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A Novel Flow-Injection Potentiometric Detector for Determination of Tamoxifen in Anticancer Drug Formulations

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A flow-injection potentiometric detector was reported for the rapid determination of tamoxifen (TAM), by using a poly(vinyl chloride) (PVC) membrane electrode containing tamoxifen-phosphomolibdate (TAM-PM) ion-pair as electroactive component. The potentiometric quality of the electrode response was optimized. Under the optimized condition (carrier solution: 5.0×10^{-7} M TAM, injection volume: 20 μL, flow-rate: 2.0 mL min^{-1}, tubing length: 10 cm), the calibration plot for TAM was linear over the concentration range of 1.0×10^{-4}–1.0×10^{-2} M with a sub-Nernstian slope of 37.7 mV per decade of TAM activity and detection limit of 4.2×10^{-5} M. The interference effects of some common alkaline metal, alkaline earth metal, heavy metals, organic and pharmaceutical molecules on electrode response were investigated and selectivity coefficients were calculated. The relative standard deviation of the electrode response for thirteen replicate measurements of 5.0×10^{-4} M TAM was 1.81%. The flow-through detector system revealed sampling rates of approximately 90 injections per hour depending on the TAM concentration of the injected sample. The offered potentiometric flow-injection system was applied for the determination of TAM in pharmaceutical preparations and the obtained results were compared with High-Performance Liquid Chromatography (HPLC) results.

Keywords: Flow-Injection Analysis, Ion-Selective Electrode, Pharmaceutical Analysis, Potentiometric Detector, Tamoxifen Determination, Anti-Cancer Drug.

1. INTRODUCTION

Tamoxifen (TAM) (Fig. 1) is a non-steroidal drug used in the treatment and prevention of breast cancer.[1] US Food and Drug Administration (FDA) offered TAM use as a chemopreventive in women at high risk in 1978, and today it is prescribed for these kinds of patients.[2] Its primary mechanism of action is competitive inhibition of the estrogen α-receptor, thereby preventing growth of malignant breast cells. TAM administration generally decreases the risk of recurrence of breast cancer and improves the survival rates of the patients.[3] Moreover, with the significant grow in the use of TAM, the drug monitoring is of great importance. Because it has many side effects and causes damage in liver DNA.[4,5]

Various analytical techniques such as High Performance Liquid Chromatography (HPLC),[2,6–8] Gas Chromatography (GC),[9–11] Capillary Electrophoresis (CE),[12–14] Thin Layer Chromatography[15] and electrochemical methods[16] have been applied for the tamoxifen determination until now. However these techniques are very cost-effective due to the used sophisticated instruments and expendable supplies, and require time-consuming and laborious analysis process. Therefore there has been a growing demand for the development of new alternative techniques exterminating the aforementioned disadvantages.

Potentiometric ion selective electrodes are frequently preferred analytical devices for the analysis of many clinical, environmental, industrial and pharmaceutical species due to a number of advantages they offer, such as adequate selectivity, wide working range, low limit of detection, high accuracy, short analysis time, practical measurement process, simple design, low cost, no irreversible effect on the tested material, no prior separation from the sample matrix and applicability to colored and turbid samples.[17] One reason of widespread use of ISEs may be attributed mainly to their utilization in flow-through

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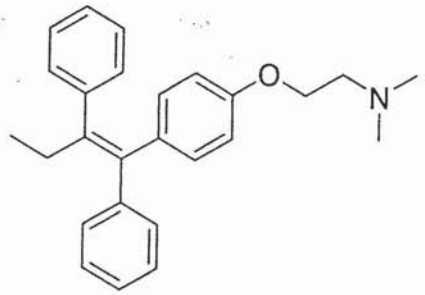


Fig. 1. Chemical structure of tamoxifen.

applications as detectors in automatic analyzers, continuous monitoring systems.[18,19] Flow injection analysis (FIA) is a simple, rapid, and versatile technique with widespread application in quantitative chemical analysis. FIA measurements offer many advantages such as flexibility, reproducibility, considerable decrease in sample consumption, higher sampling-rate, easy switching from one analysis to another, low cost, facility of automation, etc. over steady state and manual analysis methods.[20] The automation allows to make human work more effective and to avoid routine operations. Further, the transient nature of the signal in FIP may decrease the interferent effects of the foreign ions if their response is slower than the target analyte.[21] Flow Injection Potentiometry (FIP) as a combination of flow injection analysis and potentiometry holds all the advantages provided by both FIA and potentiometry. Therefore, FIP has found vast applications in diverse fields including clinical,[22,23] environmental,[24] industrial[25,26] pharmaceuticals[27,28] and agricultural analysis.[29,30]

In the current study, for the first time, we have attempted to develop a feasible and practical flow injection potentiometric detection system for the determination of TAM contents of some anticancer drug formulations. The potentiometric performance characteristics and applicability of the proposed detection system to real pharmaceutical samples were investigated.

