ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1471–1477

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Determination of dipyridamole by modified extraction–gravimetry with a surface acoustic wave resonator sensor

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Received for review 3 October 1995

Abstract

A simple and sensitive extraction–gravimetric method for the determination of dipyridamole is presented. The method is based on the extraction of free dipyridamole with chloroform, after neutralization with a basic agent, followed by measurement of the frequency shift response of the specially designed surface acoustic wave resonator sensor after evaporation of the extractant from the surface of the resonator. The frequency shift response was proportional to the amount of dipyridamole in the range 0.065–1.12 μg. Experimental parameters and the effect of interfering substances on the assay of dipyridamole were also examined in this study. The method was applied to the determination of dipyridamole in tablets.

Keywords: Dipyridamole; Extraction; Surface acoustic wave resonator; Surface acoustic wave sensor; Tablets

1. Introduction

Dipyridamole (DPM) {2,6-bis(diethanolamino) - 4,8 - dipiperidinopyrimido[5,4-d]pyrimidine} introduced in 1959 as a coronary vasodilator, is a drug that is widely used for its inhibitory effect upon blood platelet functions and for the treatment of heart diseases [1,2]. The drug can be easily absorbed into the bloodstream by the human body. Commercially available dosage forms are coated tablets and injections. Tablets are more widely used and contain 25 or 75 mg of DPM. The common recommended methods for DPM assay in pharmaceutical products have been non-aqueous titration (using perchloric acid) [3,4] and redox titrimetry (using potassium bromate as titrant) [4]. The drug has also been determined using chromatographic [5–9], spectrophotometric [10–13], colorimetric [14], spectrofluorimetric [15,16] and polarographic [2] methods and by adsorptive stripping voltammetry [17]. However, some of these methods are expensive, time-consuming or have limited sensitivity. The present investigation was prompted by the need to develop more reliable, precise and less time-consuming methods for quantitative analysis of the drug.

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PII S0731-7085(96)01724-4

The surface acoustic wave (SAW) device, which possesses characteristics such as small size, low cost, reliability, and great sensitivity to mass loading effects, is widely used in gas sensors [18–20] but there have been no studies concerning its application in pharmaceutical analysis. In the present paper, a new method is proposed for the determination of DPM based on the use of a polymer-coated SAW resonator sensor. The method is simple and sensitive and enables submicrogram amounts of DPM to be determined.

2. Material and methods

2.1. Apparatus

The complete experimental assembly is represented in Fig. 1(A). It consists of four parts: a gas-flowing system; an oscillator circuit system; a sensor system; and a measurement system. The SAW device comprised 62 MHz one-port resonators, fabricated on y-cut z-propagation $LiNbO_3$ crystals with aluminum metallization and mounted on two-pin round TO-5 headers with epoxy and gold wirebonds (Zhuzhou Radio Factory, Hunan, People's Republic of China). The aluminum interdigital transducer electrodes (IDTs) were 1350 nm in thickness. The resonators were designed with an acoustic aperture and a pathlength of several wavelengths. At the centre of each separate $LiNbO_3$ crystal chip there were 20 pairs of IDTs with 500 reflectors placed on each side. The polymer-coated SAW resonator sensor (with a sample cell, see below for its fabricaiton) was mounted horizontally in the central part of the measurement chamber and connected to an oscillator built in this laboratory [21], which was supplied by a d.c. voltage regulator. Frequencies due to the deposited DPM were measured with a digital counter (model SC7201, Iwatsu, Japan). The measuring chamber was a hermetically-sealed box made of sheet copper (length × breadth × height = 6 × 4 × 4 cm^3) and topped with a Plexiglass cover on which a rubber plug for sample injection was fixed. A rotor flow meter was used to measure the flow rate of nitrogen. A 1 μl microsyringe was calibrated and used for the sample injection.

