Determination of Benzydamine Hydrochloride in Serum and Urine by Using a Benzydamine Ion-Selective Piezoelectric Sensor

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An ion-selective piezoelectric sensor (ISP) was developed for the rapid determination of benzydamine hydrochloride in serum and urine. The ISP device was fabricated by coating a PVC membrane containing benzydamine phosphotungstate on one electrode of a thickness-shear mode piezoelectric quartz crystal. The selective adsorption of benzydamine ion across the membrane caused a decrease in the oscillating frequency; also the logarithm of the frequency shift has a linear relation to the logarithm of the concentration of benzydamine over the ranges 2×10^{-7} – 8×10^{-3} M with a detection limit of 2.0×10^{-7} M at pH 7.0. Influencing factors were investigated and optimized. The results for real samples obtained by the proposed method were in agreement with those by the conventional non-aqueous titrimetric method.

Keywords Ion-selective piezoelectric sensor, benzydamine, pharmaceuticals

Benzydamine hydrochloride 1-benzyl-3-[3-(dimethyl-amino)propoxy]-1*H*-indazole is a common drug of antipyretic, anti-inflammatory and analgesic effects.¹ For the purpose of a pharmacokinetics study and quantitative control, a measurement of benzydamine's concentration in body fluids is necessary in a clinical assay. A number of analytical methods for the assay of benzydamine have been proposed, *e.g.*, a non-aqueous titration method¹, which suffers from a tedious time-consuming operation process, and is interfered by many organic impurities. Ultraviolet spectroscopy² has a narrow response range and lacks selectivity. Fourier-transform near-infrared spectroscopy³ requires more special and expensive instruments. Liquid chromatography, especially high-performance liquid chromatography (HPLC), with peroxyoxalate chemiluminescence or spectrophotometric detection^{4,5} has been proven to be sensitive and less susceptible to interference. However, a tedious sample clean-up procedure, including extraction and purification, is always required before sample injection onto the HPLC. The all-solid-state benzydamine ISE method⁶ has the advantage in that its equipment as well as the reagent required are simpler and less expensive without any sacrifice of sensitivity or precision. A drawback, however, is that its response slope is affected by the background solution to some extent.

Since a glass electrode for pH measurements was discovered at the beginning of the present century^{7,8}, ion-selective electrodes have developed to be one of the most useful tools for rapid analysis or on-line monitor-

ing in contemporary analytical chemistry. Freiser *et al.*^{9,10} proposed ion-selective electrodes based on incorporating the cation in a plasticized PVC film for the determination of pharmaceutical compounds; however, there are still some limitations to the sensing membranes of such electrodes. Thus, the development of a rapid specific method other than potentiometry for ion-selective sensing is necessary.

Using piezoelectric quartz crystal (PQC) sensors has been an efficient method in analytical chemistry owing to their sensitive frequency response. When an AT-cut PQC was immersed into solution, the frequency is shifted along with changes in such properties as the conductivity, viscosity, density and temperature of the solution as well as the mass adsorbed/desorbed on the quartz plate. By sealing one face of the crystal and using a background solution and a thermostated water bath, the frequency response of PQC can be considered to only arise from a mass change of the crystal surface; it is a well-known quartz crystal microbalance (QCM) that was commonly employed to measure a minute mass change based on the Sauerbrey equation.¹¹ Compared with the conventional detection methods, such as mass-response microgravimetry, QCM exhibits high sensitivity in mass change and a rapid response. On the other hand, it is simple to fabricate and convenient to operate. It is not surprising that the QCM has recently been used so widely, *e.g.*, for affinity-based chemical sensing in liquid phases¹²⁻¹⁴, especially, for biochemical and physiological studies in life science.¹⁵⁻¹⁸

Neither PQC's mass response nor pure PVC's adsorption exhibits selectivity. When a PVC membrane con-

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taining a certain chemical activant is immobilized on the PQC's surface, some properties of the membrane will be changed, and the PQC sensor will be more useful in an aqueous solution. Various response mechanisms of solid-membrane ion-selective electrodes (ISEs) at an insoluble (scarcely soluble) salt/aqueous interface have been reported by some research groups.¹⁹⁻²¹ When a modified electrode was immersed into a certain related aqueous solution, a minute portion of a scarcely soluble salt could be dissolved into solution; that is to say, a few ions would diffuse away from the film. Generally speaking, there is always a kind of ion which is preferentially dissolved and diffuses away from the film, which related to the radius and charge number of the ion. The preferential ion dissolution generates some "charge vacancies" in the film and causes charge separation at the solid/aqueous solution interface, which leads to the destruction of equilibrium, including the charge and activity of ions between the solution and the film. To fill the "charge vacancies" and to neutralize the charges at the surface of the film and to establish a new equilibrium, selective adsorption is carried out at the film surface.

