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Amperometric Metronidazole Sensor Based on the Supermolecular Recognition by Metalloporphyrin Incorporated In Carbon Paste Electrode

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Abstract An amperometric metronidazole (MTZ) sensor using a glycosylated metalloporphyrin as a recognition element, which was incorporated in a carbon paste electrode, is reported. For the preparation of a MTZ-sensitive active material, 5, 10, 15, 20-tetrakis [2-(2, 3, 4, 6-tetraacetyl-β-D-glucopyranosyl)-1-O-phenyl]porphyrin (T(*o*-glu)PPH_2) and its Mn(III) complex MnT(*o*-glu)PPCl were synthesized from the reaction of pyrrole with ortho-acetylglycosylated benzaldehyde by Lindsay's method. The MnT(*o*-glu)PPCl-modified electrode showed excellent selectivity toward MTZ with respect to a number of interferents and exhibited stable response. The calibration graph obtained with the proposed sensor was linear over the range of 2.9×10^{-3}-5.8×10^{-8} M/L, with a detection limit of 5.8×10^{-8} M/L for MTZ. Cyclic voltammetric measurements indicated that MnT(*o*-glu)PPCl included in graphite-epoxy resin matrices could efficiently mediate electron transfer from the base electrode to MTZ causing a decrease of reduction potential for MTZ detection. The sensor could be regenerated by simply polishing with an alumina paper, with an excellent reproducibility (RSD=1.6%). The experimental conditions such as pH and applied working potential were optimized. The prepared sensor is applied for the determination of MTZ in pharmaceutical preparations and the results agreed with the values obtained by the pharmacopoeia method.

Key words: Metronidazole; Amperometric sensor; Metalloporphyrin

Introduction

Due to the selective toxicity to anaerobic bacteria and protozoarium, metronidazole (MTZ) has been found to be clinically useful in a variety of anaerobic and protozoarium infection, particularty,

Trichomonas Vaginalis and *Amoebic Dysentery* [1]. It can kill or inhibit the majority of anaerobic bacteria when the metronidazole concentration in serum is in the range from 2 to 8 μg/ml [2]. Therefore, the determination of trace levels of MTZ is very necessary in clinics.

Various methods, such as non-aqueous titration [3], spectrophotometry [4], high-performance liquid choromatography [5], polarography [6] and adsorptive stripping voltammetry [7], have been developed for this purpose. However, there is a drawback of insufficient selectivity in the aforementioned procedures for MTZ determination. As far as voltammetric approaches were concerned, a high applied potential for reduction of MTZ for producing electrochemical singal is usually required. Developing new, sensitive, selective and simple methods for MTZ determination is of great importance in pharmaceutics and clinics.

Recently, synthetic metalloporphyrins have attracted attention in relation to the chemical and biological recognition [8, 9]. As far as the application in electrochemical approaches is concerned, porphyrins have been used as the ionophores in potentiometric sensors. Some of them were employed as electroactive components in the membrane of ion-selective electrodes, and their potentiometric response to anions has been interpreted by a dissociation ion-exchange mechanism or metal-ligand interaction mechanism [10, 11]. Simon [12] and Meyerhoff [13] reported the methods using Co(II)TPPCl and Mn(III)TPPCl as recognition element for SCN^- and salicylate determination with a potentiometric finish. Some efforts have also been focused on the current utility of such compounds as the active material in the development of amperometric electrodes coated with porphyrin. Frey [14] and Duony [15] used TPP-coated glassy carbon electrode for the detection of heavy metalion (Cu^{++}) and electropolymerized protoporphyrin based graphite carbon electrode for dopamine determination. However, these procedures suffer from the insufficient stability of the modified porphyrin film on the electrode. To the best of our knowledge, no efforts have been made on the application of the drug electrode incorporated with metalloporphyrin in carbon paste electrode for the detection of imidazole analogous species. In a previous report, it was demonstrated that porphyrin cobalt (II) displays a selective affinity toward imidazole via a strong metal ion-nitrogen ligation reaction [16]. Inspired by the success of the above outlined molecular recognition research, the present authors tried to develop a drug sensor for MTZ assay with a voltammetric finish. In the present work, we make the first attempt to design and use a Mn(III) coordinating glycosylated porphyrin MnT(*o*-glu)PPCl -based voltammetric sensor for selective MTZ detection that does not require any specific preparation of the analyte. The approach used MnT(*o*-glu)PPCl as a MTZ-bonding site and the nitro group of MTZ as a electrochemical signal producing source. The MTZ sensor has been fabricated by including an active material MnT(*o*-glu)PPCl in epoxy resin-graphite matrices, which acts as a working electrode. The prepared MTZ sensor was characterized in terms of selectivity, sensitivity, and reproducibility. A relatively low applied potential and improved selectivity for MTZ detection has been realized.