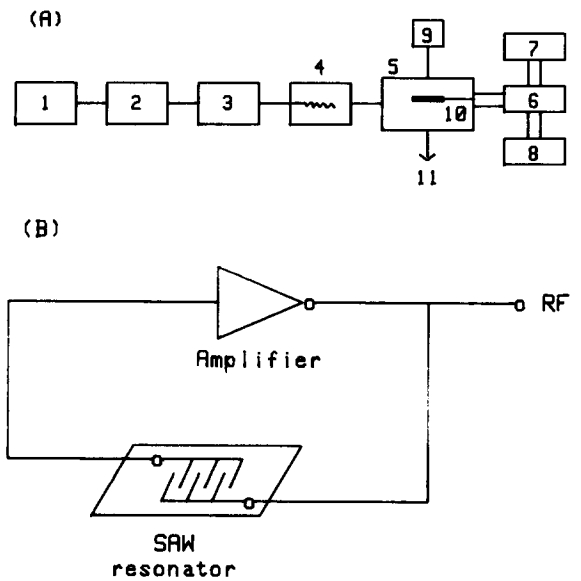

Fig. 1. (A) Flow diagram of the experimental apparatus for the modified extraction–gravimetric determination: (1) nitrogen cylinder; (2) drying tower containing anhydrous calcium chloride and silica gel; (3) rotor flow meter; (4) heating compartment; (5) measuring chamber; (6) frequency counter; (7) d.c. voltage regulator; (8) oscillator; (9) microsyringe for sample injection; (10) SAW resonator sensor; (11) nitrogen outlet. (B) The construction of the surface acoustic wave resonator sensor.

2.2. Fabrication of the surface acoustic wave resonator sensor

The construction of the surface acoustic wave resonator sensor is shown in Fig. 1(B). As the aluminium electrode is very susceptible to chemical reactions, a tiny coating was needed to prolong its useful life. Polyvinylidene fluoride (PVF) was selected as the coating material for the SAW resonator using the following procedure. PVF powder (70 mg) was mixed with 1.0 g of *N*, *N*-dimethylformamide (DMF) and gently heated on a water bath (60°C) with constant stirring. One drop of the PVF colloid was added on to the resonator, which was placed in the centrifuge at 1000*g* for 2 min. The coated resonator was dried at 80°C for 1 h. After cooling, the SAW resonator was assembled into the oscillator. The observed

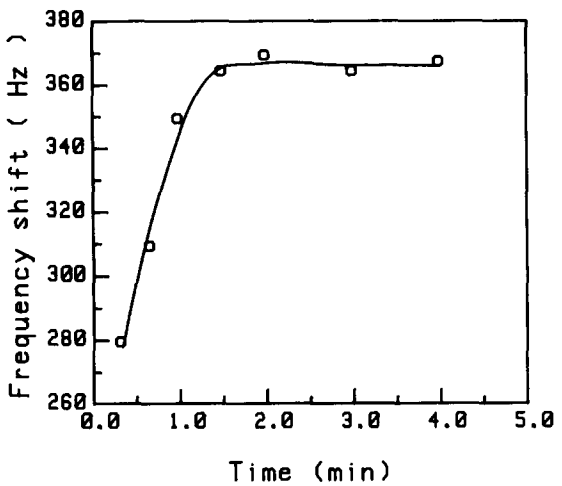

Fig. 2. A plot of frequency shift response vs. measurement time after the sample had been injected on to the SAW resonator sensor. 0.65 μg of DPM and 15°C were selected for this experiment.

frequency change was about −8.5 kHz. A PVF frame was fabricated around the IDTs of the SAW resonator and attached with epoxy glue to the bottom face to form a sample cell. The active area of the sensor was about 0.15 cm^2. In the air, the frequency of the SAW resonator sensor appeared at 62.04 MHz, which is close to the calculated value of 62.05 MHz.

2.3. Reagents and solutions

All reagents were of analytical grade. Chloroform, which was used as the extractant for dipyridamole, was saturated with water by repeated mixing. Distilled-deionized water was used. Dipyridamole was of pharmacopoeial quality [4]. A standard solution of DPM 6.50 mg ml^{-1} was prepared. Other standards were prepared from this solution by serial dilution.

2.4. Calibration graph

100 ml of the standard solution of DPM was mixed with 1.0 ml of 0.2 M sodium hydroxide. The mixture was shaken for 2 min with 2.0 ml of chloroform. After phase separation and removal of the upper aqueous layer with a dropper, 1 μl of the organic layer was applied with a 1 μl microsyringe on to the SAW resonator sensor in the measuring cell, through which a stream of dried nitrogen was passed at a flow rate of 80 ml min^{-1}. After 3 min, the oscillator frequency (F_1) was recorded and the frequency shift response calculated from the equation: $\Delta F = F_0 - F_1$, where F_0 is the frequency measured with the SAW resonator sensor with a blank under the same experimental conditions. A calibration graph of frequency shift response against dipyridamole content was constructed. After each measurement chloroform (about 10 μl) was injected on to the coating to remove the remaining free DPM. The solvent was absorbed with a filter paper. The rinsing procedure was repeated again. After 3 min, the SAW resonator sensor was ready for the next measurement.

