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Detection of Colchicine using *p*-Sulfonated Calix[4]arene Modified Electrode

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Complex characteristics of *p*-sulfonated calix[4]arene (SC4A) and colchicine were examined using various techniques. Electrochemical impedance spectroscopy results enabled observation of the colchicine-SC4A interaction, and indicated that SC4A had high sensitivity to colchicine with a detection limit (S/N = 3) of 2×10^{-9} mol L^{-1}. Cyclic voltammetry results indicated that the structural matching effect and the electrostatic interaction were the dominant stabilizing factors for the host-guest complexes of colchicine and SC4A. Molecular mechanics simulation showed that the benzene ring of colchicine entered the SC4A cavity. The sensor enabled selective determination of colchicine even in the presence of common interferences. The results indicated that it was more difficult to oxidize the non-electrochemically active complexes. This study showed that *p*-sulfonated calix[4]arene can be used in the detoxification of colchicine poisoning and may be effective in the clinical treatment of colchicine poisoning in the future.

Keywords: *p*-Sulfonated calix[4]arene, Colchicine, Electrochemical impedance spectroscopy, Cyclic voltammetry, Detoxification.

INTRODUCTION

Calixarenes are cup-like organic macrocyclic molecules in which phenol rings are connected at *ortho*-condensation of *para*-substituted phenols by formaldehyde. Calixarene has no electrochemical activity, however, modified calixarene derivatives with electroactive groups may be used to form selective functionalized electrodes [1-3]. Inclusion complexes have found pharmaceutical applications because of increased aqueous solubility of drugs and the stability of host-guest complexes [4] during the oxidation or reduction of host and guest molecules. Water soluble calix[n]arene derivatives have received considerable attention in recent years because they have both a hydrophobic environment with the favourable properties and also hydrophilic heads which enable them to form water soluble encapsulated complexes.

There are many advantages to using calixarenes as host molecules among these advantages are weak London forces, hydrogen bonding and dipole-dipole moments, which play important roles in complex formation and drug release. A new electrochemical sensor, based on *para*-sulfonatocalix[6]arene-modified silver nanoparticles was fabricated using a one-step electrodeposition approach [5]. Varmira *et al.* [6] modified a novel enzymatic electrochemical biosensor for use in determination tyrosine in some food samples. Raoof *et al.* [7] studied the application of electrocatalytic reduction of H_2O_2 with *p*-isopropyl calix[6]arene matrix using silver nanoparticles supported on a glassy carbon electrode. Calixarene derivatives are not cytotoxic and have antiviral activity at concentrations from 10 to 50 μmol L^{-1}. Sulfonated calixarene has high electrochemical activity that does not require the presence of other electrochemically active groups on the host calixarene.

Colchicine is an alkaloid present in plants of *Liliaceous colchicum* and has many pharmacological uses, such as antigout, antitumor and antihepatitis, it also has a specific effect on some cancers. Moreover, colchicine is a low-cost alkaloid that has been used in medicine for many years. It is used mainly in the treatment of acute gouty arthritis [8,9] and is also valuable for other diseases, like tumors [10], Familial Mediterranean fever and hepatic cirrhosis. However, colchicine has negative effects on the digestive tract and these effects may be fatal. Colchicine is a main component of day lilies and is non-toxic, but is metabolized *in vivo* to produce toxic colchicine derivatives. Methods used to determine colchicine have been

widely reported and include fluorescence spectrophotometry [11,12], circular dichroism spectroscopy [13] and electrochemistry [14]. However, most of the methods used to determine colchicine have focused on *in vivo* metabolism of colchicine to more toxic substances, whereas effective inhibition of oxidation reaction has been less studied.

