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Determination of Anti Colon Cancer Drug, Irinotecan by Fast Fourier Transforms Continuous Cyclic Voltammetry

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In this work a novel method for the determination of irinotecan, an anti colon cancer drug, in flow-injection systems has been developed. The fast Fourier transform continuous cyclic voltammetry (FFTCV) at gold microelectrode in flowing solution system was used for determination of irinotecan in its pharmaceutical formulations. The developed technique is very simple, precise, accurate, time saving and economical, compared to all of the previously reported methods. The effects of various parameters on the sensitivity of the method were investigated. The best performance was obtained with the pH value of 2, scan rate value of 65 V/s, accumulation potential of 50 mV and accumulation time of 0.55 s. The proposed method has some advantages over other reported methods. The method was linear over the concentration range of 1850-53000 pg/ml (r=0.9933) with a limit of detection and quantitation 35.5 and 200 pg/ml, respectively.

Keywords: Irinotecan, Ultra-microelectrode, Fast Fourier Transform Cyclic Voltammetry, Flow injection

1. INTRODUCTION

Irinotecan, Fig. 1, is a drug used for the treatment of cancer. Irinotecan is a topoisomerase 1 inhibitor, which prevents DNA from unwinding. Chemically, it is a semisynthetic analogue of the natural alkaloid camptothecin. Its main use is in colon cancer, particularly in combination with other chemotherapy agents. This includes the regimen FOLFIRI which consists of infusional 5-fluorouracil, leucovorin, and irinotecan. Irinotecan was approved by the U.S. Food and Drug Administration (FDA)

in 1994. During development, it was known as CPT-11 [1]. Irinotecan is activated by hydrolysis to SN-38, an inhibitor of topoisomerase I. This is then inactivated by glucuronidation by uridine diphosphate glucoronosyltransferase 1A1 (UGT1A1). The inhibition of topoisomerase I by the active metabolite SN-38 eventually leads to inhibition of both DNA replication and transcription [1].

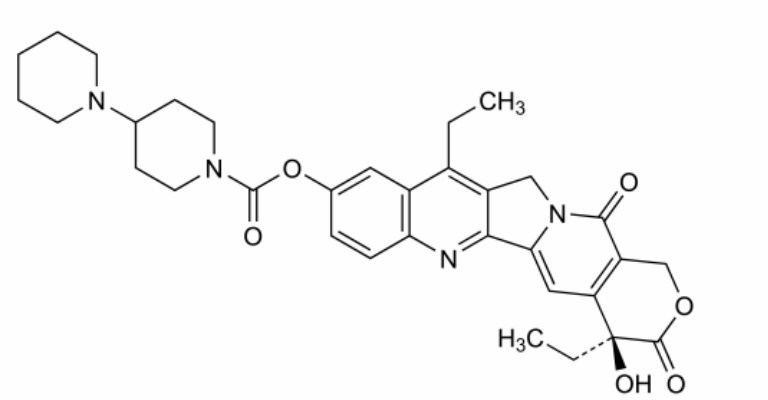


Figure 1. Chemical Structure of irinotecan

The general method for determination of irinotecan is high performance liquid chromatography (HPLC) [2-5]. However, this method is very expensive and time consuming. Along with instrumentation methods, electrochemistry methods open a new trend in determination of the pharmaceutics. During the past decade, our research group introduces many different electrochemical methods for monitoring drugs in pharmaceutical formulations and biological fluids [6-17].

The method which introduced in this paper is very sensitive, inexpensive and fast for detection of irinotecan. This technique has been further stimulated by the advent of UMEs [18-25]. Some of their special characteristics are their steady state currents, their higher sensitivity due to the increased mass transport and their ability to be used in solutions with high resistance. For instance, UMEs have been applied as sensors in various techniques such as flow injection analysis [26,27], cardiovascular monitoring and organic compounds analysis [28-30]. This study aims to introduce a novel method for the fast determination of losartan ultra trace amounts in its pharmaceutical preparation.

2. EXPERIMENTAL PART

2.1. Reagents

All of the solutions were prepared in double-distilled deionized water, using analytical grade reagents. The reagents used to prepare the eluent solution for flow-injection analysis were obtained from Merck Chemicals. In all of the experiments, solutions were made up in the background electrolyte solution, and were used without removal of dissolved oxygen. Irinotecan standard powder was a gift from quality control of drug and food (Teharan, Iran). Irinotecan vial containing 20 mg/ml irinotecan was purchased from a local pharmacy.