2.5. Tablet assay

A sample of 20 tablets was weighed and finely powdered. A portion of the powder was weighed out accurately (about 200 mg) and put into a 100 ml beaker. About 50 ml of 0.02 M HCl was added and the solution was shaken for 5 min. This suspension was then transferred to a 100 ml standard flask and diluted to volume with 0.02 M HCl. The suspension was filtered through a dry filter and the first 15 ml of the filtrate was discarded. The middle portion of the filtrate was analyzed as described above and the content of DPM was calculated from the calibration graph.

3. Results and discussion

Wohltjen [22] used the following formula to relate the frequency changes directly to mass changes assuming that changes in mass are the only transduction mechanism:

$$\Delta F = (k_1 + k_2)F^2 h\rho - k_2 F^2 h(4\mu/V_R^2)\,[(\lambda + \mu)/(\lambda + 2\mu)] \quad (1)$$

In this equation, F is the fundamental resonant frequency of the sensor oscillator (MHz), ΔF is the change in frequency due to the film (Hz), k_1

Table 1
Influence of the extracting solvent on the assay of DPM[a]

Extracting solvent	Frequency shift response (Hz)				
	1	2	3	Average	SD
Chloroform	490	503	482	492	10.6
Carbon tetrachloride	379	350	368	366	14.6
1,2-Dichloroethane	398	394	423	405	15.7
Diethyl ether	405	366	380	384	19.8
Toluene	338	357	330	342	13.9

[a] Three determinations of 0.81 μg of DPM.

and k_2 are material constants dependent on the piezoelectric substrate with units of $cm^2\ s\ g^{-1}$, h is the thickness of the film (cm), ρ is the film density ($g\ cm^{-3}$), V_R is the Rayleigh wave velocity in the substrate ($cm\ s^{-1}$), λ is the Lame constant, and μ is the shear modulus of the interface material ($dyn\ cm^{-2}$).

The first term in Eq. (1) yields the frequency shift resulting from mass loading whereas the second term describes the effect of changes in the elastic properties of the film on the resonant frequency. If the coating is a soft, rubbery material, the changes in frequency attributable to modulus changes are minimal and the second term in Eq. (1) can be ignored (for example, with $\mu = 10^7 - 10^{10}$ dyn cm^{-2} and a density of about 1 g cm^{-3}, if the second term is ignored when calculating the SAW frequency change, the resulting error is unlikely to exceed 10%) [23]. Therefore, Eq. (1) is simplified to

$$\Delta F = (k_1 + k_2)F^2 \cdot \Delta m\, A^{-1} \quad (2)$$

The film mass per unit area, $\Delta m\, A^{-1}$, replaces $h\rho$; both terms have the same units. Eq. (2) predicts a linear decrease in ΔF with increasing mass per unit area. It also shows that the absolute mass response of the SAW device depends on the physical properties of the piezoelectric substrate and on the square of the operating frequency.

When values for the material constants for y,z-$LiNbO_3$ substrate ($k_1 = 1.730 \times 10^{-7}$ $cm^2\ s\ g^{-1}$, $k_2 = 0$; values derived from Khlebarov et al. [24] and from Auld [25] respectively) are inserted, the corresponding surface acoustic wave equation becomes:

$$\Delta F = 1.73 \times 10^5 \cdot F^2 \cdot \Delta m\, A^{-1} \quad (3)$$

The theoretical sensitivity of the present SAW resonator sensor (the centre frequency of the sensor is 62.04 MHz) is calculated to be 0.67 Hz $ng^{-1}\ cm^{-2}$. If the active area of the sensor is assumed to be about 0.15 cm^2 and the signal-to-noise ratio ($S/N = 9$) is assumed to be three, this sensitivity results in a minimum detectability of about 6.0 ng. It can be seen from Eq. (3) that the SAW resonator sensor has a sensitive response to the mass of analyte deposited on to the SAW resonator.