In the present study, a sensor was developed for the simple, sensitive and selective determination of colchicine in urine samples. The experimental parameters together with the analytical application and selectivity behaviour of *p*-sulfonated calix[4]-arene were investigated in detail. In addition, analytical application in actual samples and anti-interference performance of the sensor are discussed. The interaction mechanism of *p*-sulfonated calix[4]arene and colchicine was studied using electrochemistry. *p*-Sulfonated calix[4]arene effectively inhibited the oxidation of colchicine (Fig. 1). This research shows that *p*-sulfonated calix[4]arene can be used in the detoxification of colchicine poisoning and may be effective in the clinical treatment of colchicine poisoning in future.

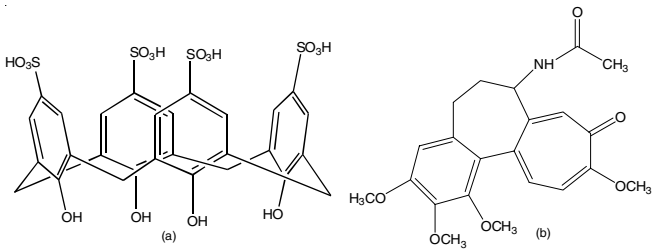

Fig. 1. Structure of (a) *p*-sulfonated calix[4]arene and (b) colchicine

EXPERIMENTAL

A scanning electron microscope (SEM, JSM-7500F) was used to observe the nanostructures and morphology of the prepared compounds. FTIR spectra were recorded over the range of 4000-400 cm^{-1} on a Spectrum One FTIR spectrometer (Agilent 600 spectrometer). Laser Raman spectra was recorded using a Renishaw Raman microscope system equipped with a 785 nm laser line, an electrically refrigerated CCD camera, and a notch filter to eliminate the elastic scattering. The spectra shown in this paper were obtained by using a 50× objective. Spectral scanning conditions were chosen to avoid sample degradation and the reported spectra are single scans.

All the electrochemical data were carried out using a LK2005A electrochemical workstation (Tianjin, China). A traditional three-electrode system was used. A modified gold electrode was used as the working electrode, a saturated calomel electrode (SCE) was used as reference electrode and a platinum wire was used as a counter electrode. All the pH values were measured with a pHS-3TC digital precision pH meter (Shanghai, China). Molecular modeling was used for optimization at the B3LYP/6-31G(d) level of density functional theory using the Gaussian 03 program.

Colchicine (content > 98.0 %) was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). *p*-Sulfonated calix[4]-arene was prepared according to a published procedure [15] and the compound was identified using IR, ^1H NMR and elemental analysis. All other reagents were of analytical grade, and were used as received. The colchicine and *p*-sulfonated calix[4]arene stock solution of 1.0×10^{-4} mol L^{-1} were prepared in double-distilled water respectively. A buffer solution of KH_2PO_4-Na_2HPO_4 (0.1 mol L^{-1}) was prepared.

RESULTS AND DISCUSSION

SEM analysis: Fig 2a shows SEM images of *p*-sulfonated calix[4]arene (SC4A), which has irregular quadrate-shaped molecular islands. As seen in Fig. 2b, colchicine grows in a regular mass structure. It is obvious from the image that in the complex formation, colchicine was enclosed in SC4A and became sliced because of the presence of colchicine molecules in them (Fig. 2c).

FTIR analysis: FTIR spectra of SC4A, colchicine and SC4A-colchicine complex are shown in Fig. 3. Fig. 3a shows that peaks of $-C_6H_5$, S=O and O-H shift from 1670 cm^{-1} to 1300 cm^{-1}, 1200 cm^{-1} and 1050 cm^{-1}, 3400 cm^{-1}, respectively. Fig. 3b shows peaks in the range from 1600 to 1300 cm^{-1} that correspond to aromatic ring stretching. These peaks were assigned to $-CH_3$ and $-CH_2$ stretching at 3260, 3059, 2937, and 2845 cm^{-1}. The strong peak in the region of 3434 cm^{-1} was assigned to N-H stretching. The IR spectra shown in Fig. 3a and 3c are very similar and this suggests that the absorption peaks of colchicine disappeared and formed the complexes.