2. EXPERIMENTAL DETAILS

2.1. Reagents

Membrane solvent; tetrahydrofuran (THF) and membrane components; high molecular weight poly(vinylchloride) (PVC) and o-nitrophenyl octyl ether (NPOE), were all obtained from Sigma-Aldrich (Bucks, Switzerland).

Graphite powder (<20 μm) from Sigma-Aldrich (Bucks, Switzerland), Epoxy (Macroplast Su 2227) from Henkel (Istanbul, Turkey) and hardener (Desmodur RFE) from Bayer AG (Darmstadt, Germany), were obtained and used in the preparation of conductive solid contact.

Tamoxifen and the ion-pairing agent sodium phosphomolibdate hydrate were purchased from Sigma-Aldrich (Bucks, Switzerland).

All chemicals and nitrate salts of the cations used in selectivity studies were in analytical grade. Methanol used in the preparation of all TAM solutions was also obtained from Merck (Darmstadt, Germany). Two drugs containing various amount of tamoxifen (10 and 20 mg in per tablet) analyzed in the present study were purchased from a local pharmacy.

2.2. Apparatus

The potentiometric measurements were conducted by using a laboratory-made computer-controlled high-input impedance multi-channel potentiometric measurement system. The system has a home-made software program. A Gamry (Warminster, PA, USA) saturated silver/silver chloride reference electrode was used throughout the measurements. The pHs of the tamoxifen solutions were fixed by using a bench top Jenco Ins. (San Diego, CA. USA) model 6307 pH meter. Thermo Scientific (Waltham, MA, USA) Dionex UltiMate 3000 HPLC-DAD system was employed as the reference method for the comparison of the results obtained from flow injection potentiometry. Thermo Scientific manual glass injector of 100 μL was employed thoroughly for chromatographic injections. Chromatographic separation was achieved with a Dionex Acclaim 120 C18 reversed-phase analytical column (150 mm $\times$ 4.6 mm i.d., 5 μm). In flow injection potentiometric system, the pump of Dionex ICS-1100 ion chromatography system (Sunnyvale, CA, USA) was used to propel the carrier and analyte to the detector.

2.3. Synthesis of Ion Pair, Electrode Fabrication and Flow Injection System Set-up

TAM-PM ion pair was obtained by mixing 25 mL 10^{-2} M tamoxifen solution (acidified with HCl equivalent to tamoxifen) with 25 mL 10^{-2} M solutions of sodium phosphomolibdate monohydrate. The solutions were prepared in methanol due to the insolubility of tamoxifen in water. After digestion overnight, the formed precipitate was centrifuged, washed with double distilled water and methanol until chloride free and dried at room temperature. The elemental analysis was performed for the synthesized ion pair. The observed (%) was C = 31.43; H = 3.13; N = 1.40 and the calculated (%) was C = 31.86; H = 3.06; N = 1.43. The results confirm that the chemical composition of Tamoxifen-PM has a molar ratio of 3:1.

For the preparation of the flow-through all-solid state electrode, the similar steps described in our previous works[23,31] were followed. The electrode was prepared at two steps. At the first step, a mixture of conductive material consisting of 50% (w/w) graphite, 35% (w/w) epoxy, and 15% (w/w) hardener was prepared by mixing in sufficient THF. This conductive material were filled into a 100 μL pipette tip and a copper wire dipped into the conductive material from the one side for the electrical connection and kept at 30 °C overnight. At the second step,

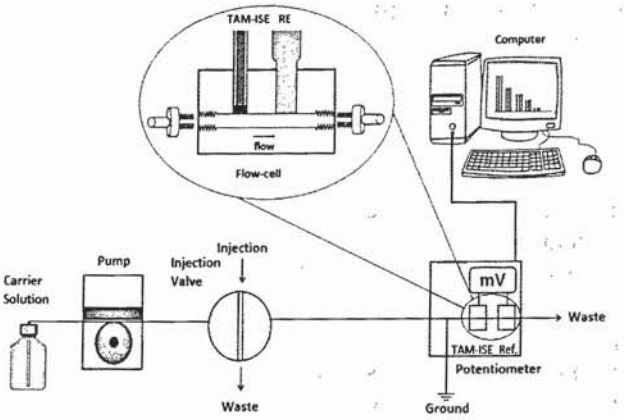


Fig. 2. Schematic representations of the currently used flow injection potentiometric system set up and flow-through detector.

