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Nanoparticles Based Carbon Paste Electrodes for the Determination of Flupentixol Dihydrochloride: Application to Pharmaceutical Analysis and Pharmacokinetic Study

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Five different carbon paste electrodes were developed and applied for the electrochemical determination of flupentixol dihydrochloride. Sensor 1 was fabricated using a mixture of graphite powder and multiwall-carbon-nanotubes. Different additives were used to improve the performance and the sensitivity of the carbon paste electrode. Sensor 2 was developed using chitosan in addition to the graphite powder and the multiwall-carbon-nanotubes while sensor 3 was fabricated with the addition of calix[4]arene ionophore. Copper nanoparticles were incorporated in the membrane paste of sensor 4. Sensor 5 was fabricated using a mixture of copper nanoparticles, multiwall-carbon-nanotubes and calix[4]arene in a graphite paste. The studied carbon paste electrodes exhibited the best performance characteristics with slopes of 29.7, 28.8, 30.2, 30.7 and 30.8 (mV / concentration decade) and linear concentration ranges of 1.0×10^{-5} - 1.0×10^{-2}, 1.0×10^{-6} - 1.0×10^{-1}, 1.0×10^{-7} - 1.0×10^{-2}, 1.0×10^{-9} - 1.0×10^{-2} and 1.0×10^{-10} - 1.0×10^{-2} mol $L^{-1}$for sensors 1, 2, 3, 4 and 5, respectively. The sensors linear ranges deviated from their ideal ranges after 40, 46, 58, 63 and 82 days for sensors 1, 2, 3, 4 and 5, respectively. All the proposed sensors were successfully used for the determination of flupentixol dihydrochloride in bulk, tablets dosage forms and human plasma samples.

Keywords: Flupentixol; copper nanoparticles; multiwall-carbon-nanotubes; pharmacokinetics; dissolution.

1. INTRODUCTION

Flupentixol (FLP) (EZ)-2-[4-[3-[2-(trifluoromethyl) thioxanthen-9-ylidene] propyl] piperazin-1-yl] ethanol is a thioxanthene derivative and is used as antipsychotic drug. FLP has been administered

as dihydrochloride salt orally for the treatment of mild to moderate depression, with or without anxiety. The injection dosage form is administered once every two or three weeks for the treatment of schizophrenia and other psychotic diseases especially for patients suffer frequent relapses of illness [1, 2].

Several analytical methods were applied for the determination of flupentixol dihydrochloride (FLP.2HCl) either alone or in combination with other antidepressants. Also, few methods were published for the determination of FLP.2HCl in biological fluids. The reported methods are spectrophotometric analysis [3,4], Spectrofluorimetric methods [5–7], Flow-injection chemiluminescence [8], Densitometric analysis [9], RP-HPLC [10–13], LC-Tandem MS [14–16], GC [17] and finally voltammetric analysis of FLP.2HCl which was based on measuring the oxidation of FLP.2HCl by the use of glassy carbon electrode. This method suffered from very narrow linearity range with high LOD and limited linearity range [18].

Mainly most of these reported methods required sophisticated instruments, extensive steps of sample pretreatment and expensive chemicals. No electrochemical method was published for the determination of FLP.2HCl. Electrochemical techniques are considered to be sensitive in the determination of drugs in pharmaceutical dosage forms, milk, urine or plasma with high accuracy and selectivity.

The proposed work compares some novel potentiometric ion-selective electrodes (ISEs) used for the first time in the determination of FLP.2HCl. These ISEs are carbon paste electrodes (CPEs) modified with either multiwall-carbon-nanotubes (MWCNTs), chitosan (CS) and calix[4]arene, copper nanoparticles (CNPs) or mixture of them trying to increase their selectivity, accuracy and sensitivity to be used in the determination of FLP.2HCl in bulk, pharmaceuticals and in human plasma samples which requires very sensitive method that reaches ng mL^{-1} level.

Carbon nanotubes (CNTs) are electroactive polymers recently used in sensors fabrication due to their dimensional and chemical compatibility with different molecules. CNTs are characterized by their great ability to catalyze reactions and enhance electron-transfer reactions between molecules and electrode substrates. Additionally, single wall (SWCNTs) and multiwall-carbon-nanotubes (MWCNTs) are also characterized by their high surface area and good electronic properties that make them widely applied in electroanalytical studies [19].

The selectivity of the ISEs is greatly affected by the incorporation of a suitable ionophore in the membrane matrix. The ionophore is responsible for the formation of a strong and reversible complex with the ion of interest [20]. CS is a low-cost natural biopolymer that has excellent film forming ability, high heat stability, mechanical strength and biocompatibility. The CS-based electrodes are widely used in the potentiometric analysis. They were used to measure pH [21], cadmium(II) and mercury(II) [22] and chromate ion [23].

Metal nanoparticles are characterized by high effective surface area, enhancement of mass transport and electric conductance. They are applied for the determination of many electroactive species [24].

This research aimed to develop new, sensitive and stable carbon paste electrodes (CPEs) for the determination of FLP.2HCl in different media. CPEs were fabricated using graphite powder, MWCNTs and other additives to improve the electrical response, sensitivity, selectivity and linearity

range. The most sensitive electrodes would be used for the determination of dissolution profile of FLP.2HCl, measuring the plasma concentration after oral administration of a single dose of 1.5 mg of FLP and calculating the main pharmacokinetic parameters.

2. EXPERIMENTAL

2.1. Instrumentation

CLEAN digital ion analyzer PH 600, model 007747 (China). Ag/AgCl double junction reference electrode, model Z113107-1EA batch 310 (Sigma-Aldrich). Magnetic stirrer, Heidolph MR Hei-Standard, model 100818877.

2.2. Chemicals and reagents

The FLP.2HCl reference standard (Batch No. T0161104002) was supplied by Mediphar Laboratories Dbayeh- Lebanon. Its potency is certified to be 99.6%. Deanxit® tablets are manufactured by H.Lundbeck A/S Ottiliavej 9, 2500 Valby, Denmark.

The chemicals and reagents are of analytical grade. Tetrahydrofuran (THF) (Fisher Scientific, UK). Dioctyl phthalate (DOP) and CS (Acros Organics, USA). Spectroscopic graphite powder (1-2 micron), MWCNTs powder (DXL 110-170 nm ×5-9 μm), calix[4]arene and nitrophenyl octyl ether (NPOE) (Aldrich, USA). (Acros Organics, USA). β-alanine, phosphoric acid, acetic acid and L-ascorbic acid (Fluka Chemie Gmbh, Germany). Potassium chloride (Merck, Darmstadt, Germany). Sodium chloride, calcium chloride, boric acid, sodium hydroxide, copper chloride dihydrate (Prolabo, Pennsylvania, USA). Dioctyl adipate (DOA) and Dibutyl phthalate (DBP) (Fluka, USA).