2.2. Background electrolyte (BGE)

The running buffer or BGE was made by addition of 8.7 ml of phosphoric acid (85% w/v) into a 1000 ml volumetric flask and dilution to a constant volume with distilled water. The pH was adjusted to 2.3 with sodium hydroxide and all solutions were freshly prepared and filtered using a Millipore filter (0.45 µm) each day.

2.2. Standard stock solutions

A standard stock solution of irinotecan (1mg/ml) was prepared in water. This solution was freshly prepared each day.

2.3. Standard solutions for FIA

Aliquots of standard stock solution of irinotecan were dispensed into 10 ml volumetric flasks and the flasks made up to volume with the running buffer to give final concentrations range of 1850-53000 pg/ml.

2.4. Assay of formulation

The samples were prepared by transferring 1 ml of the vial into 50 ml volumetric flask and filled up with 0.05 M phosphoric acid, reaching an initial concentration of 2000 pg/ml.

2.5. Electrode preparation

Gold UMEs (with a radius of 12.5 µm) were prepared by sealing metal micro-wires (Good fellow Metals Ltd., UK) into a soft glass capillary. The capillary was then cut perpendicular to its length to expose the wire. Electrical contacts were made using silver epoxy (Johnson Mat they Ltd., UK). Before each experiment the electrode surface was polished for 1 minute using extra fine carborundum paper and then for 10 minutes with 0.3 µm alumina. Prior to being placed in the cell the electrode was washed with water. In all measurements, an Ag(s)|AgCl(s)|KCl(aq, 1M) reference electrode was used. The auxiliary electrode was made of a Pt wire, 1cm in length and 0.5 mm in diameter.

2.6. Flow injection setup

The equipment for flow injection analysis included a six roller peristaltic pump (LKB 2115 Miltiperpex Co.) and a four-ways injection valve (Supelco Rheodyne Model 5020) with a 50 µl sample injection loop. Solutions were introduced into the sample loop by means of a plastic syringe. The

electrochemical cell used in flow injection analysis is shown in Fig 2. The volume of the cell was 100 µl. In all experiments described in this paper, the flow rate of eluent solution was 100µl/s.

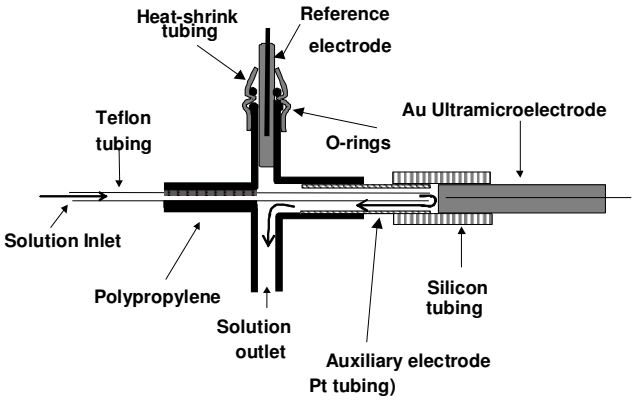


Figure 2. The electrochemical cell used in flow injection analysis

2.7. Data Acquisition and Processing

All of the electrochemical experiments were done using a setup comprised of a PC PIV Pentium 900 MHz microcomputer, equipped with a data acquisition board (PCL-818HG, Advantech. Co.), and a custom made potentiostat. All data acquisition and data processing programs were developed in Delphi 6® program environment.

