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Ion-selective piezoelectric sensor for niacinamide assay in serum and urine

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Abstract

An ion-selective piezoelectric (ISP) sensor was successfully applied for the determination of niacinamide in serum and urine. By coating a polyvinylchloride membrane containing niacinamide–silicotungstate on one electrode of a thickness-shear mode piezoelectric quartz crystal, the ISP device can adsorb niacinamide selectively. The amount of coating applied to the crystal was calculated from the Sauerbrey equation by monitoring the frequency change. The logarithm of the frequency shift was linear with the logarithm of niacinamide concentration over the range from 1.0×10^{-9} to 1.0×10^{-3} M with a detection limit of 1.0×10^{-9} M at pH 7.0. Influencing factors were investigated and optimized. The results for real samples obtained by the proposed method were in good agreement with those obtained by the conventional methods.

Keywords: Ion-selective piezoelectric sensor; Niacinamide

1. Introduction

Niacinamide, the amide of nicotinic acid, namely vitamin PP, occurs in living cells as an essential substance for metabolism and is mainly from food. But in the process of food production, niacinamide is liable to lose and deficiency of niacinamide in human body may cause arrythmia [1], so it is used as food additives and dietary supplement. On the other hand, excessive niacinamide may have influence on human, too. Therefore, in the interest of pharmacokinetics study, quantitative control and bromatology, it is necessary to establish a reliable method for the determination of niacinamide in body fluids in a clinical assay.

A great variety of methods are available for the determination of niacinamide such as spectrometry [2–7], fluorimetry [8,9] etc. The spectrometric method suffers from low sensitivity especially when the concentration of niacinamide is very low, hence, its applicability is limited. In fluorimetry, use of dangerous reagents is involved and the resulting compound is unstable. Liquid chromatography method (LC) [10] is simpler, faster and more sensitive than colorimetric and fluorometric methods. However, a preliminary sample clean-up procedure by either extraction or ion-ex-

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change column chromatography is tedious. The ion-selective electrode method [11] has the advantage of its simplicity, and less expensive equipment is required, but its response slope is usually affected by the background solution to some extent. Therefore, to advance a rapid specific method other than ion-selective electrode potentiometry is necessary.

Since the first application of a piezoelectric sensor was reported in 1964 by King [12] after Sauerbrey derived the equation describing the frequency to mass relationship [13], many reports have been published by using piezoelectric quartz crystal (PQC) as the sensoring element. The PQC itself is not a selective sensor, and it may give response to the mass change caused by any loaded substances. Surface modification of the PQC with a chemically selective reagent leads to a useful sensor that selectively adsorbs the detected ion. Selective adsorption/desorption process of the component ions of insoluble salts at their solid/aqueous interface were studied by several research groups and some fundamental characteristics of the adsorption mechanism of solid film were offered. These theoretical results were proved to agree closely with experiments and applied for real samples detection [14–16]. Hence, in the last few years application of PQC has been extended to food industry [17], chromatography [18], biotechnology and clinical diagnosis [19–21].

Based on the above-mentioned principle, a novel all-solid-state niacinamide ion-selective piezoelectric sensor was proposed for the determination of niacinamide and applied to real sample assay.

2. Experimental

2.1. Apparatus

A schematic diagram of the proposed sensor device is illustrated in Fig. 1. The frequency changes of the ion selective piezoelectric sensor were measured by a universal frequency counter. The piezoelectric quartz crystal used was a 9 MHz AT-cut crystal (12.5 mm diameter) having silver electrodes (6 mm diameter) on both sides. The AT-cut refers to quartz wafers cut at $+35°15'$ angle from the z-axis. 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 be in contact with the aqueous sample solution. The crystal holder was directly connected to an IC-TTL oscillating circuit. The IC-TTL is the name of a kind of feedback circuit device, which was designed and made by us. Detailed description can be found in a previous paper [22]. The oscillating circuit was supplied by a d.c. voltage regulator, and the working voltage was set at 5 V. The crystal, the crystal holder and the detection cell (volume 10 ml) were placed in a thermostated water bath (25 ± 0.1°C). A computer was used for data analysis.

