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ARTICLE TYPE

Novel screen printed potentiometric sensors for the determination of oxicams

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The construction and performance characteristics of new sensitive and selective sensors based on funcntiolized multi-walled carbon nanotubes/β-cyclodextrin nanocomposite (FMWCNTs/β-CD) was demonstrated for potentiometric determination of different anti-inflammatory agents including lornoxicam, meloxicam, piroxicam and tenoxicam. Screen printed sensors (SPEs) modified with FMWCNTs/β-CD composite, hyamine (Hy) and 2-fluorophenyl 2-nitrophenyl ether (*f*-PNPE) showed proper electroanalytical performances with Nernstian compliance range between 61.2 to 52.6 mV decade^{-1} activity and detection limit 6.0×10^{-7} mol L^{-1} for different oxicam derivatives. Modification with carbon nanotubes composite as sensing material remarkably improved the potential stability and lifetime of the fabricated sensors. The proposed sensors offer a simple analytical tools for determination of different oxicam derivatives in their pharmaceutical formulations under batch and flow injection analysis (FIA) conditions.

Keywords: Oxicam; Screen printed potentiometric sensor; Carbon nanotube composite; Flow injection analysis; Pharmaceutical analysis.

1. Introduction

The project that produced the novel anti-arthritic and anti-inflammatory agent piroxicam (PXM, 4-hydroxy-2-methyl-N-2-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, Feldene; Pfizer) began in 1962 and led to the product launching into key European markets in 1980 [1]. Later, other oxicam derivatives including; tenoxicam (TXM, 4-hydroxy-2-methyl-N-(pyridine-2-yl)-2H-thieno [2,3-e]-1,2-thiazine-3-carboxamide-1,1 -dioxide) and meloxicam (MXM, 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2H-1,2-benzo-thiazine-3-carboxamide -1,1-di -oxide) were widely prescribed medication. Lornoxicam (LXM 6-chloro-4-hydroxy-2-methyl-N-2-pyridyl-5H-thieno-[2,3-e]-1,2-thiazine-2-carboxamide-1,1-dioxide) is a recent non-steroidal drug of the oxicam class with analgesic, anti-inflammatory and antipyretic properties with a relatively short elimination half-life, which may be advantageous from a tolerability standpoint [2].

Different official and non-official methods for analysis of oxicams (OXMs) were found in literature [3, 4]. Bibliography data [3] indicate that only liquid chromatographic method with UV [5] or MS detection [6] have been used for LXM determination. In addition, some few spectrophotometric [7] and voltammetric ones [8] can also be found in literature.

Nevertheless, most of these methods require expensive apparatus and involve several manipulation steps before the final result of analysis. Electrochemical methods are elegant approaches in analytical chemistry and an interest in developing electrochemical-sensing devices for environmental monitoring, clinical assays or process control is growing rapidly [9-11]. Potentiometric methods using ion selective electrodes (ISEs) have the advantages of simplicity, short analysis time with adequate precision and accuracy [12, 13]. To the best of our knowledge, no ISE was reported in literature for potentiometric determination of either LXM or MXM, and only polyvinyl chloride (PVC) sensors were found for PXM and TXM [14, 15]. More recently, carbon paste electrode (CPE) based on multi-walled carbon nanotubes/β-cyclodextrin composite (MWCNTs/β-CD-CPE) was introduced for potentiometric determination of PXM [16].

Conventional PVC and CPEs are inconvenient for large-scale routine analysis taking into account their construction, size, necessity of conditioning before measurements and the requirement for sterilization or cleaning to avoid infection or contamination. Several clinical and environmental applications would benefit from the availability of low-cost disposable sensors. Screen printing seems to be one of the most promising technologies allowing sensors to be produced on a large-scale with the advantages of optimized manufacturing repeatability, long shelf-lifetime and application the field measurements with portable small instruments [17, 18].

Carbon nanotubes (CNTs) are excellent sensor materials due to their high conductivity, chemical inertness and large surface area. Incorporation of CNTs in electrode matrices improves the conductivity and transduction of the chemical signal to electrical signal, which in turn improved the dynamic working range and

response time [19]. Moreover, defects in the graphite structure, at both end and side walls of CNTs, enables the functionalization of CNTs via either covalent or noncovalent modifications to catalyze electron transfer kinetics. Functionalization of nanotubes with guest structures will lead to new composite materials possessing the properties of each component, or even with a synergistic effect, which consequently improve the sensor performance [16, 19, 20].

The aim of the present work is to introduce disposable screen printed electrodes (SPEs) incorporated with FMWCNTs/β-CD composite as potentiometric sensors that can be used in drug quality control. The developed methods are simple, rapid, accurate, precise and sensitive for the determination of different oxicam derivatives in various dosage forms under batch and FIA conditions.

2. Experiamental

2.1. Reagents

All reagents were of the analytical grade and bidistilled water was used throughout the experiments. Cyclodextrin derivatives including; α-CD (**I,** Aldrich), γ-CD (**II,** Bio Basic Inc.), native β-CD (**III**, Sigma), heptakis 2,6-di-O-methyl-β-CD (**IV**, Aldrich), and heptakis 2,3,6-tri-O-methyl-β-CD (**V**, Aldrich) were used as sensing ionophores. Multi-walled carbon nanotubes (MWCNTs, Aldrich) were used for preparation of β-cyclodextrin composite (FMWCNTs/β-CD) as described in details elsewhere [16].

Hyamine®1622 (Hy, Fluka), cetylpyridinium chloride (CPC, Fluka), hexadecyltrimethylammonium bromide (HTMAB, Fluka), tri-dodecyltrimethylammonium bromide (TDTMAB, Sigma), didodecyl-dimethylammonium bromide (DDMAB, Fluka) and Septonex (Sp, Hlohovec, CZ) were tested as ionic sites.

The tested electrode plasticizers were as following; *o*-nitrophenyl octyl ether (*o*-NPOE, Sigma), 2-fluorophenyl 2-nitrophenyl ether (*f*-PNPE, Fluka), dioctylphthalate (DOP, Sigma), dioctylsebacate (DOS, Avocado) and tricresylphosphate (TCP, Fluka). Polyvinyl chloride (PVC, relative high molecular weight, Aldrich) and graphite powder (synthetic 1-2 µm, Aldrich) were used for preparation of the printing ink.