Experimental

Materials and Reagents

Metronidazole, albendazole and E-44 epoxy resin were obtained from Shanghai Chemicals (Shanghai, China) and used as received from the supplier. Doubly distilled water was used throughout

all experiments. Before use, dichloromethane and benzaldehyde were subjected to simple distillation from K_2CO_3. Pyrrole was distilled under atmospheric pressure with CaH_2. $TPPH_2$ was synthesized by Adler's method [17].

Synthesis of T(o-glu)PPH_2

The synthesis of T(*o*-glu)PPH_2 was performed according to a known procedure[18]. A appropriate amount (9 g) of freshly synthesized ortho-(2,3,4,6-tetraacetyl-β-D-glycosylphenyl)-benzaldehyde, 1.4 g of freshly distilled pyrrole and 1 ml of $BF_3{\cdot}Et_2O$ were dissolved in 1.6 L of CH_2Cl_2 and stirred under nitrogen atmosphere at room temperature for 24 h. After addition of 3.4 g of dichlorodicyanobenzoquinone, the mixture was refluxed for a further 2 h, then 20 g of silica was added and the solvent evaporated. The residue retained on silica was purified by column chromatography (silica CH_2Cl_2/acetone 7:1). After removing of the solvent, 1.03g of T(*o*-glu) PPH_2 (yield, 20.5%) was obtained. Anal. calc. for $C_{100}H_{102}N_4O_{40}$: C, 60.1; H, 5.1; N, 2.8. Found: C, 59.8; H, 5.0; N, 3.1. UV-VIS (CH_2Cl_2): λ_{max}: 418, 516, 544.5, 587.5, 654 nm. HNMR ($CDCl_3$)(ppm): 8.84 (4H, pyrrole); 8.67 (4H, pyrrole); 7.86 (4H, benzene); 7.78 (4H, benzene); 7.70 (4H, benzene); 7.51 (4H, benzene); 4.79(4H, glucose); 4.68 (4H, glucose); 4.62 (4H, glucose); 4.14 (12H, glucose); 3.68 (4H, glucose); –1.20~-0.25, 0.5~1.3, 1.51~2.19 (48H, CH_3CO-), –2.81 (2H, NH). The structure of glycosylated porphyrin is shown in Fig.1.

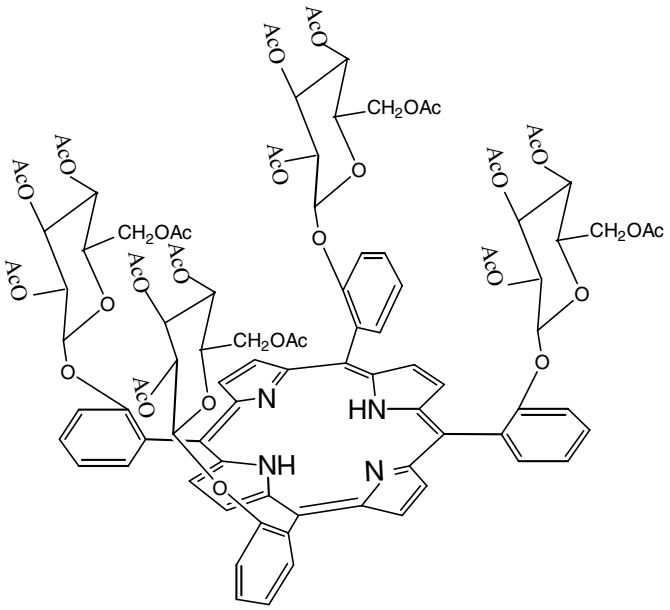

Figure 1. Structure of glycosylated porphyrin.

