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Short communication

Electrochemical investigation of interaction between mitomycin C and DNA in a novel drug-delivery system

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Abstract

A novel drug-delivery system was developed by loading the anticancer drug, mitomycin C (MC) into an oil/water system with the aim of investigation by electrochemical sensing the interaction between the drug and DNA in microemulsion phase. The physical and physicochemical properties (droplet size, pH, viscosity, conductivity and refractive index) of this microemulsion were examined. The electrochemical detection of the interaction between MC and double-stranded DNA (dsDNA) in microemulsion phase was performed by using differential pulse voltammetry (DPV) in combination with a disposable sensor, pencil graphite electrode (PGE). The magnitude of guanine oxidation signal was monitored before and after interaction between MC and dsDNA. The effect of different experimental parameters, such as MC concentration, MC interaction time with dsDNA, and dsDNA concentration were also studied to find the optimum analytical performance based on electrochemical detection of this interaction in microemulsion phase.

Keywords: Electrochemical drug–DNA interactions; Biosensor; DNA; Microemulsion; Mitomycin C; Drug-delivery system

1. Introduction

Many electrochemical approaches have been developed for analyzing nucleic acids and their interactions after the electroactivity of nucleic acids was discovered in the 1950s [1,2]. The investigation on drug–DNA interactions has a great importance in understanding the mechanism of action of antitumor, antiviral drugs and some carcinogenic compounds. The interactions of some anticancer drugs with DNA have been studied by a variety of techniques [3–6].

Electrochemical DNA biosensors play an important role in pharmaceutical, clinical, environmental and forensic applications, because they provide rapid, simple and low-cost detection of specific nucleic acid sequences. In recent years, there has been a growing interest for design of electrochemical DNA biosensors that exploit interactions between surface-confined DNA and target drugs for their rapid screening [1,2,7–15]. However, there has been no data in literature concerning evaluation by electrochemical sensing of the interaction between DNA and anticancer drugs loaded into a microemulsion drug carrier system.

Microemulsion can be defined as a drug-delivery system of water, oil and surfactants, which is a transparent, optically isotropic and thermodynamically stable liquid solution [16]. Droplet diameter in stable microemulsions is usually within the range of 5–140 nm. Microemulsions are excellent drug-delivery systems because of their improved drug solubilization, comfort intravenous injections, long shelf-life, easy preparation and improved bioavailability in comparison with solid forms and emulsions [17]. They are ideal carrier systems for peptides, steroids, hormones, diuretics, antibiotics, antineoplastic agents and other poorly soluble drugs [18]. There have been recent studies presenting the preparation of various types of microemulsions in combination with nanoparticles [19,20] or magnetic particles [21] and their application in drug-delivery systems [22,23].

Mitomycin C (MC), an anticancer antibiotic drug, which was isolated from *Streptomyces caespitosus*, is used in clinical

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anticancer chemotherapy, especially for gastrointestinal cancer [24]. In this study, a novel microemulsion-based drug-delivery system was prepared by loading MC into an oil/water system with the aim of investigation of the interaction between MC and dsDNA in the microemulsion phase using a disposable pencil graphite electrode (PGE) and differential pulse voltammetry (DPV) and monitoring the guanine oxidation signal before and after interaction between MC and dsDNA. The effect of different experimental parameters, such as MC concentration, its interaction time with dsDNA, and dsDNA concentration were also studied to find the optimum analytical performance.

2. Experimental

2.1. Apparatus

The particle size distribution and average droplet size of oil/water (o/w) microemulsion were measured by Zetasizer 3000 HS_A (Malvern, UK). Viscosity of microemulsion was measured on Brookfield DV II Viscometer with Rheocalc V2.4 software (Middleboro, USA). The pH and conductivity of the internal oil phase and o/w microemulsion were measured by Mettler Toledo instrument (Switzerland) at room temperature. Refractive indexes of internal oil phase and o/w microemulsion were measured by Apigo (Japan).

The magnitude of guanine oxidation signals was investigated by using differential pulse voltammetry (DPV) with an AUTOLAB-PGSTAT 302 electrochemical analysis system and GPES 4.8 software package (Eco Chemie, The Netherlands). The three-electrode system consisted of disposable working electrode (pencil graphite electrode, PGE, Tombo, Japan), an Ag/AgCl reference electrode (Model RE-1, BAS, W. Lafayette, USA) and a platinum wire as the auxiliary electrode.

