RESEARCH ARTICLE

Carbon nanotube/polyurethane modified hollow fiber-pencil graphite electrode for in situ concentration and electrochemical quantification of anticancer drugs Capecitabine and Erlotinib

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A sensitive electrochemical sensor has been designed for in situ preconcentration and determination of anticancer drugs Capecitabine (CPT) and Erlotinib hydrochloride (ETHC) based on a pencil graphite electrode modified with multivalued carbon nanotube—polyurethane (MWCNT-PUFIX) nanocomposite that was supported with a piece of polypropylene hollow fiber (HF-PGE). The electrochemical behavior of CPT and ETHC on the MWCNT-PUFIX/HF-PGE modified electrode was investigated by differential pulse voltammetry (DPV) techniques and the obtained results confirmed its efficiency for sensing of CPT and ETHC. The synthesized nanocomposite was characterized by infrared spectroscopy and scanning electron microscope. After optimization of some effective parameters on the method efficiency including pH, nanocomposite amount, the type of organic solvent, scan rate and the effect of some additives, the mentioned sensor presented suitable results for determination of CPT and ETHC with the linear ranges from 7.70 to 142.00 μM and 0.11 to 23.50 μM and detection limits of 0.11 and 0.02 μM , respectively. Also, the fabricated sensor has shown good performance in analysis of CPT and ETHC in biological samples.

KEYWORDS

Capecitabine, Erlotinib, hollow fiber, multiwalled carbon nanotube, polyurethane foam

1 | INTRODUCTION

In the developed countries the second cause of death is cancer that is steadily increasing [1]. The recent statistics estimate that in the next three years the number of cancer patients will increase to more than 15 million [2]. Capecitabine (CPT), that chemically known as 5'-deoxy-5-fluoro-N4-pentyloxycarbonyl-cytidine (Scheme 1A) and Erlotinib hydrochloride (ETHC) with chemical formula; N (3-ethynylphenyl) [6,7 bis (2methoxyethoxy) quinazolin-4-amine] (Scheme 1B), are used as a first-line treatment for dif-

ferent forms of cancer such as head and neck, lung, pancreatic, ovarian, colorectal, and breast cancers [3,4].

The first step to emerging better treatments is the selection of good strategies for challenging. Testing the drug candidates using robust methods is the next critical step to improving treatments. These methods not only classify the best drug candidates, but also predict whether their anticancer effects are high enough to merit clinical assessment.

Until now various methods such as HPLC, LC/MS, LC/MS/MS, HPTLC, UPLC, RP-HPLC, spectrophotometry, spectrofluorimetry, and electrochemical analysis have been reported for the estimation of these drugs [5–10].

Liquid chromatography is a rapid and simple method for studying drugs in pharmaceutical and clinical samples, but they need expensive devices and high purity solvents, which can restrict their use [11]. Also the electrochemical methods can be seen in the literature because of their comfortable

Abbreviations: CPT, Capecitabine; DPV, differential pulse voltammetry; ETHC, Erlotinib hydrochloride; FT-IR, furrier transform of infrared spectroscopy; HF-PGE, hollow fiber pencil graphite electrode; MWCNT-PUFIX, multivalued carbon nanotube–polyurethane; PUF, polyurethane foam; ZPC, zero point of charge

SCHEME 1 Chemical structure of (A) Capecitabine, and (B) Erlotinib hydrochloride

properties such as short analysis time, low cost, and sensitivity. The application of electrochemical methods in the analysis of pharmaceuticals has significantly improved over the latest few years. The renewed attention in electrochemical techniques can be attributed in part to more sophisticated instrumentation such as more precise and selective electrodes. Furthermore, a large number of electroanalytical sensors are available for quantification of pharmaceuticals and related compounds [12,13]. An important category of these sensors is based on electrodes modified by nanocomposites. Polymer-modified electrodes are among these.

An important class of polymers is polyurethane foam (PUF) that, as biomaterials, due to their biocompatibility and comfortable physical properties have played a special part in industrial activities, agriculture, medicine, etc. Polyurethanes are derived from the poly-condensation of di-isocyanates (hard segments with $-N=C=O$ groups) and di- or polyols (soft segments with $-OH$ groups) [14].