2.2. Authentic samples

Different oxicam authentic sample were kindly provided from National Organization for Drug Control and Research, Giza, Egypt. Stock drug solutions (10^{-2} mol L^{-1}) were prepared by dissolving the appropriate amount of the active ingredient in 5×10^{-2} mol L^{-1} NaOH solution and kept at 4°C.

2.3. Pharmaceutical preparations

Zeficam® tablets (Eva Pharma, Egypt, 8 mg LXM/tablet), Moxen® tablets (EGDT, Egypt, 7.5 mg MXM/tablet), Tenoxil® tablet (Sigma/MPC, Egypt, 20 mg TXM/tablet) and Dispercam® tablets (MUP Cairo, Egypt, 20 mg PXM/tablet) were purchased from local drug stores. Ten tablets were weighed, grinded, and an accurate weight of the powder equivelant to one tablet was dissolved in 5×10^{-2} mol L^{-1} NaOH. The sample was filtered, completed to 25 mL and analyzed using the proposed method and compared with the official method for each drug [21-24].

2.4. Biological samples

Aliquots of the biological fluid (2 mL urine or plasma, obtained from healthy male 12 h after an intake of one Zeficam- tablet) were spiked with standard LXM solution. Spiked samples were treated with 0.1 mL of 70% perchloric acid, diluted to 10 mL with water, vortexed for 1.0 min and centrifuged for 10 min at 13 000 rpm. Supernatants were adjusted to pH 10 with NaOH and diluted to 25 mL with water and analyzed according to the proposed protocol and official method [24].

2.5. Apparatus

Potentiometric measurements were carried out using a 692-pH meter (Metrohm, Herisau, Switzerland). A single line flow injection system, composed of four channel peristaltic pump (MCP Ismatec, Zurich, Switzerland), sample injection valve (ECOM, Ventil C, Czech Republic) and continuous flow cells adapted for screen printed electrodes [25], was constructed for FIA measurements. 46-Range Digital Multimeter (Radioshack, China) with PC interface was used for potentiometric measurements in case of FIA and response time measurements.

2.6. Procedures

2.6.1. Sensor construction

The potentiometric bielectrode strips were printed on a ceramic support (dimensions 5 × 35 mm) using silver- and graphite-based inks for reference and working electrodes, respectively [26]. The ion-sensing cocktail containing 1.0 mg β-CD (**V**), 1.0 mg Hy, 360 mg *f*-PNPE and 240 mg PVC dissolved in 6 mL tetrahydrofuran was printed on the surface of the graphite/PVC conducting track and left to dry at 50 °C for 24 h. In alternative electrode matrix, 20 mg of MWCNTs were added to the aforementioned matrix, sonicated for 30 min and typically printed on the conducting carbon track. For the nanocomposite based matrices, 2.0 mg of FMWCNTs/ β-CD, 1.0 mg Hy, 360 mg *f*-PNPE and 240 mg PVC were dissolved in 6 mL THF and sonicated for 30 min before printing of the sensing membrane. All the fabricated electrodes were directly used in potentiometric measurements after preconditioning in 10^{-3} mol L^{-1} oxicam solutions for 10 min.

2.6.2. Sensor calibration

For batch measurements, the developed sensors were calibrated by immersing the bielectrode strip in different oxicam solutions covering the concentration range from 10^{-7} to 10^{-2} mol L^{-1} at 25 °C. The potential readings were recorded and plotted against drug concentration in logarithmic scale.

Operating FIA measurements, 50 µL of freshly prepared drug solutions covering the range from 10^{-6} to 10^{-2} mol L^{-1}, were injected in the flowing 5×10^{-2} mol L^{-1} NaOH stream with flow rate of 12.6 mL min^{-1} [25]. The corresponding peak heights were recorded and used to draw the calibration graphs.

2.6.3. Potentiometric determination of oxicams in pharmaceutical preparations and biological samples

Different OXM derivatives were potentiometrically determined using the developed sensors by potentiometric titration and FIA measurements. Aliquots of the sample solutions containing 2.0 to 11.0 mg of oxicam derivatives were titrated against standardized Hy solution [27] using the fabricated OXM bielectrode strip as

indicator electrode. Potential readings were plotted against the titrant volume to estimate the end point.

Under FIA conditions, 50 µL of the sample solutions were injected in the flowing NaOH stream and the peak heights were compared to those obtained from injecting standard solutions of the same concentration. The obtained recoveries were compared with the official methods for each drug.

3. Results and discussion

Chemically modified electrodes (CMEs), were suggested for improving sensitivity and selectivity of the electroanalytical methods through using of species capable of molecular recognition [28]. Different macrocyclic molecules; such as crown ethers, calixarenes, phthalocyanins, porphyrins and cyclodextrins (CDs), have been proposed as sensing ionophores, where CDs were by far the most commonly used [29]. Cyclodextrins are distinguished by the number of glucopyranose units that form the troncoconic structure, which can be 6, 7 or 8, and are referred to as α-, β- and γ-cyclodextrin [30]. Hydroxyl groups of the glucopyranose subunits of the CD molecule are orientated to the exterior of the molecule; therefore the CD exterior is hydrophilic, whereas the central cavity, lined with skeletal carbon and ether oxygen atoms of the glucopyranose units, is relatively hydrophobic. The lipophilic cavity of a cyclodextrin molecule provides a micro environment which an appropriately sized nonpolar drug molecule, or more often nonpolar parts, can enter to form an inclusion complex [31].

3.1. Characterization of LXM/β-CDs inclusion complexes

Phase solubility studies, on different oxicam and cyclodextrin derivatives, were performed according to Higuchi and Connors [32] and the apparent stability constant ($K_{1:1}$) was calculated [33]. Stability constant of LXM-*β*-CD inclusion complex was higher than that with either *γ*-CD or α-CD (values were 10.959, 6.400 and 3.700 M^{-1} for *β*-CD, *γ*-CD and α-CD, respectively). Considering the size of α-, β- and γ-CDs, α- has the smallest cavity (4.7 Å) which may be unable to include the lornoxicam molecule with the cavity, while β- and γ-CDs have larger cavities that enables them to include the LXM inside their cavities [34, 35].

The stability constants for different OXM derivatives with β-CDs (**III-V**), assuming a 1:1 stoichiometry are listed in Table 1. Phase solubility experiments at 25°C showed an increase of the drug solubility in a linear form as a function of CDs concentration indicating formation of a soluble complex of the A_L type. Stability constants of different oxicam derivatives were of lower values compared to finding by other investigators [36-38] which may be attributed to the high pH value of the complexation medium used (5×10^{-2} mol L^{-1} NaOH) [36].