Raman analysis: Raman spectroscopy is a non-destructive tool that is often used to evaluate the quality of many materials. As seen in Fig. 4a, Raman spectrum of SC4A exhibits a prominent peak at 1368 cm^{-1}. For colchicine , the spectrum (Fig. 4b) shows bands from 100 cm^{-1} to 1650 cm^{-1}. Fig. 4c shows a broad peak at 1368 cm^{-1}, which indicates that complexes formed. Raman spectra provided further evidence of interaction between SC4A and colchicine in the composite as seen in Fig. 4c.

EIS analysis: Electrochemical impedance spectroscopy (EIS) is an informative, non-destructive method and is used to

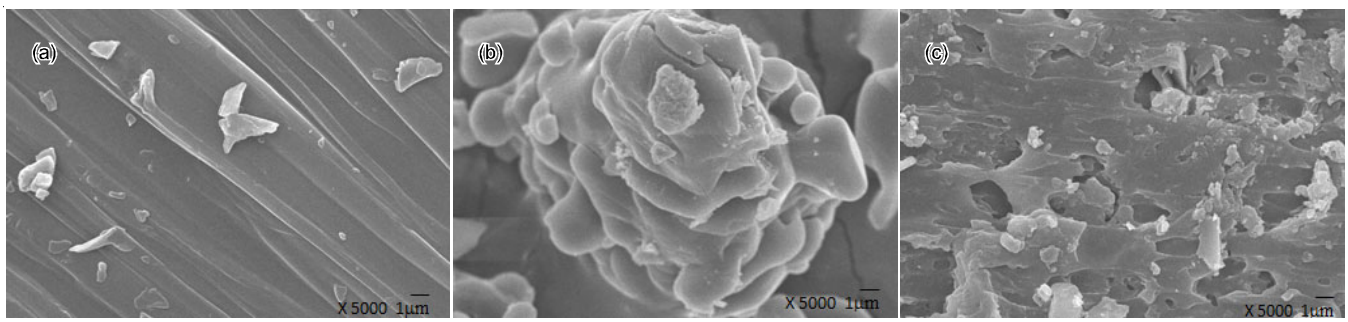

Fig. 2. SEM images of (a) SC4A, (b) colchicine and (c) SC4A- colchicine complex

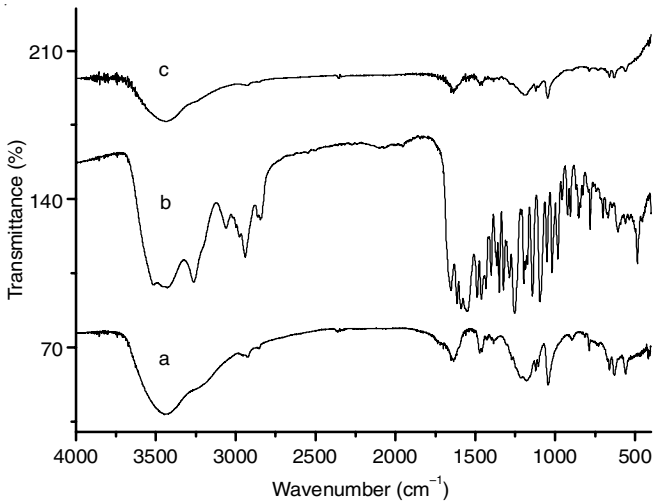

Fig. 3. Infrared spectra of (a) SC4A, (b) colchicine and (c) the SC4A-colchicine complex