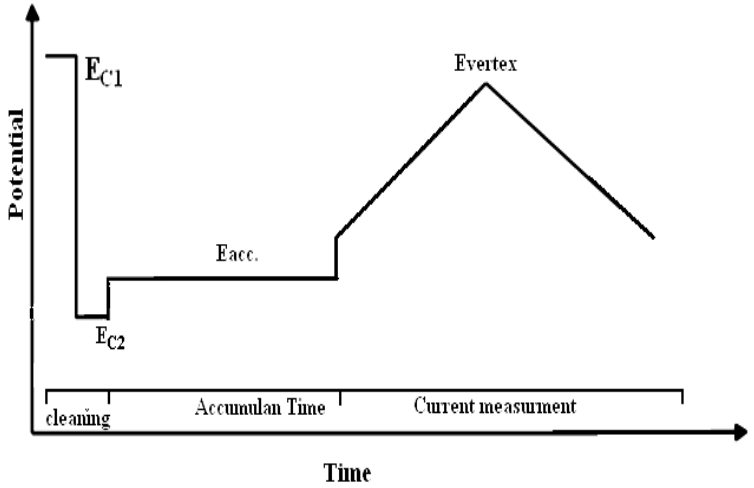


Figure 3. The diagram of applied waveform potential during cyclic voltammetric measurements

In Fig. 3, the diagram of applied waveform potential during cyclic voltammetric measurements is shown. The potential waveform consists of three parts; a) Potential steps, E_{c1} and E_{c2} (which are used for oxidizing and reduction of the electrode surface, respectively), by which electrochemical cleaning of the electrode surface takes place, b) E_c, where accumulation of analyte takes place, c) the final, part potential ramp, in which current measurements take place. Signal calculation in this method is established based on the integration of net current changes over the scanned potential range. It must be noted that in this case, the current changes (result of injected analyte) at the voltammograms can be caused by various processes, which take place at the electrode surface. Those processes include; a) oxidation and reduction of adsorbed analyte, and b) inhibition of oxidation and reduction of the electrode surface by the adsorbed analyte. Indeed, in order to see the influence of the adsorbed analyte on the oxidation and reductions peaks of the gold surface, the scan rate must be set at very high rates (e.g. >20 V/s)

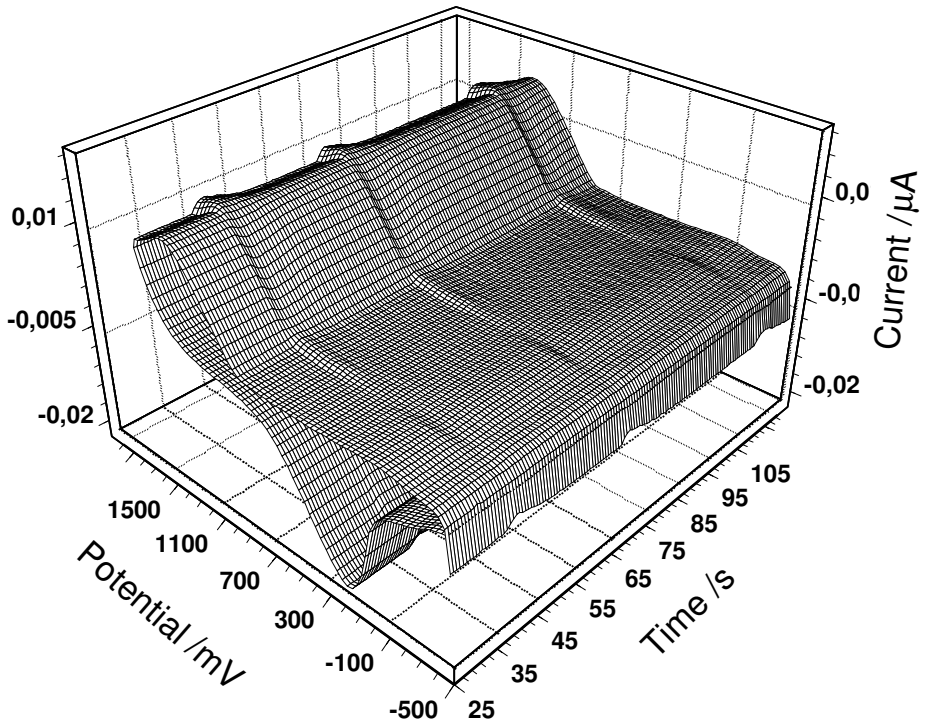
However, during the scan, some of the adsorbed analyte molecules are desorbed. Depending on the rate of those processes and scan rate, the amount of the desorption analyte molecule (during the scan) can be changed. The important point here is that part of the adsorbed analyte molecule still remaining on the electrode surface that can inhibit the red/ox process of the electrode surface. In this method, ΔQ is calculated based on the all current changes at the CVs [31-36]. However, the selectivity and sensitivity of the analyte response expressed in terms of ΔQ strongly depends on the selection of the integration limits. One of the important aspects of this method is application of a special digital filtration, which is applied during the measurement. In this method at the first, a CV of the electrode was recorded and then by applying FFT on the collected data, the existing high frequency noises were indicated. Finally, by using this information, the cutoff frequency of the analog filter was set at a certain value (where the noises were removed from the CV).