PUFs sorbents with functional groups are especially useful in trace analysis, preconcentration, and separation of analytes [15]. Although conventional PUFs have some excellent properties for trapping and separation of specific analytes but, the capacities of these PUFs are very low [16]. Therefore, in order to improve their properties, nanoparticles with specific characteristics are used in the polymer matrix. These can react with functional groups already present on the polymer chains [17]. The incorporation of nanoparticles with their large surface area and fine dimensions, as well as very close contact between particles and the polymer matrix, could alter foam morphology and its properties [15,18,19].

On the other hand, due to matrix effects in real samples, a pretreatment step for separation and preconcentration of analytes before analysis is essential. Nanocomposites have been employed as an efficient absorbent for this purpose [20].

Owing to difficulties in using the electrodes for field analysis in electrochemical methods, the development and application of single-use electrodes are widely desirable. The important note is that disposable electrodes only once used therefore not being affected by the contaminated carryover problems [21,22].

The present paper describes a novel disposable MWCNT-PUFIX/HF-PGE sensor, based on pencil graphite electrode

PRACTICAL APPLICATION

In this work, we used a new, simple, affordable, disposable, and applicable sensor for in situ preconcentration and electrochemically screening of anti-cancer drugs Capecitabine and Erlotinib. In the present work, we decided to introduce an innovative disposable hollow fiber-based carbon nanotube-polyurethane modified-electrochemical sensor. So that, the new sensor plays the role of the drug trap and simultaneously serves as the working electrode or an electrochemical sensor for simultaneous detection of Capecitabine and Erlotinib. The homemade electrode has been designed for extraction, preconcentration, back-extraction, and electrochemically detection of the analytes. The work can be considered as a novel and simple method for a wide range of drug analytes due to the flexible nature of the nano-carriers.

Moreover, the proposed method allows the very effective and enriched recuperation of the analytes into one single extract. Ease of preparation, low background current, high sensitivity, stability, and small loading of nanocomposite can create new potentials and applications for fabricating robust sensors for many important species.

modified with MWCNT-PUFIX nanocomposite covered with hollow fiber and an innovative approach for determination of Capecitabine and Erlotinib drugs. This sensor acts as working electrode in differential pulse voltammetry technique for the tracing amounts of the mentioned drugs in real biological samples.

2 | MATERIALS AND METHODS

2.1 | Instruments

Electrochemical experiments were performed by a Metrohm Model 797 Computrace (Switzerland). A conventional three-electrode cell was used with an $Ag/AgCl/3\text{ M KCl}$ as the reference electrode, a Platinum wire as the counter electrode and the designed sensor was used as a working electrode. The dispersing process of MWCNT and PUF were performed by an Ultrasonic Processor, model UP 400S (Germany) with 60% amplitude and 0.5 Sec cycle. The functional groups of the nanocomposite in fabrication steps were studied with a Shimadzu FTIR-8400 Fourier transform infrared spectrophotometer (Japan). The pH measurements were carried out using a Metrohm pH meter 827 (Switzerland).

2.2 | Chemical and reagents

The anti-cancer drugs, Capecitabine (CPT) and Erlotinib hydrochloride (ETHC) was awarded from Osvahpharma Co. (Tehran, Iran). Chitosan powder (CAS Number 9012-76-4) of ~ 5 kDa was obtained from Sigma-Aldrich (Switzerland).

The polypropylene hollow fiber membrane was obtained from Membrana (Wuppertal, Germany, wall thickness 200 nm, the inner diameter was 600 nm). The multiwalled carbon nanotubes with 10–40 nm diameters, 1–25 μ m length, core diameter 5–10 nm, with 98% < purity were purchased from Research Institute of the Petroleum Industry, Tehran, Iran.

All organic solvents and all reagents in analytical grade were purchased from Merck and Sigma-Aldrich (Darmstadt, Germany). The stock solutions were prepared by dissolving a calculated amount of drugs in ethanol/water solution.