3.1. Factors affecting the dipyridamole assay

Factors affecting the frequency measurement were investigated in order to select the optimum conditions for the determination of dipyridamole with the SAW resonator sensor. To avoid adsorption of moisture from the surrounding air and to speed up the volatilization of the organic solvent (chloroform), all measurements were carried out in a stream of nitrogen, previously dried with anhydrous calcium chloride and silica gel, at a flow rate of 80 ml min^{-1}. Study of the influence of temperature on the determination of DPM showed that temperature had no significant influence within the tested range of 10–45°C. Volatilization of the chloroform from the analyte

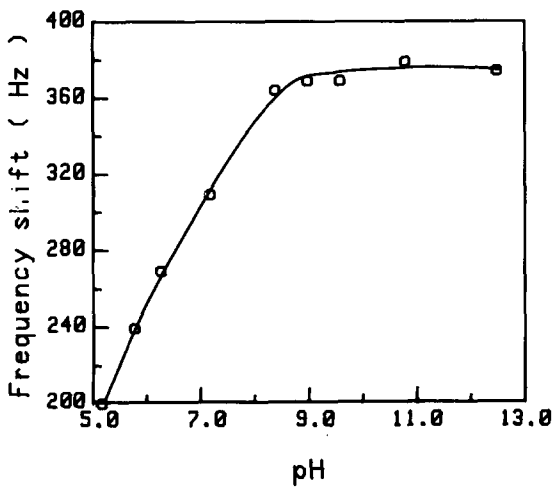

Fig. 3. Influence of pH on the measurement of 0.65 μg of DPM.

Table 2
Recover of dipyridamole in the assay

Test No.	Taken (μg)	Found (μg)				Recovery (%)
		1	2	3	Average[a]	
1	0.065	0.060	0.058	0.069	0.062	95.4
2	0.16	0.155	0.172	0.161	0.163	101.9
3	0.48	0.490	0.466	0.458	0.471	98.1
4	0.65	0.639	0.630	0.660	0.643	98.9
5	0.81	0.856	0.822	0.825	0.834	103.0
6	0.96	0.968	0.964	0.992	0.975	101.6

[a] Each value represents the mean of three measurements

was complete within 1.5 min at 15°C. As shown in Fig. 2, the frequency shift response was unchanged after 1.5 min. Therefore, the assay of DPM by this method can be accomplished at room temperature without any need for heating to evaporate the chloroform.

Table 3
Influence of foreign substances on the assay of 0.48 (μg) DPM

Foreign substance	Analyte found (μg)			
	1	2	3	Average[c]
None				0.480
Sodium chloride[a]	0.470	0.493	0.465	0.476
Zinc sulphate[b]	0.494	0.485	0.506	0.495
Copper sulphate[b]	0.490	0.513	0.504	0.502
Magnesium chloride[a]	0.482	0.510	0.495	0.496
Ammonium acetate[a]	0.485	0.502	0.477	0.488
Nickel sulphate[b]	0.460	0.465	0.480	0.468
Manganous sulphate[b]	0.465	0.474	0.495	0.478
Potassium iodide[a]	0.478	0.478	0.500	0.485
Lead nitrate[b]	0.460	0.464	0.485	0.470
EDTA[a]	0.512	0.490	0.493	0.498
Calcium chloride[a]	0.466	0.471	0.484	0.474
Glucose[a]	0.475	0.495	0.471	0.480
Sucrose[a]	0.462	0.460	0.478	0.468
Urea[a]	0.488	0.484	0.506	0.493
Thiourea[a]	0.476	0.495	0.480	0.484
Soluble starch[a]	0.465	0.480	0.462	0.469
Glycine[a]	0.502	0.485	0.488	0.492
Glutamic acid[a]	0.454	0.450	0.470	0.458
Alanine[a]	0.488	0.475	0.472	0.478
Lysine[a]	0.497	0.513	0.495	0.502
DL-aspartic acid[a]	0.455	0.452	0.472	0.460

[a] Concentration = 0.05 M.
[b] Concentration = 0.02 M.
[c] Each value represents the mean of three measurements.