Moreover, the apparent stability constants of the four investigated oxicam derivatives with β-CDs (**III-V**) lie in the following order: 2,3,6-tri-O-methyl β-cyclodextrin **(V)** > 2,6-di-O-methyl β-cyclodextrin **(IV)** > native β-cyclodextrin **(V)**. All the studied β-CD derivatives have the same cavity diameter; however, the parent β-CD has height (8A˚), therefore part of the drug could still be outside the nanocage (vide infra). Upon methylation, the height of β-CD increased (about 11A˚), which enhance more penetration of the drug inside the cyclodextrin cavity. Furthermore, the hydrophobic part provided by the methyl groups increases the hydrophobicity of the cavity [39]. Such results come in accordance with other investigators where methyl β-CD and hydroxypropyl β-CD showed higher values of stability constant compared to native β-CD [40, 41].

Table 1: Stability constants of different oxicam and β-CD inclusion complexes

Oxicam	β-CD (**III**)	β-CD (**IV**)	β-CD (**V**)
PXM	2.897	4.249	5.904
TXM	2.432	4.035	4.883
MXM	5.351	6.807	7.326
LXM	10.959	12.095	20.118

In addition, different oxicam derivatives showed variant stability constants with cyclodextrin which may be related to their structures and the part of the drug molecule which enter the CD cavity. Molecular modelling studies [42] show that the minimum energy configuration gives favourable interaction energy between the β-CDs and the oxicam molecule when the conjugated rings of the drugs are inside the hydrophobic bucket-like cavity of β-CDs and the third ring is exposed to the solvent.

3.2. Optimal sensor matrices compositions

3.2.1. Effect of sensing material

Various CDs derivatives (**I-V**) were tested as molecular recognition elements for oxicam potentiometric sensors. Lower Nernstian responses were obtained via incorporation of α- or γ-CDs as sensing ionophore, while electrodes incorporated with different β-CDs ionophores (**III-V**) showed reasonable Nernstian responses (Fig. 1a). Sensors incorporated with methylated β-CD **(V)** showed the superior performance with Nernstian slope value of 52.0±1.6 mV $decade^{-1}$ in the concentration range from 10^{-5} to 10^{-2} $molL^{-1}$ LXM. Such variation in sensitivity can be explained on the basis of the stability constants of LXM/β-CDs inclusion complexes and fitting of LXM in the CD cavities (see 3.1). Moreover, β-CD **(V)** contents within the electrode matrices were varied from 0.5 to 5.0 mg and incorporation of 1.0 mg of the aforementioned ionophore was sufficient to get the proper performance.

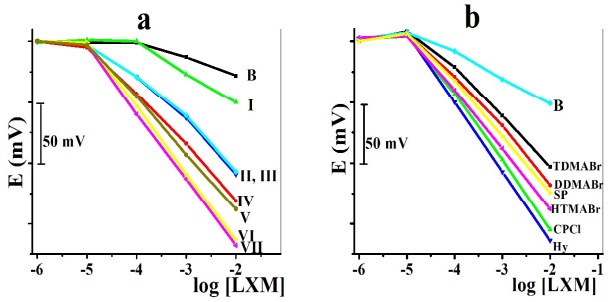

Fig. 1: Effect of sensing ionophores and ionic sites nature on lornoxicam electrode performance.

3.2.2. Effect of ionic sites

It is well established that lipophilic ionic additives promote the interfacial ion-exchange kinetics and decrease the membrane resistance by providing mobile ionic sites in the membrane matrix [43, 44]. Cyclodextrins behave as neutral carrier

ionophores and therefore addition of cataionic sites to their electrode matrices is required. From different ionic sites tested, Hy exhibited the best performance compared with CPC, DDMAB, HTMAB, TDMAB or Sp, which may be attributed to the difference in their lipophilicities (Fig. 1b). Furthermore, the content of Hy was changed from 0.0 to 5.0 mg and addition of 1.0 mg was the selected.

3.2.3. Effect of membrane plasticizer

Nature of the membrane plasticizer has crucial roles on the electrode performance through its affect on the mobility of ionophore molecules and the state of the formed inclusion complexes between the sensing ionophore and analyte [45, 46]. In the present work, five membrane plasticizers with different dielectrical contestants, were tested namely; *f*-PNPE, *o*-NPOE, TCP, DOS and DOP (ε = 50, 24, 17.6, 5.2 and 4.7, respectively). Application of less polar plasticizers lowered the Nernstian responses compared with reasonable sensitivity observed for electrodes containing high polar plasticizer. Sensors fabricated using *f*-PNPE showed the highest slope value (57.9±1.2 mV decade^{-1}) in the concentration range from 10^{-6} to 10^{-2} mol L^{-1} LXM.

3.2.4. Nanomaterial and nanocomposite based electrodes

It has been shown experimentally that the introduction of CNTs into a polymer matrix improves the electric conductivity which in turn improved the dynamic working range and response time [19, 47, 48]. For sake of more sensitivity, different quantities of MWCNTs (ranging from 0 to 50 mg) were incorporated into the electrode matrix containing β-CD (**V**) as sensing material. Improved sensitivity and potential stability were achieved via addition of 20 mg MWCNTs (slope value was 58.1±0.8 mV decade^{-1}).

Moreover, the FMWCNT-β-CD composite was used as a sensing ionophore instead of free β-CD (**V**) or MWCNTs. The effect of the nanocomposite contents within the electrode matrix was tested in the concentration range from 0.5 to 10 mg and addition of 2.0 mg was sufficient to get Nernstian slope 60.5±0.6 mV decade^{-1} in the concentration range from 10^{-6} to 10^{-2} mol L^{-1} of LXM.

3.3. Sensor performances

The potentiometric response characteristics of the developed sensors towards different oxicam derivatives were evaluated according to the IUPAC recommendation [49] (Table 2 and Fig. 2a). Data recommended application of FMWCNTs/β-CD nanocomposite as sensing material compared with those incorporated with free β-CD (**V**) or carbon nanotube which can be explained on the basis of the synergistic effect between MWCNTs and β-CD within the composite structure [16]. From tested oxicam derivatives, sensors showed higher sensitivity towards lornoxicam which may be explained on the basis of the stability constants of β-CD (**V**) with oxicam derivatives (Table 1, Fig. 2b).