Synthesis of MnT(o-glu)PPCl

An appropriate amount of DMF (35 ml) solution containing 140 mg of T(o-glu)PPH_2 was stirred under refluxing with the addition of 0.7 g of $MnCl_2{\cdot}H_2O$ for 6 h. TLC (silica gel, CH_2Cl_2) showed the complete disappearance of the starting material and UV-VIS spectroscopy showed the absence of a

non-metallized porphyrin ring. After evaporation of the solvent in vacuum, column chromatography gave 170 mg of MnT(o-glu)PPCl (yield 79%). UV-VIS (CH_3OH): λ_{max}: 403, 425, 514, 575, 651 nm.

Apparatus

Electrochemical experiments were carried out using a Perkin Elmer Electrochemical Analyzer System connected with an Echem M 270 data storage system. Cyclic voltammetric (CV) and amperometric measurements were performed with a conventional three-electrode system consisting of SCE as a reference electrode, a Pt counter electrode (2.7 cm^2) and a working electrode mentioned later. All experimental solutions were thoroughly deoxygenated by bubbling nitrogen through the solution for at least 10 min. Unless otherwise indicated, voltammetry and amperometry were performed at 80 mV/s in 40 mM/L Britton-Ribinson (BR) buffer, pH 4.3 at 25^0C.

Sample Preparation

An amount of 10 pharmaceutical tablets of MTZ were ground into powder. The resulting powder was then extracted with 25 ml of BR buffer solution (pH 4.3). After being extracted, the extract filtered was into a calibrated flask and then diluted to 1000 ml for determination.

*Preparation of Mnt(*O-*Glu)TTCl Modified Electrode*

An appropriate amount of MnT(*o*-glu)TTCl (15 mg) was dissolved in 5 ml of acetone following an addition of 1 g of graphite and mixed thoroughly. The acetone in the mixture was left to evaporate. The resulting graphite powder (0.45 g) absorbing MnT(*o*-glu)TTCl and 0.55 g of epoxy resin were mixed throughout. The paste was then squeezed into a poly(vinyl chloride) (PVC) tube of 6 mm diameter to a depth of 1 cm contacting with a screwing nut at the other end of the tube. A MnT(*o*-glu)TTCl-modified electrode was obtained and could be used after aging for 6 h.

Renewal of the Sensor Surface

The sensor surface can be regenerated by turning the nut to extrude 0.1 mm-thick outer MnT(*o*-glu)TTCl-graphite-epoxy resin matrix layer and polishing with an alumina paper (0.05 μm) wetted with water to formed a smooth, shiny surface. The renewed surface was finally cleaned with doubly distilled water.

Measurement with Prepared Sensor

The determination procedure is as follows. A sensor was incubated in a sample solution containing MTZ for 30 min at room temperature. After washing with BR buffer solution (pH 7.0), the incubated electrode was used for voltammetric measurement with a three-electrode sytem in a 25 ml BR buffer (pH 4.3) and the reduction peak current was recorded. The current response derived from the reduction of the nitro group of MTZ binding to the electrode surface was taken as the measure of the concentration of MTZ.