a solvent polymeric membrane was prepared from 3.0% (w/w) TAM-PM ion pair, 67.0% (w/w) NPOE and 30.0% (w/w) PVC by solving in 3 mL THF and its 25 μL aliquot was carefully deposited on the surface of the another side of the pipette tip at three steps. The coated surface was left to be dried at laboratory conditions overnight. The flow-through electrode was conditioned by soaking into 10 mM acidic TAM solution for at least 6 h before use. Then the conditioned electrode and the reference electrode were inserted on the flow-line of the flow-cell made of PMMA. When not in use, the electrode was stored in air. The flow injection system set-up utilized in the current study was basically illustrated in Figure 2.

All potentiometric measurements were carried out at ambient conditions by using the following cell assembly:

Ag/AgCl reference electrode | test solution | PVC membrane | solid contact | Cu wire.

2.4. Chromatographic Measurements

In chromatographic separations, 1% triethylamine (in deionized water, pH 8.0) and methanol (11:89, v/v) solution was used as mobile phase. Throughout the chromatographic measurements, a sample loop of 20 μL was utilized to introduce the standards and samples to the HPLC system. Elution was isocratic at a flow-rate of 1.0 mL min^{-1} at 20 °C. The mobile phase, standard solutions and samples were filtered through 0.45 μm Polyvinyl Difluoride (PVDF) filters before introducing the HPLC system. Tamoxifen was monitored at 260 nm wavelength.

3. RESULTS AND DISCUSSION

3.1. Optimization of FIA Conditions

It is well-known that various system parameters such as type and concentration of carrier solution, flow-rate, injection volume, tubing length etc. affects the analytical performance of the measurement system in flow injection analysis studies. Therefore, these parameters are required to be optimized. Preliminary, we tried to select suitable carrier solution. We decided to use 5.0×10^{-7} M methanolic solution of Tamoxifen at pH = 5.75 as a carrier with regards to baseline stability, reproducibility, linear range and detection limit because the solubility of tamoxifen is low in water. During the optimization process, we have kept the tubing length and injection volume constant as 10 cm (from injection valve to the inlet of detection cell) and 20 μL, respectively.

The dependency of the peak heights on flow rate was studied using the electrode response to 1.0×10^{-3} M TAM. As the flow rate increased, the peaks became narrower and increased in height to near plateau at a flow rate of 2.0 mL min^{-1}. As can be seen in Figure 3, at flow-rates above the 2.0 mL min^{-1}, some decreases were seen in peak heights. Thus, a flow rate of 2.0 mL min^{-1} was selected as an optimum value for further studies. High flow-rate increases sampling rate and decreases the total time of analysis. However, at the same time, high flow-rate increases reagent consumptions and decreases sensitivity of the detection system above a critical flow-rate.

3.2. Potentiometric Performance Characteristics of the TAM Sensor in FIA

Standard methanolic TAM solutions in the concentration range of 5.0×10^{-6}–1.0×10^{-2} M were injected into the carrier solution and the potentiometric signals were recorded. For each concentration of the standard solution, five injections were performed. The obtained peaks and related calibration plot were illustrated in Figure 4. The calibration plot was linear over the concentration range of 1.0×10^{-4}–1.0×10^{-2} M with a slope of 37.7 ± 0.7 per decade of TAM concentration. The R-squared (R^2) value is 0.9998. Furthermore; detection limit of the electrode was calculated from the intersection of the two extrapolated segments of the calibration plot as 4.2×10^{-5} M according to Ref. [32].

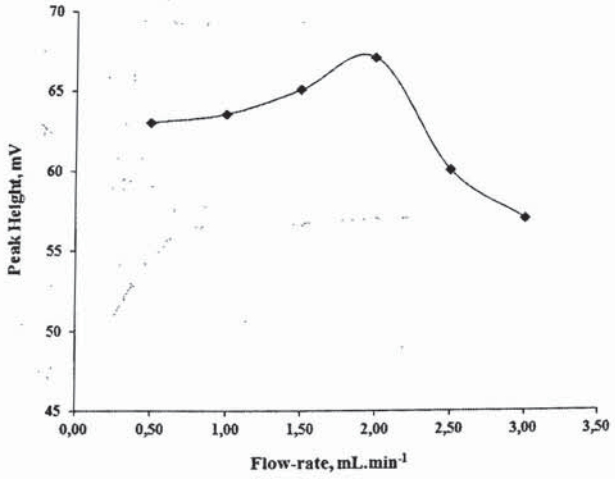


Fig. 3. Peak heights depending on flow-rate.

