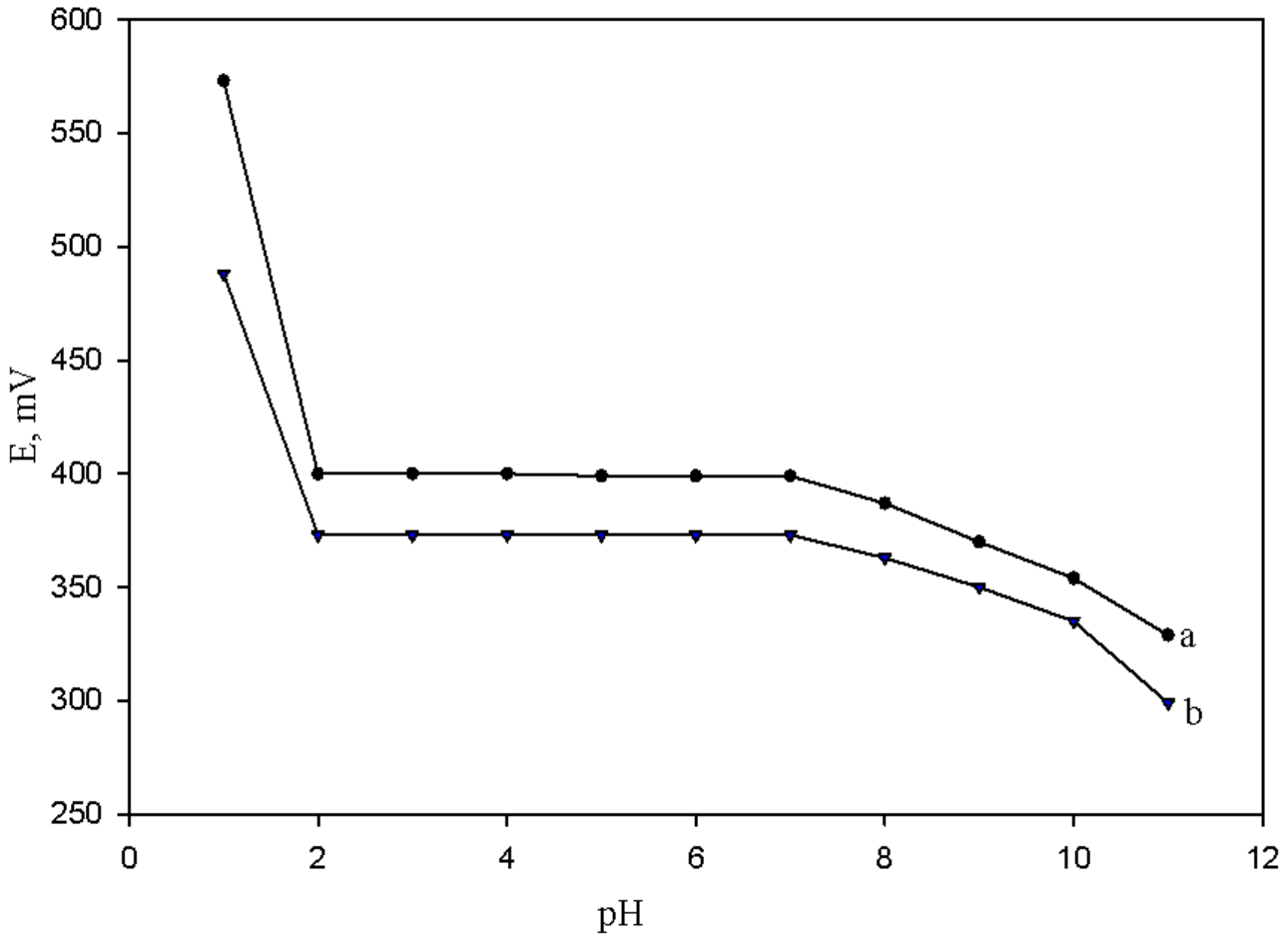

Fig.5: Effect of pH of the test solutions on the potential response of CGS a) 1.0×10^{-3} and b) 1.0×10^{-4} mol L^{-1} of HCQS solution

3. 4 Lifetime:

The sensor exhibited a good stability in the response up to a period of 25 days. Indeed, the mean drift of slope observed over this period was found to be less than 0.02 mV dec $^{-1}$.

