Full Paper

Electrochemical Studies of Oxidation of Lomefloxacin and Interaction with Calf Thymus DNA at Nano-SnO_2/DHP Modified Electrode

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Abstract
A nanoparticle thin film modified electrode has been constructed using a glassy carbon electrode (GCE) coated with a nano-tin oxide/dihexadecylphosphate (nano-SnO_2/DHP). In pH 6.0 phosphate buffer solutions (PBS), lomefloxacin (LMF) appeared as an anodic peak with peak potential of 1.35 V at nano-SnO_2/DHP modified electrode. In comparison with a bare GCE or a nano-SnO_2 modified electrode, the nano-SnO_2/DHP modified electrode exhibited an enhanced effectiveness for the oxidation of LMF. Cyclic voltammetry (CVs) coupled with fluorescence and UV/vis absorbance spectra techniques were used to study the interaction of LMF with Calf thymus DNA (ctDNA) in phosphate buffer solutions (PBS). The interaction of LMF and ctDNA could result in a considerable decrease in the peak currents and positive shift in the peak potential, as well as changes of fluorescence, UV/vis adsorption spectra and gel electrophoresis. All the acquired data showed that the new adduct between LMF and ctDNA was formed. Electrochemistry coupled with spectroscopy techniques could provide a relatively easy way to obtain useful information about the molecular mechanism of LMF-ctDNA interactions.

Keywords: Lomefloxacin, ctDNA, Nano-SnO_2, Dihexadecylphosphate, Modified electrode

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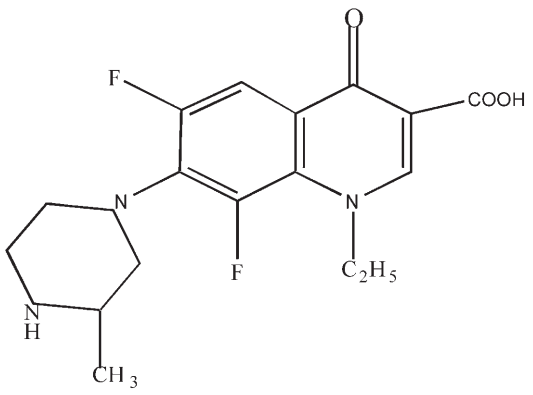
1. Introduction

Lomefloxacin (LMF) [1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylix acid] (Scheme 1) is one of the third generation synthetic antibacterial fluoroquinolone, which exhibits high activity against a broad spectrum of gram-negative and gram-positive bacteria through inhibition of their DNA gyrase, is often used as important drugs for the treatment of respiratory tract, urinary tract, skin and skin-structure infections.



Scheme 1. The structure of LMF

Generally, the determination of LMF is performed with high performance liquid chromatography method [1, 2]; microbiological assay method [3]; micellar electrokinetic capillary chromatographic [4]; chemiluminescent [5]; spectrophotometric [6] and photochemical-fluorimetric [7] methods. However, these methods have some limitations in terms of simplicity, sensitivity, or stability. In recent years, there are some reports about detection of LMF by electrochemical techniques [8–14].

Recently nano-materials have attracted much attention and have been widely used in material, electronic, physical fields for various purposes due to their unique properties. Nano-SnO_2 possesses excellent photoelectronic properties, high gas sensitivities and a short response time as well as relatively higher conductivity than TiO_2 and SiO_2 [15–17]. These new kinds of inorganic nanomaterials exhibit tunable porosity, high thermal and chemical stability. Up to now, some inorganic oxide nanomaterials have been investigated in fundamental studies of protein-electrode interactions and the development of biosensing devices [18–29]. The nanoporous structures of these inorganic oxide films greatly enhance the active surface area available for protein binding. These films facilitate direct transfer process between biomolecules and electrodes.