2.3 | Synthesis of the nanocomposite (MWCNT-PUFIX)

Polyurethanes are an important class of polymeric sorbents for some organic and inorganic analytes due to its high available surface area and extremely low cost. In addition, it is stable in acids and bases, it will not change its structure when heated up to 180°C. By means of its high basicity decreases its sorption capacity, low basicity polyurethane must be prepared. Thus, must be fabricated the polyurethane containing a polyhydroxyl functional group, which has a high sorption capacity [23,24].

Polyhydroxy polyurethane was prepared by replacing the primary amine with a hydroxyl group. Because the hydroxyl groups act as selective receptors with high bond stability for a wide range of target analyte. For this aim, the commercial polyurethane foam (PUF) was shaved so fine, then 1 g of shaved PUF were soaked in HCl 3 M solution for 24 h. This mixture was washed with distilled water and was placed into a 0.1 M HCl solution, then cooled in an ice bath.

Introduction of the ionic groups by the reaction of ethyl iodide with the amine led to phase separation as the results of the exclusion of the ionic hard segments from the soft segments. On the other hand, the polyurethane polyhydroxylated displayed a single phase behavior. Thus, solution of polyurethane is stabilized by the addition of an organic halogen-containing compound such as ethyl iodide. The stabilized matrices are protected against degradations on aging, even at high temperature. Therefore, the PUF was stirred strongly and 10 mL of ethyl iodide was added dropwise to it. The product for 24 hours was left in the fridge. PUFIX was air dried and then blended in an agate mortar.

Multiwalled carbon nanotube was functionalized by sonication in the mixture of nitric and sulfuric acid (70:30) for 6 h by 60% amplitude and 0.5 S cycles.

To prepare nanocomposite, the synthesized PUFIX and functionalized MWCNT were added to 40 mL ethanol and refluxed for 6 h at less than 60°C. The black powder MWCNT-PUFIX was obtained which was washed with distilled water and acetone, respectively, followed by air-drying. Prepared MWCNT-PUFIX was blended in an agate mortar [17]. Scheme 2, shows the steps of MWCNT-PUFIX nanocomposite synthesis.

FTIR spectrums of primary PUF, PUFIX, initial, and functionalized multiwalled carbon nanotube was investigated. Also, Scanning Electron Microscope (SEM) imaging of MWCNT-PUFIX was carried out.

2.4 | Fabrication of the MWCNT-PUFIX/HF-PGE electrode and LSSPME procedure

To remove impurities, polypropylene hollow fiber was cut into segments of 15.0 mm length, and washed with distilled water and acetone, respectively, and dried in air. One end of the hollow fiber segment was closed by heating. Besides graphite pencil rod with O.D., 0.5 mm was cleaned with water and acetone and air dried.

Graphite pencil rod was placed in the dispersed mixture of synthesized MWCNT-PUFIX in organic solvent and mixed again, by an ultrasonic processor for 10 min with cycle 0.5 s and 60%. The Graphite pencil rod was inserted carefully into the hollow fiber segment. This one-use MWCNT-PUFIX/HF-PGE electrode was placed in a voltammetry cell and use as a working electrode and it also was performed simultaneously as a preconcentration agent, before analyzing the drugs without carryover.

After fabrication the one-use MWCNT-PUFIX/HF-PGE sensor, it is the time to use the fabricated device for separation, preconcentration, and electrochemical determination of anticancer drugs in one step as a new approach. Liquid-solid phase microextraction procedure was conducted as follows:

The solution of Erlotinib and Capecitabine in phosphate buffer (0.05 M) was added to the voltammetry cell as a supporting electrolyte in pH of 7.0. The MWCNT-PUFIX/HF-PGE electrode was inserted in the voltammetry cell as the working electrode in the three electrode system. The peak current of drugs was recorded by voltage scanning in the range of 0 to +1.5 V. Scheme 3 shows the steps well.

Its mechanism is such that, the analyte molecules penetrate inside the fiber through the fiber wall pores and adsorb by the nanocomposite at the electrode surface. Finally, the extracted analyte is detected by the electrochemical reaction [25–28].