3. 5 Selectivity of the sensor:

Bakker protocol, an excellent approach to confirm the selectivity behaviorof potentiometric sensors toward various interfering species, was applied [41, 42]. The influence of some inorganic cations, sugars and amino acids on the HCQS sensor was investigated graphically by plotting the potential response of the sensor for different interferents against their varying concentration. As shown from the calibration curves (Fig. 6), except for HCQ cation there is no significant response of the sensor for all interferents tested and the results reflect a very high selectivity of the investigated sensor for the HCQ cation. The low interference of inorganic cati-ons may be due to the differences in ionic size and consequently their mobilities and permeabilities as compared with HCQ+. In the case of non-ionic species, the high selectivity of the sensor is mainly attributed to the difference in polar-ity and to the lipophilic nature of their molecules relative to HCQ^{+}.

The mechanism of selectivity is mainly based on the stereospecificity and electrostatic environment, and is dependent on how much matching is present between the location of the lipophilic sites in the two competing species in the bath-ing solution side and those present in the receptor of the ion-exchanger [47].

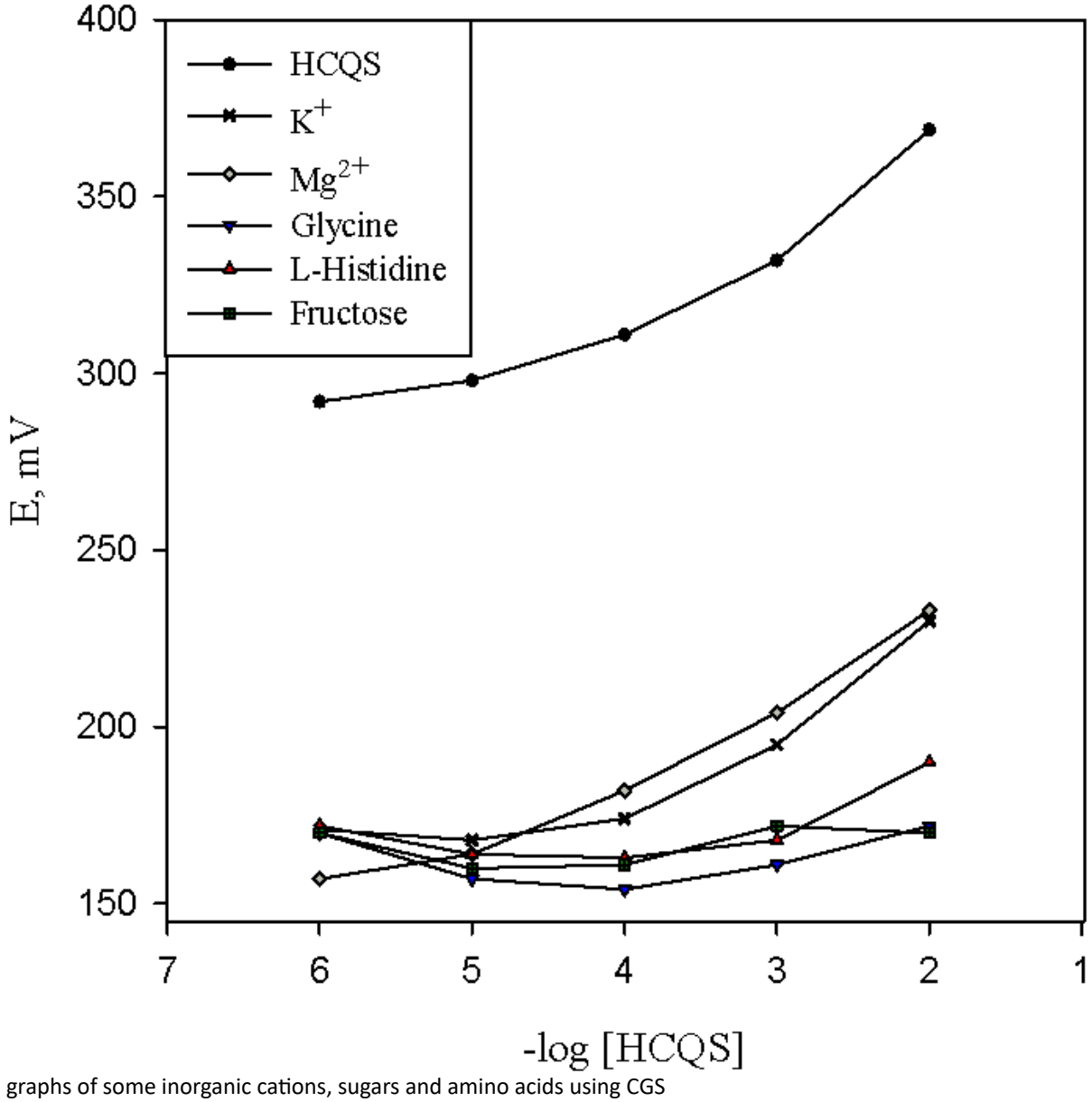

Fig. 6: Calibration graphs of some inorganic cations, sugars and amino acids using CGS

3. 6 Analytical applications:

The investigated sensor was successfully used for the potentiometric determination of hydroxychloroquine sulfate in pure solutions by applying standard addition method. The obtained average recovery and relative standard deviation values are summarized in Table 2, which reflect the high accuracy and precision of the sensor.

Table 2. Determination of hydroxychloroquine sulfate in pure solution applying the standard addition method

Sample	Standard addition method			
	Taken mg	Found mg	Mean Recovery %	RSD %
Pure solution	CGS			
	0.22	0.21	99.00	0.35
	2.17	2.12	97.72	0.51
	21.69	21.26	98.00	0.71