The antibacterial mechanism of the third generation synthetic antibacterial fluoroquinolone has once thought to

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be through inhibition of bacteria DNA gyrase, so many scientists (such as biologists, chemists) are struggling to seek for the interaction mechanism. Now there are some reports about norfloxacin interaction with DNA [30–38]. From these studies, norfloxacin had been reported to probably associate near the minor groove of double stranded DNA in a non-classical manner with possibility of partial intercalation. A. Radi et al. thought the binding model of levofloxacin and pefloxacin to DNA were based on electrostatic binding and intercalation under their conditions [39, 40]. The steric structure of a small molecule determines the binding properties such as affinity and binding site. It has been suggested that a planar structure is an important feature needed for efficient intercalation [41]. LMF is also a member of the quinolone antibiotics whose chromophore is identical to that of norfloxacin and has a chromophore plane. However there are few reports about the interaction between LMF and ctDNA. In this work, we study the electrochemical behavior of LMF at nano-SnO_2/DHP film modified electrode. We hope to get a novel result at nano-modified electrode. Experiment result showed the nano-SnO_2/DHP film could facilitate the electron transfer of LMF and the electrode surface. Furthermore we adopted CVs, fluorescence, UV/vis absorbance spectra and agarose gel electrophoresis techniques to study the LMF and ctDNA interaction. As a result, our study showed that adduct of LMF and ctDNA was similar to that of norfloxacin-DNA and it may be partially inserted between ctDNA bases.

2. Experimental

2.1. Reagents

Calf thymus DNA (sodium salt, type I) was obtained from Sigma (USA) and was used without further purification, and its purity was confirmed by UV/vis absorption, which produced A_{260}/A_{280} of approximately 1.8–1.9, suggesting the DNA sample was free of proteins. The stock solution of DNA was directly dissolved in water and stored at 4 °C. The DNA concentration per nucleotide (DNA-P) was determined spectrophotometrically [42] at 260 nm by using the extinction coefficient 6600 cm^{-1} M^{-1}. LMF was purchased from Chinese National Institute for the Control of Pharmaceutical and Biological Products (content > 90.0%) and used without further purification. Nano-SnO_2 were donated from Professor Ni Yonghong (Anhui Normal University) and used without further treatment. SnO_2 nanocrystals was synthesized by the hydrothermal synthesis using $SnCl_4 \cdot 5H_2O$ as the precursor at pH 1.0 at 150 °C and reacted 24 hours. Dihexadecylphosphate (DHP) was purchased from Fluka. Other chemicals were of analytical reagent grade. All solutions were prepared with twice-distilled water.

2.2. Apparatus

Cyclic voltammograms (CVs) were obtained on a CHI 660A electrochemical workstation (Shanghai ChenHua Instruments, China) with a three-electrode system. A platinum wire was used as auxiliary electrode, a saturated calomel electrode (SCE) as a reference electrode, a bare or nano-SnO_2/DHP film modified glassy carbon electrode (GCE) ($\varnothing = 3.2$ mm) as a working electrode, respectively. All potentials were reported to the SCE.

The UV/vis absorbance spectra were acquired on U-3010 spectrofluorometer (Hitachi, Japan) equipped with a quartz micro-colorimetric vessel of 1 cm path length.

Fluorescence spectra and relative fluorescence intensities were measured on a model F-4500 fluorescence spectrophotometer (Hitachi, Japan) equipped with a xenon lamp, dual monochromators, and a 1 cm × 1 cm quartz cell. The slit-width for both excitation and emission was set at 5 nm.

Agarose gel electrophoresis was attained on a general WC horizontal electrophoresis trough and UV-2000 analysis instrument (Shanghai Tianneng Company, China).

2.3. Electrode Preparation

The bare GCE was successively polished in the Al_2O_3 slurry from 0.3 to 0.5 μm before modification. Then it was rinsed with double distilled water and sonicated in 1:1 nitric acid, acetone and double distilled water for 10 min, respectively. Then continuous cyclic scanned for 15 circles in 1.0 M sulfuric acid solution between −0.1 and +1.6 V with a rate of 100 mV/s and allowed to dry at room temperature.