Since the crystal structure of a polycrystalline gold electrode, strongly depends on the condition of applied potential waveform [37], therefore various potential waveforms were examined in order to obtain a reproducible electrode surface (or a stable background signal). In fact, application of cyclic voltammetry for determination of electroactive compound mainly face to low stability of the background signal, due to changes occurring in the surface crystal structure during oxidation, and reduction of the electrode in each potential cycle. In this work, after examination of various potential wave forms, the best potential waveform for obtains a stable background during the measurement was the waveform shown in Fig. 3. As mentioned above, in this work, the potential waveform was continuously applied during an experiment run where the collected data were filtered by FFT method before using them in the signal calculation.

The electrochemical oxidation process of gold surface started with electrosorption of hydroxyl ion, which at more positive potentials formation of gold oxide and undergoes structural rearrangement [38]. The surface oxidation can be initiated by adsorption of water molecule and then at more positive potential AuOH forms leading to the formation of a two-dimensional phase of gold oxide;

$$2Au + 3H_2O \longrightarrow Au_2O_3 + 6e + 6H^+$$

(a)



(b)

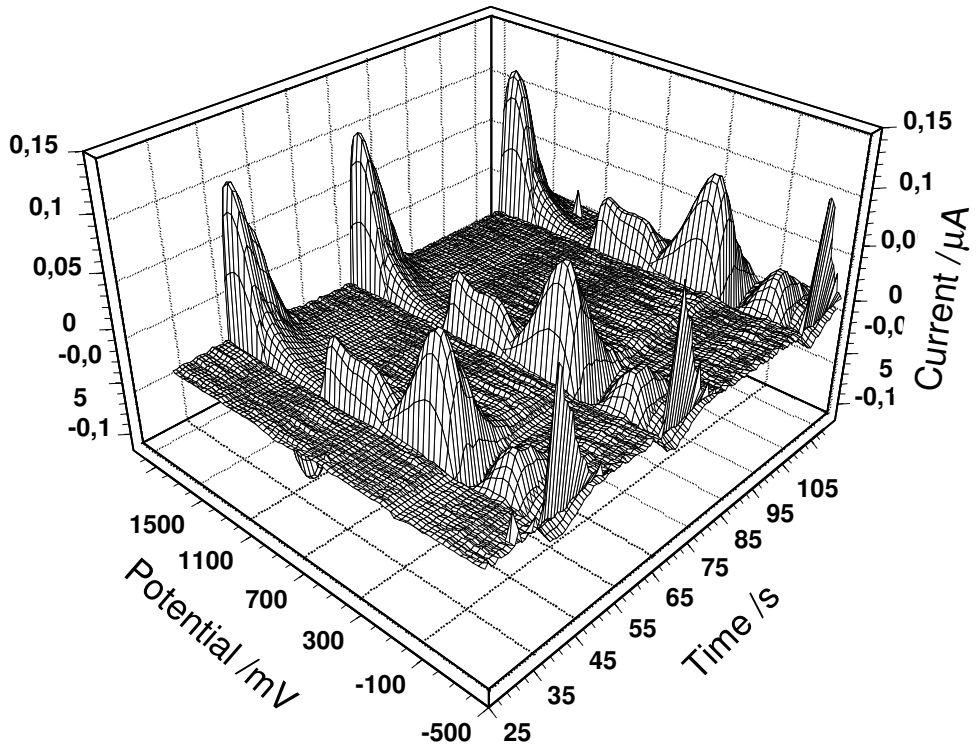


Figure 4. a) A sequence of CVs recorded during the flow analysis for determination of the drug. The volume of the injection was of 50 µL of 5.0×10^{-6} M irinotecan (in 0.05 M H_3PO_4) into the eluent solution containing 0.05 M H_3PO_4**;** b) the absolute current changes in the CVs curves after subtracting the average background 4 CVs (in absent of the analyte)