Results and Discussion

Selection of Active Materials for Recognition Element in Electrode

The characteristic performance of the prepared electrodes incorporated with different porphyrins including MnT(*o*-glu)TTCl, T(*o*-glu)TTH_2 and FeTPPCl were investigated by amperometry. The results of these electrodes are showed in Table 1. It can be observed that the electrodes based on MnT(*o*-glu)TTCl and FeTPPCl exhibit better response characteristic than that based on T(*o*-glu)TTH_2. The current response could be originated from the reduction of the nitro group of MTZ binding to the electrode. It is thought that the interaction of MTZ with porphyrin was via the coordination with metalloporphyrin. T(*o*-glu)TTH_2 does not possess central metal capable of coordinating with MTZ. Therefore, the poor amperometric response characteristics of T(*o*-glu)TTH_2 modified electrodes are expected as compared to those of MnT(*o*-glu)TTCl and FeTPPCl. In view of the significant selectivity of the MnT(*o*-glu)TTCl modified electrodes compared with FeTTPCl-modified electrode as described in the next section, MnT(*o*-glu)TTCl was used as the MTZ-sensitive carrier in all further studies.

Table 1 Response characteristics of the modified electrodes incorporated with different active materials

Electrode*	Modifier	Working range (M/L)	Slope(μA/decade)
1#	$FeTPPH_2$	2.6×10^{-8}—1.3×10^{-3}	27.5 ± 1.8 μA
2#	T(o-glu)PPH_2	Sluggish response	------
3#	MnT(o-glu)PPCl	5.8×10^{-8}—2.9×10^{-3}	25.2 ± 1.3 μA

* The amount of the active materials in all electrodes is 2%.

Response Characteristics of the MTZ Sensor

The prepared electrodes were examined with the MTZ containing solution and BR buffer. Figure 2 shows the cyclic voltammograms obtained with a MnT(*o*-glu)TTCl-graphite-epoxy resin based electrode and a unmodified electrode incubated in the MTZ solution. One can notice that a reduction peak (-408 mV) derived from nitric group of the MTZ binding to the electrode appears and the reduction peak current values are concentration-dependent (Fig. 2, curve A, B, C). The unmodified electrode with the same incubation shows a sluggish response (Fig. 2 curve D).

Electrochemical methods have been employed to study the reduction of nitroimidazole[18]. The reduction of nitroimidazole is a complex process involving the formation of the corresponding amine accompanied by a six-electron transfer [19]. A relatively high applied working potential (> 500 mV) is required in voltammetric studies of these compounds with typical glass carbon or carbon paste electrodes [20, 21]. Brett, et. al reported a novel electrochemical method in which the metronidazole was reduced at a DNA- modified glassy carbon electrode at –472 mV. This improvement in applied potential was ascribed to the improvement of the conductivity of the electrode induced by DNA [22]. In the case of present study, a reduced reduction potential has been realized. It might be assumed that

the metalloporphyrin could mediate electron transfer. On the other hand, owing to the preconcentration of the MTZ at the modified electrode, an amplified reading of current response can also be obtained.

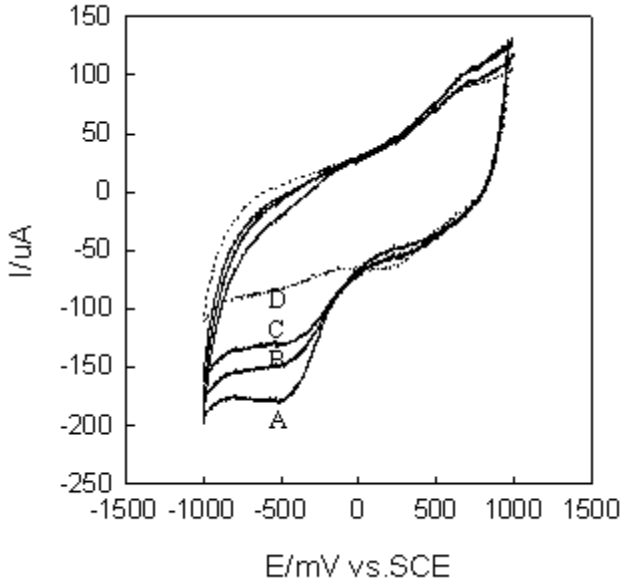

Figure 2. Cyclic voltammograms obtained with a MnT(*o*-glu)TTCl-modified electrode after incubation in MTZ solutions of concentration (A): 5×10^{-4} M/L, (B): 1×10^{-5} M/L, (C): 5×10^{-7} M/L, and a unmodified one incubated in 5×10^{-4} M/L MTZ (D).