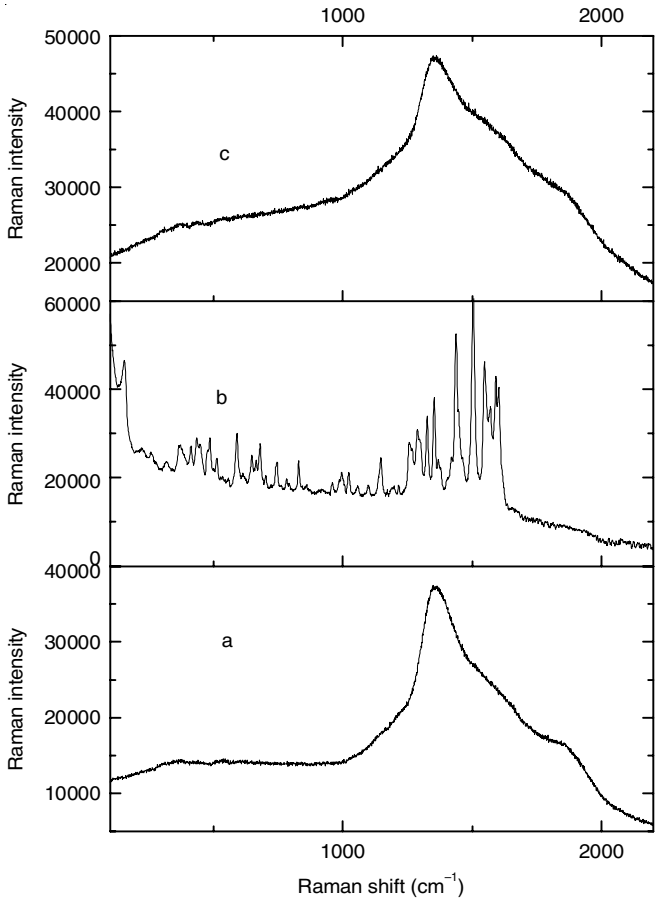

Fig. 4. Raman spectra of (a) SC4A, (b) colchicine and (c) the SC4A-colchicine complex

monitor the preparation process and to determine properties of the modified electrode [16-18]. The experimental impedance spectra were fitted by computer simulation using an equivalent electronic circuit based on Randles-Ershler theoretical model [19,20]. Fig. 5a shows the Nyquist plot of bare gold electrode, and that of SC4A modified gold electrode in PBS solution. The EIS of bare gold electrode is a nearly straight line and this suggests a diffusion-controlled step in the electrochemical process. With the modification of electrode using SC4A, the semi-circle diameter increased remarkably and the impedance increased. The respective semi-circle diameter corresponds to the electron transfer resistance at the electrode surface. After suitable circuit and calculation, the diameter of semi-circle was $R_1 = 6.59$ kΩ cm^2. As seen in Fig. 5b, a Randles circuit shows the impedance spectra. This result suggests that SC4A can act as an inert electron and mass transfer-blocking layer, which also clearly proves that SC4A is successfully immobilized on the electrode surface.

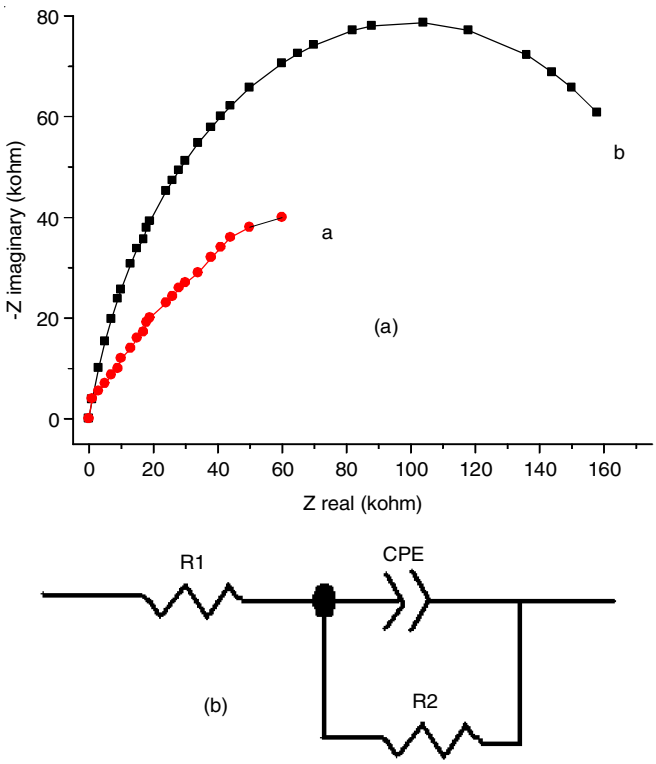

Fig. 5. (a) Nyquist plots of the bare gold electrode and the SC4A modified gold electrode in PBS solution; (b) Randles circuit

