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Review

Electroanalytical Techniques used in Determination of Tamoxifen

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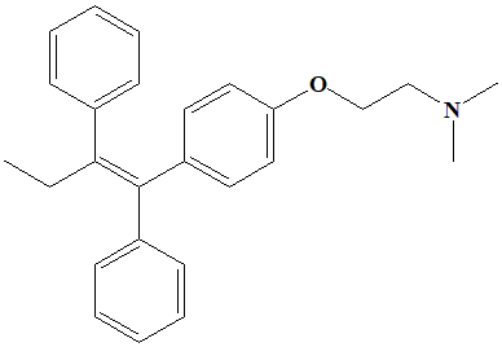
Abstract- Tamoxifen (TAM) is a selective estrogen receptor modulator used in the treatment of breast cancer, women's infertility and some other endocrine diseases. TAM is a generic medication that is prescribed relatively a lot. Due to the impact of this medication and its side effects, screening TAM level in biological samples and in pharmaceutical formulations are of great importance. Various analytical techniques are developed for the detection or monitoring TAM levels in different matrices. Since TAM chemical structure is able to undergo electrochemical oxidation, electrochemical techniques due to their remarkable features are also considered as analytical methods. Here, electroanalytical measurements of TAM will be reviewed.

Keywords- Tamoxifen; Non-steroidal antiestrogen; Electrochemical determination; Sensors; Biosensor

1. INTRODUCTION TO TAMOXIFEN

Tamoxifen (TAM) with chemical name, 1-p-beta-Dimethylaminoethoxyphenyl-trans-1,2-diphenylbut-1-ene (Scheme 1) is a selective estrogen receptor modulator used in the treatment of breast cancer, women infertility and some other endocrine diseases. TAM is a non-steroidal antiestrogen which is used in treatment of estrogen receptor positive breast cancers [1]. It is used alone or as an adjuvant in these treatments. Although TAM sometimes is not the preferred treatment for breast cancers due to the side effects, and compliance with other medications such as anastrozole [2], it is the oldest and the high-consumption selective estrogen receptor modulator (SERM).



Scheme 1. Chemical structure of TAM

TAM is also recommended for hormone-sensitive breast cancer in both women and men [3]. It bonds to estrogen receptors and suppress the growth of breast tumors. TAM can be hydroxylated to α-hydroxytamoxifen which is then glucuronidated or undergoes sulfate conjugation by sulfotransferase 2A1 [4-8]. The resulting metabolites, 4-hydroxy TAM (afimoxifene) and N-desmethyl-4-hydroxytamoxifen (endoxifen), have 30 to 100 times more affinity for estrogen receptors than TAM alone [9]. However, TAM and its metabolites block growth factor proteins in breast tissue cells [10]. This complex is able to inhibit the effects of estrogen and suppresses the DNA synthesis in cell proliferation [11-14]. TAM is mainly excreted in the feces and urine.

2. IMPORTANCE OF DETERMINATION OF TAM

Since TAM's approval in 1998, it has been prescribed to treat millions of women and men diagnosed with hormone-receptor-positive breast cancer. U.S. Food and Drug Administration (FDA) recommends TAM for treatment of women and men early or even advanced stage breast cancer and other metastatic hormone-receptor-positive disease. Furthermore, TAM is used to reduce breast cancer risk in women are at higher-than-average risk. While TAM is not effective

on hormone-receptor-negative breast cancer, it is an aromatase inhibitor which is the first hormonal therapy medicine choice for postmenopausal women. TAM reduces the risk of breast cancer coming back up to 50%, and reduces the risk of a new cancer developing in the other hormone-receptor-positive breast cancers [15].

Besides the profits of TAM as medication, it can cause some side effects such as blurred or decreased vision or even blindness, irregular menstrual periods, bladder pain, decrease in the volume of urine, bloody or cloudy urine, chills, confusion, cough, difficult or labored breathing, dizziness, fainting, fast heartbeat, fever, increased clear or white vaginal discharge, lower back or side pain, pale skin, rapid weight gain, stopping of menstrual bleeding, tightness in the chest and tingling of the hands or feet [16]. High doses of TAM in advanced metastatic cancer patients can causes in acute neurotoxicity, hyperreflexia, unsteady gait, and dizziness [17].

TAM has been widely prescribed as an oral nonsteroidal antiestrogen drug [18,19]. It is prepared through several methods for the prevention and treatment of breast cancer.