Selectivity

Eight possible interfering substrates were used to evaluate the selectivity of the MTZ sensor. The current recorded for each interfering substrate at a concentration of 0.5 mM/L in the presence of 5×10^{-5} M MTZ was used as an indicator for the sensor selectivity in comparison with the MTZ readings alone. The results of the interference investigation are listed in Table 2. From the data in Table 2, one can observe that hexacyanoferrate(II), Fe^{3+}, Cu^{2+}, ascorbic acid, albendazole, NO_3^- and *o*-aminophenol do not cause any interference (≤ 3% change) under the experimental conditions. The most obvious interfering species is imidazole, it seems that imidazole can compete the coordinated sites, and thus, interferes in the determination of MTZ.

Table 2 Possible interferences tested with the MnT(o-glu)TTCl-electrode.

Interferent	**Current ratio** [a]
Hexacyanoferrate(II)	0.98
Ascorbic acid	1.00
Albendazole	1.00
o-aminophenol	0.99
Fe^{3+}	1.01
Cu^{2+}	1.03
imidazole	1.12
NO_3^-	1.00

[a] Ratio of currents for mixtures containing 0.5 mM interfering substrate and 5×10^{-5} M MTZ compared to that for 5×10^{-5} M MTZ.

As far as the substitution in the benzene rings is concerned, the glucopyranosyl group bound at the benzene rings of MnT(*o*-glu)TTCl formed a three-dimensional cage which might act as a molecular recognition host for MTZ (see Fig. 3). On the other hand, the glucopyranosyl group might cause a steric hindrance for the analyte to approach the metalloporphyrin. Therefore, although albendazole (an imidazole analogous species) has the potential of coordinating with MnT(*o*-glu)TTCl, it can not get into the cavity and coordinate with the central metal.

Figure 3. Skeleton of the interaction of MTZ with MnT(*o*-glu)TTCl.

The above reasoning is the basis of choosing MnT(*o*-glu)TTCl based electrode exhibiting response characteristics similar to but a selectivity better than (see Table 2) those of $FeTPPH_2$ based electrode (data not shown in Table 2).

Effect of pH

The effect of the electrolyte pH on the response performance of the MTZ sensor was investigated by recording the reduction peak potential of MTZ at a concentration of 10^{-4}M/l over a pH range of 2.0-12. Fig. 4 is a plot of E_P vs. pH for the reduction of metronidazole. It shows that the slope is 59 mV/decade which confirms that the mechanism is pH dependent in acidic and neutral media, although for pH values higher than 8 the reduction in pH independent. From the inset in Fig. 4, one can see that the reduction current decreases with the increase of pH values up to 7.9, then tend to stabilize. However, a lower pH less than 3.0 could cause a decrease of the current response.

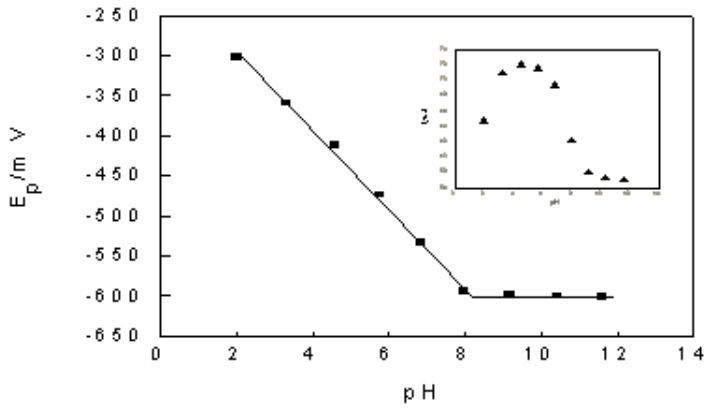

Figure 4. Effect of pH on the reduction potential and peak current of MTZ.

