Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 1192–1196
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Catalytic action of copper (II) ion on electrochemical oxidation of metformine and voltammetric determination of metformine in pharmaceuticals

Xin-Juan Tian, Jun-Feng Song *

Institute of Analytical Science, Northwest University, Xi'an 710069, China

Received 3 December 2006; received in revised form 10 April 2007; accepted 15 April 2007
Available online 20 April 2007

Abstract

The catalytic effect of Cu(II) ion toward the oxidation of metformine (MET) have been observed in $NH_3{\cdot}H_2O$–NH_4Cl buffer (pH 8.9; 0.1 M). The oxidation peak current of imino-group in guanidino-group of MET at 0.95 V at carbon paste electrode (C/PE) in the presence of 2.0×10^{-4} M Cu(II) ion was increased by about 20 times and the peak potential was unchanged compared with that in the absence of Cu(II) ion. Moreover, the oxidation peak current of MET at multiwalled carbon nanotube paste electrode (MWCNT/PE) was further increased by about three times compared with that at C/PE in the same medium. Based on the catalytic oxidation peak of MET by Cu(II) ion at MWCNT/PE, a voltammetric method for the determination of MET is developed. The peak current of the catalytic oxidation peak was proportional to MET concentration in the range of 2.0×10^{-7} to 1.0×10^{-5} M. The detection limit was 6.7×10^{-8} M.

Keywords: Metformine; Cu(II) ion; Oxidation; Carbon nanotube; Voltammetry

1. Introduction

Metformin (MET) is one of most commonly prescribed medications for type II diabetes. It is the drug of choice in obese diabetic patients [1]. Multiple pharmacological mechanisms of MET have been studied. On the one hand, through activation of tyrosine kinases and AMP-activated protein kinase, MET leads into an increase in cellular glucose transport and a decrease in hepatic glucose production, cholesterol and triglyceride synthesis [2–6]. On the other hand, MET can combine with many transition metal ions and these coordination compounds can improve or change its pharmacological function and biochemical process [7–12]. For instance, the complexes of MET with endogenous metals, Zn^{2+}, Cu^{2+}, Fe^{3+} and others, can inhibit cysteine proteases, and oxovanadium (IV)-MET coordination compounds is used as insulin-enhancing agents from a synergistic action.

In this work, the catalytic action of Cu(II) ion toward the oxidation of MET at carbon paste electrode (C/PE) was studied, and a voltammetric method for the determination of MET using multiwalled carbon nanotube paste electrode (MWCNT/PE) is proposed, and is applied to MET determination in pharmaceutical formulations.

2. Experimental

2.1. Reagents and apparatus

A 1.0×10^{-2} M stock standard aqueous solution of metformin hydrochloride (purity >98.9%, Shandong Keyuan Pharmaceutical, China) was prepared and stored under refrigeration at 4 °C. Working standard solutions of MET were prepared by diluting the stock solution with water before use. Multiwalled carbon nanotubes (MWCNT, purity ≥95%, 10–20 nm diameter, 5–15 μm length, Shenzhen Nanotech Port, China) were further purified by stirring in 2 M nitric acid for 20 h in order to remove metal ions which maybe present [13]. Graphite powder was of chemical pure grade (Beijing Chemical Reagent Factory, China). Silicon oil was of chromatographic pure grade (Chengdu Chemical Reagent Factory, China). All chemicals used were of analytical reagent grade. Twice-distilled water was used throughout.

* Corresponding author. Tel.: +86 29 8830 3448; fax: +86 29 8830 3448.
E-mail address: songjunf@nwu.edu.cn (J.-F. Song).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.04.014

A CHI 660 electrochemical workstation (CH Instrument Inc., USA) and a JP-303 polarographic analyzer (Chendu Instrument Factory, China) were used for voltammetric study and measurements. A three-electrode set-up was equipped with homemade C/PE or MWCNT/PE working electrode, a saturated calomel reference electrode (SCE) and a platinum-wire counter electrode. All the potentials quoted in this work were relative to the SCE. UV-2550 UV–vis spectrophotometry (Shimadzu Corporation Assembled, China) were used for the spectrophotometric determination of MET content in the samples. All the measurements were performed at room temperature.