Tamoxifen citrate is rapidly metabolized through hydroxylation, demethylation and conjugation, giving rise to several metabolites with a similar pharmacological property to TAM. The metabolites are excreted as conjugates in the bile, and little TAM is eliminated as unchanged drug. Excretion is mainly via the faeces.

TAM is not an easy biodegradable compound. In aqueous environment, TAM may be adsorbed to solids or sediments. Tamoxifen citrate is an ionisable compound. The octanol-water distribution coefficients values are less than 4.5 but more than 3. In the normal environmental pH range (pH 5–9) TAM, the risk of bioaccumulation of TAM in aquatic organisms is low [20]. The Predicted Environmental Concentration (PEC) / Predicted No Effect Concentration (PNEC) ratio is 0.47, which means use of TAM is predicted to present a low risk to the environment.

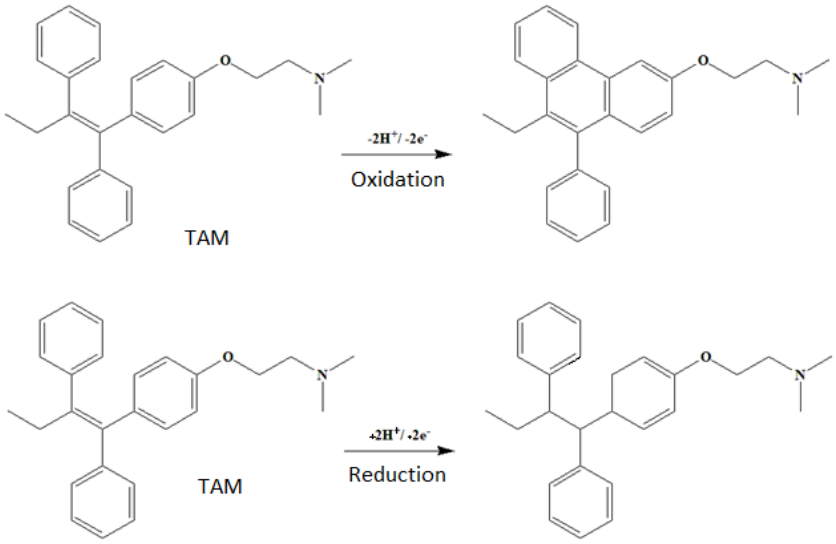
Due to the rather high prescription and use of this drug and its side effects on the human body and the environment and the need to determine the level of this drug in formulations and in biological fluids, there is an urgent need to measure the amount of TAM. In this regards, different analytical methods have been developed to investigate and determine TAM and its metabolites in biological and pharmaceutical formulations.

Analytical techniques, such as spectrophotometry [21-23], high-performance liquid chromatography (HPLC) [24,25], liquid chromatography–mass spectrometry (LC–MS) [26], thin layer chromatography (TLC) [27], gas chromatography (GC) [28], gas chromatography–mass spectrometry (GC–MS) [29,30], ion chromatography [31,32], capillary electrophoresis [33-40], fluorescence and phosphorescence methods [41-44], and electrochemical methods, such as polarography [45], potentiometry [46], and voltammetry [47-49] have been used to analyze and control the dosage of TAM in different samples.

Among these analytical methods, electrochemical techniques offer advantages of simplicity, cost effectiveness, portability, and fast response time.

3. ELECTROCHEMICAL DETERMINATION OF TAM

Researches on the electrochemical determination of anticancer drugs grow rather fast and wide because most of these types of medications have electroactive groups, such as OH and NH_2. Valuable pharmacology information on electroactive anticancer drugs can be obtained through electrochemical tools. TAM, as seen in Scheme 2, can be an electroactive compound that can undergo electro-oxidation on the surface of electrodes.



Scheme 1. Double electron transfer of TAM oxidation on glassy carbon electrode [48,51]

Literature survey resulted 15 reports on determination of TAM in variety of matrixes through electrochemical methods [45-58]. The most important features of each measurement have been listed in Table 1.

The first electrochemical determination of TAM dates back to 1987 [45] when FijaŁek et al. used direct current polarography and cyclic voltammetry to study TAM electrochemical behavior. They observed two reduction signals. The first reduction wave had a diffusive-adsorptive character, and the second one was a catalytic wave. The reduction processes consume two electrons and two protons. They also concluded that oxidizing the reduced bond is very small, an anodic peak seen on the cyclic curves is perhaps produced by oxidation of phenanthrene derivative of TAM. They finally determined TAM contents of some tablets by their proposed method with good accuracy.