Based on the aforementioned basic principle, an all-solid-state benzydamine ion-selective piezoelectric sensor was constructed and applied to a real sample assay by exploiting a modified 9 MHz AT-cut piezoelectric quartz crystal.

Experimental

Apparatus

A schematic diagram of the present sensing device is illustrated in Fig. 1. The frequency changes produced by adsorption were measured with a universal frequency counter (Model SC-72001, Iwatsu, Japan). The piezoelectric quartz crystal (JA-5, made in Peking Factory No. 707) used was a 9-MHz AT-cut crystal (12.5-mm diameter) having silver electrodes (6-mm diameter) on both sides. As shown in Fig. 1, the quartz crystal was fixed in a detection cell made of PTFE, in which only one side of the quartz crystal was allowed to have contact with the aqueous sample solution. The crystal holder was directly connected to an IC-TTL oscillating circuit, whose design was given in a previous paper.²² The oscillating circuit was supplied by a d.c. voltage regulator (JWY-30B Model, Shijiazhuang Electronic Factory No. 4), and the working voltage was set at 5 V. A computer (Model 4192A, Hewlett-Packard) was used for data analysis, and temperature control ($25 \pm 0.1^\circ\text{C}$) was achieved using a constant-temperature water bath.

Reagents

All chemicals used were of analytical grade or chemical-pure grade. Benzydamine hydrochloride, obtained from Peking Medicine Factory (China), was of >99.0% purity. Double-distilled water was used throughout.

Fig. 1 Schematic diagram of the benzydamine ISP sensor modified with a benzydamine phosphotungstate PVC membrane.

Standard solution

A stock standard solution of benzydamine hydrochloride was prepared by dissolving benzydamine hydrochloride in double-distilled water and consequent diluting with a 0.1 M NaNO_3 background solution of pH 7.0.

Preparation of ion-pair complexes

A 0.01 M solution of phosphotungstate acid was slowly dropped into 50 ml of a 0.01 M solution of benzydamine hydrochloride under stirring until the precipitation was completed. Then, the precipitate was filtered off on a porosity-4 sintered-glass crucible and washed several times with double-distilled water; after adding several drops of ethanol, it dried under a vacuum.

ISP sensor construction

The ISP sensor was constructed as follows: benzydamine phosphotungstate (BPT), prepared by the aforementioned method, was dissolved in a minimum amount of dibutyl phthalate; the concentration of this resulting solution was 0.002 M. A 0.34 ml portion of this solution of ion-pair complex was mixed with 3 ml of a 1% solution of poly(vinylchloride) (PVC) in tetrahydrofuran, a drop of which was spread on the surface of a Ag electrode of the quartz crystal. Tetrahydrofuran was slowly evaporated at room temperature from a mixture placed on the surface of the Ag electrode. A transparent uniform membrane was formed on the crystal surface. When not in use, the modified sensor can be stored in air.

Procedure for examining the ion-selective sensor

A modified electrode was activated by immersing into a 0.001 M solution of standard benzydamine hydrochloride for 30 min; it was then washed with double-distilled water until a steady frequency was achieved. To eliminate the effect of the membrane thickness, the same modified sensor was used repeatedly to complete a series of measurements. A 0.1 M NaNO_3 solution (pH=7.0) was used as a background

solution.