The reduction of nitroimidazole takes place in a similar way to that of nitrobenzene under anaerobic condition or low oxygen pressure. The formation of the nitro derivatives (R-NO) and hydroamine (R-NHOH) requires a total of four electrons and four protons. The lower the pH of the electrolyte, the more favourable for the reduction of the nitronidazole. However, the lower pH is unfavourable for the stabilization of the metal coordinated with porphyrin. This behaviour is in agreement with previous studies using a dropping mercury electrode [22].

Regeneration, Stability and Repeatability of MTZ Sensor

The prepared MTZ sensor could be regenerated by simply polishing. The calculated RSD of the response error of the renewable electrode was 2.1% (10 regenerations and measurements).

The sensors were stored in a refrigerator with a nice retention of electrochemical response for at least two months. The repeatability of the prepared MTZ sensors was evaluated with ten amperometric measurements in 5×10^{-5} M MTZ solutions. The sensor exhibited a good repeatability with a standard deviation of 1.6%.

Preliminary Analytical Application

Fig. 5 shows calibration curve obtained at pH 4.3 with the prepared MTZ sensor. It was linear over the range 2.9×10^{-3}-5.8×10^{-8} M/L, with a detection limit of 5.8×10^{-8} M/L for MTZ. On this basis, the proposed procedure was applied for the direct determination of MTZ in commercially available pharmaceutical tablets. The sample solutions prepared as described in the experimental section were ten-fold diluted with a BR buffer solution of pH 4.3 followed by cyclic voltammetric measurements using the prepared MTZ sensor. Table 3 shows the results compared with those obtained by the pharmacopoeia method (UV-visible spectroscopy). It indicates that the concentrations of MTZ determined by the sensor method are in good agreement with those by the pharmacopoeia method. An obvious advantage of the proposed procedure is the simple sample preparation with even turbid samples.

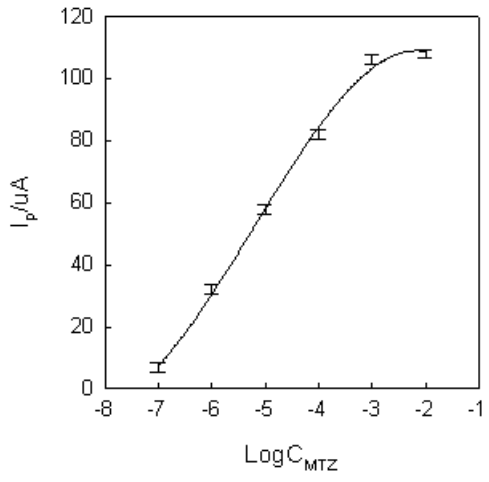

Figure 5. Calibration graph for the MTZ sensor obtained with the MTZ sensor incubated in MTZ solutions of different concentrations followed by voltammetric measurements. Operating potential: - 408 mV vs. SCE.

Table 3 MTZ determination with the MnT(*o*-glu)TTCl modified electrode in the pharmaceutical tablets.

Sample [a]	Sensor method (mg/tablet)	UV-visible spectroscopy
1#	4.96±0.07 [b]	4.93
2#	5.01±0.05	4.99
3#	4.97±0.04	4.98

[a] The sample solutions were obtained by diluting the stock solution prepared as described in the experimental section.
[b] Mean±SD of six measureme

Conclusion

The amperometric sensor detection for MTZ offers the advantages derived from the selectivity owing to incorporation of metalloporphyrin in the carbon paste electrode.

he proposed procedure can overcome the drawback of the direct amperometric approach requring relatively highly applied potential for the reduction of metronidazole. The simplicity, sensitivity and low analytical cost make it capable of replacement of the officially recommended procedure.

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Sample Availability: Available from the authors.