2.3. Procedure

2.3.1. Standard solution of FLP.2HCl

The preparation procedure was conducted at room temperature and the standard solutions were stored at -5 ◦C when not in use. The FLP.2HCl stock solution (1×10^{-1}mol.L^{-1}) was prepared by weighing 12.686 g of FLP.2HCl in a 250 ml volumetric flask, dissolving it and completing the volume to the mark with deionized water. Working standard solutions (1×10^{-8} to 1×10^{-2} mol.L^{-1}) were prepared by suitable dilutions of the stock solution with deionized water.

2.3.2. Preparation of copper nanoparticles (CNPs).

In a 250 ml conical flask, 50 ml aqueous solution of 0.1M $CuCl_2.2H_2O$ was added followed by dropwise addition of 50 ml of 0.2M L-ascorbic acid solution with continuous magnetic stirring and heating the solution at 80°C. Then, 30 ml of 1M sodium hydroxide solution was slowly added with

constant stirring and heating at 80°C for 2 h. The color of the solution changed gradually from yellow to dark brown. The solution was allowed to settle overnight. The formed precipitate was separated by vacuum filtration and washed with deionized water and ethanol 3 times each. The precipitate was dried at room temperature and stored in a stoppered glass vial for further use [25].

2.3.3. Fabrication of carbon paste electrodes (CPEs)

The carbon paste electrodes (CPEs) were prepared by proper mixing of the spectroscopic graphite powder (1-2 μm) and the suitable plasticizer (ratio of graphite powder to the plasticizer was 60:40 w/w of the total weight of components of 0.35 gram) in a small mortar until homogenously mixed. The effect of the addition of variable percent of MWCNTs, CS, calix[4]arene and CNPs was studied to reach the most stable, selective, sensitive and rapid response electrode for the effective determination of FLP.2HCl. The Teflon part of the electrode body was filled with the membrane paste. A new surface was obtained by moving the steel screw forward through the electrode body and a clean filter paper was used to polish and get a new shiny surface.

2.3.4. Sensors selectivity

The potentiometric selectivity coefficient ($K_{pot}^{A,B}$) was calculated for the most sensitive and rapid response sensors towards some interfering substances using a separate solution method by applying the following equation [26] and matched potential method [27, 28].

$$\text{Log } K_{pot}^{A,B} = [(E_B - E_A)/ (2.303RT/Z_AF)] + [1-(Z_A/Z_B)] \log [A]$$

Where K_{pot} is the potentiometric selectivity coefficient. E_A is the potential measured for 1×10^{-3} mol L^{-1} FLP.2HCl solution, E_B is the potential measured for 1×10^{-3} mol L^{-1} interfering solution. Z_A and Z_B are the charges of FLP.2HCl and the interfering substance, respectively. $2.303RT/Z_AF$ is the slope of the calibration plot (mV/ concentration decade). [A] is the activity of FLP.2HCl.

2.3.5. Water layer test

The water layer test was performed to study the effect of the presence of water layer between the electroactive membrane and the transducer [29]. The potential of each of the studied electrodes was alternately recorded after conditioning in 1×10^{-3} mol L^{-1} FLP.2HCl solution followed by 1×10^{-3} mol L^{-1} melitracen hydrochloride solution and again in 1×10^{-3} mol L^{-1} FLP.2HCl solution.

2.3.6. Potentiometric determination of FLP.2HCl

The potentiometric determination of FLP.2HCl was carried out using the proposed electrodes through the standard addition method [30]. The change in potential was recorded after the addition of a small volume of standard FLP.2HCl solution 1×10^{-2}mol L^{-1} to 50 ml of samples of different concentrations. The change in potential reading was recorded for each increment.

2.3.7. Potentiometric determination of FLP.2HCl in pharmaceutical formulation

Forty tablets of Deanxit® were used to determine the FLP.2HCl concentration in a pharmaceutical formulation. Each tablet was accurately weighed and then, all tablets were finely powdered. Part of the powder equivalent to 12.686 g FLP.2HCl was weighed and transferred to a 250 ml volumetric flask. Around 100 ml deionized water was added and the flask was sonicated for about 15 min. The solution was filtered and completed to the volume with deionized water to obtain a 1×10^{-1} mol L^{-1} aqueous solution of FLP.2HCl. The required concentrations from 1×10^{-8} to 1×10^{-2} mol L^{-1} FLP.2HCl were prepared by diluting the stock solution. The potentials of these solutions were measured using the studied electrodes and the corresponding concentrations were calculated for each sensor from its specific regression equation.

2.3.8. Dissolution test of FLP.2HCl tablets

One tablet of Deanxit® containing FLP.2HCl equivalent to 0.5 mg FLP was added in the dissolution medium of 900 ml 0.1N HCl and maintained at 37±0.5°C at 100 rpm for 45 min. At specified time intervals, a 10 mL sample was withdrawn from the dissolution medium and replaced with fresh dissolution medium. The potential reading corresponding to the amount of FLP.2HCl released at different time intervals was measured using sensor (3) and sensor (4).

2.3.9. Potentiometric determination of FLP.2HCl in spiked human plasma and pharmacokinetic determination using sensor 5

In each of 5ml volumetric flask, 1.5 ml of human plasma was added and spiked with the FLP.2HCl working solution to provide concentration range 0.125 - 2.5 $ng.ml^{-1}$ which is equivalent to 2.46×10^{-10} - 4.9×10^{-9} mol L^{-1} and then the volume was completed to 5 ml with a britton-robinson buffer of pH=6. The content of each volumetric flask was shaken for 1 min and transferred to a 10 ml beaker. The sensor 5 was immersed in these solutions to measure their corresponding potential and then, washed with water between measurements. The plasma concentration of FLP.2HCl was calculated using the regression equation of sensor 5.

A pharmacokinetic study of a single oral dose of FLP.2HCl equivalent to 1.5 mg FLP [16] was held using 5 healthy male subjects under fasting condition. Blood samples were collected in a heparinized tube at 0 h pre-dose and at 1, 2, 3, 4, 5, 5.5, 6, 6.5, 7, 9, 12, 24, 48, 72 h post dose. Plasma samples (2ml for each time interval) were immediately separated by centrifugation at 1600 rpm for 15 min and stored at -20°C until analysis. The corresponding concentrations of plasma samples of the five volunteers were measured using sensor 5. The main pharmacokinetic parameters were calculated.

3. RESULT AND DISCUSSION

Ion selective electrodes (ISE) became a routine tool of analysis in the clinical and environmental determination of certain ions. The conventional ISE has significant drawbacks that limit

its usage. It requires the vertical position of the electrode to avoid leakage of the internal solution which needs refilling by time. So it would be reasonable to use internal solution free sensor [31]. CPEs become widely used since the 1980s. They are characterized by their very low background current, high electrode activity at the carbon paste surface and also at the carbon paste bulk and the ability to regenerate the carbon paste surface which can extend its the lifetime [32].

Nowadays, nanomaterials have been widely used in the synthesis of ISE. They have several advantages of being used in electrochemical sensors such as signal amplification, permit large surface area for immobilization of the analyzed molecules and increasing the binding sites to the target molecule. CNTs are one of the interesting materials because of their unique electronic conductivity, high electrochemical stability and sensitivity [33] that can decrease the electrode response time and increase the electrode surface area of various electroactive substances. CPEs modified with MWCNTs were applied for the determination of several drugs and organic molecules [34–36].