For preparation of a nano-SnO_2 and DHP film modified GCE, a nano-SnO_2 suspension was obtained by dispersing 5.0 mg nano-SnO_2 into 10 mL DHP alcohol solution. 10 μL of the suspension was dropped onto the surface of clean GCE. Then solvent was evaporated in the air, the final electrode was taken as nano-SnO_2/DHP/GCE. A DHP/GCE (casting a 10 μL DHP solution), a nano-SnO_2/GCE (dropping 10 μL of the nano-SnO_2 suspension) and a bare GCE were taken to compare. All measurements were performed in a 10 mL electrolytic cell with 5 mL solutions, where oxygen was removed with high-purity nitrogen for 10 min. All measurements were performed under a nitrogen atmosphere.

3. Results and Discussion

3.1. Electrochemical Behaviors of LMF at the Nano-SnO_2/DHP Film Modified Electrode

Figure 1 depicted the CVs of LMF at the nano-SnO_2/DHP/GCE (curve d), nano-SnO_2/GCE (curve e), DHP/GCE (curve a) and bare GCE (curve b) in 0.1 M PBS (pH 6.0) respectively. At a bare GCE (curve b) and DHP/GCE (curve a), the electrochemical responses of LMF were very poor. However at nano-SnO_2 modified electrode (curve c),

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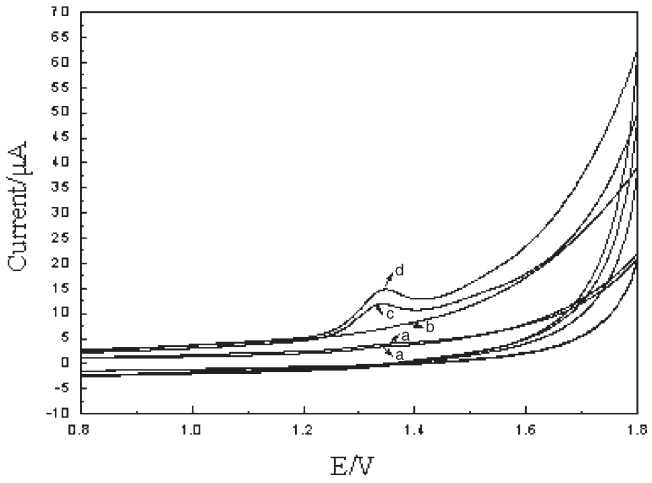

Fig. 1. Cyclic voltammograms of 1×10^{-5} M LMF on modified GCE in 0.1 M PBS at pH 6.0: a) DHP film modified GCE; b) the bare GCE; c) nano-SnO_2 modified GCE; d) nano-SnO_2/DHP modified GCE. The scan rate is 100 mV/S.

it showed a good electrochemical response, the peak current was enhanced; meanwhile the oxidation peak negatively shifted. The reason may ascribe to the great surface area of nano-SnO_2 [43] and catalyzing action to oxidation of LMF. At the nano-SnO_2/DHP electrode (curve d), the anodic peak current was higher than that at the nano-SnO_2 modified electrode. The reason could be that nano-SnO_2/DHP film has negative charge, so it can attractive positive charge LMF ($pK_a = 7.14$) in pH 6.0 PBS. Meanwhile it can provide a favorable microenvironment for LMF and facilitating the electron exchange [43]. From the CVs we can conclude the electrode reaction of LMF at nano-SnO_2/DHP film modified electrode is irreversible.

In addition, the effect of the scan rate on the peak current of LMF was investigated. Figure 2 shows the CVs of LMF at the various scan rates in 0.1 M PBS (pH 6.0). The anodic peak currents are proportional to scan rates from 80 to 220 mV/S (inset in Fig. 2). Linear regression equation was obtained as $i_{pa} = 2.504 + 0.045$ V (mV/s) with a correlation coefficient $r = 0.9986$; meanwhile we also investigated the effect of accumulation time on the peak current and the peak current increased with the accumulation time prolonging till five minutes, which suggests that the electrode reaction of LMF at nano-SnO_2/DHP film modified electrode is an adsorption-controlled process.