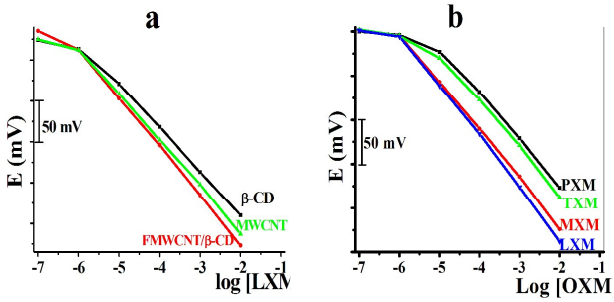

Fig. 2: Electrochemical performances of oxicam screen printed sensors.

Screen printing technology offers the advantages of high fabrication reproducibility. The average Nernstian slope values for ten printed electrodes within the same batch were 59.0 ± 0.5 mV decade^{-1} with standard potential of -246.6±1.8 mV.

Operational lifetimes of the fabricated electrodes were tested by performing day-to-day calibration with storage of electrodes at 4°C when not used. SPEs showed useful shelf lifetime for 16 weeks during which the Nernstian slopes did not change significantly (±1 mV decade^{-1}). Shorter lifetimes (12 weeks) were noticed in case of sensor incorporated with free ionophores due to leakage of the sensing material to the measuring solution.

One disturbing drawbacks of solid contact electrodes was the potential drift and the poor adhesion of the sensing membrane to the metal substrate [50] due to penetration of water and ions through the sensing membrane with formation of undefined layer between sensing membrane and conductor.

Table 2: Analytical performances [a] of different oxicam screen printed sensors

Sensors	LXM			MXM	TXM	PXM
	Free β-CD	CNT-β-CD	FMWCNT/β-CD	FMWCNT/β-CD	FMWCNT/β-CD	FMWCNT/β-CD
Concentration range (mol L^{-1})	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-5}-10^{-2}	10^{-5}-10^{-2}
Slope (mV decade^{-1})[a]	57.9±1.2	58.1±0.8	60.5±0.6	54.5±0.9	53.5±2.4	52.6±3.2
R	0.99973	0.99973	0.99987	0.9996	0.998	0.9964
Limit of detection (LOD, mol L^{-1})	1.0×10^{-6}	1.0×10^{-6}	6.0×10^{-7}	1.0×10^{-6}	5.0×10^{-6}	4.0×10^{-6}
Limit of quantification (LOQ mol L^{-1})	1.0×10^{-5}	7.0×10^{-6}	3.0×10^{-6}	1.0×10^{-5}	5.0×10^{-5}	4.0×10^{-5}
Response time (s)	4	3	2	2	2	2
Lifetime (week)	12	12	16	16	16	16
Reproducibility (mV per day)	±0.6	±0.5	±0.3	±0.8	±0.8	±0.7

[a] Results are average of five different calibrations

In the proposed sensor fabrication protocol, the sensing layer contain the same polymer matrix of the conducting track, and co-polymerization of both PVC matrices (sensitive membrane and conducting track) during electrode fabrication will hinder formation of such internal water layer and improve the electrode potential stability. Thus, the fabricated electrodes can directly be

used in measurements after preconditioning in 10^{-3} mol L^{-1} OXM solutions for 10 min. In addition, incorporation of MWCNTs enhances the hydrophobicity of the membrane, which contributes to the more stable potential signal by elimination of undesirable water layer at the interface [51]. Even though the use of the SPEs allows a single use of the sensor, it can be reliably applied up to ten times without significant losses of the sensitivity.

The dynamic response times of the fabricated sensors were tested by measuring the time required to achieve a steady state potential (within ±1 mV) after sudden increase in the oxicam concentration from 1×10^{-6} to 1×10^{-3} mol L^{-1}. Sensors incorporated with FMWCNTs/β-CD nanocomposite showed fast response time (about 2 s) compared with 3 and 4 s for those contain free ionophore or free MWCNTs.

The influence of pH on the electrode response was checked by recording the electrode potential readings for oxicam solutions containing 10^{-4} to 10^{-2} mol L^{-1} at different pH values (pH 6–12). The electrode responses were pH independent in the range from 8-11. The better performances at higher pH value may be explained on the bases of the stability constants of OXM tautomeric forms with β-CD. Oxicams usually presents in neutral, zwitterionic, anionic (deprotonated) and cationic (protonated) forms. The optimized structures of the inclusion complexes reveal an overall affinity ranking for the OXM guest molecule in the following order: deprotonated form > enolic form > zwitterionic form > protonated form [36, 52].

In pharmaceutical analysis, it is important to test the selectivity of the method towards the excipients which are usually added to the pharmaceutical preparations, such as glucose, starch, talc, lactose, sucrose. Selectivity of the prepared potentiometric sensors towards different species was tested applying Matched Potential Method (MPM) [53]. Results revealed high selectivity toward LXM in the presence of other interferents, additives and fillers introduced in pharmaceutical formulations (Table 3). Some metal cations such as Fe (III), Cr (III), Cd (II), Cu (II) and U which form organometalic complexes with oxicams [54] are expected to interfere, but they are not present in the aforementioned drug formulations.

Table 3: Potentiometric selectivity coefficients of lornoxicam - screen printed sensors under batch and FIA conditions.

Interferent	-log $K_{A,B}$			
	Batch	FIA		Batch
Li^+	3.20	3.30	Maltose	3.60
NH_4^+	3.40	3.50	Starch	3.80
Ca^{2+}	2.80	3.00	Sucrose	3.10
Mg^{2+}	3.50	3.7 0	Glucose	2.00
Ni^{2+}	2.10	2.45	Fructose	2.20
Co^{2+}	1.80	2.00	Glycine	2.40
Phosphate	3.80	4.00	Caffeine	3.30
Citrate	2.95	3.10	Cysteine	2.80

[a] Average of five measurements

3.4. Analytical applications

3.4.1.Potentiometric titration

Different oxicam derivatives were titrated against standardized hyamine solution [27] using sensors incorporated with FMWCNT/β-CD composite (Fig. 3). It can be noticed that the best titration curve was obtained in the case of LXM compared with other oxicam derivatives (the total potential change values were 220, 204, 172 and 155 mV for LXM, MXM, TXM and PXM, respectively). Under the optimum conditions, titration curves were symmetrical with well-defined potential jumps (ΔE ranged from 120 to 220 mV) allowing the determination of less 0.8 mg LXM.