Also, metal nanoparticles become one of the most exciting fields in analytical chemistry. Generally, metal nanoparticles have excellent conductivity and catalytic properties which enhance the electron transfer between the analyte molecule and the electrode surface and increase the rate of the electrochemical reaction.

In a trial to increase the stability, sensitivity and the selectivity of CPEs different neutral ionophores were used such as chitosan and calix[4]arene. The ionophores chemical structures are represented in fig.1. They are characterized by a number of lipophilic groups that minimize the leaching rate from the membrane to the sample solution [37].

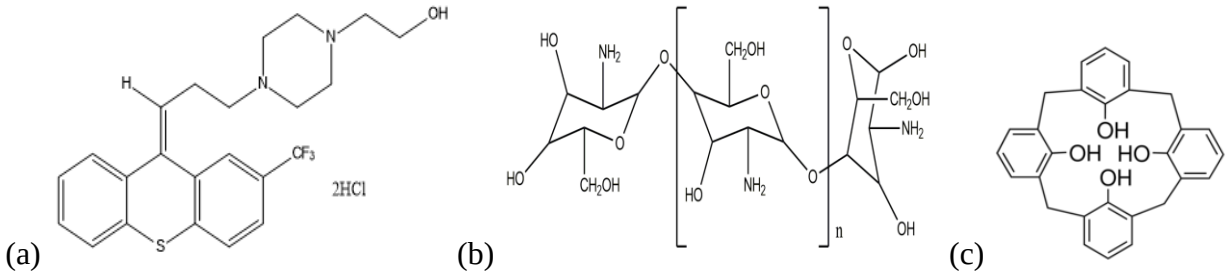

Figure 1. Chemical Structure of (a) FLP.2HCl, (b) β-Cyclodextrin and (c) Calix[4]arene.

3.1. Characterization of CNPs

CNPs were synthesized by chemical reduction method using L-ascorbic acid as reducing and capping agent to control the growth of nanoparticles and to avoid their oxidation and aggregation. UV-VIS spectroscopy, double beam spectrophotometry (Jenway 6800, path length 1cm, spectral range 200-800nm) was used for the estimation of CNPs. As shown in fig.2. the absorption peak reported around 570 nm proves the formation of CNPs [38].

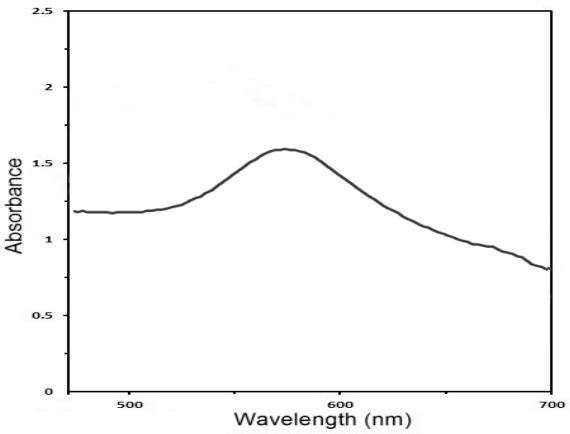

Figure 2. UV-VIS absorption spectrum of CNPs

Fourier transform-IR (FT-IR) spectrometry was also used to study the interaction between L-ascorbic acid and Cu^{2+} ion solutions. Table 1 represents the FT-IR data interpretation of both solutions that proved the attachment of hydroxyl groups to the surface of CNPs. This confirmed that L-ascorbic acid acted as reducing and capping agent to increase the stability and the dispersion of CNPs.

Table 1. FT-IR data of L-ascorbic acid and Cu2+ ion solutions

	Frequency cm^{-1}	Reason
L-ascorbic acid solution	1670	The stretching vibration of the c-c double bond
	1319	The peak of enol hydroxyl
Cu^{2+} solution after reaction with L-Ascorbic acid	3428	The peak of hydroxyl group
	1715	oxidized ester carbonyl groups
	1678	conjugated carbonyl groups

3.2. Design and synthesis of sensors under study:

Five CPEs were synthesized with different compositions. Different trials were done trying to reach to the optimum percent of each ionophore and CNPs in addition to the nature and amount of the plasticizer to obtain the best performance characteristics. As being reported in table 2, the best composition for sensor 1 was found to be 50% graphite powder, 10%MWCNTs and 40% DOP. The optimum composition of sensor 2 was 50% graphite powder, 10%MWCNTs, 10% CS and 30% NPOE. For sensor 3 it was found to be 46% graphite powder, 10%MWCNTs, 8% calix[4]arene and 36% DOP. In order to increase the sensitivity of the proposed CPEs, CNPs were used in the fabrication of sensors 4 and 5. Sensor 4 was composed of 47% graphite powder, 10%MWCNTs, 8% CNPs and 35% NPOE, while sensor 5 was formed of 40% graphite powder, 9%MWCNTs, 8% CNPs, 7% calix[4]arene and 36% NPOE.

Table 2. Optimizing the composition of modified Carbon based electrodes (CPE) and their slopes at 25°±1.

Electrode no.	Composition % (w/w)					Slope mV/decade	Linearity range (mol L^{-1})	Response time (sec)	LOD[c] (mol L^{-1})	RSD%[d]
	Graphite powder	Plasticizer	MWCNTs[a]	Ionophore	CNPs[b]					
1	57	40% NPOE	3	-	-	25.6	1.0×10^{-4} - 1.0×10^{-2}	25	4.1×10^{-5}	2.44
2	62	35% DOP	3	-	-	25.9	1.0×10^{-4} - 1.0×10^{-2}	26	1.6×10^{-5}	2.15
3	55	42% NPOE	3	-	-	26.1	1.0×10^{-4} - 1.0×10^{-2}	24	3.7×10^{-5}	1.43
4	55	40% DOP	5	-	-	25.5	1.0×10^{-5} - 1.0×10^{-3}	18	4.8×10^{-6}	1.33
5	53	40% DOP	7	-	-	26.8	1.0×10^{-5} - 1.0×10^{-2}	15	2.6×10^{-6}	1.27
6	50	40% DOP	10	-	-	27.6	1.0×10^{-5} - 1.0×10^{-2}	15	1.5×10^{-6}	1.19
7	50	35% DOP	10	5% chitosan	-	27.8	1.0×10^{-5} - 1.0×10^{-1}	13	3.3×10^{-6}	0.96
8	51	32% NPOE	10	7% chitosan	-	26.7	1.0×10^{-5} - 1.0×10^{-2}	16	1.7×10^{-6}	1.26
9	50	30% NPOE	10	10% chitosan	-	28.8	1.0×10^{-6} - 1.0×10^{-1}	8	2.8×10^{-7}	0.88
10	40	35% NPOE	10	15% chitosan	-	25.4	1.0×10^{-4} - 1.0×10^{-2}	20	3.7×10^{-5}	1.83
11	53	34% NPOE	10	3% CX[4][e]	-	28.5	1.0×10^{-6} - 1.0×10^{-2}	15	5.2×10^{-8}	0.57
12	52	33% DOP	10	5% CX[4]	-	28.7	1.0×10^{-6} - 1.0×10^{-1}	12	4.7×10^{-8}	0.67
13	46	36% DOP	10	8% CX[4]	-	30.2	1.0×10^{-7} - 1.0×10^{-2}	8	3.2×10^{-8}	0.93
14	45	35% NPOE	10	10% CX[4]	-	29.1	1.0×10^{-6} - 1.0×10^{-2}	10	4.1×10^{-7}	1.01
15	50	37% DOP	10	-	3	28.8	1.0×10^{-7} - 1.0×10^{-2}	12	6.4×10^{-8}	0.83
16	50	35% NPOE	10	-	5	29.7	1.0×10^{-8} - 1.0×10^{-2}	10	2.6×10^{-9}	0.96
17	47	35% NPOE	10	-	8	30.7	1.0×10^{-9} - 1.0×10^{-2}	6	3.6×10^{-10}	0.76
18	43	37% NPOE	10	-	10	29.5	1.0×10^{-8} - 1.0×10^{-1}	10	2.7×10^{-9}	1.24
19	40	39% DOP	8	5% CX[4]	8	29.1	1.0×10^{-10} - 1.0×10^{-3}	8	4.9×10^{-11}	0.93
20	40	36% NPOE	9	7% CX[4]	8	30.8	1.0×10^{-10} - 1.0×10^{-2}	5	2.5×10^{-11}	0.57
21	40	39% NPOE	6	8%CX[4]	7	30.2	1.0×10^{-10} - 1.0×10^{-2}	8	4.6×10^{-11}	0.77