An example of recorded CVs is shown in Fig. 4 (a, b). Fig. 4a. shows a sequence of CVs recorded during the flow analysis for determination of the drug. The volume of the injection was of 50 µL of 5.0×10^{-6} M irinotecan (in 0.05 M H_3PO_4) into the eluent solution containing 0.05 M H_3PO_4. The time axis of the graph represents the time of the flow injection experiment. In the absence of irinotecan, the shape of the CV curves is typical for a polycrystalline gold electrode in acidic media [38]. Fig. 4b shows the absolute current changes in the CVs curves after subtracting the average background 4 CVs (in absent of the analyte). As can be seen, this way of presentation of the electrode response gives more details about the effect of adsorbed ion on currents of the CV. The curves show that current changes mainly take place at the potential regions of the oxidation and reduction of gold. When the electrode-solution interface is exposed to irinotecan, which can adsorbed on the electrode, the oxide formation process becomes strongly inhibited. In fact, the inhibition of the surface process causes significant change in the currents at the potential region, and as a consequence the profound changes in the shape of CVs take place. Universality of the detector in this mode is very advantageous for chromatographic analysis, where a mixture of compounds presents in sample.

It must be noted that, theoretically, in this method, the analyte response can be affected by the thermodynamic and kinetic parameters of adsorption, the rate of mass transport and electrochemical behavior of the adsorbed species. The free energy and the rate of adsorption depend on the electrode potential, the electrode material, and to some extent, on the choice of the concentration and type of supporting electrolyte. By taking points into consideration, in order to achieve maximum performance of the detector, the effect of experimental parameters (such as; pH of the supporting electrolyte, potential and time of the accumulation and potential scan rate) must be examined and optimized [39-43].

3. RESULTS AND DISCUSSION

3.1. Optimizing the experimental parameters

The effect of eluent pH on performance of the detector was examined the results are shown in Table 1. As shown, the best S/N ratio was obtained between pH 2-3. In addition, the results shows that at pH values higher than 9 noises level in the baseline (ΔQ vs. Time), is higher up to 12% compared to acidic solutin .

Table 1. Effect of pH on the response of the microelectrode

pH	2	4	6	8	10	12
S/N	120	105	90	84	76	79

For investigation on the influence of scan rates and the eluent flow rate on the sensitivity of the detector response, solutions having a concentration of 5.0×10^{-7} M irinotecan were injected. At different scan rates (from 5 to 140 V/s) and the eluent flow, the responses of the detector to the injected sample were recorded. The results are presented in Fig. 5. As it is clear from the Fig. 5, the detector exhibits the maximum sensitivity at 65 V/s of scan rate and 3 mL/min of the flow rate. The effects of the sweep rate on the detection performance can be taken into consideration from three different aspects: first, speed in data acquisition, second, kinetic factors of adsorption of the irinotecan, and finally the flow rate of the eluent which controls the time window of the solution zone in the detector. The main reason for application of high scan rates, is prevention from desorption of the adsorbed irinotecan during the potential scanning, (because under this condition, the inhibition outcome of the adsorbed irinotecan on the oxidation process can take place.