2.2. Chemicals

Isopropanol, Tween 80, Oleic Acid (Riedel de Haen), Span 80 (Fluka) and fish-sperm double-stranded DNA (dsDNA, as lyophilized powder) were obtained from Sigma–Aldrich Company (Germany). Other chemicals were of analytical reagent grade. dsDNA stock solution (100 mg/l) was prepared with TE solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) and kept frozen. More dilute solutions of DNA were prepared with acetate buffer solution (ABS; 0.5 M, pH 4.8) containing 20 mM NaCl.

2.3. Procedure

Each measurement involved the immobilization of the nucleic acid/detection cycle at a freshly pretreated PGE surface. All experiments were performed at room temperature ($25.0 \pm 0.2\,^{\circ}C$).

2.4. The preparation of O/W type microemulsions

Oleic acid was used as the oil phase and Span 80 (S 80) and Tween 80 (T 80) were used as surfactants. The hydrophilic lipophilic balance (HLB) value of microemulsions was characteristic of o/w type. Isopropanol was used as co-surfactant. To investigate the microemulsion formation regions, phase diagrams were constructed by titration of a series of surfactant/co-surfactant (Sur/CoSur)—oil mixtures with distilled water at room temperature $25.0 \pm 0.2\,^{\circ}C$ under moderate magnetic stirring with 150 rpm. The percentages of oil, water and Sur/CoSur ratios were then calculated and the boundaries of the microemulsion domains were determined for different values of Sur/CoSur ratios. Pseudoternary phase diagrams were constructed to obtain the concentration ranges of the components resulting in large existence areas of microemulsion. The optimum microemulsion formulation was detected from the gravity center of the microemulsion formulation area.

2.5. Loading of an anticancer drug, MC into microemulsion (M-MC)

The required amounts of S 80, T 80, oleic acid and isopropanol were mixed by magnetic stirring at 150 rpm. An aliquot of MC dissolved in the required amount of water was added to the mixture to obtain a final concentration of 2.0 μg/ml.

2.6. The preparation of disposable electrodes

The renewable PGE was used in the voltammetric measurements for the electrochemical detection of DNA [25]. A Tombo pencil was used as a holder for the graphite lead. Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part. The pencil was held vertically with 14 mm of the lead extruded outside (10 mm of which was immersed in the solution).

2.7. Immobilization of dsDNA onto PGE surface

PGEs were pretreated by applying +1.40 V for 30 s in ABS without stirring. A 10 μg/ml dsDNA was immobilized onto the pretreated PGEs by applying +0.5 V for 5 min. After immobilization of DNA, each PGE was rinsed with ABS for 10 s. The oxidation signals of guanine were measured in ABS by using DPV.

2.8. Interaction of MC in the microemulsion phase with dsDNA-modified PGE

dsDNA-modified PGE was immersed into the microemulsion solution containing 2.0 μg/ml concentration level of MC by stirring for 5 min without applying any potential [15]. After the interaction, the electrode was rinsed with 0.05 M Tris buffer solution (TBS, pH 7.0) for 10 s. The oxidation signal of guanine was taken by using DPV in the blank ABS.

Repetitive measurements were carried out by renewing the surface and repeating the above assays by using the electrochemical transducer.

2.9. Voltammetric transduction

The oxidation signal of guanine was measured by using DPV in ABS by scanning from +0.20 to +1.40 V at 50 mV pulse amplitude and 30 mV/s scan rate. The raw voltammograms were treated by using the Savitzky and Golay filter (level 2) included in the General Purpose Electrochemical Software (GPES) of Eco Chemie with moving average baseline correction using a "peak width" of 0.01 V [13,14,25].

3. Results and discussion

Firstly, the physical and physicochemical properties of the novel drug-delivery system (optimum conditions for its droplet size, pH, viscosity, conductivity and refractive index) were determined.

The pH of the microemulsion and its internal phase was 7.0 and 6.1, respectively, suitable for physiological conditions.

The HLB value of the microemulsion (Span 80:Tween 80 (2:3, w/w)) was 10.72. The composition of the microemulsion with optimum values of the constituents is shown in Table 1.

After loading of MC into the microemulsion, there was no opalescence noted, indicating that this system retained its stability when the drug was added. The average particle size of microemulsion with MC (M-MC) was 107.3 ± 0.4 nm and the polydispersity index was 0.702 (shown in Fig. 1). The w/o M-MC showed a single peak in the size distribution curve shown also in Fig. 1, with no evidence of multiple peaks or large droplets. No visible oil droplets could be observed on the surface of the microemulsion samples. It is known that the particle size and size distribution are among the most important characteristics of emulsions for stability and *in vivo* fate of emulsion and their interactions in the biological cells [26]. Thus, our system was optimized for the subsequent electrochemical studies.