[a]: Multiwall-carbon-nanotubes.

[b]: Cupper nanoparticles.

[c]: Limit of detection.

[d]: Relative standard deviation (5 determinations).

[e]: Calix[4]arene.

By comparing the performance characteristics of the five sensors as reported in table 2, it was found that the response time was greatly enhanced from 15 to 8 s after the incorporation of calix[4]arene. However, the use of CS as a selective ionophore did not greatly enhance either the electrical response of the electrode or the linear concentration range as expected. This may be explained by the hydrophilic nature of CS that caused the penetration of the aqueous layer in the membrane which would be investigated later by applying the water layer test.

The dynamic working range, LOD and the slope were improved after the addition of CNPs in sensors 4 and 5. The slopes reached nearly the ideal value of a divalent cation (30 mV/ concentration decade) and also the stability was greatly enhanced to be 63 and 82 days for sensors 4 and 5, respectively with wider concentration ranges.

The studied CPEs exhibited the best performance with slopes of 29.7, 28.8, 30.2, 30.7 and 30.8 (mV / concentration decade) with linear concentration ranges of 1.0×10^{-5} - 1.0×10^{-2}, 1.0×10^{-6} - 1.0×10^{-1} , 1.0×10^{-7} - 1.0×10^{-2} , 1.0×10^{-9} - 1.0×10^{-2} and 1.0×10^{-10} - 1.0×10^{-2} mol $L^{-1}$for sensors 1, 2, 3, 4 and 5, respectively.

Four different plasticizers were tested to select the optimum one to be used in the fabrication of CPEs. This was done by using DOP, NPOE, DBP and DOA and comparing the resulting slope, linear

concentration range and LOD. As reported in table 3, it was found that DOP was the most suitable plasticizer for sensors 1 and 3 but the suitable plasticizer for sensors 2, 4 and 5 was found to be NPOE.

Table 3. Effect of different plasticizers on the characteristics of the proposed carbon paste FLP.2HCl-electrodes

Electrode composition	Plasticizer	Slope mV/decade	Linearity range (mol L^{-1})	LOD[a] (mol L^{-1})	RSD[b]%
50% graphite powder + 10%MWCNTs[c] + 40% plasticizer	DOA	25.2	1.0×10^{-4} - 1.0×10^{-2}	2.0×10^{-5}	1.44
	DOP	27.6	1.0×10^{-5} - 1.0×10^{-2}	1.5×10^{-6}	1.19
	DBP	25.3	1.0×10^{-3} - 1.0×10^{-1}	2.5×10^{-4}	1.68
	NPOE	26.5	1.0×10^{-4} - 1.0×10^{-1}	2.7×10^{-5}	1.24
50% graphite powder + 10%MWCNTs+ 10% Chitosan + 30% plasticizer	DOA	26.5	1.0×10^{-4} - 1.0×10^{-1}	2.4×10^{-5}	1.23
	NPOE	28.8	1.0×10^{-6} - 1.0×10^{-1}	2.8×10^{-7}	0.88
	DBP	25.4	1.0×10^{-5} - 1.0×10^{-2}	6.4×10^{-5}	1.88
	DOP	26.7	1.0×10^{-5} - 1.0×10^{-1}	4.9×10^{-6}	0.93
46% graphite powder + 10%MWCNTs+ 8% CX[4][d] + 36% plasticizer	DOA	28.4	1.0×10^{-5} - 1.0×10^{-1}	6.6×10^{-6}	1.55
	DOP	30.2	1.0×10^{-7} - 1.0×10^{-2}	3.2×10^{-8}	0.93
	DBP	27.4	1.0×10^{-5} - 1.0×10^{-2}	1.5×10^{-6}	1.76
	NPOE	26.6	1.0×10^{-5} - 1.0×10^{-1}	1.1×10^{-6}	1.98
47% graphite powder + 10%MWCNTs+ 8% CNPs[e] + 35% plasticizer	DOA	28.4	1.0×10^{-6} - 1.0×10^{-2}	3.7×10^{-7}	1.33
	DOP	26.9	1.0×10^{-7} - 1.0×10^{-2}	1.6×10^{-8}	0.86
	DBP	29.8	1.0×10^{-8} - 1.0×10^{-2}	2.4×10^{-9}	0.93
	NPOE	30.7	1.0×10^{-9} - 1.0×10^{-2}	3.6×10^{-10}	0.76
40% graphite powder + 9%MWCNTs+ 8% CNPs + 7% CX[4] + 36% plasticizer	DOA	28.5	1.0×10^{-9} - 1.0×10^{-2}	3.8×10^{-10}	1.38
	DOP	29.8	1.0×10^{-7} - 1.0×10^{-1}	1.6×10^{-8}	0.83
	DBP	29.4	1.0×10^{-8} - 1.0×10^{-1}	3.2×10^{-9}	0.86
	NPOE	30.8	1.0×10^{-10} - 1.0×10^{-2}	2.5×10^{-11}	0.57

[a]: Limit of detection.

[b]: Relative standard deviation (5 determinations).

[c]: Multi-wall carbon nanotubes.

[d]: Calix[4]arene.

[e]: Cupper nanoparticles.

3.3. Effect of soaking time and the lifespan of the studied electrodes

As reported in table 4, the effect of soaking of the studied sensors in 1×10^{-2} mol L^{-1} FLP.2HCl was studied by measuring their slopes at different time intervals. They attained their maximum slope values after 12, 24, 12, 10 and 6 h conditioning time for sensors 1, 2, 3, 4 and 5, respectively.