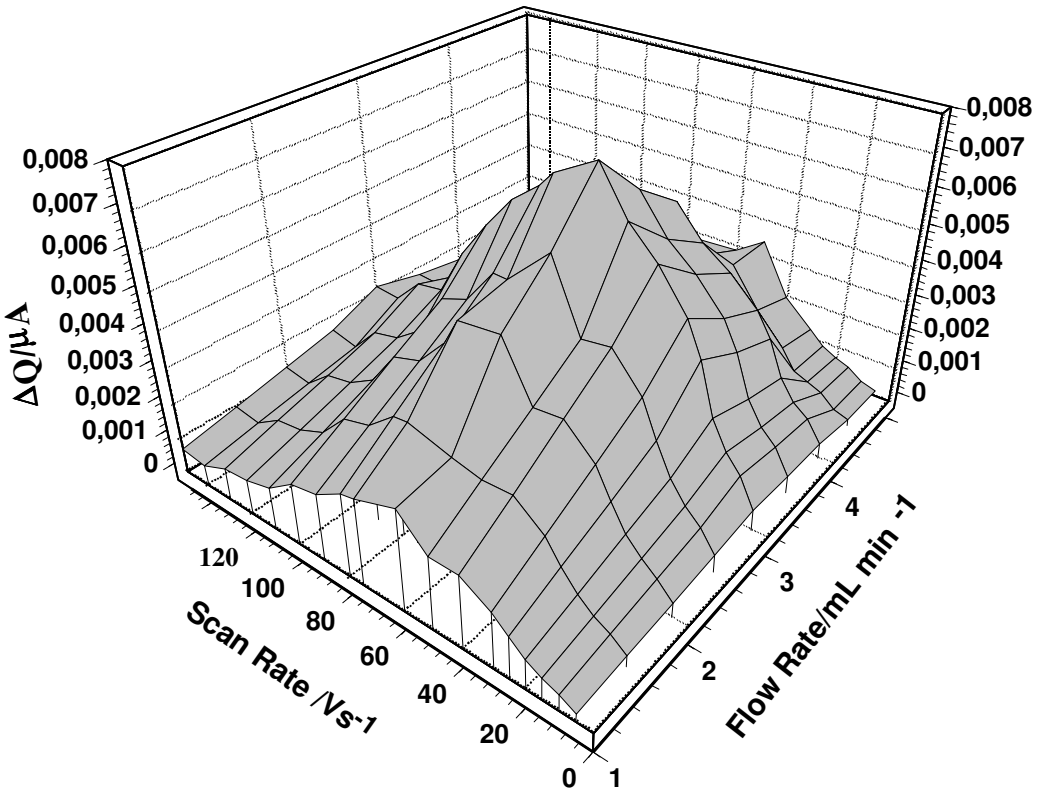


Figure 5. The influence of scan rates and the eluent flow rate on the sensitivity of the detector response of the solutions having a concentration of 5.0×10^{-7} M irinotecan

Indeed, the use of this detection method in conjunction with fast separation techniques such as capillary electrophoresis also requires the employment of high scan rates. From this point of view, checking how the sensitivity of the method is affected by the sweep rate is necessary. To detect the amount of the adsorbed analyte on the electrode surface, high sweep rates must be employed, so that the potential scanning step is short in comparison with the accumulation period. Notably, when the accumulation of irinotecan occurs at a potential that is very larger or smaller than E_i, this is very

significant in this detection method. However, sensitivity of the detection system mainly depends on the potential sweep rate mainly due to kinetic factors in adsorption, and instrumental limitations .

Due to this fact that any changes in the parameters related to adsorption process shows a strong dependence upon the applied potential and the time and the potential of accumulation strongly affect the sensitivity of the measurement. Therefore, the influence of the accumulation potential and time on the response of the method for the injection of a solution of 5.0×10^{-7} M irinotecan, in 0.05 M H_3PO_4, was studied. Fig. 6 shows the detector response over the accumulation potential ranges 300 to -600 mV and accumulation time range from 0.05 s to 0.95 s. Based the figure, accumulation potential 50 mV at time 550 ms was chosen as the optimum condition. Because, the surface of the electrode becomes saturated with the irinotecan within 550 ms time window .