Optimization of experimental conditions: The experimental parameters together with the analytical application and selectivity behaviour of *p*-sulfonated calix[4]arene were investigated. Influences of supporting electrolytes were investigated in $HClO_4$, H_2SO_4, HNO_3, CH_3COOH-CH_3COONa buffer, phosphate buffer solution and B-R buffer solution, respectively. The results show that voltammetry peaks were observed in all of these electrolyte solutions, but among these solutions, the electrochemical responses in 0.1 mol L^{-1} PBS solution had the best shape and largest stripping peak current. Therefore, this supporting electrolyte was used in subsequent experiments.

The pH of a solution is an important parameter that affects the stability and performance of the modified electrodes. For electroanalytical purposes, both maximal and stable currents are necessary, thus, we studied the effects of pH on the response of the modified electrodes. The SC4A-modified gold electrode was analyzed using impedance spectroscopy at different values of pH ranging from 6.1 to 7.8 (Fig. 6). The results show that a maximum current is observed at a pH of 7.3 for colchicine detection in 0.1 mol L^{-1} PBS. Therefore, pH 7.3 was selected as the optimal pH for subsquent studies.

Colchicine-SC4A interaction: Different concentrations of colchicine were injected into the PBS buffer solution to study

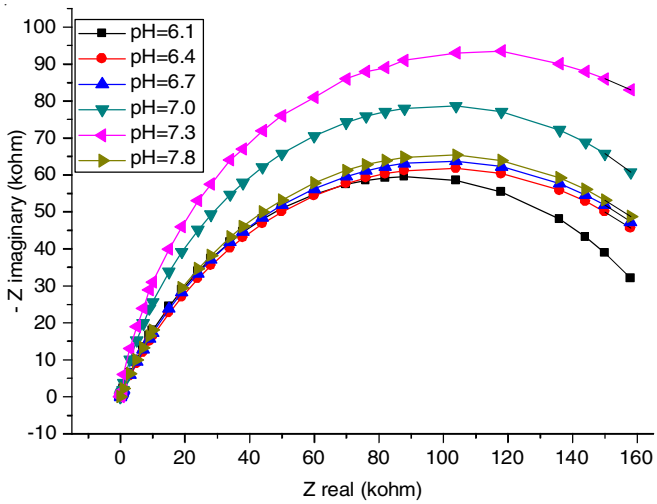

Fig. 6. Nyquist plots of the gold electrode modified by SC4A in 0.1 mol L^{-1} PBS at different pH values from 6.1 to 7.8

SC4A-colchicine interactions. Fig. 7 shows the Nyquist plots for different concentrations of colchicine detected using SC4A in 0.1 mol L^{-1} PBS buffer at pH 7.3. The diameter of semicircle increased with an increase in colchicine concentration, and this indicates that colchicine complexion to SC4A increased the resistance of the layer. *p*-Sulfonated calix[4]arene had the best sensitivity with a minimum detectable concentration of 8×10^{-9} mol L^{-1}. Based on the signal-to-noise ratio of 3 (S/N), the detection limit was 2×10^{-9} mol L^{-1}.