The longer conditioning time required for sensor 2 may be attributed to the presence of CS ionophore which has certain hydrophilic nature that may affect the response of the membrane to some extent.

The electrode lifespan is the period in which the electrode is optimally functioning until at least one of the performance characteristics deviates from its ideal value. The studied CPEs showed a longer life-span than other conventional ISEs. The slope values of the proposed sensors started to decrease

from its maximum values by nearly 10% and their linear ranges deviated from their ideal ranges after 40, 46, 58, 63 and 82 days for sensors 1, 2, 3, 4 and 5, respectively. The overall performance characteristics of the studied electrodes are represented in table 5.

Table 4. Effect of soaking time on the performance of the proposed carbon paste modified electrodes at 25°C±1

Electrode composition	Soaking time	Slope (mV/decade)	Usable concentration range (mol L^{-1})	Response time (sec.)	Electrode composition	Soaking time	Slope (mV/decade)	Usable concentration range (mol L^{-1})	Response time (sec.)
CPE 1 50% graphite powder + 10%MWCNTs+ 40% DOP	0.5 h	24.1	1.0×10^{-4} - 1.0×10^{-2}	20	CPE 4 47% graphite powder + 10%MWCNTs+ 8% CNPs + 35% NPOE	1 h	29.8	1.0×10^{-7} - 1.0×10^{-2}	10
	1 h	24.4	1.0×10^{-4} - 1.0×10^{-2}	18		2 h	30.1	1.0×10^{-7} - 1.0×10^{-2}	8
	2 h	25.7	1.0×10^{-4} - 1.0×10^{-2}	20		10 h	31.1	1.0×10^{-9} - 1.0×10^{-2}	6
	12 h	27.9	1.0×10^{-5} - 1.0×10^{-2}	15		10 h	30.7	1.0×10^{-9} - 1.0×10^{-2}	5
	24 h	26.7	1.0×10^{-5} - 1.0×10^{-2}	15		2 days	30.8	1.0×10^{-9} - 1.0×10^{-2}	5
	6 days	27.3	1.0×10^{-5} - 1.0×10^{-2}	16		10 days	31.2	1.0×10^{-9} - 1.0×10^{-2}	6
	11 days	27.1	1.0×10^{-5} - 1.0×10^{-2}	17		23 days	30.9	1.0×10^{-9} - 1.0×10^{-2}	6
	28 days	27.8	1.0×10^{-5} - 1.0×10^{-2}	15		35 days	31.1	1.0×10^{-9} - 1.0×10^{-2}	6
	40 days	27.1	1.0×10^{-5} - 1.0×10^{-2}	15		49 days	31.3	1.0×10^{-9} - 1.0×10^{-2}	5
	53 days	25.7	1.0×10^{-4} - 1.0×10^{-1}	20		63 days	30.7	1.0×10^{-9} - 1.0×10^{-2}	6
	65 days	24.8	1.0×10^{-4} - 1.0×10^{-1}	22		75 days	28.4	1.0×10^{-8} - 1.0×10^{-2}	12
CPE 2 50% graphite powder + 10%MWCNTs+ 10% Chitosan + 30% NPOE	0.5 h	26.5	1.0×10^{-5} - 1.0×10^{-2}	15	CPE 5 40% graphite powder + 9%MWCNTs+ 8% CNPs + 7% CX[4] + 36% NPOE	1 h	28.6	1.0×10^{-8} - 1.0×10^{-1}	10
	5 h	27.3	1.0×10^{-5} - 1.0×10^{-2}	12		6 h	30.8	1.0×10^{-10} - 1.0×10^{-2}	5
	10 h	28.5	1.0×10^{-5} - 1.0×10^{-1}	10		12 h	30.9	1.0×10^{-10} - 1.0×10^{-2}	6
	24 h	28.8	1.0×10^{-6} - 1.0×10^{-1}	8		24 h	31.1	1.0×10^{-10} - 1.0×10^{-2}	5
	2 days	29.0	1.0×10^{-6} - 1.0×10^{-1}	9		2 days	31.0	1.0×10^{-10} - 1.0×10^{-2}	5
	12 days	28.8	1.0×10^{-6} - 1.0×10^{-1}	9		11 days	30.7	1.0×10^{-10} - 1.0×10^{-2}	6
	20 days	28.9	1.0×10^{-6} - 1.0×10^{-1}	10		20 days	31.1	1.0×10^{-10} - 1.0×10^{-2}	5
	33 days	29.1	1.0×10^{-6} - 1.0×10^{-1}	9		40 days	30.8	1.0×10^{-10} - 1.0×10^{-2}	5
	46 days	29.1	1.0×10^{-6} - 1.0×10^{-1}	9		82 days	31.0	1.0×10^{-10} - 1.0×10^{-2}	5
	54 days	29.1	1.0×10^{-5} - 1.0×10^{-2}	15		95 days	28.9	1.0×10^{-8} - 1.0×10^{-2}	12
	60 days	28.3	1.0×10^{-4} - 1.0×10^{-2}	25		120 days	28.5	1.0×10^{-7} - 1.0×10^{-1}	15
CPE 3 46% graphite powder + 10%MWCNTs+ 8% CX[4] + 36% DOP	0.5 h	29.3	1.0×10^{-5} - 1.0×10^{-1}	10					
	2 h	29.5	1.0×10^{-5} - 1.0×10^{-1}	13					
	6 h	30.4	1.0×10^{-6} - 1.0×10^{-1}	5					
	12 h	30.2	1.0×10^{-7} - 1.0×10^{-2}	6					
	2 days	29.9	1.0×10^{-7} - 1.0×10^{-2}	6					
	8 days	29.8	1.0×10^{-7} - 1.0×10^{-2}	5					
	16 days	30.6	1.0×10^{-7} - 1.0×10^{-2}	6					
	25 days	30.5	1.0×10^{-7} - 1.0×10^{-2}	5					
	39 days	30.6	1.0×10^{-7} - 1.0×10^{-2}	5					
	58 days	30.2	1.0×10^{-7} - 1.0×10^{-2}	5					
	62 days	27.9	1.0×10^{-6} - 1.0×10^{-1}	8					
	70 days	27.5	1.0×10^{-5} - 1.0×10^{-1}	8					

Table 5. Electrochemical Performance characteristics of the investigated FLP.2HCl carbon paste modified electrodes.