It is notable that, after placing the working electrode in a three-electrode system and scanning was performed instantly, no peak was observed. As the simultaneous

SCHEME 2 The steps of MWCNT-PUFIX nanocomposite synthesis

SCHEME 3 The process of preparation and use of MWCNT-PUFIX/HF-PGE for preconcentration and determination of CPT and ETHC. In order to clarify the role of the fabricated working electrode, reference electrode (Ag/AgCl in 3 M KCl) and the counter electrode (the platinum wire) are not represented in the scheme

preconcentration and extraction processes are ongoing, just after five consecutive scans the analyte peaks were observed and quantified.

Effective parameters such as pH of donor phase (from 3.0 to 11.0), different supporting electrolytes with different concentrations, the nanocomposite concentration, different solvents for dispersion of nanocomposite, scan rate, the effect of adding triton X-100, and chitosan to donor phase were optimized. Finally, calibration curve was obtained under optimized parameters and LOD and LOQ and real samples were investigated.

3 | RESULTS AND DISCUSSION

3.1 | Characterization of synthesized nanocomposite

Furrier transform of infrared spectroscopy (FT-IR) was performed to verify the functional groups in all steps of synthesis. The FT-IR spectrums of sequential steps of the synthesis of nanocomposite are illustrated in Figs. 1–3.

As can be seen in spectrums of PUF and PUFIX, several bands stretch at 3402.2, 3270.6, 2770.0, and 1580.4 cm^{-1} due to free NH, –NH of urethane group, aliphatic hydrocar-

FIGURE 1 FT-IR spectrum of (A) PUF (B) PUFIX and (C) SEM imaging of PUF (www.engii.org)

FIGURE 2 FT-IR spectrum of (A) MWCNT (B) functional MWCNT and (C) SEM imaging of MWCNT (www.carbonnano.co.kr)

bon, and urethane ($-\text{NHCOO}-$) groups, respectively. Also at 1724.2 , two bands at 1527.5 and 1410 and finally 1072.3 were observed, which are related to carbonyl group that is shifted to lower energy because of the conjugated NO_2 group and C-O stretching vibration, respectively (Fig. 1).

In the following, the infrared spectra of initial and functionalized MWCNT were studied. The band stretches at 2751.2 indicates the presence of aliphatic hydrocarbon, at 1645.2 for $\text{C}=\text{C}$ bond, also bands at 1519.8 and 1415.2 due to the presence of NO_2 results of nitric acid and sulfuric acid used for functionalized (Fig. 2) [29].

Some of the bonds in the MWCNT and PUFIX are disappeared in the spectrum of MWCNT-PUFIX (Fig. 3), reflecting joining of these groups with the surface functional groups of carbon nanotubes [30]. The IR spectrum of MWCNT-PUFIX, indicate NH of the urethane group at 3261.2 , the carboxyl group at 1716.5 and, the NO_2 group at 1519.8 and 1418.3 with the slight shift in the absorption bands that indicated that they are involved the binding. Also, the presence of tertiary amino group approved by a band at 1255.3 cm^{-1} and $\text{C}=\text{C}$ bands

at 1645.2 cm^{-1} [24,31]. By the SEM image, the morphology of the MWCNT-PUFIX was examined. As can be seen in Fig. 3, multiwalled carbon nanotubes twisting around the foam nanoparticles (with $r \approx 53$), creating the perfect platform for analyte transport to the nanocomposite and increase extraction.

3.2 | Optimization of experimental parameters

3.2.1 | The effect of supporting electrolyte

The supporting electrolyte by large ionic strength and conductivity is widely used in electrochemical measurements to eliminate the transport of electroactive species via ion migration in the electric field, to maintain constant ionic strength, constant pH, etc. [32]. The electrochemical studies of the CPT and ETHC were carried out at Robinson buffer, phosphate buffer, and sodium perchlorate by differential pulse voltammetry technique (DPV) in pH 7.0. The voltammograms were obtained in the range of 0 to $+1.5 \text{ V}$ for drugs solution.