2.2. Reagents

All reagents used were of analytical grade, except the niacinamide–silicotungstate (niacinamide–ST) and niacinamide–phosphotungstate

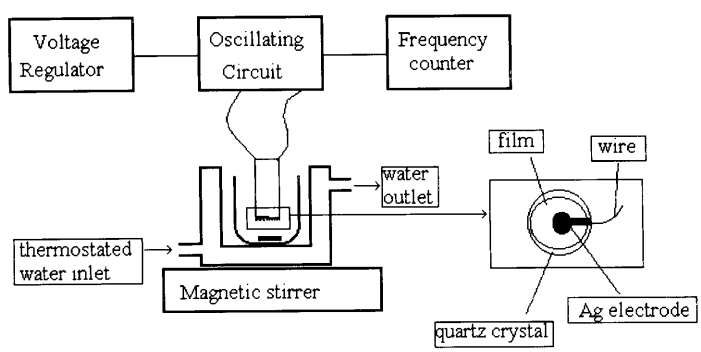

Fig. 1. Schematic diagram of the niacinamide ISP sensor modified with a niacinamide–ST PVC membrane.

(niacinamide–PT), which were synthesized in our laboratory. Niacinamide, obtained from Shanghai Medical Factory (China), was of pharmacopoeial quality [1]. Double-distilled water was used throughout.

2.3. Preparation of standard solutions

Niacinamide (1.2212 g) was dissolved in double-distilled water and the solution was diluted to volume in a 100-ml standard flask, to give a 0.1 M niacinamide stock standard solution of niacinamide. Working standards were made by successive dilution of the 0.1 M solution with a 0.1 M NaCl background solution of pH 7.0.

2.4. Preparation of ion-pair complexes

Niacinamide–ST was prepared by mixing 20 ml of 0.01 M silicotungstate acid and 40 ml of 0.01 M niacinamide under stirring until the precipitation was completed. Then, the precipitation 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 was evaporated under a vacuum, resulting in a white product. niacinamide–PT was prepared in a similar way.

2.5. Electrode fabrication

The procedure for the surface modification of the Ag electrode on the ISP was as follows: 10 mg of the ion-pair complex (niacinamide–ST or niacinamide–PT), 100 mg of the powder of polyvinylchloride (PVC), and 0.2 ml of dibutylphthalate were mixed thoroughly and dissolved in 10 ml of tetrahydrofuran (THF). A small portion of thus obtained solution was spread on the surface of the Ag electrode of the PQC under rapid rotation. Then the coated crystal was kept at 40°C for 6 h to evaporate the THF, leaving a transparent uniform film of coating on the surface. When not in use, the modified sensor was stored in a desiccator.

2.6. Process of preconditioning

The ISP sensor was preconditioned by immersing into a 1.0×10^{-5} M solution of standard niacinamide solution for 12 h. Then it was washed with double-distilled water until the frequency was close to the value after modification when put in air, and fluctuated only 1 Hz within 3 min. The same modified sensor was used repeatedly to complete a series of detection to avoid the effect of the membrane thickness. A 0.1 M NaCl solution (pH 7.0) was used as a background solution.

2.7. Measuring procedure

Before the experiment 30 min was required to allow the oscillator to stabilize. After a steady resonant frequency (f_1) in the background solution was achieved, a series of standard sample solution was injected from low concentration to high concentration. After each addition, the resonant frequency (f_2) of the ISP sensor was recorded and the respective frequency shift was calculated:

$$\Delta f = f_2 - f_1 \quad (1)$$

A calibration curve $\log(-\Delta f)$ vs. $\log C$ was made, where C is the niacinamide concentration. Then, the concentration of the unknown sample was calculated by using the calibration curve method or standard addition method.

3. Results and discussion

3.1. Theoretical discussion

Piezoelectric device consists of an oscillating quartz crystal incorporating an adsorbent on its surface. When any ion is adsorbed into or desorbed from the modified membrane, or when ions with different molecular weight exchange with one another across the membrane, the surface mass change in the modified membrane can be measured by an ISP sensor, even though this change may be very small.