	Carbon paste based electrodes				
	CPE 1	CPE 2	CPE 3	CPE 4	CPE 5
Slope (mV/decade)[a]	27.6	28.8	30.2	30.7	30.8
LOD (mol L^{-1})[b]	1.5×10^{-6}	2.8×10^{-7}	3.2×10^{-8}	3.6×10^{-10}	2.5×10^{-11}
Response time (Sec.)	15	8	8	6	5
Working pH range	2.5-5	2.5-6	3-6	3.5-6	3-6
Concentration range (mol L^{-1})	1×10^{-5}-1×10^{-2}	1×10^{-6}-1×10^{-1}	1×10^{-7}-1×10^{-2}	1×10^{-9}-1×10^{-2}	1×10^{-10}-1×10^{-2}
Stability (days)	40	46	58	63	82
Average recovery%±SD[a]	98.83±0.73	99.50±0.78	99.29±0.73	99.42±0.74	99.12±0.71
Correlation coefficient	0.998	0.998	0.998	0.998	0.999
Repeatability (SD_r)	0.86	1.22	1.08	0.95	0.77
Intermediate precision	1.03	1.37	1.22	1.16	1.22
Ruggedness [c]	100.05±0.67	99.45±0.44	98.67±0.89	97.99±0.56	100.01±0.56

a: the average of five determinations.

b: Limit of detection (measured by the intersection of the extrapolated arms of the potential profile figures for each sensor).

c: The average recovery percent of determining (10^{-5}, 10^{-4} and 10^{-3}M for the proposed sensors using Mettler Toledo MP225digital ion analyzer instead of clean PH 600 digital ion analyzer.

3.4. Dynamic response time of the studied sensors

The time required for the electrodes to reach a stable potential reading after increasing the concentration of FLP.2HCl 10 fold was decreased with the use of MWCNTs than other conventional ISEs. They improved the performance of the electrodes through increasing their conductivities. This was also most probably due to the fast exchange kinetics of the association–dissociation of FLP.2HCl with the ionophores at the solution–membrane interface. Also, the incorporation of CNPs had a significant effect on the response time that reached ≈ 5 s. The electrodes potentials remained unaffected when measuring the concentrations of FLP.2HCl from low to high and from high to low as graphically represented in fig.3.

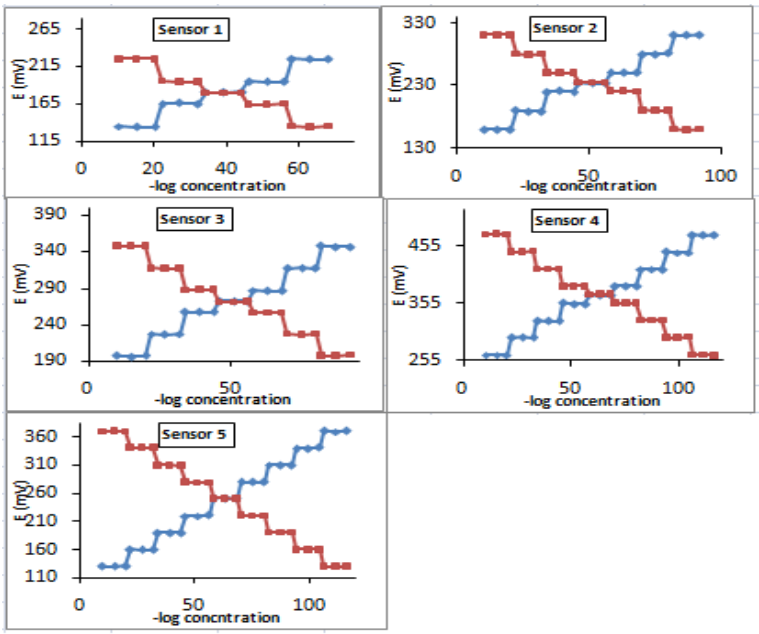

Figure 3. The dynamic response time of the proposed carbon paste based sensors by changing FLP.2HCl concentration from low to high and from high to low.

3.5. Effect of pH and temperature

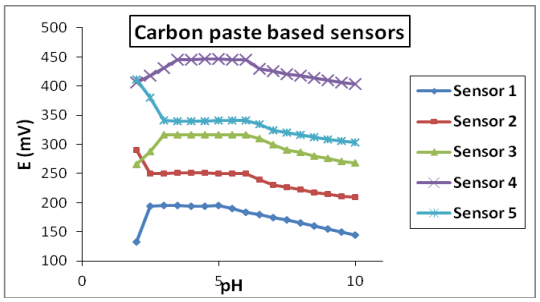

Figure 4. Effect of pH on the response characteristics of the proposed chitosan and carbon paste sensors using 1×10^{-3} mol L^{-1} FLP.2HCl solution for each electrode.

The effect of pH on the response of the proposed sensors was studied over the pH range of 2-10. As shown in fig.4, the electrodes potentials were nearly the same within the pH range of 3-7. Therefore, pH 6 was used as the pH of choice for the electrodes assembly. Moreover, it was noted that, above pH 7, non-nernestian slopes were observed. This can be attributed to the formation of the free FLP base (pKa = 8.4) in the test solution.

Upon studying the effect of temperature, it was found that the CPEs potentials slightly increased with increasing temperature with thermal stability up to 60°C without significant change in the electrodes performances. The calibration graphs obtained for each sensor at different temperatures were parallel and the limit of detection, slope and response time were almost of the same values by increasing the temperature up to 60°C.

3.6. Effect of the water layer

The presence of the water layer between the ion selective membrane and the transducer may result in the diffusion of O_2 or CO_2 through the membrane. O_2 can favor redox side-reactions while CO_2 can change the pH of the interface, which may affect the electrode response. Certain potential drift was observed in sensor 2 response when replaced from 1×10^{-3} mol L^{-1} FLP.2HCl solution to 1×10^{-3} mol L^{-1} melitracen.HCl solution. This may be due to the hydrophilic properties of CS ionophore which facilitated the penetration of aqueous solution through the membrane. This drift was not observed in the case of other sensors as shown in fig.5. As their potentials dropped fast into the negative direction and maintained a stable value. When the electrodes were removed from melitracen.HCl solution, the potential returned to their initial values. This means that no water layers were detected due to the high hydrophobic character of these membranes.

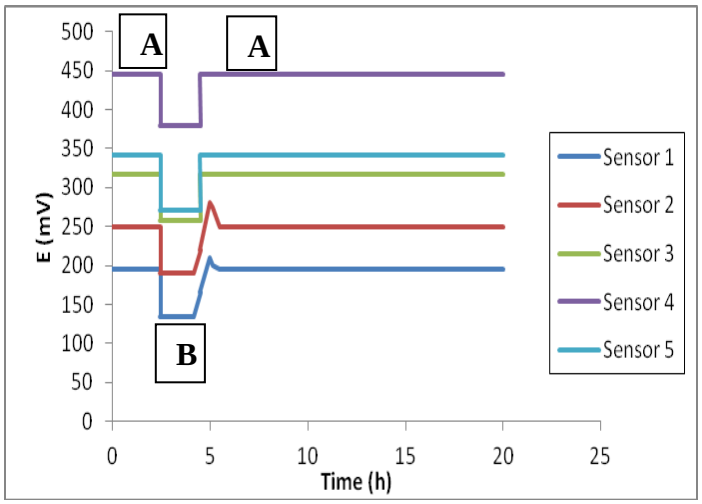

Figure 5. Water layer test of FLP.2HCl selective electrodes. Area A: solution of 1×10^{-3}mol L^{-1} FLP.2HCl. Area B: solution of 1×10^{-3}mol L^{-1} melitracen hydrochloride.