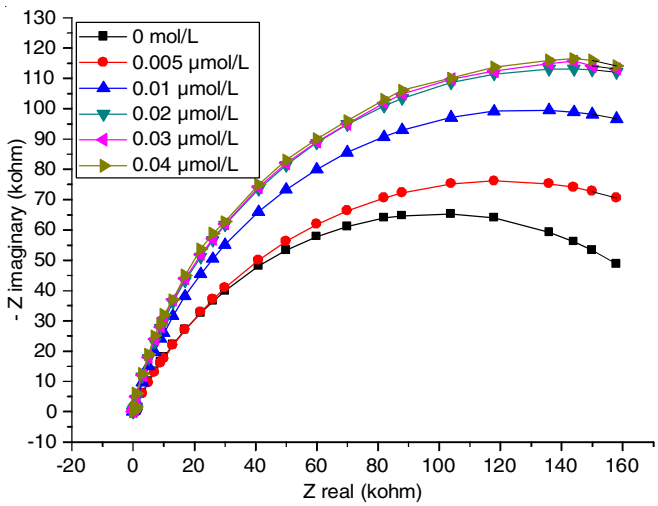

Fig. 7. Nyquist plots of different colchicine concentrations detected using SC4A in PBS buffer at pH 7.3

Cyclic voltammetry: Cyclic voltammetry is also a valuable and convenient tool for monitoring the barrier of a modified electrode. Therefore, it was chosen as a factor for investigating changes in the behaviour of the modified electrode. To further examine colchicine and SC4A immobilization on the gold surface, cyclic voltammetry curves were recorded in a 5 mmol L^{-1} solution of redox couple $(Fe(CN)_6)^{4-/3-}$ prepared in PBS buffer. The scanning potential was varied between –0.5 V and 1.4 V at a pH of 7.3 in 0.1 mol L^{-1} PBS.

Colchicine has electrochemical activity. Fig. 8 shows the cyclic voltammogram of colchicine changed with SC4A at pH 7.3. The electrochemical behaviour of colchicine-SC4A

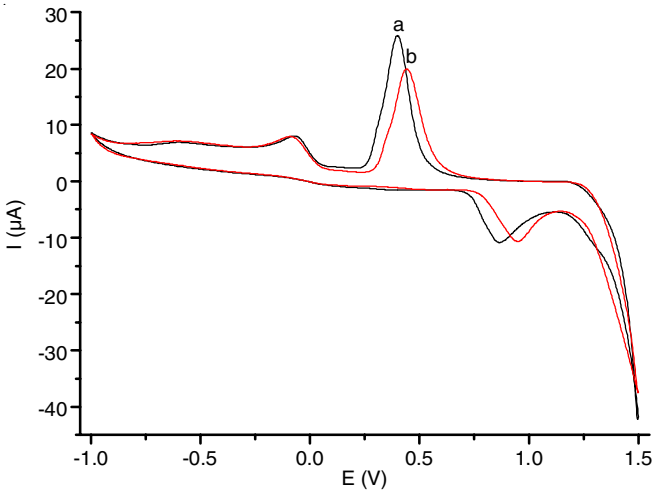

Fig. 8. Cyclic voltammograms of colchicine on the SC4A modified electrode (a) colchicine and (b) the SC4A-colchicine complex in 0.1 mol L^{-1} KH_2PO_4-Na_2HPO_4 buffer solution at 25 °C; Scan rate: 0.1 V/s

complex was studied *in vivo*. The oxidation and reduction peaks of colchicine were not symmetrical, which indicates that colchicine had an irreversible voltammogram. Colchicine had a sensitive oxidation peak in the range of +0.5 V to +1.5 V and the peak potential was + 0.86 V. However, the cyclic voltammogram of *p*-sulfonated calix[4]arene did not have an oxidation peak in this range. When *p*-sulfonated calix[4]arene was added to colchicine, the oxidation peak current of colchicine decreased correspondingly and the oxidation peak potential showed a positive shift. These results indicate that non-electrochemically active complex that formed was more difficult to oxidize [21]. Therefore, using *p*-sulfonated calix[4]arene to reduce toxicity or to detoxify colchicine should be considered.