RSD: Relative standard deviation (four determinations)

4. Comparison with reported methods:

This study was compared with some previously published data [23]. Results of this study showed the wider linear range ($9.3x10^{-5}$-$1.0x10^{-2}$ mol L^{-1}) and near Nernstian slope (28.5 mV/ decade). It is less expensive than those methods. The data are given in Table 3, thus proving that it is a good [HCQS] ion selective electrode for the pure drug and with high accuracy, and precision.

Table 3. Comparison of the proposed HCQS ion-selective electrode method with published methods

Method	LR mol L^{-1}	LOD mol L^{-1}	r2	RSD %	Ref
Spectrophotometry	4.0×10^{-6}–2.0×10^{-5}	2.3×10^{-5}	0.9999	0.36	[22]
Pulse differential voltammetry	2.0×10^{-5}–5.0×10^{-4}	2.6×10^{-5}	0.9999	0.46	[22]
Potentiometric sensor CGS	$9.3x10^{-5}$-$1.0x10^{-2}$	$4.7x10^{-5}$	0.9997	0.49	[P.W]

r^2: Correlation coefficient

P.W: Present work

Conclusion

The proposed sensor based on PTA as the electroactive compound can be used as an interesting alternative analytical tool for determination of hydroxychloroquine in pure solutions. The sensor showed a Nernstian slope of 28.5±0.2 mV $decade^{-1}$, a wide concentration range from 9.3×10^{-5} to 1.0×10^{-2} mol L^{-1}, a low detection limit of 4.7×10^{-5} mol L^{-1} and a short response time (≤ 10 s) over the pH range 2.0-7.0. The proposed potentiometric method offers the advantages of simplicity, adequate selectivity, accuracy (mean recovery reached 98.24%), precision (RSD reached 0.49) and low cost.

Acknowledgment

It is our pleasure to express deep gratitude and sincere appreciation to Chemistry Department, Faculty of Science , Beni-Suef University, Egypt.

Declaration

On behalf of all Co-Authors, the corresponding Author, states that this research work is original and has not been published in whole or in part elsewhere.

Authorship (author(s) contribution or attribution)

M. M. Khalil and S. M. Mostafa suggested the problem and wrote the manuscript.

A. A. Masoud carried the experimental part of the manuscript.

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