For the majority of piezoelectric research in analytical chemistry or biochemistry AT-cut crys-

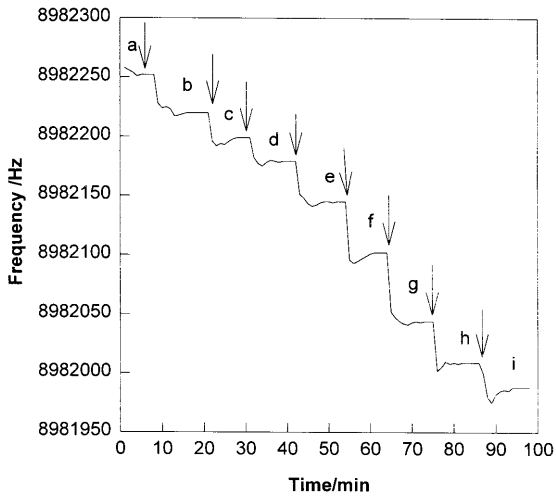

Fig. 2. Course of the observed frequency response of the niacinamide–ST ISP sensor with sample-solution injections. Final concentration of niacinamide (M): (a) 0, (b) 1.0×10^{-9}, (c) 1.0×10^{-8}, (d) 1.0×10^{-7}, (e) 1.0×10^{-6}, (f) 1.0×10^{-5}, (g) 1.0×10^{-4}, (h) 5.0×10^{-4}, (i) 1.0×10^{-3}.

tal has usually been used due to its low temperature coefficient from -55 to 85°C [23]. The idea that adding mass to the crystal surface decreases the oscillation frequency was first utilized by Sauerbrey [13] who derived an expression relating the change in the frequency to the mass of material deposited as follows:

$$\Delta f = -f^2 \Delta m / N \rho A \quad (2)$$

where Δf is the frequency change caused by the deposited material, f is the fundamental frequency of the quartz crystal oscillating in the thickness shear mode, Δm is the change in mass, N is the frequency constant, ρ, is the density of the quartz crystal and A is the area coated. The symbol of minus indicates that as the mass loading increases, the frequency of the crystal oscillating decreases. Therefore, the mass change can be expressed by the frequency shift. When an ISP sensor is dipped into a niacinamide solution, an equilibrium is established between the membrane and the solution. At a low concentration (≤ 0.01 M), the relationship between the mass change of the modified membrane and the tested ion concentration, can be expressed by the Freundlich isotherm [24] using the equation

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

where n and K_f are constants and $n > 1$. Combining Eqs. (2) and (3) and substituting for the various constants for the oscillating quartz crystal, we get

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

For a given AT-cut crystal, the area A is a constant, so Eq. (4) can be simplified as

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

where $K' = 2.26 \times 10^6 f^2 K_f / A$, is a constant too. The logarithmic form of the above equation is

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

3.2. Sensor performance

A number of experiments were carried out using the ISP sensor. Fig. 2 shows a typical course of the response of the ISP sensor modified with ion-pair complex niacinamide–ST to change the concentration in niacinamide in the sample solution. With the increment of the concentration in niacinamide, the amount of the adsorbed substance increases, and the frequency of the sensor gradually decreases. After each addition of the analyte, there exists competitive adsorption on the surface of crystal, and the resonant frequency becomes stable after equilibrium is established.

After completing detection of a series of niacinamide ion concentration, the adsorbed niacinamide species can be washed off the modified membrane. The ISP may be recovered by washing with double-distilled water until the frequency of the ISP sensor gradually increase to reach a value (f_0) close to the steady oscillating frequency (f_1) in the background solution obtained after the precondition. In general, there always exists a little difference between f_0 and f_1 finally, which is mainly due to the adsorption of niacinamide ions at the sensor surface or the probability of the dissolution of activants in the renewed niacinamide ion-free background solution.

Table 1
Comparison of the niacinamide–ST ISP and the niacinamide–PT ISP sensors

niacinamide–ST ISP sensor					niacinamide–PT ISP sensor				
Added (mg)	Found[a] (mg)	R[b] (%)	M[c] (%)	SD	Added (mg)	Found[a] (mg)	R[b] (%)	M[c] (%)	SD
0.145	0.146 ± 0.002	100.7			0.122	0.123 ± 0.004	100.8		
0.292	0.290 ± 0.005	99.3			0.246	0.250 ± 0.003	101.6		
0.441	0.443 ± 0.004	100.5	100.2	0.62	0.358	0.358 ± 0.005	99.7	100.5	0.78
0.601	0.605 ± 0.003	100.7			0.492	0.491 ± 0.005	99.8		
0.643	0.642 ± 0.002	99.8			0.520	0.523 ± 0.007	100.6		

[a] Mean values ± standard deviations ($n = 3$).
[b] R = recovery.
[c] M = average recovery.