Ten years later, Wang et al [50] introduced an adsorptive stripping potentiometry for trace determination of TAM. In this method, TAM was first accumulated on an electrochemically treated glassy carbon electrode through adsorption at -0.1 V for 4-min in 0.05 M BR buffer (pH 4) containing 20% methanol and then was measured by chronopotentiometry. The

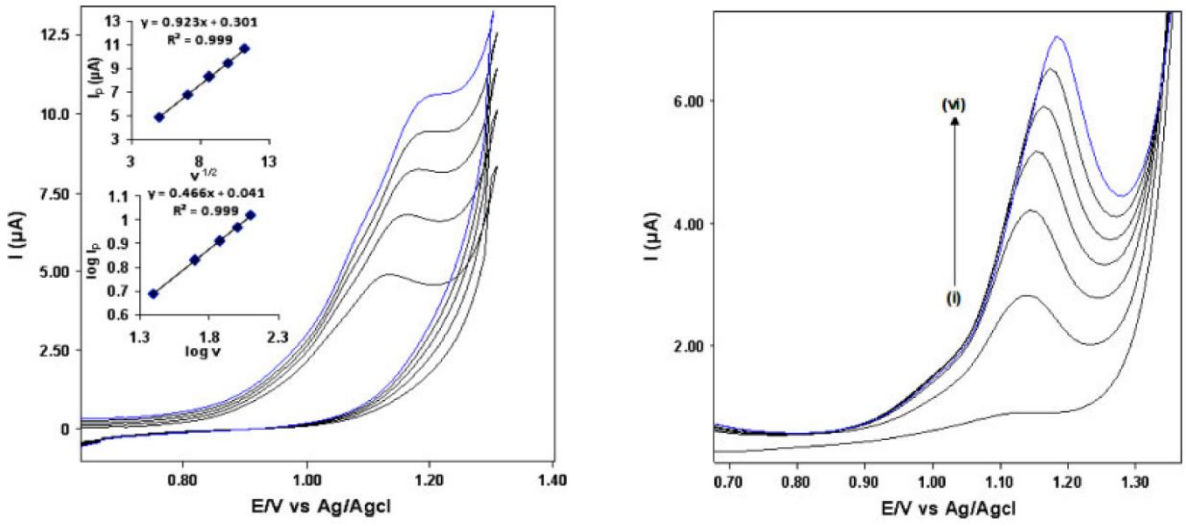
chronopotentiometric operation effectively removes the large background contribution inherent to the GCE.

Far after in 2008, Guo et al [51] proposed a single-sweep voltammetric method for the determination of TAM. Their method had the advantage of both accumulation of TAM on a carbon paste electrode and speed of single-sweep voltammetry. In an acetate buffer (pH 4.1)/methanol (85:15 v/v) solution, an irreversible oxidation peak of TAM was seen at 1.1 V (vs. SCE). The second-order derivative peak current of TAM and its concentration plots were linear with a detection limit of 1.0×10^{-10} M without any preconcentration.

In a report on 2009 by Norouzi et al [52] determination of TAM in urine and plasma samples was done by fast Fourier transform square wave voltammetry (FFT-SWV) using a gold microelectrode in a flow-injection system. Using sensitive square wave voltammetry, the determination was performed by measuring the changes in admittance voltammogram of a gold ultramicroelectrode (in 0.05 M H_3PO_4 solution) after adsorption of TAM on the electrode surface. The best response was obtained in a frequency of 600 Hz and 0.05 M phosphate buffers (pH 2.0). The best performance was obtained with the pH value of 2, pulse amplitude 25 mV, frequency 600 Hz, accumulation potential of -100 mV, and accumulation time of 0.5 s. Moreover, using the discrete fast Fourier transform method, background subtraction and two-dimensional integration of the electrode response over a selected potential range and time window increased signal-to-noise ratio significantly which led to a low detection limit.

In 2011, Guo et al [53] found that oxidation peaks of a sequence of calf thymus dsDNA and TAM overlapped with each other. Hence, they used zero-current potentiometry to determine TAM. For this purpose, the dsDNA was immobilized on the surface of a carbon paste which connected in series between a counter and a reference electrode. Interaction of dsDNA and TAM molecules caused a change in interfacial potential at the dsDNA/CPE and solution interface. Linear sweep potential was applied to the dsDNA/CPE and the corresponding I-E curve was recorded. Interfacial potential offset applied potential partially, making the I-E curve displace along potential axis. Zero-current potential where circuit current I was equal to zero in the I-E curve was measured to be sure about the displacement of the I-E curve. In this way, the thermodynamic binding constants of a 1:1 interaction between dsDNA and TAM was obtained $(6.85\pm0.20)\times10^{6}$ M^{-1}. Such potentiometric method was independent of the changes in redox potential or current of both dsDNA and TAM themselves.