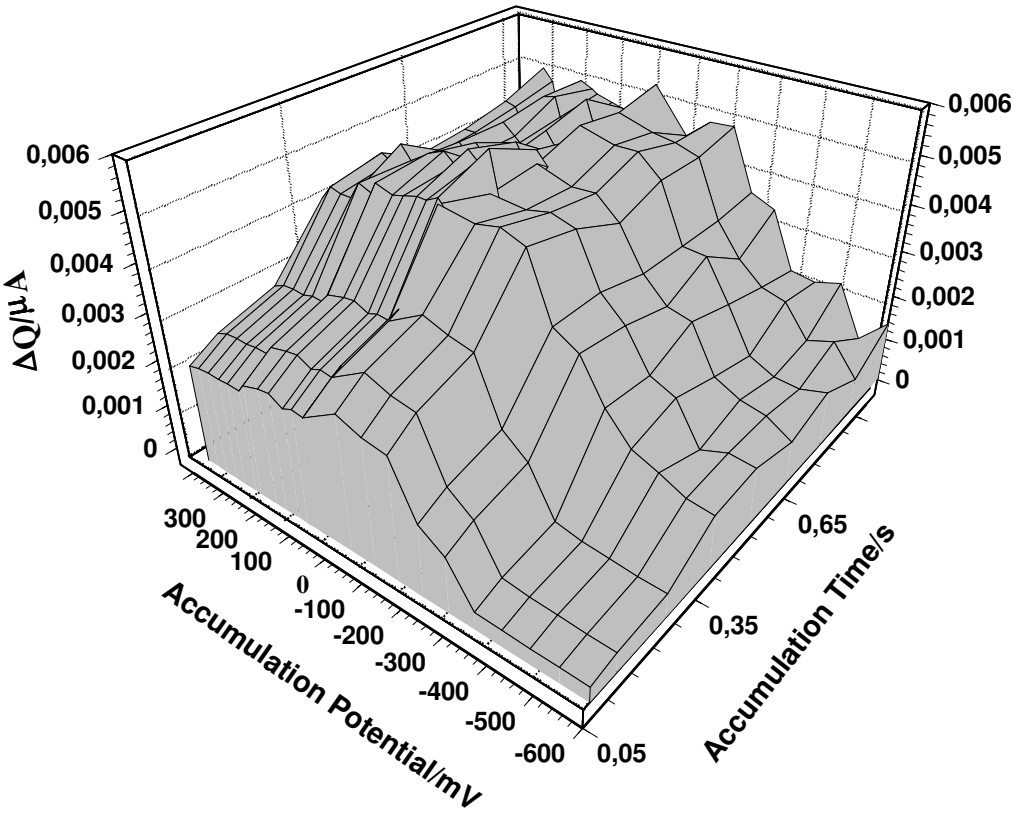


Figure 6. The detector response over the accumulation potential ranges 300 to -600 mV and accumulation time range from 0.05 s to 0.95 s

On the electrode, the accumulation of irinotecan takes place during the accumulation step (assuming that an appropriate potential is selected). In fact, the difference in the time of saturation of the various compounds can be related to the existing differences in their kinetics of the electron transfer and mass transport. As mentioned above, the surface of the gold ultramicroelectrode is very small, and in a very short time the surface of the electrode can be saturated.

After optimization the parameters, the calibration graph was prepared by injection of irinotecan in concentration between 1850-53000 pg/ml that it is presented in Fig. 7, and as it's clear, the electrode will be saturated in high concentrations and the response of electrode is independent of concentration.