3.3. Effect of ion-pair complexes

Niacinamide sensitive ISP sensor of all-solid-state construction with different ion-pair complexes were tested to compare their response functions. Table 1 gives the results with two kinds of such ion-pair complex materials tested as coating for adsorption of niacinamide. The average recovery was 100.2% and the S.D. was 0.62 for the niacinamide–ST ISP, while for the niacinamide–PT ISP was 100.5% and 0.78, respectively. The results indicated that the niacinamide–ST ISP gave higher sensitivity, so it was used for all subsequent work.

3.4. Influence of pH

pH dependence of the responses of the sensor was examined by measuring the frequency changes of the ISP sensor in the niacinamide solution. The pH was adjusted by adding appropriate volume of 0.1 M hydrochloric acid or 0.1 M sodium hydroxide solution using a 50 μl microsyringe. It is seen from Fig. 3, pH values between 5 and 8 no significant change in the frequency response was observed. Taking the situation of the human body into account, pH 7 was adopted in this work.

3.5. Thickness of membrane

In most cases, as the amount of coating material increases, the frequency shift increases, and the sensitivity is raised, too. However, the response time and recovery time of the ISP sensor are also prolonged. In addition, when the amount of coating material increases to a certain value, the response sensitivity of the sensor will not increase any more because of the saturation of the coating material on the crystal surface. An over-thick film may lead to ceasing of oscillation of the ISP device or change its mode of oscillation [23]. On the other hand, an over-thin film causes a long-term shift in the response frequency as well as a narrow linear range. In this paper, a membrane coating equivalent to 10 000 Hz frequency shift has been adopted.

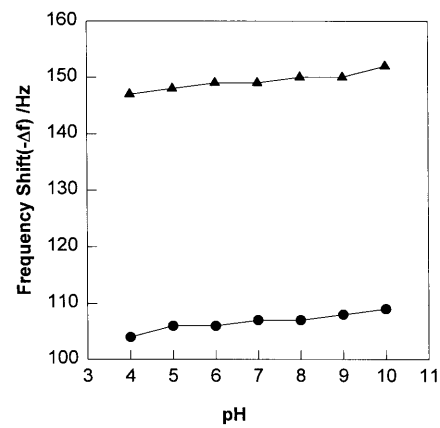

Fig. 3. Influence of the solution pH on the frequency response of the sensor: (●) 1.0×10^{-6} M niacinamide; (▲) 1.0×10^{-5} M niacinamide.

Table 2
Ion selectivity of a niacinamide ISP sensor[a]

Interfering ion	$K_{ij} = \Delta f_i/\Delta f_n$	$K = K_{ij}M_n/M_i$
Copper sulfate	0.027	0.020
Calcium nitrate	0.040	0.030
Glucose	−0.053	No interference
Isoniazid	−0.020	No interference
Ammonium chloride	0.027	0.061
Vitamin C	0.040	0.028
Chlorphenamine maleate	0.060	0.019
Lactose	0.027	0.010
Promethazine hydrochloride	0.047	0.018
Urea	0.020	0.047
Trimethoprim	0.033	0.014
Benzydamine hydrochloride	−0.067	No interference
Tetramethylammonium iodide	0.047	0.077
Tetraethylammonium iodide	0.080	0.075
Tetrapropylammonium iodide	0.127	0.083
Tetrabutylammonium iodide	0.187	0.094
Cetyltrimethylammonium iodide	0.273	0.117

[a] K, response selectivity coefficient; f_i, frequency response of the sensor to 1.0×10^{-3} M interfering ion; f_n, frequency response of the sensor to 1.0×10^{-3} M niacinamide; M_i, molecular weight of the interfering ion; M_n, molecular weight of niacinamide.

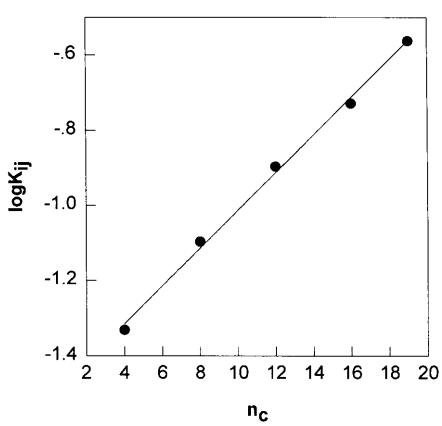

Fig. 4. Relationship between $\log K_{ij}$ and number of carbon atoms in the symmetrical quaternary ammonium ions (n_c).