In another reports in 2011 by Jain et al [54], electrochemical behavior of TAM at gold electrode was studied through cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (Fig. 1). They found that TAM was oxidized in a single two-electron, irreversible and diffusion-controlled wave. Linear calibration plots are obtained over the concentration range 1.0-5.0 and 1.0-6.0 μgmL^{-1} in 1.0 M KCl and Britton Robinson buffers (pH 2.51) respectively. The procedure has been applied to the assay of TAM in tablets with mean percentage recoveries of 99.98%.



A **B**

Fig. 1. A) Cyclic voltammograms of 6.0 μgmL^{-1} TAM citrate in 1.0% TX-100 at different scan rates 25, 50, 75, 100 and 125 mV s^{-1}; B) DPVs for TAM citrate at different concentrations in BR buffer pH 2.5, (i) Blank (ii) 1.0 (iii) 2.0 (iv) 3.0 (v) 4.0 (vi) 5.0 (vii) 6.0 μgmL^{-1}. Reprinted with permission from [54]

Sharma et al [55] in 2012 investigated the electro-oxidative behavior of TAM and 4-hydroxytamoxifen, one of the TAM metabolite, by CV, differential-pulse adsorptive anodic stripping (DPAdAS) and square-wave adsorptive anodic stripping voltammetry (SWAdAS). Like other studies, anodic oxidation peak of TAM was corresponded to the cyclization reaction to form the phenanthrene derivative and the mechanism of oxidation was based on controlled potential electrolysis and isolation of the oxidative product. Oxidative stripping analysis was successfully used for the determination of TAM in a pharmaceutical formulation, human urine and serum. Since 4-hydroxytamoxifen was oxidized at more positive potentials than TAM, separated from the TAM stripping peak, and its adsorption to the glassy carbon electrode is less than TAM.

In 2013, a TAM amperometric biosensor was reported by Radhapyari et al [56]. The biosensor was prepared by horseradish peroxidase immobilization on a polyaniline modified platinum electrode. CV was used to monitor the electro-catalytic reduction of TAM under diffusion-adsorption controlled conditions. The proposed biosensor demonstrated excellent electro-analytical properties with sensitivity of 1.6 μA ngmL^{-1}.

Yarman and Scheller [49] in 2014 were introduced an electrochemical MIP sensor for TAM. MIP was synthesized by electropolymerisation of an *O*-phenylenediamine–resorcinol mixture directly on the electrode surface in the presence of TAM as a template molecule. Electro-

polymerisation of the monomers in the presence of TAM generated a film which completely suppressed the reduction of ferricyanide. Removal of TAM gave an increased ferricyanide signal significantly. The decrease of the ferricyanide peak of the MIP electrode depended linearly on the TAM concentration between 1 and 100 nM.

Kanberoglu et al [46] in 2015 reported a novel TAM selective potentiometric sensor based on ionic feature of TAM. A plastic membrane composed of (w/w) 3% TAM-phosphomolibdate ion-pair, 32% poly(vinylchloride), and 65% 2-nitrophenyloctylether was used to made the indicator electrode. In the pH range of 2-6, the sensor responded in the concentration range of 9.1×10^{-6}- 1×10^{-3} M of TAM with a sensitivity of 42.9±0.3 mV/decade within 25 s.

Kanberoglu et al [57] also used the membrane sensor in a flow-injection system for rapid determination of TAM. The flow-system proposed sampling rates of approximately 90 injections per hour depending on the TAM concentration of the injected sample.