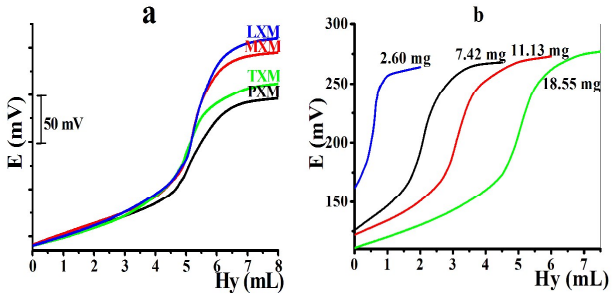

Fig. 3: Potentiometric titration of, a) 5 mL 10^{-2} mol L^{-1} of different OXM derivatives, and b) different LXM concentrations with 10^{-2} mol L^{-1} Hy using FMWCNT/β-CD based screen printed sensors as indicator electrode.

3.4.2.Electrode response under FIA conditions

One of the promising advantages of ISEs is their incorporation in flow injection systems for sake of automation and high sampling frequency [55]. Figure 4 showed flow injection peaks from the LXM electrode when 50 µL of LXM solutions covering the concentrations range from 10^{-6} to 10^{-2} mol L^{-1} were injected in 5×10^{-2} mol L^{-1} NaOH flowing stream (12.6 mL min^{-1}). Calibration graphs were linear in the concentration range from 10^{-6} to 10^{-2} mol L^{-1} with Nernstian slopes of 61.2±0.7mV $decade^{-1}$. Reproducibility was evaluated from repeated 10 injections of 50 µL LXM solution (10^{-3} mol L^{-1}); the average peak heights were found to be -70.2±1.2 mV. The fabricated electrodes showed fast response time (2 s) and stable potential readings which improve the residence time (ranged between 10 and 25s) and the sampling output (100 sample h^{-1}).

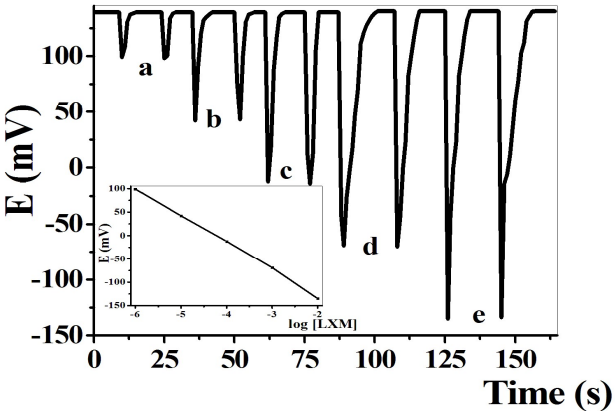

Fig. 4: FIA potentiometric determination of lornoxicam using FMWCNT/β-CD-SPE: a) 1×10^{-6}, b) 1×10^{-5}, c) 1×10^{-4}, d) 1×10^{-3}, and e) 1×10^{-2} mol L^{-1} via injection of 50 µL sample at flow rate 12.6 mL min^{-1}.

3.4.3. Potentiometric determination of oxicam derivatives in pharmaceutical preparations and biological fluids

The obtained satisfactory sensitivity and selectivity of the fabricated sensors made the proposed methods suitable for the routine quality control analysis of different oxicam derivatives in their pharmaceutical formulations and biological fluids under FIA and potentiometric titration modes. Results (Table 4) clearly indicated satisfactory agreement between the drug contents in pharmaceutical samples determined by the developed sensor and official methods [21-24]. The time required for sample analysis was short in case of FIA (about 2 min) with the advantage of accuracy and automation feasibility. Lornoxicam is absorbed from gastrointestinal tract and is characterized by a rapid rate. The peak blood concentration is reached after approximately 1–2 h [56]. Lornoxicam is found in the plasma in unchanged form and as its metabolite. 5-Hydroxylation is the main metabolic pathway, which accounts for up to 95% of total intrinsic lornoxicam clearance. Preliminary tests showed that hydroxylation of the pyridyl ring of lornoxicam molecules does not affect the complexation with cyclodextrin [56].

Table 4: Potentiometric determination of oxicam derivatives their pharmaceutical preparations and biological fluids

Sample	Potentiometric titration				FIA			
	Official (mg)	Proposed (mg)	Recovery[a] (%)	RSD	Official (µg)	Proposed (mg)	Recovery[a] (%)	RSD
Zeficam	2.10	2.07	98.6	1.5	0.74	0.73	99.1	1.1
	6.30	6.25	99.2	1.4	7.44	7.43	99.9	1.3
	10.50	10.46	99.6	1.7	74.36	75.48	101.5	1.4
Spiked urine	2.10	2.05	97.6	2.0	0.74	0.73	98.2	1.9
	6.30	6.21	98.5	1.9	7.44	7.34	98.7	1.6
Spiked plasma	2.10	2.06	98.1	2.2	0.74	0.72	97.9	1.7
	6.30	6.22	98.8	2.0	7.44	7.33	98.6	1.4
Moxen	2.11	2.07	98.0	1.2	0.70	0.69	98.7	1.0
	6.33	6.25	98.7	1.6	7.03	6.97	99.1	1.2
	10.55	10.57	100.2	1.7	70.28	70.06	99.7	1.3
Tenoxil	3.20	3.17	99.0	1.3	6.74	6.68	99.1	1.0
	6.40	6.39	99.8	1.4	67.40	66.66	98.9	1.3
	11.00	10.91	99.2	1.6				
Dispercam	3.30	3.24	98.2	1.7	6.62	6.53	98.7	1.0
	6.60	6.53	99.0	1.8	66.20	65.87	99.5	1.1
	9.90	9.86	99.6	1.5				

[a] Mean recovery and relative standard deviations of five replicate of the same concentration. Average recoveries were calculated according to the official method for each drug [21-24].

3.5. Method validation

Validation of electroanalytical methods is used to confirm the applicability of the proposed procedure for a specific test like other analytical methods [57]. Accuracy, precision, linearity, specificity, limits of detection (LOD) and quantification (LOQ) were achieved using a standard LXM stock solution.