3.6. Influence of temperature

The temperature of the detection cell can influence the frequency response of the piezoelectric crystal sensor dramatically, so all the measurements should be performed under constant temperature. In this work, the detection cell was kept at 25 ± 0.1°C by using a thermostated water bath and the temperature of environment was about 16°C.

3.7. Selectivity

The effect of potential interfering elements and other commonly met compounds on the response of the proposed ISP sensor was examined by measuring the selectivity coefficient by the separation solution method.

All measurements were carried out in a aqueous solution containing 0.1 M NaCl (pH 7.0), and the chloride ion and sodium ion did not seriously interfere with the response of the present ISP sensor. At first, we defined $K_{ij} = \Delta f_i/\Delta f_n$ as the response-selective coefficient, here Δf_i is the frequency shift response of the ISP sensor to 1.0×10^{-3} M solution of the interfering ion, Δf_n is the frequency shift response to 1.0×10^{-3} M solution of niacinamide. Taking the molecular weight into account, we can acquire the equation for the response selectivity coefficient (K), and K can express selectivity more precisely,

$$K = K_{ij}M_n/M_i$$

where M_i is the molecular weight of the interfering ion and M_n is the molecular weight of niacinamide. Differences in the selectivity coefficient of more than 0.05 were regarded to result from interference. Table 2 shows the results of some interfering ions. We can see that (1) there is no significant interference from copper sulfate, calcium nitrate, glucose, isoniazid, vitamin C, benzydamine hydrochloride, chlorphenamine maleate, lactose, urea, promethazine hydrochloride, trimethoprim; (2) for symmetrical quaternary ammonium ions, there is a regular change of selectivity with the number of carbon atoms in the ion. Hence, a plot of $\log K_{ij}$ vs. the number of carbon atoms in the quaternary ammonium ion gives a

straight line (Fig. 4). Least squares analysis of this line gives

$$\log K_{ij} = -1.52 + 0.050 \log n_c \quad (r = 0.997)$$

(r is the correlation coefficient, n_c is the number of carbon atoms)

A possible explanation for this was given by Martin et al. [25]. As for the homologues of surfactants, the greater the number of carbon atoms, the stronger the adsorbability.

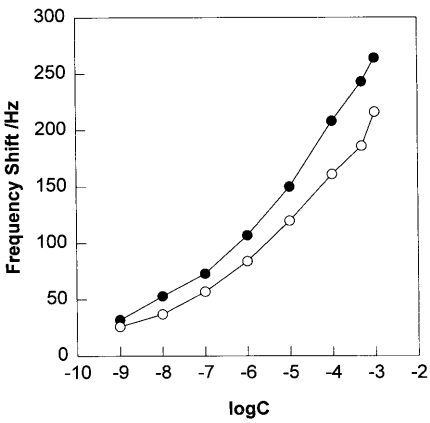

Fig. 5. Frequency shift vs. concentration of niacinamide plots for the niacinamide ISP sensor with different ion-pair complex: (●) the niacinamide–ST ISP sensor; (○) the niacinamide–PT ISP sensor (C is the concentration of niacinamide).

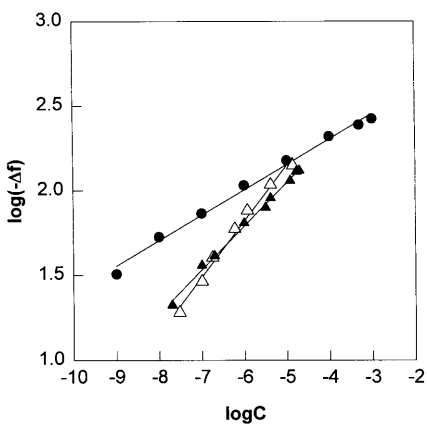

Fig. 6. Calibration graph of the niacinamide–ST ISP sensor: (●) in 0.1 M NaCl background of pH 7.0; (▲) in urine (containing 0.1 M NaCl, pH 7.0); (△) in serum (containing sodium citrate in case of agglutination, pH 7.0) (C is the concentration of niacinamide).