Fig. 2 shows the representative voltammograms of guanine oxidation signals measured by disposable PGE. A gradual decrease was observed at the oxidation signal of guanine after interaction between MC and DNA. This decrease at the guanine signal was attributed to the binding of MC to the bases in double helix of DNA; this phenomenon could be explained by the shielding of oxidizable groups of electroactive bases, such as guanine while MC interacts with DNA at electrode surface [15].

The histogram in Fig. 3 shows the changes of the guanine signal before and after the interaction of dsDNA with different concentration level of MC as 1, 2, 5 and 10 μg/ml. Gradual decrease of the guanine oxidation signals was observed after the

Table 1
Composition of microemulsion formulation

Formulation	Composition	(%)
Oil	Oleic acid	3.87
Surfactant (2:3)	Span 80	6.97
	Tween 80	10.45
Co-surfactant	Isopropanol	17.43
Water	Distilled water	61.28

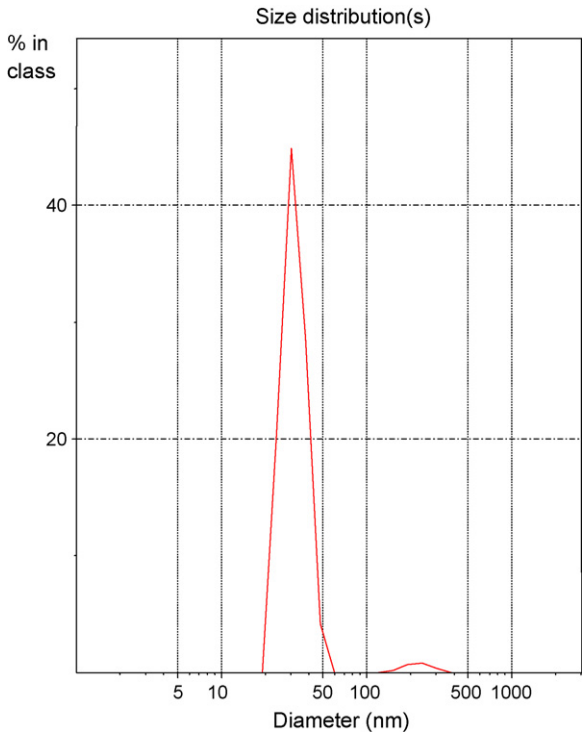

Fig. 1. The particle size distribution of o/w microemulsion.

interaction of MC with DNA. The largest decrease was observed at 2.0 μg/ml; this was found the optimum concentration for this study.

The effect of interaction time of MC with dsDNA on guanine oxidation signal was also studied and the representative histogram is shown in Fig. 4. A gradual decrease was observed till 5 min with no further change between 5 and 10 min. Accordingly, the optimum interaction time for MC loaded into the microemulsion with dsDNA was 5 min, in agreement with the results obtained in an earlier study, where surface-confined DNA with different types of carbon electrodes was used [15].

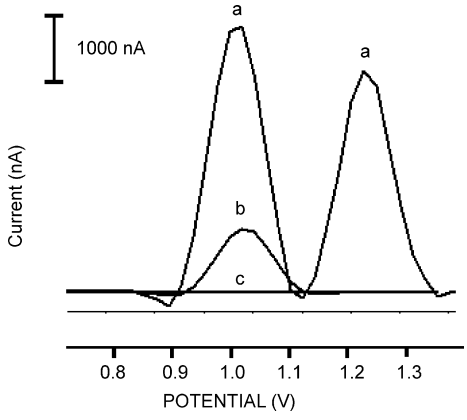

Fig. 2. Differential pulse voltammograms for the interaction of 2 μg/ml MC with 10 μg/ml dsDNA at PGE surface; guanine oxidation signal: (a) before interaction, (b) after interaction of MC with dsDNA and (c) bare electrode. PGE pretreatment +1.4 V for 30 s, dsDNA immobilization at +0.5 V for 5 min, MC accumulation by stirring for 5 min without applying any potential, measurement in ABS scanning between +0.2 and +1.4 V, 50 mV/s scan rate.