3.7. Selectivity coefficient of the studied CPEs

The potentiometric selectivity of the studied electrodes was measured relative to other ions which might be present with FLP.2HCl in the solution. The selectivity coefficient was measured using the separate solution method and the matched potential method. The represented results in table 6 reveal the high selectivity of all the studied electrodes for the FLP.2HCl in the presence of inorganic cations, amino acids, sugars and other co-administered pharmaceutical drugs e.g., melitracen.HCl. This may be attributed to the differences in ionic size, mobility or permeability of the interfering ions to the membrane as compared with FLP.2HCl.

Table 6. Selectivity coefficients and tolerance values for FLP.2HCl carbon paste modified electrodes

Interferent	Carbon paste electrodes									
	CPE 1		CPE 2		CPE 3		CPE 4		CPE 5	
	SSM	MPM	SSM	MPM	SSM	MPM	SSM	MPM	SSM	MPM
Na^+	5.21	4.35	3.54	3.44	5.43	5.34	4.36	4.13	3.65	3.76
NH_4^+	4.66	3.97	4.25	4.31	4.77	4.66	3.76	3.56	3.48	3.56
K^+	4.68	4.08	4.82	4.66	5.29	5.18	5.46	5.27	5.65	5.45
Mg^{2+}	5.13	4.89	4.56	4.61	4.53	4.46	4.52	4.33	5.23	5.32
Ca^{2+}	3.67	3.45	3.94	3.76	3.87	3.93	5.38	5.21	6.34	6.09
Ba^{2+}	4.37	4.08	3.74	3.65	3.74	3.54	5.44	5.56	4.72	4.58
Cu^{2+}	4.85	4.27	4.55	4.37	4.62	4.73	5.38	5.42	4.10	3.94
Al^{3+}	3.67	3.41	3.26	3.38	4.69	4.52	6.01	5.95	3.49	3.53
Sr^{2+}	5.36	5.13	2.76	2.65	3.82	3.72	3.54	3.62	3.42	3.51
Fe^{2+}	4.32	4.45	4.17	3.94	3.56	3.65	3.44	3.63	4.69	4.57
L-Alanine	3.67	3.31	4.22	3.92	2.55	2.69	3.47	3.38	4.83	4.73
Glucose	5.47	5.13	4.65	4.58	2.64	2.87	2.43	2.38	3.58	3.64
Lactose	4.90	4.81	3.89	3.95	2.38	2.55	3.76	3.54	3.43	3.51
Propylene glycol	3.54	3.67	3.64	3.56	2.17	2.31	4.62	4.53	3.75	3.65
Melitracen	3.76	3.94	3.55	3.47	2.28	2.17	4.15	4.21	3.52	3.71
Ampicillin	2.44	2.32	3.67	3.39	2.67	2.87	4.29	4.16	3.69	3.53
Moxifloxacin	3.28	3.54	3.18	3.26	3.47	3.53	3.18	3.34	3.72	3.82
Pazufloxacin	2.67	2.81	4.22	4.02	3.98	3.87	3.28	3.19	4.18	4.20

[a] SSM: Separate solution method.

[b] MPM: Matched potential method.

3.8. Application of the proposed sensors

3.8.1. Potentiometric determination of FLP.2HCl in pharmaceutical tablets

The proposed sensors were applied for the analysis of FLP.2HCl in pharmaceutical Deanxit® tablets. The results represented in table 7 show the high recovery percentages of FLP.2HCl that prove the applicability of the sensors for the determination of FLP.2HCl. Statistical analysis of the results was done using t-test and F-test. No significant differences were detected between the results of the proposed methods and those obtained from the reported method [3] which is based on the simultaneous

spectrophotometric determination of FLP.2HCl and melitracen.HCl using simultaneous equation method.

Table 7. Determination of FLP.2HCl by applying the standard addition method using modified carbon paste electrodes with statistical comparison of the obtained data with the official method

	CPE 1			CPE 2			CPE 3			CPE 4			CPE 5		
	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD
Pure solution	5×10^{-4}	97.67	0.67	5×10^{-2}	98.53	0.77	6×10^{-4}	100.16	0.63	6×10^{-6}	98.44	0.99	3×10^{-8}	98.79	0.74
	5×10^{-5}	98.79	0.74	3×10^{-3}	99.61	0.86	5×10^{-5}	98.52	0.48	5×10^{-5}	99.94	0.67	5×10^{-4}	98.32	0.64
	1×10^{-4}	99.08	0.93	5×10^{-4}	100.21	0.94	1×10^{-6}	98.49	0.82	1×10^{-4}	100.12	0.82	1×10^{-6}	99.29	0.57
	5×10^{-3}	99.67	1.03	1×10^{-5}	100.26	0.82	1×10^{-4}	99.37	0.77	1×10^{-7}	98.78	0.57	1×10^{-5}	98.96	0.89
	7×10^{-3}	98.95	0.86	7×10^{-3}	98.88	0.65	5×10^{-3}	99.89	0.93	5×10^{-8}	99.84	0.65	4×10^{-9}	100.23	0.94
Average± SD	98.83 ± 0.73			99.50 ± 0.78			99.29 ± 0.77			99.42 ± 0.76			99.12 ± 0.71		
n	5			5			5			5			5		
Variance	0.53			0.61			0.59			0.58			0.51		
F-test (5.19)[a]	2.62			2.28			2.35			2.40			2.73		
Student t test (2.262)[a]	1.44			0.29			0.64			0.42			0.95		
Deanxit Tablet® (0.5 mg flupentixol)	5×10^{-3}	100.03	0.88	5×10^{-2}	98.35	0.87	5×10^{-4}	99.45	0.88	6×10^{-7}	98.47	1.04	6×10^{-7}	99.56	0.66
	5×10^{-4}	98.03	0.69	3×10^{-5}	98.76	0.96	1×10^{-5}	98.72	0.75	5×10^{-6}	99.23	0.97	5×10^{-4}	98.67	0.74
	1×10^{-2}	99.31	0.83	5×10^{-4}	99.34	0.65	1×10^{-4}	100.15	0.94	1×10^{-5}	99.54	1.13	1×10^{-9}	99.43	0.89
	1×10^{-3}	99.05	0.56	1×10^{-5}	99.51	0.43	5×10^{-6}	98.14	1.08	1×10^{-4}	100.21	0.86	1×10^{-8}	98.21	0.56
	1×10^{-4}	100.1	0.75	1×10^{-4}	100.34	0.59	5×10^{-4}	99.65	0.95	5×10^{-8}	100.18	0.63	5×10^{-5}	100.12	0.97
Average± SD	99.30 ± 0.84			99.26 ± 0.76			99.22 ± 0.79			99.53 ± 0.72			99.20 ± 0.76		
n	5			5			5			5			5		
Variance	0.71			0.58			0.62			0.52			0.58		
F-test (5.19)[a]	2.10			2.57			2.40			2.87			2.57		
Student t test (2.262)[a]	1.17			1.28			1.33			0.84			1.38		

a The values into parentheses are the corresponding theoretical values of t and F at the 95% confidence level.