This may be attributed due to the interaction between the $-NH_2^+$- of colchicine and the $-SO_3^-$ of calixarene, high electron density and the cavity size of SC4A. Therefore, the structural matching effect and electrostatic interaction were the dominant stabilizing factors for the host-guest complex of colchicine and SC4A.

Interference study: To evaluate the selectivity of constructed colchicine sensor, the influences of several interfering agents *e.g.*, urea, sucrose, galactose, boric acid, oxalic acid, L-lysine, L-asparagine, glycine, phenylalanine, glutathione, L-cysteine, ascorbic acid, Mg^{2+}, Ca^{2+}, Na^+, K^+ and lactase on the determination of 1×10^{-5} mol L^{-1} colchicine under the optimal experimental conditions were investigated. The results are listed in Table-1. The tolerance limit was computed as the maximum concentration of the interfering agent that caused a relative error of approximately ± 5 % in determination the colchicine concentration. The data shows that current *p*-sulfonated calix[4]-arene modified electrode was highly selective for detecting colchicine.

Reproducibility and stability of SC4A modified gold electrode: The reproducibility of SC4A gold modified electrode was respectively estimated using eight modified electrodes that were prepared under the same conditions and used to detect the current response of 1×10^{-6} mol L^{-1} colchicine. The results show that these electrodes had satisfying reproducibility with relative standard deviations (RSD) of 1.38 % for SC4A for 50

TABLE-1
EFFECT OF POTENTIAL INTERFERING SPECIES ON THE DETERMINATION OF COLCHICINE AT A CONCENTRATION OF 1×10^{-5} mol L^{-1}

Interference	Interference concentration (10^{-5} mol L^{-1})	Found[a] (10^{-5} mol L^{-1})	Relative error (%)	Recovery (%)
Mg^{2+}	0.01	1.02	+2.00	102
Ca^{2+}	0.01	1.03	+3.00	103
Na^{+}	0.01	1.05	+5.00	105
K^{+}	0.01	0.95	–5.00	95
L-lysine	0.01	0.98	–2.00	98
L-asparagines	0.01	1.01	+1.00	101
Ascorbic acid	0.01	1.04	+4.00	104
Glycine	0.01	0.99	–1.00	99
Phenylalanine	0.01	0.96	+4.00	104
L-cysteine	0.01	1.025	+2.50	102.5
Urea	0.001	1.008	+0.80	100.8
Sucrose	0.001	0.988	–1.20	98.8
Lactase	0.001	1.016	+1.60	101.6
Galactose	0.001	1.028	+2.80	102.8
Boric acid	0.001	1.015	+1.50	101.5
Oxalic acid	0.001	1.017	+1.70	101.7
Glutathione	0.001	0.986	–1.4	98.6

[a]Average of 5 times of determination.

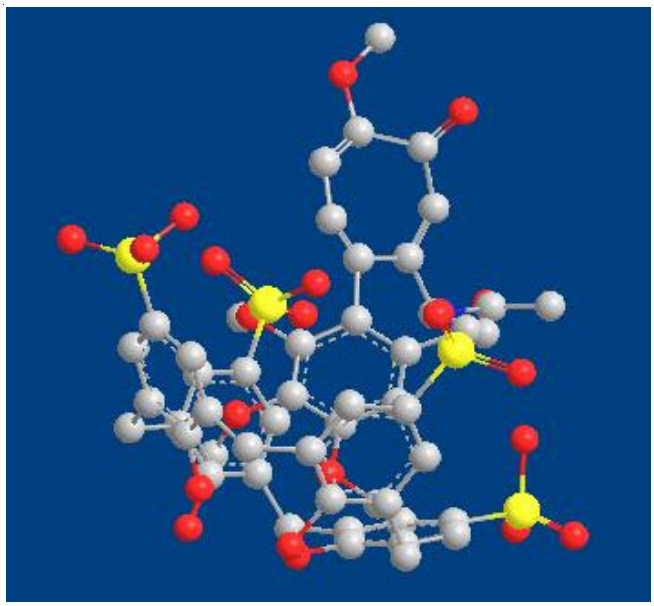

Fig. 9. Lowest energy structure of the colchicine and SC4A complex using ball and stick model determined using molecular dynamics simulation with direct minimization

successive measurements. The storage stability of each electrode was studied by storing them at 4 ºC in a refrigerator when not in use and intermittently measured [22]. After 5 days, the SC4A gold modified electrode still retained 96.8 % response. After 30 days, the response current of the modified electrode remained at around 91.3 %.