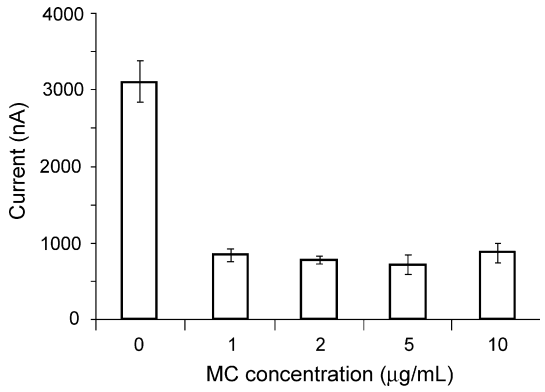

Fig. 3. Histograms of guanine oxidation signal obtained from interaction of dsDNA with MC in various concentrations changing between 0 and 10 μg/ml. The other conditions are same as in Fig. 2.

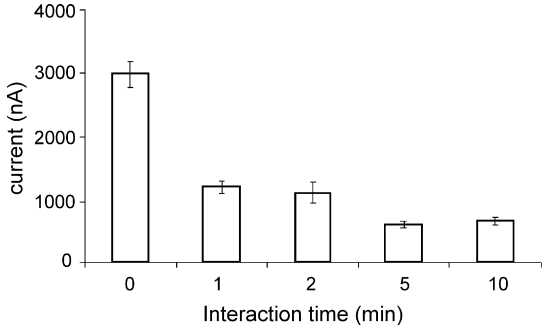

Fig. 4. The effect of MC interaction time with dsDNA-modified PGE based on the changes at guanine oxidation signal at different interaction times of MC changing between 0 and 10 min. The other conditions are same as in Fig. 2.

In Fig. 5, the changes of the magnitude of guanine oxidation signals are presented before and after the interaction of 2.0 μg/ml MC with dsDNA in various concentrations from 0.1 to 10 μg/ml. A great decrease of about 36% was obtained after interaction of MC with 2.0 μg/ml concentration level of dsDNA in comparison to the guanine signal measured in the absence of MC. When the concentration of dsDNA was increased up to 5 and 10 μg/ml, a higher decrease of about 37% and 46%, respectively, was obtained. In the previous study on interaction between MC and DNA at the surface of carbon paste electrode (CPE) [15], a 52% decrease of guanine signal in the presence

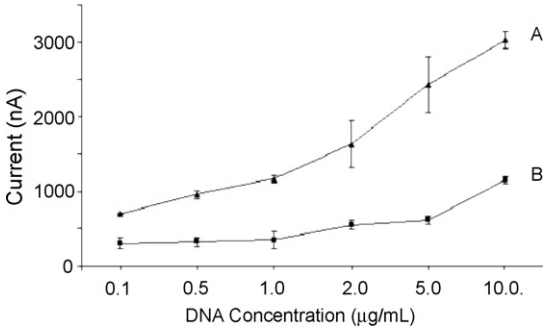

Fig. 5. Oxidation signals of guanine obtained (A) before and (B) after interaction of 2 μg/ml MC with dsDNA in different concentrations; (a) 0.1 μg/ml, (b) 0.5 μg/ml, (c) 1 μg/ml, (d) 2 μg/ml, (e) 5 μg/ml and (f) 10 μg/ml dsDNA. The other conditions are same as in Fig. 2.

of the same amount of dsDNA and 2.5 times higher concentration level of MC was found. However, the interaction process of MC–DNA was performed in this study in microemulsion phase with less amount of MC.

A series of three repetitive DPV scans of guanine for interaction between 2.0 μg/ml MC and 10 μg/ml concentration level of dsDNA at PGE surface resulted in reproducible results, such as a mean response of 928 nA, with a relative standard deviation of 5.9% ($n = 3$). The sensitivity of the method based on the interaction of DNA with MC in microemulsion phase (LOD = 0.23 μg/ml; $S/N = 3$; 5 min accumulation time) and the reproducibility are much better than in the case of using interaction at the surface of carbon paste electrode [15].

The results obtained with the new drug carrier encourage us to use this system for *in vitro* cell studies under the scope of monitoring the mechanism for drug–DNA interaction and exploring its application in chemotherapy.

4. Conclusion

Electrochemical detection of MC interaction with dsDNA by using PGEs is experimentally convenient, sensitive, very simple, easier and faster in comparison to other electrochemical detection methods reported in literature by using different transducers [10–12,15,27–29], such as carbon paste electrode, glassy carbon electrode, gold electrode and mercury drop electrode. The detection limit is lower, the method is faster and less laborious.

In addition, this is the first study in literature where electrochemical sensing of interaction between an anticancer drug in a drug carrier system (in our case in microemulsion) and DNA was investigated. The results are of potential use for developing and optimizing drug-delivery systems loaded with DNA-targeted molecules for application in chemotherapy and diagnosis.

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