The accuracy of the proposed method using sensors incorporated with FMWCNT/β-CD composite was investigated by the determination of LXM in spiked samples prepared from serial concentrations of LXM reference standards. The results summarized in Table 5 show high accuracy of the proposed method, as indicated by the percentage recovery values. The statistical analysis of the results using student's t-test and F-test showed nosignificant differences between them regarding accuracy and precision (Table 4).

Intra-day and inter-day precisions were assessed using three concentrations and five replicates of each concentration. The relative standard deviations were found to be very small indicating reasonable repeatability and reproducibility of the proposed method as shown in Table 5.

The specificity of the method was examined by observing the interference caused by the common excipients of the pharmaceutical formulation. It was found that these compounds did not interfere with the results of the proposed method as shown in Table 3.

Linear relationship was present between the electrode potential (mV) and the log [LXM]. The regression data, correlation coefficients (r) and other statistical parameters are presented in Table 2. The values of LOD that are presented in Table 2 indicate that the sensors under investigation are highly sensitive, selective and can be applied in determination of small amounts of LXM. The LOQ was determined by establishing the least concentration that can be measured according to ICHQ2 (R1) recommendations [58], below which the calibration range is non-linear, and was found to be 5.0×10^{-6} mol L^{-1}.

Conclusions

The present work describes the fabrication of novel disposable screen printed sensor based FMWCNT/β-CD for potentiometric determination of different anti-inflammatory agents including lornoxicam, meloxicam, piroxicam and tenoxicam. Sensors showed Nernstian compliance in the concentration range from 10^{-6} to 10^{-2} mol L^{-1} with fast response time (2 s) and long operational lifetime (16 weeks). Improved sensitivity and selectivity were achieved by incorporation of carbon

nanotubes/β-CD composite and carbon nanotube within the electrode matrix. The fabricated sensors showed better performance compared with previously published oxicam sensors [14-16] in term of sensitivity, operational lifetime and the simple fabrication protocol for mass production. The proposed method is suitable for routine analysis of different oxicam derivatives in pure and four different dosage forms with average recoveries comparable to the official methods. These results may be the base for further research leading to improvement of the analytical parameters for preparation of simple drug potentiometric sensors with the possibility of commercialization of such disposable sensors.

Table 5: Evaluation of accuracy and precision of lornoxicam - screen printed sensors

Sample	Official (mg)[a]	Intra-day				Inter-day			
		Proposed (mg)	Recovery (%)	SD	RSD	Proposed (mg)	Recovery	SD	RSD
LXM	2.10	2.07	98.6	0.09	1.52	2.02	96.4	0.12	1.65
	6.30	6.25	99.2	0.06	1.25	6.19	98.2	0.0.9	1.40
	10.50	10.46	99.6	0.04	1.00	10.66	101.5	0.08	1.35
Zeficam	2.11	2.07	98.0	0.10	1.42	2.08	98.7	0.15	1.78
	6.33	6.25	98.7	0.08	1.36	6.27	99.1	0.11	1.65
	10.55	10.57	100.2	0.07	1.17	10.51	99.7	0.09	1.33

[a] Mean recovery and relative standard deviations of five replicate of the same concentration. Average recoveries were calculated according to the official method for each drug [24].

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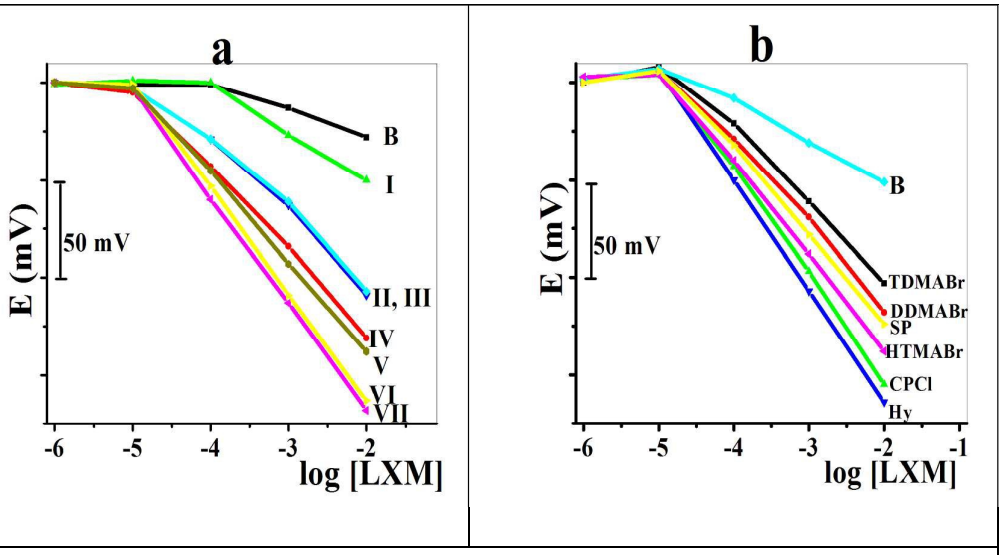


Fig. 1: Effect of sensing ionophores and ionic sites nature on lornoxicam electrode performance.

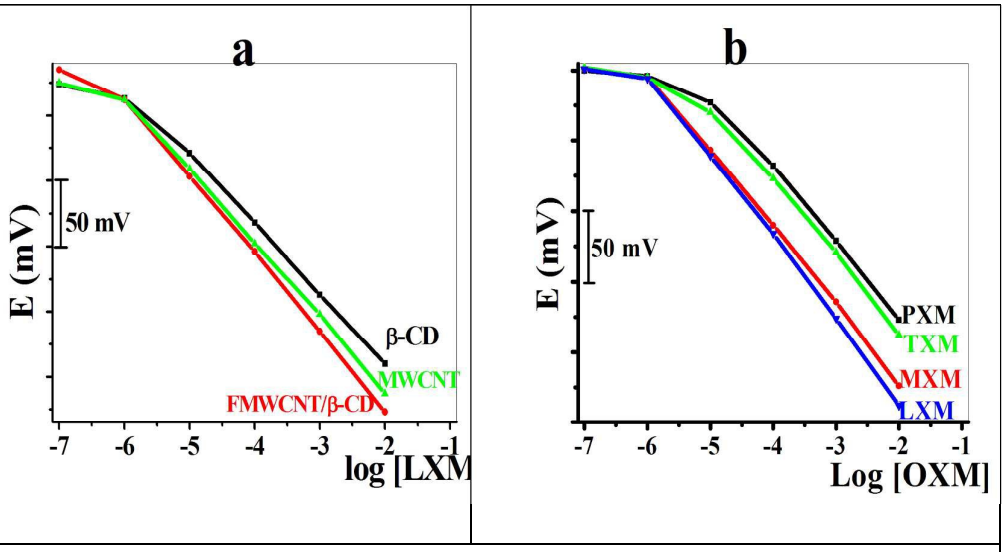


Fig. 2: Electrochemical performances of oxicam screen printed sensors.