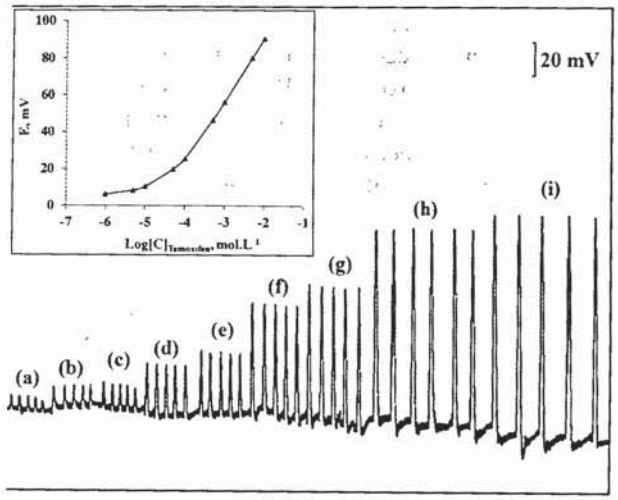


Fig. 4. Injections of TAM at various concentrations. A:1.0×10^{-6}, B:5.0×10^{-6}, C:1.0×10^{-5}, D:5.0×10^{-5}, E:1.0×10^{-4}, F:5.0×10^{-4}, G:1.0×10^{-3}, H:5.0×10^{-3}, I:1.0×10^{-2}. Inset: Calibration curve of the TAM-selective detector versus log a_{TAM}.

3.3. Effects of Sample pH on the Peak Heights

The pH dependence of the FIP response of the electrode was investigated by injecting 20 μL of 1.0×10^{-3} M TAM at different pHs (between 1.03 and 8.05) into the carrier solution (Fig. 5). The pH of 100 mL TAM solution was adjusted by the addition of appropriate volume of 2 M HCl. The potentiometric response profile of the electrode depending on different pH values was illustrated in Figure 5. As can be seen, the potentiometric response does not show a considerable deviation for 1.0×10^{-3} M constant TAM over the pH range of 2.0–6.0 in which the proposed FIP system can be used safely to obtain analytically meaningful measurements for the respective determination. At pH values lower than 2.0, peak heights increase gradually with pH which may be due to the penetration of the hydronium ion into the membrane. At pH higher than 8.0, peak heights decrease gradually which can be attributed to the formation of the free base of the TAM and disappearance of the protonated species sensed by the electrode.

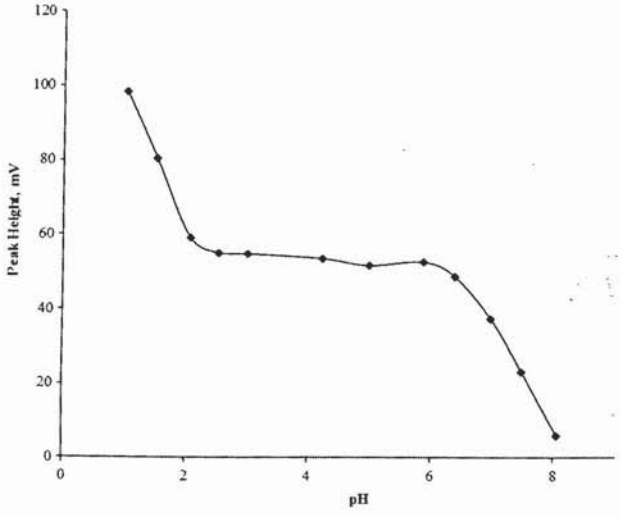


Fig. 5. pH effect on the response of the TAM-selective detector.

3.4. Investigation of Interference Effects

The selectivity coefficients of the flow injection TAM detector were calculated according to the separate solution method (SSM) ($E_A = E_B$)[33] by using 1.0×10^{-4} M TAM concentration and the interferent peak concentration which produced the equal peak heights to 1.0×10^{-4} M TAM. In preparation of the solution of unsolved species in the methanol, 10% (v/v) water:methanol solvent mixture were used. The obtained selectivity coefficients are presented in Table I. The results show that the electrode exhibits selective potentiometric response towards TAM with respect to some common alkaline, alkaline earth, heavy metal, organic and pharmaceutical molecules. Higher selectivity of the electrode toward TAM can be related to the fast ion-exchange kinetic between TAM and selective PVC membrane containing TAM-FM ion pair compared to the other studied species.

3.5. Repeatability of the Electrode

To evaluate the repeatability of the current FIA system, thirteen injections of 5.0×10^{-4} M TAM were performed and the obtained peaks were illustrated in Figure 6. The relative standard deviation was calculated as 1.81%. As seen, the proposed FIA system offers very repeatable and stable measurements.