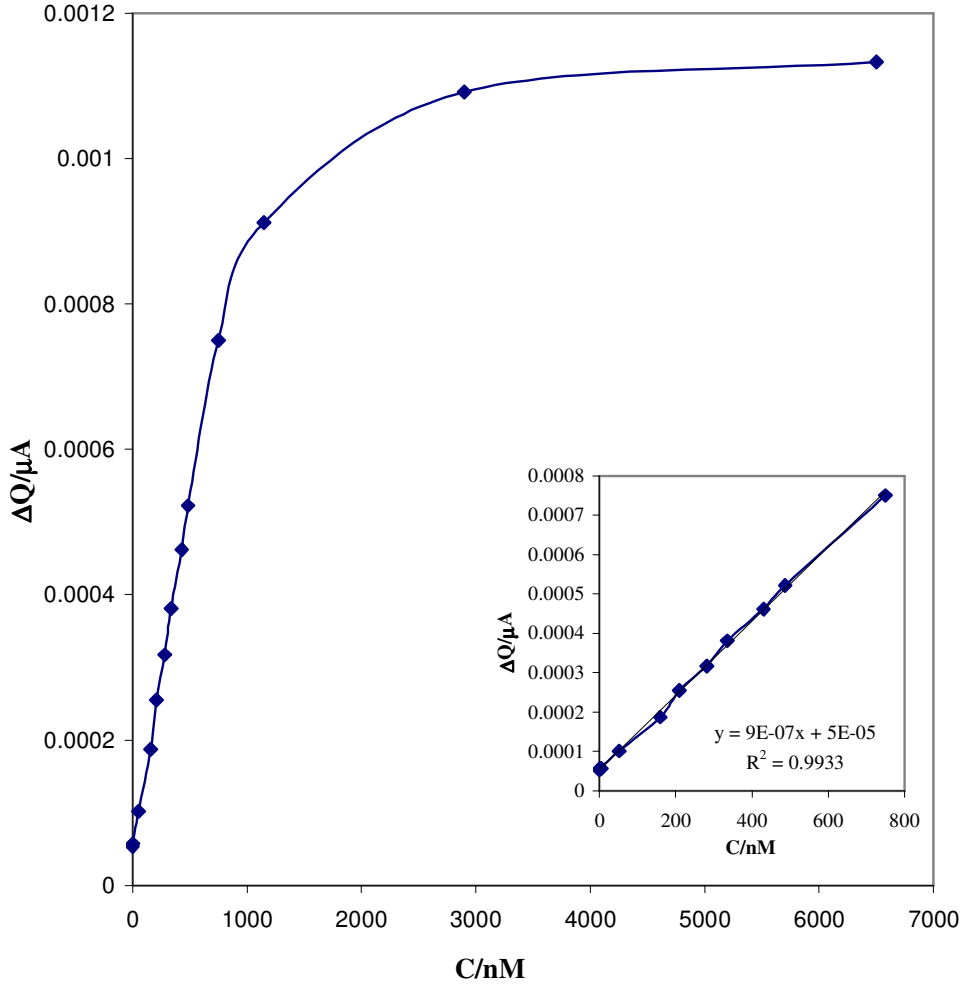


Figure 7. The calibration graph of the irinotecan injection in different concentration

3.2. Validation

The method was validated with respect to parameters including linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, ruggedness/robustness, recovery and selectivity [44-46].

3.3. Linearity

The Linearity was evaluated by linear regression analysis, which calculated by the least square regression method [44-46]. The calibration curves constructed for irinotecan were linear over the

concentration range of 1850–53000 pg/ml. Peak areas of irinotecan were plotted versus its concentration and linear regression analysis performed on the resultant curve. A correlation coefficient of R=0.9933 with %R.S.D. values ranging from 0.28–3.50 % across the concentration range studied were obtained following linear regression analysis. Fig. 7 shows the calibration graph that obtained for the monitoring of irinotecan in a 0.05 M H_3PO_4.

3.4. LOQ and LOD

The LOQ and LOD were determined based on a signal-to-noise ratios and were based on analytical responses of 10 and 3 times the background noise, respectively [47] The LOQ was found to be 200 pg/ml with a resultant %R.S.D. of 0.25% (n=5). The LOD was found to be 35.5 pg/ml.

3.5. Precision

Precision of the assay was investigated with respect to both repeatability and reproducibility. Repeatability was investigated by injecting nine replicate samples of each of the 1920, 5000 and 53000 pg/ml standards where the mean concentrations were found to be 1900, 5095 and 52910 pg/ml with associated %R.S.D.'s of 3.62, 1.43 and 0.25, respectively.

Table 2. The influence of the changes in the experimental conditions on the performance of the FIA system

Parameter	**modification**	**Irinotecan (% recovery)**
pH	2.0 2.3 2.5 3.0	101.2 102.1 99.6 100.5
flow rate ml/min	2.8 3.0 3.2	99.4 100.3 101.5
buffer composition (M)	0.04 0.05 0.06	101.2 100.7 99.3
Lab. Temperature (ºC)	20 25 30	101.6 99.5 100.3

3.6. Accuracy

Accuracy of the assay was determined by interpolation of replicate (n = 6) peak areas of three accuracy standards (1900 and 5000 pg/ml) from a calibration curve prepared as previously described. In each case, the percent relevant error and accuracy was calculated. The resultant concentrations were 1850.1±50 pg/ml and 5057±50.50 pg/ml with percent relevant errors of 3.5 and 1.17 %, respectively.

3.7. Ruggedness

The ruggedness of the method was assessed by comparison of the intra- and inter-day assay results for irinotecan undertaken by two analysts. The %R.S.D. values for intra - and inter – day assays of irinotecan in the cited formulations performed in the same laboratory by the two analysts did not exceed 4.2%, thus indicating the ruggedness of the method. Also the robustness of the method was investigated under a variety of conditions such as small changes in the pH of eluent, in the flow rate, in the buffer composition and in the laboratory temperature. As can be seen in table 2, the percent recoveries of irinotecan were good under most conditions and did not show a significant change when the critical parameters were modified.

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