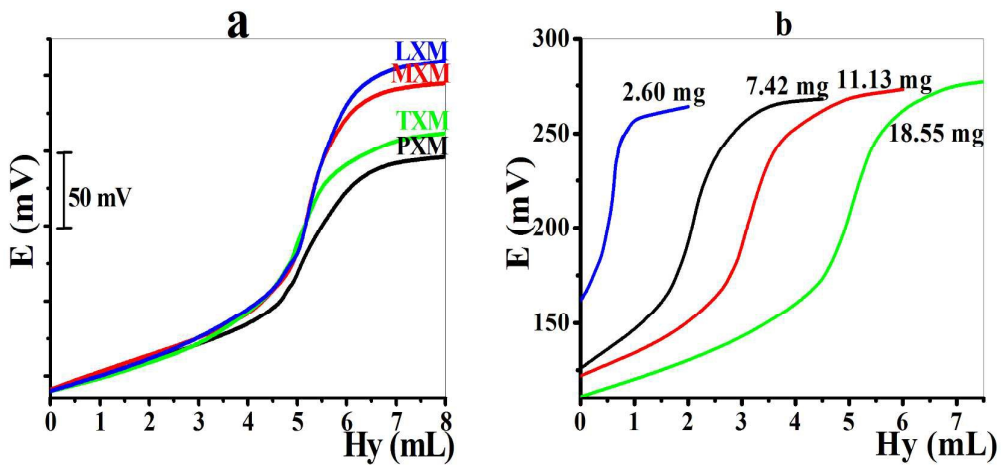


Fig. 3: Potentiometric titration of, a) 5 mL 10^{-2} mol L^{-1} of different OXM derivatives, and b) different LXM concentrations with 10^{-2} mol L^{-1} Hy using FMWCNT/β-CD based screen printed sensors as indicator electrode.

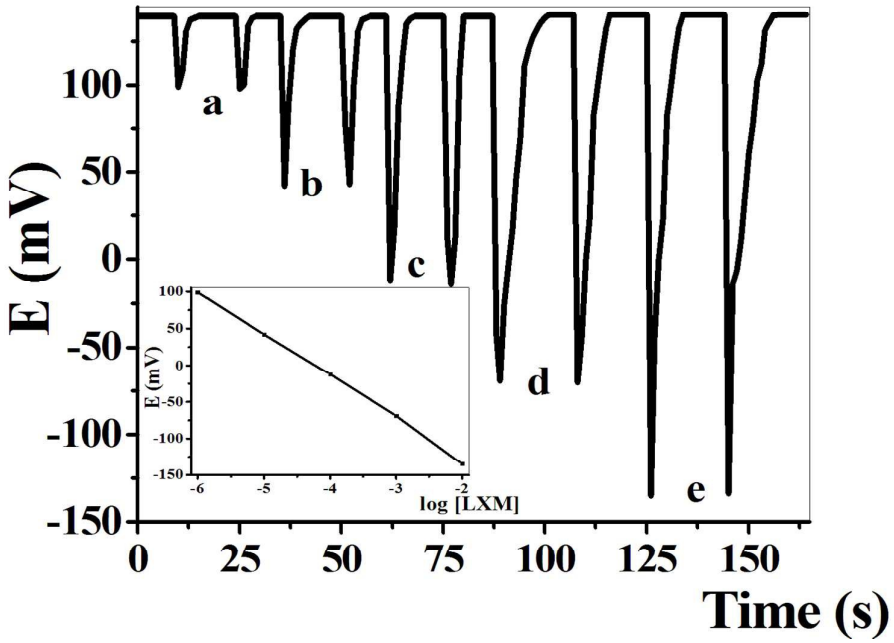


Fig. 4: FIA potentiometric determination of lornoxicam using FMWCNT/β-CD-SPE: a) 1×10^{-6}, b) 1×10^{-5}, c) 1×10^{-4}, d) 1×10^{-3}, and e) 1×10^{-2} mol L^{-1} via injection of 50 µL sample at flow rate 12.6 mL min^{-1}.

Table 1: Stability constants of different oxicam and β-CD inclusion complexes

Oxicam	β-CD (**III**)	Methylated β-CD (**IV**)	Methylated β-CD (**V**)
PXM	2.897	4.249	5.904
TXM	2.432	4.035	4.883
MXM	5.351	6.807	7.326
LXM	10.959	12.095	20.118

Table 2: Analytical performances [a] of different oxicam screen printed sensors

Sensors	LXM			MXM	TXM	PXM
	Free β-CD	CNT-β-CD	FMWCNT/β-CD	FMWCNT/β-CD	FMWCNT/β-CD	FMWCNT/β-CD
Concentration range (mol L^{-1})	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-5}-10^{-2}	10^{-5}-10^{-2}
Slope (mV $decade^{-1}$)[a]	57.9±1.2	58.1±0.8	60.5±0.6	54.5±0.9	53.5±2.4	52.6±3.2
R	0.99973	0.99973	0.99987	0.9996	0.998	0.9964
Limit of detection (LOD, mol L^{-1})	1.0×10^{-6}	1.0×10^{-6}	6.0×10^{-7}	1.0×10^{-6}	5.0×10^{-6}	4.0×10^{-6}
Limit of quantification (LOQ mol L^{-1})	1.0×10^{-5}	7.0×10^{-6}	3.0×10^{-6}	1.0×10^{-5}	5.0×10^{-5}	4.0×10^{-5}
Response time (s)	4	3	2	2	2	2
Lifetime (week)	12	12	16	16	16	16
Reproducibility (mV per day)	±0.6	±0.5	±0.3	±0.8	±0.8	±0.7

[a] Results are average of five different calibrations

Table 3: Potentiometric selectivity coefficients for LXM-sensor under batch and FIA conditions.