Applications of modified sensor: The proposed method was also applied in determining colchicine in urine samples. The results are summarized in Table-2. The results suggest that the modified electrode can be used for determination colchicine in urine samples with satisfactory recoveries of 95 to 104.5 %, and a relative error of not more than 5.5 %. Good recoveries of the samples indicate that proposed electrodes can be successfully used to detect colchicine in real samples. This method had good accuracy and high sensitivity.

Molecular modeling calculation: Molecular modeling calculation was used for optimization at the B3LYP/6-31G(d) [23] level of the density functional theory [24] by using Gaussian 03 program [25]. Molecular mechanics simulations were used to obtain the optimized conformation of host-guest complex (Fig. 9). The energy-minimized structure revealed that the interaction between the benzene ring of colchicine and the negative charge of sulfonyl groups of SC4A dispersed the electron cloud density, and thus SC4A could partially accommodate the benzene ring of colchicine with a tilted conformation. These results are consistent with the previous cyclic voltammetric data.

Conclusion

Numerous techniques have focused on the ability of *p*-sulfonated calix[4]arene to form complexes with colchicine. Scanning electron microscopy and Fourier transform infrared and laser Raman spectroscopies were used to study the organization and molecular structure of different layers of the electrode surface. The modifications were characterized using electrochemical impedance spectroscopy, cyclic voltammetry and molecular modeling calculation. Both *p*-sulfonated calix[n]-arene (SC4A) and colchicine have electrochemical activity, and when *p*-sulfonated calix[4]arene was added to colchicine, the oxidation peak current of colchicine decreased and the oxidation peak potential showed a positive shift. The results indicate that a non-electrochemical active complex formed that was more difficult to oxidize. Under optimal conditions, the sensor detected colchicine with a limit of detection of 2×10^{-9} mol L^{-1}. The sensor

TABLE-2
COLCHICINE CONTENT DETERMINATION IN URINE SAMPLES DETERMINED USING THE SC4A MODIFIED ELECTRODE

Content determined by local hospital (10^{-7} M)	Content determined by current method (10^{-7} M)	Relative error (%)	Added (10^{-7} M)	Found[a] (10^{-7}M)	Recovery (%)
5.05	5.12	+ 1.39	2.00	7.21	104.5
5.54	5.48	– 1.08	2.00	7.49	100.5
3.25	3.32	+ 2.15	2.00	5.30	99.0
4.26	4.28	+ 0.47	2.00	6.33	102.5
3.55	3.61	+ 1.69	2.00	5.51	95.0
3.98	3.92	–1.51	2.00	6.00	104.0
3.87	3.79	– 2.07	2.00	5.82	101.5
5.42	5.41	– 0.18	2.00	7.43	101.0
3.22	3.05	– 5.27	2.00	5.03	99.0
5.76	5.79	+ 0.52	2.00	7.76	98.5

[a]Average of 5 times of determination.

was able to selectively determine colchicine even in the presence of common interferences. Therefore, the sensor was highly selective. The sensor also shows good operational stability, sensitivity, repeatability and reproducibility. Molecular mechanics simulation showed that benzene group of colchicine partially penetrated the hydrophobic cavity of water-soluble *p*-sulfonated calix[4]arene (SC4A). The possible complexation mechanism of colchicine and SC4A may involve the structural matching effect and electrostatic interactions. Therefore, using *p*-sulfonated calix[4]arene to reduce toxicity or to detoxify colchicine should be considered.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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