FIGURE 3 (A) FT-IR spectrum of MWCNT-PUFIX. (B) SEM imaging of MWCNT-PUFIX

FIGURE 4 The Voltammogram and the chart of effect of supporting electrolyte versus current of working electrode (MWCNT-PUFIX/ HF-PGE) for CPT and ETHC extraction over the range 0 to +1.5 V vs. Ag/AgCl

In the presence of sodium perchlorate and Robinson buffer, approximately no good peaks were observed, but the peak obtained in phosphate buffer is so sharper. In the next step, phosphate buffer 0.05 and 0.1 M were examined and compared, as is clear from 4. 4, in the presence of phosphate buffer 0.05 M, the obtained peak is more profound.

3.2.2 | Effect of pH

The environment pH has an important effect on chemical reactions and it can be positive or negative role in the process. Therefore, the effect of pH on the peak current in the presence of 0.05 M phosphate buffer was studied in the pH range of 3.0–11.0. The results showed that the peak current is dependent on the pH of the donor phase. Capecitabine and Erlotinib hydrochloride were showed maximum peak intensity at the neutral pH of 7.0, as shown in Fig. 5. This may be the result of several factors; protonation and deprotonation of COOH groups in the acid-treated MWCNTs and the dispersion of acid-treated MWCNTs in water is exposed to change of pH.

Thus, the dispersion of acid-treated MWCNTs increases with increase in pH, because low pHs lead to aggregation by hydrogen bonding due to full protonation of MWCNTs.

On the other hand, at low pHs, the hydrogen bonds can occur between the protons of the CH₂ groups in the aromatic ring of drugs and the nitrogen atoms of the PUF, which can increase the extraction efficiency [20,33,34].

Moreover, the zero point of charge (ZPC) value for the MWCNT-PUFIX is in the basic region (about pH 8.0–9.0) that is based on terminal groups. At pHs lower than that of ZPC, the surface of the MWCNT-PUFIX is positively charged while at higher pHs, the surface of the MWCNT-PUFIX becomes negatively charged [17]. The best result was observed pH 7.0 that is near to body pH, and therefore the next stages were carried out at this pH.

3.2.3 | Effect of scan rate

The scan rate is an important factor with the determinative role in the process direction. The effect of scan rate

FIGURE 5 The voltammogram and the chart of the effect of pH of donor phase (the phosphate buffer 0.05 M) versus peak current of working electrode (MWCNT-PUFIX/HF-PGE) for CPT and ETHC extraction over the range 0 to +1.5 V vs. Ag/AgCl

3.2.4 | Concentration of nanocomposite

The nanocomposite concentration has the important influence on the sensor performance. Thus, different concentrations of nanocomposite (0.5–10 mg nanocomposite/mL octanol) were prepared and its effect on voltammetry peak currents of CPT and ETHC were studied and optimized.

As can be seen in Fig. 7 at first with increasing the nanocomposite concentration, current peak was increased due to increase of the binding sites for connection and the extraction of CPT and ETHC, but after that, it was decreased, especially for ETHC, as the negative impact of congestion molecules is greater than the positive effect associated with nanocomposite. The high peak current of the CPT and ETHC was observed in the presence of 2.5 and 1.0 mg/mL of the nanocomposite in octanol. Because of in the presence of ETHC, maximum yield with use 1.0 and then 2.5 mg/mL was observed, therefore this amount (2.5 mg/mL) was selected and next experiments were carried out in this concentration.

3.2.5 | Effect of organic solvent

The nanocomposite should be dispersed completely into a suitable dispersive solvent. So the solvent has an important role in nanocomposite dispersion and tension of the sensor. When depositing nanoparticles on the electrode was regular, the performance of analyte transfer process was superior. For evaluation of this effect some solvents such as 2-decanol, 1-octanol, cyclohexanol, 1-butanol, and acetone were investigated. According to the obtained results, the dispersion process failed by the solvents, acetone, and 1-butanol. While, in the presence of cyclohexanol, 1-octanol and 2-decanol, the dispersing process was successfully done and MWCNT-PUFIX/HF-PGE sensors were prepared by them. After placing the sensor in the system, no peak was observed in the presence of 2-decanol, also cyclohexanol showed a small peak