3.8. Calibration curve and application of the ISP sensor

Fig. 5 shows the calibration curves for the niacinamide determination. The results indicate that the frequency decreases along with an increment in the niacinamide concentration over the range from 1.0×10^{-9} to 1.0×10^{-3} M in a background solution of pH 7.0 containing 0.1 M NaCl. The regression equation for the niacinamide–ST ISP is

$$\log(-\Delta f) = 2.90 + 0.15 \log C \quad (r = 0.993)$$

while for the niacinamide–PT ISP is

$$\log(-\Delta f) = 2.80 + 0.15 \log C \quad (r = 0.994)$$

Obviously, the resulting linear relationship agreed well with the theoretical discussion.

In order to utilize the method to analyze human body fluids, serum (sodium citrate was added to avoid coagulation, and pH was adjusted to 7.0) and urine (containing 0.1 M NaCl, and pH was adjusted to 7.0) were examined. Fig. 6 shows plots of the logarithm of the frequency shift vs. logarithm of the niacinamide concentration in the background solution (containing 0.1 M NaCl and pH was adjusted to 7.0) and human body fluids using the niacinamide–ST ISP sensors. The response ranges were 2.0×10^{-8}–2.0×10^{-5} and 3.0×10^{-8}–1.4×10^{-5} M for serum and urine respectively. The regression equation obtained with serum sample is:

$$\log(-\Delta f) = 3.80 + 0.33 \log C \quad (r = 0.987)$$

while the regression equation obtained with urine sample is:

$$\log(-\Delta f) = 3.33 + 0.26 \log C \quad (r = 0.996)$$

where r is the correlation coefficient. All these curves were in good agreement with the Freundlich isotherm [24].

The proposed sensor was applied to a quantitative assay of niacinamide in serum and urine. The results are shown in Table 3. The average recovery was 101.2% and the S.D. was 1.60 for the niacinamide–ST ISP in serum sample, while in urine sample was 100.5% and 0.95, respectively. The results were in good agreement with those obtained from the spectrometry method.

Table 3
Determination of niacinamide in human serum and urine using the niacinamide–ST ISP sensor

Serum[a]					Urine[b]				
Added (μg)	Found[c] (μg)	Recovery (%)	Average recovery (%)	S.D.	Added (μg)	Found[c] (μg)	Recovery (%)	Average recovery (%)	S.D
1.252	1.274 ± 0.086	101.8			1.864	1.845 ± 0.080	99.0		
2.074	2.124 ± 0.096	102.4			3.892	3.953 ± 0.082	101.6		
2.622	2.584 ± 0.115	98.6	101.2	1.60	5.432	5.460 ± 0.005	100.5	100.5	0.95
3.097	3.934 ± 0.062	100.7			6.876	6.921 ± 0.078	100.7		
5.127	5.074 ± 0.086	102.4			7.465	7.526 ± 0.092	100.8		

[a] Sodium citrate was added to avoid coagulation, and the pH was adjusted to 7.0.
[b] Urine solution contained 0.1 M NaCl, and pH was adjusted to 7.0.
[c] Mean values ± S.D. ($n = 3$).

Table 4
Comparison of the proposed method and other methods for the determination of niacinamide

Method	Application	Calibration range (mM)	Detection limit (μM)	Recovery (%)	Reference
Spectrophotometry	Some pharmaceutically active amides and imides	1.3×10^{-5}–6.5×10^{-5}	1.3×10^{-2}	99.3	[7]
Photochemical fluorimetry	Vitamin B complex tablets	4.9×10^{-3}–8.2×10^{-2}	4.9	100.1	[26]
Potentiometry		8.2×10^{-1}–2.5	8.2×10^{2}	98.3	[11]
LC	Beef and pork	4.1×10^{-2}–2.1×10^{-2}	4.1×10^{1}	96.3	[2]
ISP sensor	In serum and urine	1.0×10^{-6}–1.0	1.0×10^{-3}	100.2	This paper

4. Conclusion

Table 4 gives a comparison of this novel method with other methods. It indicates that the ISP sensor provides a selective, sensitive and precise method for the determination of niacinamide. This method has some advantages over other detection methods, for example, the reagents and instruments required are cheaper and simpler than those required by the HPLC, NIR and UV methods. All those make it an attractive and promising alternative for the pharmaceutical assay compared to the other currently used methods.

Acknowledgements

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