After activation, a steady resonant frequency (f_i) in the background solution was obtained and a series of standard sample solutions was injected in order to raise the concentration of benzydamine hydrochloride in the background solution. After each addition, the resonant frequency (f_m) of the ISP sensor was measured and the respective frequency change was calculated:

$$\Delta f = f_m - f_i. \quad (1)$$

Results and Discussion

Theoretical considerations

The proposed method is based on the selective adsorption/desorption of benzydamine ion across a BPT-PVC membrane. When any ion is adsorbed/desorbed into/from the modified membrane, or several ions with different molecular weight exchange with one another across the membrane, the surface mass change of the modified membrane can be measured by an ISP sensor, even though these changes may be very small. For the AT-cut quartz crystal, considering the general case of overtone, the relation²² between the mass change with a frequency shift can be expressed as

$$\Delta f = -2.26 \times 10^6 f_0^2 h \Delta m / A, \quad (2)$$

where Δf is the change in the oscillation frequency, h the overtone order, $\Delta m/A$ the change in the mass of the membrane per unit area, and f_0 the oscillation frequency of the fundamental mode of the quartz crystal. Hence, the mass change can be expressed by the frequency shift. On the other hand, when the sensor is immersed into a benzydamine solution, an equilibrium is established between the two phases. Under a low concentration (≤ 0.01 M), the relation between the concentration of the tested ion and the adsorbed quantity, namely the mass change of the modified membrane, can be expressed by the Freundlich isotherm²³ using the equation

$$\Delta m = K_f C^{1/n}, \quad (3)$$

where n and k are constants and $n > 1$. Combining Eqs. (2) and (3), we obtain

$$\Delta f = -2.26 \times 10^6 f_0^2 K_f h C^{1/n} / A. \quad (4)$$

For a given AT-cut quartz crystal, the area A is a constant, with the following mathematical consequence:

$$\Delta f = K' C^{1/n}, \quad (5)$$

in which $K' (= 2.26 \times 10^6 f_0^2 K_f h / A)$ is a constant. Its logarithmic form is

$$\log(-\Delta f) = 1/n \log C + \log K'. \quad (6)$$

Sensor performance

As described above, a number of experiments have been carried out using the ISP sensor. Figure 2 shows a typical course of the response of a sensor modified with BPT-PVC in order to change the concentration of benzydamine ion in the sample solution. Along with an increase in the benzydamine concentration, the sensor frequency gradually decreases. In every concentration platform there exists an increasing trend in frequency, due to competitive adsorption at the sensor surface. After equilibrium is established, the resonant frequency becomes stable.

After every experiment (a series of concentrations were detected), the ISP sensor is recovered by washing with distilled water. The adsorbed benzydamine

Fig. 2 Course of the observed frequency response of the benzydamine ISP sensor with sample-solution injections. Final concentration of benzydamine hydrochloride (M): (A) 0, (B) 2×10^{-7} , (C) 2×10^{-6} , (D) 2×10^{-5} , (E) 2×10^{-4} , (F) 2×10^{-3} , (G) 4×10^{-3} , (H) 6×10^{-3} , (I) 8×10^{-3} (all containing 0.1 M NaNO_3 , pH=7.0).

Fig. 3 Influence of the solution pH on the frequency response of the sensor: (○) 2×10^{-3} M benzydamine hydrochloride; (◇) 2×10^{-4} M benzydamine hydrochloride.

Table 1 Effect of the activant concentration in a modified membrane

$C_m(\text{M})$	2×10^{-3}	7×10^{-3}	2×10^{-4}	7×10^{-4}	2×10^{-5}	7×10^{-5}
$C_L(\text{M})$	7.5×10^{-7}	4.5×10^{-7}	2×10^{-7}	6×10^{-7}	9×10^{-7}	1.1×10^{-6}

C_m : activant concentration in the modified membrane; C_L : lower limit of the linear response range of the benzydamine ISP sensor modified with BPT-PVC.

species can be easily washed off from the modified membrane.

pH effect

The pH dependence of the responses of the sensor was investigated by measuring the frequency shift of the ISP sensor in the benzydamine solution in which the pH was adjusted by adding appropriate amounts of hydrochloric acid or a sodium hydroxide solution using a 50 μL microsyringe. Figure 3 shows the influence of pH on the response of the BPT-ISP sensor in 1 mM and 0.1 mM benzydamine hydrochloride solution containing 0.1 M NaNO_3 . The pH range is where the frequency shift remains constant at $\text{pH} > 6$, while it decrease slightly at $\text{pH} < 6$. This is due to a shift of the equilibrium between benzydaminium and free benzydamine in the solution:

Taking the situation of the human body, $\text{pH} = 7$ was adopted in our work.