FIGURE 6 The effect of scan rate (10–230 mV/S) versus peak current of working electrode working electrode (MWCNT-PUFIX/HF-PGE) for CPT and ETHC extraction over the range 0 to +1.5 V versus Ag/AgCl. (Linear relationship between the peak current and the square root of scan rate)

on peak current of drugs was studied in the range of 10–230 mV/S. As can be seen in Fig. 6 the peak currents varied linearly with the square root of scan rate with a correlation coefficient of 0.9928 for Capecitabine and 0.9892 for Erlotinib, expressed for an ideal reaction of diffusion controlled electrode process [35,36]. The results showed a decrease in the analyte peak current with increasing the scan rate. This is due to the justified time needed for the process. As the speed rises, it results in time reduction and the analyte have opportunities to be transferred to hollow fiber cavities and then to nanocomposite will be declined. Therefore, the scan rate of 10 mV/S was chosen as the optimized value.

FIGURE 7 The voltammogram and the chart of the influence of nanocomposite concentration versus current of working electrode (MWCNT-PUFIX/HF-PGE) for CPT and ETHC extraction over the range 0 to +1.5 V versus Ag/AgCl, in phosphate buffer pH 7.0 as a supporting electrolyte and scan rate 10 mv/s

with a lot of background current. The analyte peak in the presence of 1-octanol solvent was sharper with the lowest background current. Therefore, 1-octanol was selected as the suitable dispersing solvent.

3.2.6 | Effect of Chitosan addition

Chitosan is a naturally abundant polysaccharide and it has been applied in numerous biomedical studies and biomaterial researches due to its nontoxicity, biocompatibility, and biodegradability [37,38].

Low molecular aqueous chitosan owns interesting interaction capacities with various molecular groups that can be involved in hydrogen bonds, electrostatic, and hydrophobic interactions.

The structure of chitosan supports interactions through various mechanisms, with amine and hydroxyl groups supporting hydrogen bonding, the protonated amine supporting interactions through an electrostatic mechanism, and the hydrophobic nature of the acetyl groups that are present in chitosan can potentially arbitrate hydrophobic interactions.

The electrochemical studies of the CPT and ETHC were continued by investigating the impact of chitosan addition. The chitosan solutions were prepared in acetic acid 5% w/v. Different concentrations of chitosan (0.005–0.0375 μg/mL) were added to the buffer solution containing CPT and ETHC as donor phase. Overall addition of chitosan showed a positive effect on extraction efficiency. Results indicate the best voltammogram at 0.09 μg/mL was obtained; therefore, the optimal amount of chitosan was added to the donor phase in later stages.

3.2.7 | Effect of triton X-100 addition

The influence of triton X-100 addition as a nonionic surfactant and surface tension reducer was investigated by

preparation and addition of 0.09 to 0.375 μg/mL solution of triton X-100 to buffer solution containing CPT and ETHC. Up to addition of 0.16 μg/mL triton X-100 showed a positive effect and extraction yield increased, but due to creation bubbles and causing disconnection of the solution and electrode, more addition of triton X-100 resulted in a decrease of extraction efficiency. Therefore, the best result was observed in the presence of 0.16 μg/mL triton X-100.

3.3 | Calibration curve and linear range

By employing DPV method, the obtained peak current of Capecitabine versus concentration was linear concentration range from 7.70 to 142.0 μM with a correlation coefficient R^2 of 0.989, and for Erlotinib, was linear concentration range from 0.11 to 23.5 μM, with a correlation coefficient R^2 of 0.985.

The LOD is defined as the analyte concentration giving a signal equal to $y_b + 3 s_b$, where y_b is the signal of the blank and s_b is its standard deviation. Similarly, the LOQ was defined as $y_b + 10 s_b$.