3.6. Analytical Application

The offered FIP system was employed for the analysis of two drugs containing various amounts of tamoxifen (10 and 20 mg in per tablet). Ten tablets for each drug were crushed and mixed well in a mortar to obtain fine powders. A powder portion equivalent to the weight of two tablets was carefully weighed for drug containing 10 mg tamoxifen and, equivalent weight to one tablet for drug containing 20 mg tamoxifen was added into two separate 20 mL methanol and the mixtures were sonicated for 5 min. The mixtures were centrifuged at 3000 rpm and supernatants were transferred into 100 mL volumetric

Table I. Potentiometric selectivity coefficients of the detector calculated according to SSM.

Interference	$\log K_{TAM,X}$	Interference	$\log K_{TAM,X}$
Na^+	−0.85	Isoniazid	−1.10
K^+	−1.30	Nicotinamide	−2.01
Mg^{2+}	−2.00	D-(−)-Fructose	−2.05
Ca^{2+}	−2.10	L-Ascorbic acid	−1.40
Zn^{2+}	−2.05	D-(+)-Maltose	−1.03
Fe^{3+}	−3.37	Sucrose	−2.09
Cu^{2+}	−1.52	Caffeine	−1.70
Tolbutamide	−1.52		

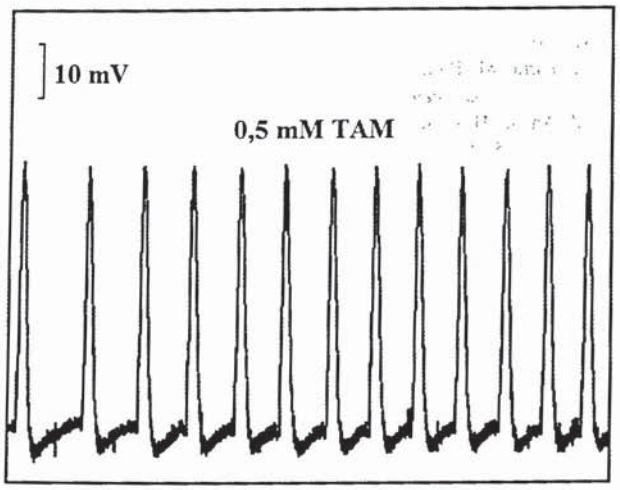


Fig. 6. Repeatability of the TAM-selective detector on optimized conditions ($n = 13$).

flasks. The solutions were acidified with the addition of HCl and the volumes of the solutions were completed to 100 mL with the additions of methanol.

For the determination of TAM contents of the tablets, standard addition method was used. Three replicate injections were performed for each tablet. Later on, 0.5 mL of 0.01 M methanolic tamoxifen solution (containing 0.01 M HCl) was added into 20 mL aliquots of drug solutions two times. After each addition, three replicated injections of the obtained solutions were performed. The changes in peak heights were recorded and they were used in the calculation of the tamoxifen concentrations of the drug solutions. Furthermore, HPLC analysis was performed for the validation of the proposed method. In HPLC analysis, methanolic solutions of tamoxifen in the concentration range of 0.1–1.0 mM were used as standards to obtain calibration plot. Peak areas were used in the construction of the calibration plots and determination of unknown concentrations of the pharmaceutical samples. The analysis results obtained from FIP and HPLC were given in Table II. When the potentiometric results compared to the HPLC results, it is clearly seen that flow injection potentiometric results are similar to the HPLC results because F and t values are lower than the critical values at 95% confidence level.

Table II. Determination of TAM contents of some anticancer drugs by the proposed FIP system and comparison of the results with HPLC results.

Sample	Potentiometric (mg)[a]	HPLC (mg)[a]	Claimed value (mg)	t-test[b]	F-test[b]
Drug A	9.3±0.8	9.7±0.5	10.0	0.63 (2.78)	2.30 (19.00)
Drug B	20.6±0.6	20.2±0.4	20.0	0.79 (2.78)	2.29 (19.00)

Notes: [a]Average of three determinations ± S.D. [b]The values in the parenthesis are the critical t and F at $P = 0.05$.

4. CONCLUSIONS

The proposed TAM-selective flow injection potentiometric detector is simple, low-cost, and easy to manipulate. It has promising potentiometric characteristics. The detector was used successfully for the determination of tamoxifen contents of some anticancer drugs. In the analysis process, no sample pretreatment or separation steps are required. In general, the proposed measurement procedure is inexpensive with regard to reagent consumption and required equipment, especially compared to the previously reported TAM determination techniques.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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