Membrane-concentration effect

The effect of different concentrations of the activant material in the PVC on the sensor response was investigated. A series of sensors with different activant concentrations in the membrane were constructed in order to study the relation between the frequency response and the different membrane concentrations. The results given in Table 1 show that the optimum concentration was 0.2 mM. Both higher and lower concentrations cause an increase in the lower limit of the linear-response range for sensors. In addition, a high concentration may lead to an instability of the response signals.

The thickness of membrane

Before starting experiments, modified ISP sensors were immersed into a background solution until a stable baseline could be easily obtained. In the process of testing, a series of measurements were completed by using the same modified ISP sensor; the detective system was kept in the same detective situation. After testing, a washed ISP sensor can easily return to its initial frequency. These treatments allow the effects due to the viscoelasticity of the coating to be neglected; the later testing results prove that this is valid. The thickness of the film has significant effects on the response. An over-thin film causes a long-time shift in the fre-

quency as well as a narrower linear range, while an over-thick film leads to low sensitivity and an instability, even non-oscillation of the QCM easily. In our work, about a 10000 Hz shift, resulting from the film, has usually been used.

Selectivity

In the assay of benzydamine hydrochloride by some aforementioned methods in the introduction section, certain substances that cause interference must be removed before any analytical measurement. Interference by some of these substances and other commonly used compounds on the response of an ISP sensor were studied using the "separated-solution" method.

All measurements were performed in solutions containing 0.1 M NaNO_3 ($\text{pH} = 7$), which indicates that the sodium cation did not seriously interfere with the response of the present ISP sensors. At first, we define $k_{ij} = \Delta f_i / \Delta f_b$ as the response-selectivity coefficient, where Δf_i is the frequency-shift response of the ISP sensor to 1×10^{-3} M of the interfering ion, and Δf_b is the frequency-shift response to 1×10^{-3} M of benzydamine ion. Differences in the selectivity coefficient of more than 0.05 were considered to result from interferences. Table 2 gives the results of some interfering cations. It can be seen that there is no significant interference from potassium, barium, magnesium, cuprum, caffeine,

Table 2 Ion selectivity of a benzydamine ISP sensor

Interfering ion	$k_{ij} = \Delta f_i / \Delta f_b$	$K = k_{ij} \cdot (M_i / M_b)$
Potassium chloride	-0.01087	
Barium chloride	0.00362	0.0082
Magnesium chloride	0.01449	0.1909
Copper chloride	0.00752	0.0353
Caffeine	0.00362	0.0052
Glucose	0.00725	0.0125
Atropine sulfas	0.02174	0.0116
Theophylline	0.03261	0.0550
Nicotinamide	0.01812	0.0456
Tetramethylammonium bromide	0.02899	0.1218
Quinine hydrochloride	0.04348	0.0415
Morphine hydrochloride	0.03624	0.0392

f_i : frequency response of the sensor to 2.0×10^{-3} M interfering ion; f_b : frequency response of the sensor to 2.0×10^{-3} M benzydamine ion; M_i : molecular weight of the interfering ion; M_b : molecular weight of the benzydamine ion.

glucose, atropine, theophylline, nicotinamide, tetramethylammonium, Morphine, and Quinine cations. When the molecular weight is considered, k_{ij} becomes $K=(\Delta f_i/M_i)/(\Delta f_b/M_b)=k_{ij}\cdot(M_b/M_i)$, where M_i , M_b are the molecular weight of the interfering actions and benzydamine, respectively. Here, K can express the selectivity more precisely.

Applications of the ISP sensor

Calibration curves for the benzydamine determination are given in Fig. 4. The results indicate that the frequency shift ($-\Delta f$) increases along with an increase in the benzydamine concentration over the range from 2.0×10^{-7} to 8.0×10^{-3} M in a background solution of pH=7.0 containing 0.1 M NaNO_3 . In order to adapt the method to the assay of human body fluids, serum (Natrii Citrs was added in the case of agglutination, and the pH was adjusted to 7.0) and urine (containing 0.1 M NaNO_3 solution, and the pH was adjusted to 7.0) were examined; the results are given in Fig. 4. The response ranges were 4.0×10^{-4} – 5.0×10^{-6} M and 2.0×10^{-3} – 3×10^{-6} M for serum and urine, respectively. All of these curves are in good agreement with the Freundlich isotherm.