In the unweighed least-squares method, it is quite suitable to use s_{xy} (RSD) instead of s_b and the value of the calculated intercept instead of y_b . Where b is the slope of the regression line, $LOD = 3 s_{xy}/b$ and $LOQ = 10 s_{xy}/b$ [39]. Therefore, the LOD and LOQ in the present study, were 0.11, 0.33 for Capecitabine and 0.023, 0.07 for Erlotinib μg/mL. Calibration curves are shown in Fig. 8.

3.4 | Repeatability of sensor

The repeatability of sensor operation was investigated in the presence of drugs in phosphate buffer solution (pH 7.0). Up to 18 times measurements of peak current were carried out

FIGURE 8 (A) DP voltammograms of different concentrations of ETHC (0.1–23.5 μM) in the presence of fixed concentration of CPT and (B) different concentrations of CPT (7.7–142.0 μM) in the presence of fixed concentration of ETHC at MWCNT-PUFIX/ HF-PGE, in BR (pH 7.0). (C) DP voltammograms for simultaneous determination of CPT (0.1–21.7 μM) and ETHC (7.9–134.0 μM) by using MWCNT-PUFIX/ HF-PGE in BR (pH 7.0)

using the electrode and the highest peak current was turned out in the fourth scan. Therefore, this peak was used in optimizations and studies. After the fourth scan, up to the eighth scan, peak current was approximately constant, but after that, it slowly felled. The mentioned sensor indicated a suitable repeatability. Table 1 shows a comparison between previously reported methods for determination of CPT and ETHC and the proposed method by using MWCNT-PUFIX/HF-PGE sensor.

3.5 | Real sample treatment

For assessment of the capability of the method and developed sensor in biological real samples, it was used to determine CPT and ETHC in a nail and urine samples.

3.5.1 | Nail sample

The nail samples were cleaned to remove all pollutants (fats and lipids), from its surface and then the solution of water and acetone (1:1) was used to remove all types of exogenous contamination [48]. A 0.01 g of clean and dried nail was weighted and it was soaked in 1.0 mL concentrated HCl for 24 h. A 0.1 mL of this mixture was treated after dilution with 25 mL of phosphate buffer 0.05 M at pH 7.0. The nail samples were investigated using the designed sensor.

The accuracy of the method was estimated by calculation of relative recovery (RR%) carried out by spiking nail samples with specific volumes of standard solutions via standard addition method. It is notable that, the high background effects and broadening peaks, makes recognition and determination of nail sample with PGE electrode impossible, but with use

TABLE 1 A comparison between previously reported method and the proposed method for CPT and ETHC determination

Analyte	Method	Linear range ($\mu\text{g/mL}$)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)	R^2	RSD%	Year	Ref.
CP	Chromatography	50–150	—	—	0.9995	0.49	2017	[11]
	Spectrophotometry UV and Chromatography	2–10	0.169	0.512	0.9997	<2	2013	[12]
	Spectrophotometry UV	—	1.03	0.312	0.999	—	2015	[40]
	Chromatography	—	0.047	0.1424	0.999	<2	2015	[40]
	Chromatography	—	0.6	2.0	0.999	4.9	2017	[41]
	Chromatography	—	0.254	0.771	—	—	2018	[42]
	Voltammetry technique by MWCNT-PUFIX/HF-PGE	7.70–142.00 (μM)	0.11 (μM)	0.33 (μM)	0.9895	<5		This work
ETHC	Chromatography	—	0.016	0.048	0.9989	—	2017	[43]
	Stability Indicating Chromatography	0.1–1.5	0.001	3.0	0.995	—	2017	[44]
	Spectrophotometry UV and Chromatography	50–150	0.01	0.04	—	<1	2013	[45]
	Chromatography	0.38–6.51	0.005	16.80	0.985	—	2017	[46]
	Spectrophotometry UV and Chromatography	10–60	0.62	2.07	0.999	—	2017	[47]
	Spectrophotometry	0.5–30	—	—	0.9992	2.3	2014	[10]
	Voltammetry technique by MWCNT-PUFIX/HF-PGE	0.11–23.50 (μM)	0.02 (μM)	0.07 (μM)	0.9851	<5		This work

TABLE 2 Analytical results for determination of Capecitabine and Erlotinib in the nail samples