3.2. Effect of pH

The effect of the solution pH on electrochemical response of LMF was investigated in the range of 4.0 – 9.0. Figure 3A shows that the anodic peak potential negatively shifts with increasing solution pH. The line indicates protons take part in the electrode process. From Figure 3B, we could also observe the peak current increasing with the pH of solution from pH 4.0 – 6.0. When pH > 6.0, the anodic peak current decreased and peak shape became worse. From the changes of peak shape and peak current of LMF, we selected pH 6.0 PBS for the supporting electrolyte.

3.3. Determination of LMF

The determination of LMF concentration was performed with differential pulse voltammetry (DPV). The oxidation peak current of LMF was selected as the analytical signal. The results show that the anodic peak current is proportional to the concentration of LMF in the range of

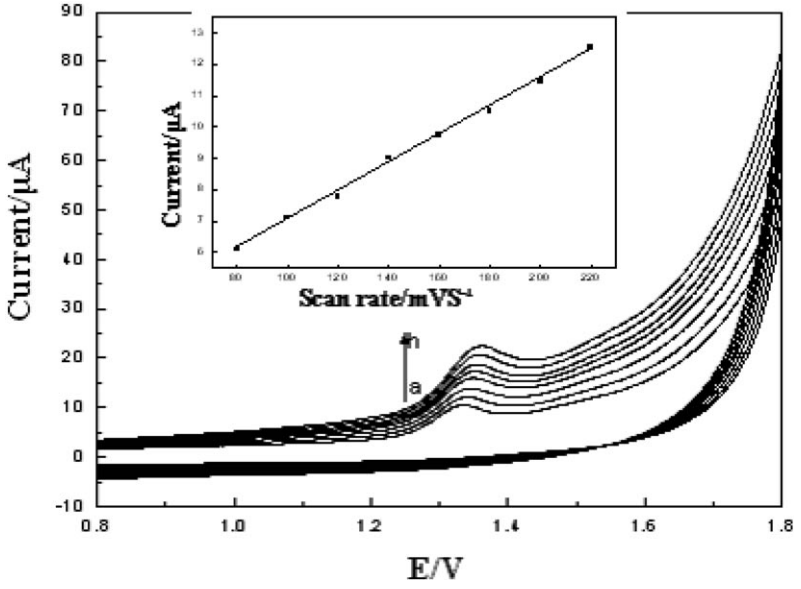

Fig. 2. Cyclic voltammograms of the nano-SnO_2/DHP modified GCE in 0.1 M PBS at pH 6.0 at various scan rates: a) 80, b) 100, c) 120, d) 140, e) 160, f) 180, g) 200, h) 220 mV/S. Inset: Relationship of the oxidation peak currents of 1×10^{-5} M LMF vs. scan rates.

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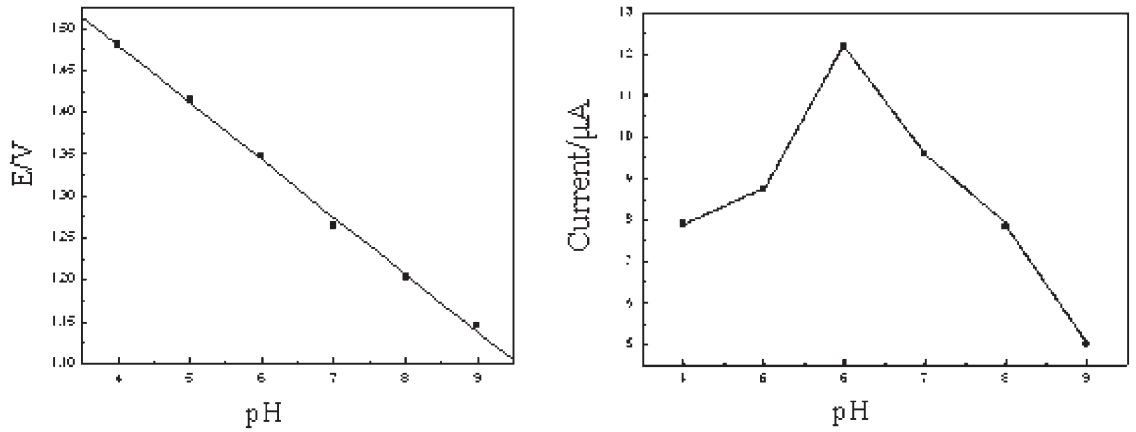