Figure 5 shows plots of the logarithm of the decrease in the frequency vs. logarithm of the benzydamine concentration in the background solution (containing 0.1 M NaNO_3 and pH 7.0) using the ISP sensors. Obviously,

Fig. 4 Frequency shift ($-\Delta f$) vs. concentration of benzydamine hydrochloride plots for the benzydamine ISP sensor. (○): in aqueous solution containing 0.1 M NaNO_3 , pH=7.0; (□): in urine (containing 0.1 M NaNO_3 , pH=7.0); (◇): in serum (containing Natrii Citrs in case of agglutination, pH=7.0).

Fig. 5 Calibration graph of the benzydamine ISP sensor in 0.1 M NaNO_3 background of pH=7.0.

the resulting linear relationship is in agreement with the theoretical conclusion discussed in section "Theoretical consideration". The regression equation is

$$\log(-\Delta f)=0.32\log C+3.34 \quad (R=0.9904),$$

where R is the correlation coefficient.

The suggested sensor was applied to a quantitative determination of benzydamine hydrochloride in various samples. The results are listed in Tables 3 and 4. The average recovery was 100.35% and the standard deviation was less than 1%; the results agree well with those obtained by the non-aqueous titration method.¹

The successful development of a method for the determination of benzydamine hydrochloride and the results obtained in a sample analysis confirm the usefulness of the proposed PVC modified ion-selective piezoelectric sensor for quantitative analysis, and indicate that this method might also find useful applications in the assay of other drugs.

For an overall evaluation of the proposed method, a comparison of this novel method with other methods (Table 5) has indicated that the ISP sensor provides a selective, sensitive and precise method for the determination of benzydamine hydrochloride. The method

Table 3 Determination of benzydamine hydrochloride using the benzydamine ISP sensor and the pharmacopeia method

ISP sensor					Non-aqueous titration ¹				
Added/mg	Found/mg	Recovery, %	Mean, %	SD	Added/mg	Found/mg	Recovery, %	Mean, %	SD
0.846	0.849	100.4	100.5	0.72	119.7	120	100.3	100.2	0.716
1.69	1.70	100.6			121.3	122	100.6		
2.527	2.521	99.8			124.4	124	99.7		
3.386	3.441	101.6			132.1	131	99.2		
4.232	4.558	99.9			134.7	136	101.0		

Table 4 Determination of benzydamine hydrochloride in human serum and urine using the benzydamine ISP sensor

Serum ^a					Urine ^b				
Added/mg	Found/mg	Recovery, %	Mean, %	SD	Added/mg	Found/mg	Recovery, %	Mean, %	SD
0.98	0.984	100.4	100.4	1.25	0.83	0.827	99.6	100.6	0.9
1.2	1.22	101.7			1.47	1.491	101.4		
2.6	2.58	99.2			2.59	2.607	100.7		

a. Natrii Citrs was added into serum solution in case of agglutination and pH was adjusted to 7.0. b. Urine solution contains 0.1 M NaNO₃ and pH was adjusted to 7.0.

Table 5 Comparisons of the proposed method and other methods for the determination of benzydamine hydrochloride

Method	Application	Calibration range/ mM	Detection limit/ mM	Mean recovery, %	Reference
UV detection	tablet	$5.2 \times 10^{-2} - 1.5 \times 10^{-1}$		100.3	2
NIR detection	powder analysis	2.28 – 6.56% (m/m)			3
HPLC detection	in plasma	$2.5 \times 10^{-3} - 1 \times 10^{-1}$	0.147		4
ISE detection	in serum and in urine	$1.5 \times 10^{-3} - 1 \times 10^1$	1.5	99.7	6
ISP sensor	in serum and in urine	$2 \times 10^{-4} - 8 \times 10^0$	0.2	100.35	this paper

proposed here has some advantages over other detection techniques, *e.g.*, the required reagents and instruments are simpler and cheaper than that required by the HPLC, NIR and UV methods. Finally, the influence of the electrical double-layer capacitance, the Faradaic impedance and the conductive properties of the modified membrane are negligible. Since no significant effect was caused by the background, a good responses can easily be obtained, and the stability of the frequency signal is better than that of the signal obtained by the potentiometric ISE method. The advantages of the new method should make it an attractive alternative for the pharmaceutical assay compared to the other methods currently in use.

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