In the proposed method, DPM was first neutralized with a basic reagent to form the free alkaloid which was then extracted into an appropriate organic solvent; measurement was made of the frequency change of the SAW resonator sensor caused by the deposition of DPM after evaporation of the solvent. From a study of the effect of different extracting solvents, it was found that chloroform gave the highest extraction of DPM and also better reproducibility (Table 1).

The pH of the aqueous phase may affect the determination of DPM (Fig. 3). Results showed that the frequency shift response becomes constant when the pH is not less than 9; at lower pH values, the frequency shift response decreases with decreasing pH. No significant differences in the frequency shift response were found between the different basic reagents tested, such as 7.0 M ammonia, 0.2 M or 2.0 M sodium hydroxide, 10% sodium carbonate and 5% sodium bicarbonate.

The effect of extraction time was examined. The results showed that extraction proceeded very quickly; equilibrium was established within 1 min. Investigation of the influence of organic to aqueous phase volume ratio demonstrted that there was no significant influence for phase volume ratios of 2:1–1:4. The organic extract was stable and the frequency shift response was constant even after 48 h when stored under dim light.

3.2. Calibration graph, reproducibility and analytical recovery

The frequency shift response of the proposed SAW resonator sensor was directly proportional

Table 4.
Analytical results for the assay of dipyridamole in tablets

Sample	n	Present method Mean value (mg per tablet)	SD	Pharmacopoeial method [4] Mean value (mg per tablet)	SD
1	3	24.7	1.3	25.2	1.4
2	3	24.0	1.5	24.6	1.5
3	3	25.5	1.4	26.1	1.4

to the content of DPM in the range 0.065–1.12 μg. The following linear regression equation for dipyridamole was obtained under the selected experimental conditions: $\Delta F = (584.3 \pm 2.9)\ W + (4.5 \pm 3.4)$ with a correlation coefficient of 0.9987 ($n = 8$), where ΔF is the frequency shift (Hz) and W is the amount of dipyridamole (μg); the slope and intercept are expressed with their 95% confidence limits. The standard deviation was 9.0 Hz (RSD = 3.2%) for five determinations of 0.48 μg of DPM. The lower detection limit, that is the calculated amount for which ΔF corresponds to three times the standard deviation, was 38 ng, which is higher than the theoretical value derived from Eq. (3) (6.0 ng). This difference may be caused by the coating on the SAW resonator.

The recovery results obtained for the DPM assay using the SAW resonator sensor are given in Table 2. The mean recovery was 99.8% and the RSD was 2.95%.

3.3. Life span and restoration of the saw resonator sensor

The life span of the SAW resonator sensor was not less than 6 months. When not in use, it should be kept in a desiccator with silica gel as the drying agent. No deterioration was caused by the extracting solvent (chloroform) used in this work.

For the resonator to be restored after the DPM assay, about 20 μl of chloroform was used to rinse the polymer-coated resonator twice with 10 μl. The free DPM left can be completely removed. The polymer-coated resonator is ready for the next assay after the restoring solvent has been absorbed by a filter paper and evaporated within 2–3 min. An increased flow rate of nitrogen may be used to accelerate solvent evaporation.

3.4. Interference

The possible interference of a number of inorganic and organic substance with the measurement of DPM has been investigated by adding 0.50 ml of a solution of the substance under test to 0.50 ml of aqueous sample solution containing 0.48 mg of DPM and following the standard procedure. The results are listed in Table 3. Calcium (II), magnesium (II), copper (II), and iron (II) produce precipitates with sodium hydroxide. These precipitates remain in the aqueous phase and therefore cause no interference with the DPM assay. Alkaloids such as cinchonine, diphenhydramine (diphenylhydramine) and chlorpheniramine can also be extracted by chloroform and cause interference. No significant interference was observed with other substances listed in Table 3.

3.5. Assay of dipyridamole tablets

Determinations of dipyridamole in samples of three batches of tablets were performed by the proposed technique and the conventional non-aqueous titration [4]. The results of a few analyses are given in Table 4. There is no significant difference among these three batches of tablets. The results of the proposed method are in good agreement with those obtained by the pharmacopoeial method, and illustrate the feasibility of determining dipyridamole in tablets by modified extraction–gravimetry with a surface acoustic resonator sensor.

Acknowledgements

This work was supported by the Natural Science Fund and State Education Commission of the People's Republic of China.

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