Fig. 3. A) Relationship between the oxidation peak potential and solution pH. B) Relationship between the oxidation peak current and solution pH.

1×10^{-8} $M^{-5} \times 10^{-5}$ M, and the linear regression equation is i_{pa} (10 μA) = 0.2773 + 0.0087 C (μM), with a correlation coefficient of $r = 0.9987$. The detection limit ($S/N = 3$) is 5×10^{-9} M. To the same surface of a nano-SnO_2/DHP film modified electrode, the relative standard deviation of 10 successive scans is 2.3% for 1×10^{-5} M LMF, indicating excellent reproducibility of the modified electrode.

Furthermore, we also investigated the stability of the modified electrode. The peak current of same concentration LMF could hardly change after storage in air for at least 3 weeks or cyclic scanning 400 circles in PBS solution to the same surface of a nano-SnO_2/DHP film modified electrode.

3.4. Studies of the Interaction Between LMF and ctDNA

3.4.1. Electrochemical Confirmation of the Interaction of LMF with ctDNA

We investigated the CVs of LMF at a nano-SnO_2/DHP / GCE in PBS (pH 6.0) in absence of ctDNA or in the presence of ctDNA. The oxidation peak current and peak potential of LMF were 6.536×10^{-6} A and 1.343 V in absence of ctDNA, respectively. However the oxidation peak current of LMF obviously decreased in the presence of ctDNA, meanwhile the oxidation peak potential positively shifted and the peak current and peak potential were 3.372×10^{-6} A and 1.420 V, respectively. Bard and co-workers [44] explained the reason of positive shifts in the peak potential was due to the hydrophobic binding form. If it was electrostatic interactions, the peak potential negatively shifts. Based on this report, the positive shifts in the peak potential of LMF should be a result of specific intercalation to ctDNA. In order to demonstrate that the decrease of current was not due to the increased viscosity of the solution or the blockage of the electrode surface by ctDNA adsorption, a special CV experiment was designed in a $K_4Fe(CN)_6$ solution with or without ctDNA. In these solutions, the $Fe(CN)_6^4$-ions did not interact with ctDNA because of electrostatic repulsion action. To compare with absence of ctDNA, the peak current was almost same and there was no shift in the peak potential in the presence of ctDNA. Therefore, there were only few effects of ctDNA addition on peak current and peak potential of the probe molecule. The great decrease of LMF peak current in CVs could be attributed to the formation of the LMF-ctDNA complex.

In addition, we designed a series experiments to confirm interaction mechanisms of LMF and ctDNA. Under condition of different pH (6.0, 8.0), we could observe that the peak currents decreased and the peak potential positively shifted at the presence of ctDNA compared with the absence of ctDNA. At pH 8.0, its peak current diminishing and the peak potential positively shifting shows that the interaction of LMF and ctDNA is not electrostatic affinity and should be an intercalating action, for the negative charge carboxylate group of LMF is thought to disfavor at pH 8. Furthermore, a more decreasing was seen at presence of ctDNA at pH 6.0, this shows that electrostatic attraction is advantaged of LMF intercalating into ctDNA.