Interferent	-log $K_{A,B}$			
	Batch	FIA		Batch
Li^{+}	3.20	3.30	Maltose	3.60
NH_4^{+}	3.40	3.50	Starch	3.80
Ca^{2+}	2.80	3.00	Sucrose	3.10
Mg^{2+}	3.50	3.7 0	Glucose	2.00
Ni^{2+}	2.10	2.45	Fructose	2.20
Co^{2+}	1.80	2.00	Glycine	2.40
Phosphate	3.80	4.00	Caffeine	3.30
Citrate	2.95	3.10	Cysteine	2.80

[a] Average of five measurements

Table 4: Potentiometric determination of oxicam derivatives their pharmaceutical preparations and biological fluids

Sample	Potentiometric titration				FIA			
	Official (mg)	Proposed (mg)	Recovery[a] (%)	RSD	Official (µg)	Proposed (mg)	Recovery[a] (%)	RSD
Zeficam	2.10	2.07	98.6	1.5	0.74	0.73	99.1	1.1
	6.30	6.25	99.2	1.4	7.44	7.43	99.9	1.3
	10.50	10.46	99.6	1.7	74.36	75.48	101.5	1.4
Spiked urine	2.10	2.05	97.6	2.0	0.74	0.73	98.2	1.9
	6.30	6.21	98.5	1.9	7.44	7.34	98.7	1.6
Spiked	2.10	2.06	98.1	2.2	0.74	0.72	97.9	1.7
plasma	6.30	6.22	98.8	2.0	7.44	7.33	98.6	1.4
Moxen	2.11	2.07	98.0	1.2	0.70	0.69	98.7	1.0
	6.33	6.25	98.7	1.6	7.03	6.97	99.1	1.2
	10.55	10.57	100.2	1.7	70.28	70.06	99.7	1.3
Tenoxil	3.20	3.17	99.0	1.3	6.74	6.68	99.1	1.0
	6.40	6.39	99.8	1.4	67.40	66.66	98.9	1.3
	11.00	10.91	99.2	1.6				
Dispercam	3.30	3.24	98.2	1.7	6.62	6.53	98.7	1.0
	6.60	6.53	99.0	1.8	66.20	65.87	99.5	1.1
	9.90	9.86	99.6	1.5				

[a] Mean recovery and relative standard deviations of five replicate of the same concentration. Average recoveries were calculated according to the official method for each drug [21-24].

Table 5: Evaluation of accuracy and precision of lornoxicam - screen printed sensors

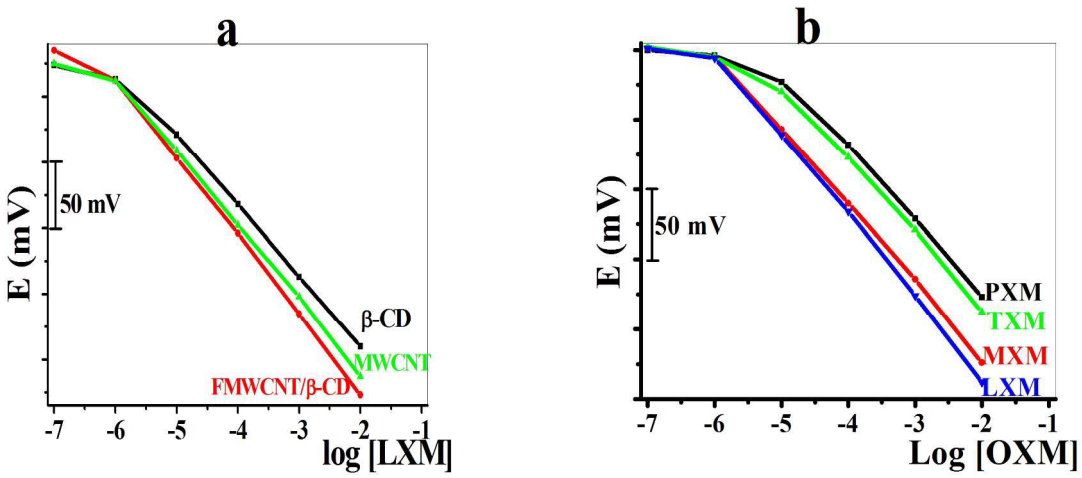
Sample	Official (mg)[a]	Intra-day				Inter-day			
		Proposed (mg)	Recovery (%)	SD	RSD	Proposed (mg)	Recovery	SD	RSD
LXM	2.10	2.07	98.6	0.09	1.52	2.02	96.4	0.12	1.65
	6.30	6.25	99.2	0.06	1.25	6.19	98.2	0.0.9	1.40
	10.50	10.46	99.6	0.04	1.00	10.66	101.5	0.08	1.35
Zeficam	2.11	2.07	98.0	0.10	1.42	2.08	98.7	0.15	1.78
	6.33	6.25	98.7	0.08	1.36	6.27	99.1	0.11	1.65
	10.55	10.57	100.2	0.07	1.17	10.51	99.7	0.09	1.33

[a] Mean recovery and relative standard deviations of five replicate of the same concentration. Average recoveries were calculated according to the official method for each drug [24].

Novel screen printed potentiometric sensors for the determination of oxicams

Elmorsy Khaled [*, a], Manal S. Kamel [a], Hassan N. Hassan [a], Sameh H. Abd El-Alim[b] and Hassan Y. Aboul-Enein [c*]

The construction and performance characteristics of new sensitive and selective sensors based on funcntiolized multi-walled carbon nanotubes/β-cyclodextrin nanocomposite (FMWCNTs/β-CD) was demonstrated for potentiometric determination of different anti-inflammatory agents including lornoxicam, meloxicam, piroxicam and tenoxicam. Screen printed sensors (SPEs) modified with FMWCNTs/β-CD composite, hyamine (Hy) and 2-fluorophenyl 2-nitrophenyl ether (*f*-PNPE) showed proper electroanalytical performances with Nernstian compliance range between 61.2 to 52.6 mV decade^{-1} activity and detection limit 6.0×10^{-7} mol L^{-1} for different oxicam derivatives. Modification with carbon nanotubes composite as sensing material remarkably improved the potential stability and lifetime of the fabricated sensors. The proposed sensors offer a simple analytical tools for determination of different oxicam derivatives in their pharmaceutical formulations under batch and flow injection analysis (FIA) conditions.



Electrochemical performances of oxicam screen printed sensors.