N.B.: The reported method Average± SD (99.67 ± 1.18), n=6 for pure solution and (100.03 ± 1.22), n=6 for pharmaceutical dosage form

3.8.2. Dissolution testing of FLP.2HCl pharmaceutical tablets

One tablet of Deanxit® containing FLP.2HCl equivalent to 0.5 mg FLP was added in the dissolution medium of 900 ml 0.1N HCl and maintained at 37±0.5°C at 100 rpm for 45 min. The potential reading corresponding to the amount of FLP.2HCl released at different time intervals was measured using the sensors 3 and 4. Fig.6. shows the release profile of FLP.2HCl at different time intervals that not less than 70% of the drug is dissolved within 30 min.

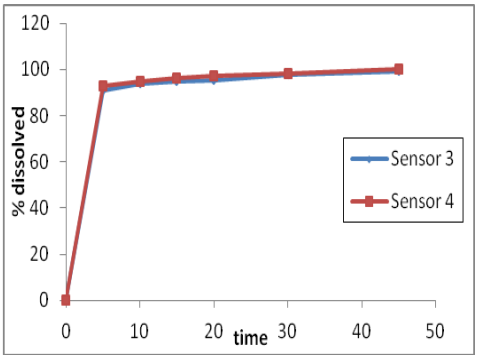

Figure 6. The dissolution profile of Deanxit® tablet (FLP.2HCl equivalent to 0.5 mg FLP) using sensor 3 and sensor 4.

3.8.3. Potentiometric determination of FLP.2HCl in spiked human plasma

The represented results in table 8 proved the applicability of the sensor 5 for the determination of FLP.2HCl in spiked human plasma with a high degree of recovery, precision and accuracy. Therefore the proposed sensor 5 could be successfully applied for the determination of FLP.2HCl in clinical trials without the need of either sophisticated instrumentation, internal standard or extensive sample preparation steps.

Table 8. Accuracy and precision of FLP.2HCl in spiked human plasma.

	Plasma concentration ($ng.ml^{-1}$)	Calculated mean plasma concentration ($ng.ml^{-1}$)*	SD[a]	CV%[b]	Recovery %	RE%[c]
Intra-day	0.125	0.125	0.0013	1.04	100.16	-0.16
	0.188	0.187	0.0011	0.61	99.68	0.32
	0.25	0.249	0.0016	0.63	99.60	0.40
	0.5	0.506	0.0114	2.25	101.20	-1.20
	1	0.992	0.0130	1.31	99.20	0.80
	2	2.028	0.0563	2.78	101.40	-1.40
	2.5	2.498	0.0239	0.96	99.92	0.08
Inter -day	0.125	0.126	0.0020	1.59	100.80	-0.80
	0.188	0.188	0.0015	0.81	100.18	-0.18
	0.25	0.248	0.0030	1.21	99.20	0.80
	0.5	0.497	0.0208	4.19	99.33	0.67
	1	0.983	0.0153	1.55	98.33	1.67
	2	2.053	0.0451	2.20	102.67	-2.67
	2.5	2.480	0.0265	1.07	99.20	0.80

* Average of 5 determinations.

[a:] SD: standard deviation.

[b:] CV%: coefficient of variation%.

[c:] RE%: relative error %.

3.8.4. Pharmacokinetic study after single oral dose of 1.5 mg FLP tablets.

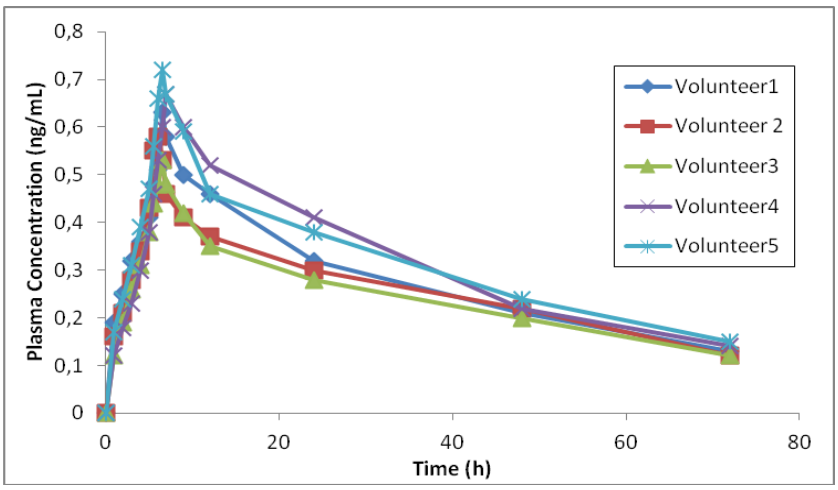

Figure 7. Plasma concentration-time curves of FLP.2HCl equivalent to 1.5 mg FLP single dose for 5 volunteers.

The high sensitivity, stability and accuracy of sensor 5 were sufficiently enough to be used in a pharmacokinetic study. Five healthy male volunteers of average age 22.5 (20-25), non-smokers, were received a single oral dose of 1.5mg FLP under fasting condition with 200 mL water. The plasma samples were prepared as under 2.3.9. The FLP.2HCl concentrations were calculated using the calibration curve linear equation which is Y= -30.08 X + 430.8, where Y is the electrode potential in mV and X is the plasma concentration in ng mL^{-1}. Table 9 represents the main pharmacokinetic parameters of the volunteers and fig.7 represents the plasma concentration-time curve of the five volunteers.

Table 9. Pharmacokinetic parameters of FLP.2HCl equivalent to 1.5 mg FLP single oral dose of five healthy volunteers.

Volunteer no.	C_{max} (ng.mL^{-1})	t_{max} (h)	AUC_{0-t} (ng mL^{-1} h^{-1})	$AUC_{0-\infty}$ (ng mL^{-1} h^{-1})	$K_{elemination}$ (h^{-1})	$T_{1/2\ elimination}$ (h)
1	0.63	6.5	20.02	26.40	0.020	33.98
2	0.58	6	18.64	25.22	0.018	38.04
3	0.53	6.5	17.45	24.33	0.017	39.76
4	0.67	7	22.49	28.75	0.022	30.99
5	0.72	6.5	22.60	30.99	0.019	36.82
Average ± SD	0.63 ± 0.07	6.5 ± 0.35	20.24 ± 2.29	27.05 ± 2.57	0.02 ± 0.002	35.92 ± 3.47

4. CONCLUSION

The proposed modified CPEs offered great advantages for the determination of FLP.2HCl with high accuracy, precision and sensitivity. They were applied for the determination of FLP.2HCl in bulk, pharmaceuticals and biological fluids. Sensor 5 was characterized with wide concentration range, high stability, shortest response time and lower LOD that reached picogram level. This may be attributed to its unique composition that gathered the advantages of MWCNTs, CNPs and calix[4]arene in one sensor. Sensor 5 was applied for the determination of real human plasma samples after oral administration of 1.5 mg single dose FLP tablet. This can be used in the routine analysis of FLP.2HCl in either quality control laboratories, bioequivalence or bioavailability studies without the need of either expensive tools or equipment.

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