Table 1. Electrochemical reports on electro-analysis of TAM

Working and Ref Electrodes	**Electrolyte and pH**	**Technique**	**Real sample**	**Linear Range**	**LOD**	**Year**
GCE vs. Ag/AgCl	0.05 M BR pH 4.0	Chronopotentiometry	Human urine	1-10 nM	4×10^{-10} M	1997 [50]
CPE vs. SCE	Acetate buffer (pH 4.1) /methanol (85:15 v/v)	Single-sweep voltammetry	Tamoxifen citrate tablets	7.0×10^{-10} ~ 3.0×10^{-8} M	1.0×10^{-10} M	2008 [51]
Gold microelectrode vs. Ag| AgCl	0.05 M PBS pH 2.0	SWV	Urine and plasma and formulation	1.0×10^{-11}-3.0×10^{-6} M	3.0×10^{-12} M	2009 [52]
dsDNA/CPE vs. SCE	PBS pH 7.0	Zero-current Potentiometry	-	2.0×10^{-7}-8.0×10^{-6} M	1.1×10^{-7} M	2011 [53]
GE vs. Ag/AgCl	1.0 M KCl/BR pH 2.51	SWV DPV	Drug formulations	1.77-8.87 μM 1.77-10.64 μM	4.59 nM 16.67 nM	2011 [54]
GCE vs. Ag/AgCl	Universal BR pH 4.2	DPAdAS SWAdAS	Human urine and serum	0.2×10^{-3}–1.5×10^{-3} M	1.98×10^{-7} M 4.75×10^{-7} M	2012 [55]
Pt/PANI/HRP vs. Ag/AgCl	0.1 M PBS pH 6.8	CV	Pharmaceutical formulation	2.69–29.6 nM	0.188 nM	2013 [56]
TAM-MIP/ GCE vs. Ag/AgCl	-	CV	Serum samples	1- 100 nM	-	2014 [49]
PVC membrane electrode vs. Ag/AgCl	pH 2-6	Potentiometry	Pharmaceutical formulations	9.1×10^{-6}-1×10^{-3} M	7.3×10^{-6} M	2015 [46]
PVC membrane electrode vs. Ag/AgCl	-	Flow-injection potentiometry	Pharmaceutical formulations	1.0×10^{-4}-1.0×10^{-2} M	4.2×10^{-5} M.	2016 [57]
Graphene CPE vs. Ag/AgCl	0.1M H_2SO_4:2.5%v v^{-1} MeOH	DPAV	Pharmaceutical and serological samples	1.34 – 13.45 μM	0.067 μM	2017 [58]
ds-DNA- GPE vs. Ag/AgCl	0.1 M BR pH 2.5	LSV	Tablet, serum and urine	8.0×10^{-7}-8.5×10^{-5} M	1.0×10^{-7} M	2017 [59]

In 2017, Deris Falahieh et al [58] studied CV of TAM by diverse electrodes in acidic electrolytes containing 10% v v^{-1} methanol (MeOH). TAM oxidation was found to be most favorable using 0.1 M H_2SO_4. The best conditions were 2.5% v v^{-1} MeOH, 0.1 M H_2SO_4, deposition potential 0.4 V, deposition time 30 s, and GCE rotating rate 400 rpm.

In another report in 2017 Moghaddam et al [59] applied DPV to study an electrochemical interaction between TAM and salmon-sperm double-stranded DNA (ds-DNA) on a graphene paste electrode. Then, they made a sensitive biosensor based on this interaction. The proposed biosensor was used for determination of TAM in tablet, serum and urine samples.

These are report on electrochemical determination of TAM. Research on the development of the new modified electrodes and sensors are seriously going on. Among electrochemical methods, now a days, biosensors based on nanomaterials play an important role in determination of pharmaceuticals, drugs, poisons and organic pollutants [60].

4. CONCLUSION AND SUMMARY

As an oral non-steroidal antiestrogen drug, TAM has been widely prescribed for the prevention and treatment of breast cancer and some endocrine disorders. Due to its side effects, and indicating the plasma levels, or its concentration in pharmaceutical formulations its determination is of great importance. TAM, as an electroactive compound, is electro-oxidized from phenyl moiety and transfer two electrons through a redox reaction. Thus, electrochemical techniques can be an alternative method for rapid and/or on-line detection of TAM. Among all reports, Norouzi et al reached the best sensitivity, detection limit and widest linear range using a gold microelectrode in a flow-injection system by fast Fourier transform square wave voltammetry. The biosensors introduced by Radhapyari et al was also one of the excellent electrochemical method for monitoring TAM concentration.

List of Abbreviations

Abbreviation	**Full name**
BR	Briton-Robinson buffer
CPE	Carbon Paste Electrode
CV	Cyclic Voltammetry
DPV	Differential Pulse Voltammetry
DPAdAS	Differential-Pulse Adsorptive Anodic Stripping
GCE	Glassy Carbon Electrode
GE	Gold Electrode
FFTSWV	Fast Fourier Transform Square Wave Voltammetry
LOD	Limit of Detection
LR	Linear Range
LSV	Linear Sweep Voltammetry
MIP(s)	Molecularly Imprinted Polymer(s)
PBS	Phosphate Buffer Solution/Saline
SWV	Square Wave Voltammetry
SWAdAS	Square-Wave Adsorptive Anodic Stripping Voltammetry
SCE	Standard Calomel Electrode
TAM	Tamoxifen

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