3.4.2. Investigation of UV/Vis Absorption Spectra

Spectroscopic techniques are very useful tools to gain important information in biological science [34, 39]. Spectroscopic investigation of the interaction of LMF with ctDNA may be helpful for clarifying the mechanism of interaction. The interaction of LMF with ctDNA in PBS (pH 6.0) could be further confirmed by UV/vis spectra. The variation of adsorption spectroscopy is presented in Figure 4. For the spectrum of free LMF, an absorption band is observed at 284 nm and 322 nm (Curve a). Peak maximum of free ctDNA occurs at 256 nm results from electronic transition of bases (Curve b). However, in the presence of ctDNA, the 322 nm absorption peak of LMF red shifted and the absorption intensity decreased (Curve c). Accompanying the binding process two-isosbestic points appeared which indicated the conformation of the DNA-LMF was homogeneous. It has been suggested that hypochromism is a consequence of interactions between the electronic states of

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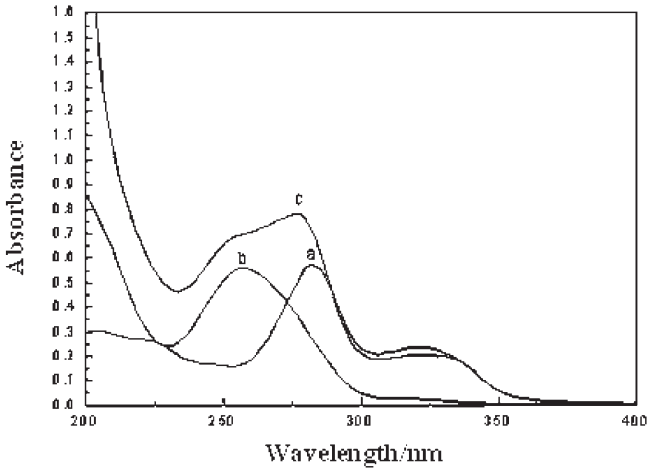

Fig. 4. UV/vis absorption spectra of 0.1 M PBS (pH6.0) containing 1.5×10^{-5} M LMF (a), containing 4.0×10^{-5} M ctDNA (b), and a mixture of 1.5×10^{-5} M LMF and 4.0×10^{-5} M ctDNA (c).

the intercalating chromophore and that of the ctDNA base pairs [45, 46]. These spectral changes, e.g., hypochromicity, red shift, and isobestic point, are consistent with intercalation of the chromophore into the ctDNA base pairs. From the observations detailed above, we surmised that the intercalation occurred. This is consistent with the electrochemical methods.

3.4.3. Fluorescence Spectra Studies

The interaction of LMF with ctDNA was also examined using fluorescence spectra. The fluorescent emission spectra of LMF and the effect of ctDNA concentrations on the fluorescence emission spectra of LMF were illustrated in Figure 5. LMF exhibited an emission maximum at 463 nm (Fig. 5a). The fluorescence emission was gradually decreased with increasing amount of ctDNA (curve b – e), showing that the LMF fluorescence was efficiently quenched upon binding to ctDNA. Norfloxacin have been

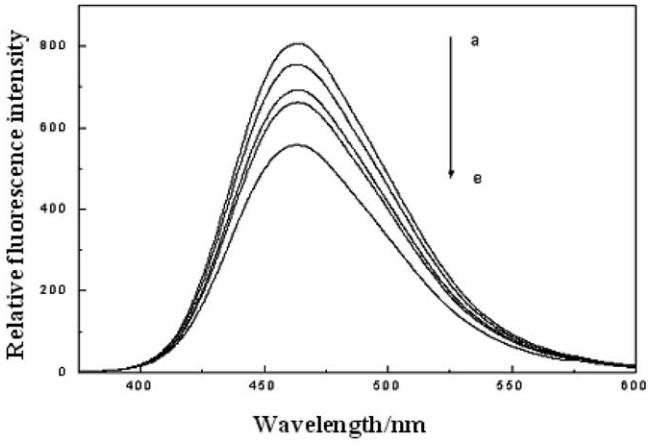

Fig. 5. Fluorescence emission of 0.1 M PBS (pH 7.0) containing 1.5×10^{-5} M LMF in the presence of ctDNA: a) 0.0, b) 2.0×10^{-5} M, c) 3.0×10^{-5} M, d) 4.0×10^{-5} M, e) 6.0×10^{-5} M.