Real sample	Spiked conc. CPT (μM)	Spiked conc. ETHC (μM)	Measured conc. CPT (μM)	Measured conc. ETHC (μM)	RR% of CPT	RR% of ETHC	RSD% of CPT	RSD% of ETHC
Nail 1	3.0	3.0	2.73	2.93	91.24	97.87	4.20	1.85
Nail 2	3.0	3.0	2.79	2.92	93.17	97.39	6.31	5.72

TABLE 3 Analytical results for determination of Capecitabine and Erlotinib in the urine samples

Real sample	Spiked CPT (μM)	Spiked ETHC (μM)	Measured CPT (μM)	Measured ETHC (μM)	RR% of CPT	RR% of ETHC	RSD% of CPT	RSD% of ETHC
Urine 1	3.0	3.0	3.01	2.76	100.40	92.14	3.97	4.08
Urine 2	3.0	3.0	2.79	2.90	93.15	96.86	6.31	3.71

*RR%, relative recovery percent.

MWCNT-PUFIX/ HF-PGE sensor, specified, and sharp peaks without background effects could be observed. As can be seen in Table 2 spiked CPT and ETHC was recognized and determined.

3.5.2 | Urine sample

Urine sample often is diluted with an optimum buffer because requires no pretreatment. Usually, a strong acid or base is added to the urine for adjusting optimum pH. The pH of urine was checked and with the help of low amount of NaOH 0.4 M adjusted to pH 7.0. Then 1.0 mL urine was diluted tenfold with the phosphate buffer (0.05 M) at pH 7 [20]. The MWCNT-PUFIX/HF-PGE sensor was applied and the LSSPME procedure was carried out. Specified amounts of CPT and ETHC (0.2, 3.0, and 20 μM) were spiked and electrochemical determinations were carried out by differential pulse voltammetry

technique. The results for spike concentration 3.0 (μM) are shown in Table 3. The relative recoveries (RR%) were above 91.0% in all cases with the standard deviations (RSD%) of less than 7.0%.

4 | CONCLUDING REMARKS

In this work, the nanostructured sensor based on MWCNT-PUFIX/HF-PGE was used as a simple, inexpensive and sensitive disposable sensor for application in a simultaneous Capecitabine and Erlotinib anti-cancer drugs preconcentration, extraction, and measurement method by the electrochemical procedure.

The hollow fiber-based sensor is disposable, although this sensor was shown to have an acceptable repeatability. Single

use of the fiber reduces the risk of cross-contamination and carry-over problems. The proposed method allows the very effective and enriched determination of the target analytes into one single step. An additional benefit to the hollow fiber-based sensor is the isolation of the inner electrode from the matrix.

In this work, we used of a new, simple, affordable, applicable, and disposable device for the first time for insitu preconcentration and simultaneous electrochemically determination of trace amounts of two anticancer drugs Capecitabine (CPT) and Erlotinib hydrochloride (ETHC).

The simultaneous measurement of the concentration of anticancer drugs with a sensitive and accurate method in biological samples is a challenge for better monitoring of drug therapy and better determine the pharmacokinetics. In the literature no reports are available for simultaneous determination of CPT and ETHC. So, it is very attractive to determine these drugs simultaneously with a sensitive sensor.

On the other hand, an extremely efficient sensor for determination of CPT and ETHC based on multivalued carbon nanotube-polyurethane(MWCNT-PUFIX) nanocomposite, for the first time, which was supported with a piece of polypropylene hollow fiber (HF-PGE). The electrochemical behavior of the drugs on the proposed sensor by CV and DPV methods, proved that nanocomposite film with large specific surface area provide a large number of active sites for the drugs entrapment. The results clearly show that the fabricated sensor is a promising platform for reliable concurrent determination of CPT and ETHC.

Mentioned sensor with regard to the proposed method, is fast and efficient, therefore it is acceptable. The method has satisfactory LOD and LOQ. Because of the electrode showed better responses to Capecitabine and Erlotinibe determination, it may find application in the analysis of real samples. Therefore, small amounts of the drugs in complex biological nail and urine matrices were determined easily with good efficiency.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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