well recorded [34, 35, 37, 38]. When norfloxacin bound to DNA or synthetic polynucleotide, the intensity of fluorescence emission was decreased without changing the shape upon binding to DNA and poly[d(G-C)$_2$] while emission maximum shifted to red with an isosbestic wavelength when bound to poly[d(I-C)$_2$] and poly[d(A-T)$_2$]. From these results and other observations including linear dichroism spectrum, norfloxacin was suggested to bind in the minor groove of poly[d(G-C)$_2$] and native DNA, probably between amine group at the 2-position of guanine base and oxygen atoms of norfloxacin with the possibility of partial intercalation [47], while, in the poly[d(A-T)$_2$] and poly-[d(I-C)$_2$] case, norfloxacin locates near the minor groove and is stabilized by the electrostatic interaction with phosphate group. The chromophore of norfloxacin and LMF is identical hence similar spectral properties for both compounds are expected if they bind to native DNA with the similar binding mode. Therefore these resulted in the above spectra changes. This is similar to that clarified in absorption spectra and the electrochemical methods.

3.4.4. Quenching Studies

Another evidence that support the intercalation mechanism is come from determining the relative fluorescence intensity of the free and bound LMF to the anionic quenchers potassium iodide. If LMF is intercalated into the helix it should be protected, owing to the base pairs above and below. Whereas single groove binding or electrostatic interaction could expose the bound molecules to the solvent much more than for intercalated species, it should provide much less protection [48]. Consistent with our expectation of intercalation, when the anionic quenchers potassium iodide were added to a solution, fluorescence quenching was found to be much smaller in the presence of ctDNA than in its absence. Simple linear behavior was apparent from the quenching curves, this indicates the interaction of LMF and ctDNA is only one major binding mode, which is as a result of the intercalation system. This is another forceful proof of intercalation mechanism.

3.4.5. Agarose Gel Electrophoresis

Agarose gel electrophoresis is a generally separating, identifying, purifying DNA method. For more confirmation the mode of LMF and ctDNA action, an agarose gel electrophoresis has also been done. The patterns are below (Fig. 6). The ctDNA is dyed by the LMF and not ethidium bromide (EB). The figure is the patterns of 1.3×10^{-5} M ctDNA and different LMF (2×10^{-6} M, 2×10^{-5} M, 2×10^{-4} M from 1 to 3). The electrophoresis speeds become slower and the patterns are more luminous with the LMF concentration increasing. The reason could be due to much more ctDNA-LMF adduct forming so that the molecular electrophoresis speeds are slower and the dyed ctDNA are more.

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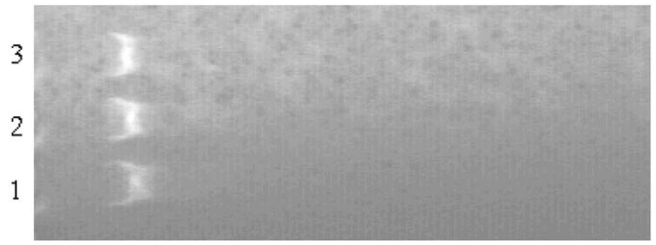

Fig. 6. The agarose gel electrophoresis patterns of 1.3×10^{-5} M ctDNA and different concentration LMF. 1) 2×10^{-6} M, 2) 2×10^{-5} M, 3) 2×10^{-4} M

4. Conclusions

In this paper, the nano-SnO_2/DHP film was cast onto the surface of GCE and the electrochemical behavior of LMF at the modified electrode was investigated. In addition to the electrochemical, spectroscopic and agarose gel electrophoresis methods were applied to investigate the interaction of LMF with ctDNA, which is an intercalation system.

5. Acknowledgement

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