2.2. Preparation and activation of the working electrodes

Preparation of C/PE and MWCNT/PE: the C/PE was prepared by thorough mixing graphite powder and silicon oil in a ratio of 5:2 (w/w) in a mortar. A portion of the resulting paste was packed firmly into a plastic tube (Φ 3 mm), in which a copper wire was inserted to produce the electrical connection. The MWCNT/PE was prepared in similar manner by mixing MWCNT powder and silicon oil in a ratio of 3:2 (w/w).

Activation of the C/PE and MWCNT/PE: to obtain a more sensitive and stable voltammetric responds, the working electrodes were activated by cyclically scanning in the potential range of −0.2 to 1.2 V at 0.1 V s^{-1} for 16 times until a stable background current was obtained.

2.3. Procedure

$NH_3{\cdot}H_2O$–NH_4Cl (pH 8.9; 0.1 M; 10 mL) buffer containing 2.0×10^{-4} M Cu(II) ion and appropriate amount of MET was transferred into a voltammetric cell, and the second-order derivative linear sweep voltammogram was recorded by applying an anode-going potential scan from 0.6 to 1.2 V at 0.1 V s^{-1} and the second-order derivative peak current of the catalytic oxidation peak of MET at 0.95 V was measured. The calibration curve was obtained by plotting the second-order derivative peak current versus MET concentration. Cyclic voltammetry was conducted in the same medium.

After each measurement, the C/PE and MWCNT/PE were cleaned in $NH_3{\cdot}H_2O$–NH_4Cl (pH 8.9; 0.1 M) buffer by potential linearly scanning from 0.6 to 1.2 V until a stable background current was obtained to remove the residuary MET and Cu(II) ion, the electrodes were then used to the next measurement after washing with water. If necessary, the electrode surfaces were renewed by cutting a part of the graphite or multiwalled carbon nanotubes paste (a thickness of 2–3 mm), then polishing and washing with water.

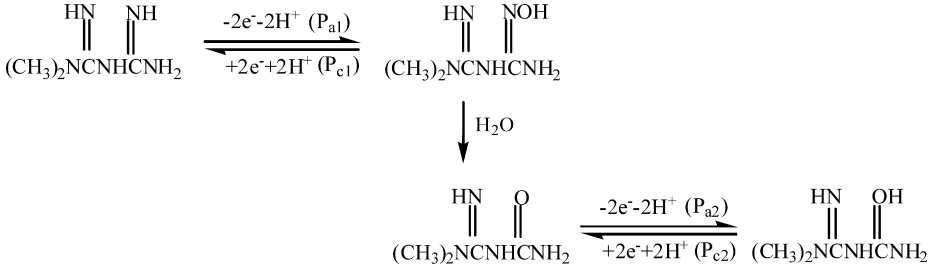
2.4. Analysis of drug samples

Ten tablets of metformin hydrochloride tablets (Shenzhen Zhonglian Pharmaceutical Co. Ltd., China) were weighed and powdered in a mortar. A portion of the powder was accurately weighed and dissolved into 100 mL volumetric flask using water. The sample solution was stored under refrigerator at 4 °C until it became clear. The supernatant liquid of the sample solution was properly diluted with water to obtain suitable final concentrations of MET. The determination of MET in the obtained samples were performed according to the experimental procedure described above. The MET content of the samples was calculated according to the calibration curve.

3. Results and discussion

3.1. Voltammetric behavior of MET in the presence of Cu(II) ion at C/PE

Cyclic voltammograms of MET in $NH_3{\cdot}H_2O$–NH_4Cl buffer (pH 8.9; 0.1 M) at C/PE was shown in Fig. 1 by curve a. Over the potential range of −0.2–1.2 V, MET exhibited two pairs of redox peaks P_1 and P_2, respectively. The oxidation peak P_{a1} appeared at 0.95 V, the corresponding reduction peak P_{c1} did at 0.81 V. And the oxidation peak P_{a2} appeared at 0.18 V, the corresponding reduction peak P_{c2} did at 0.17 V. From this, the voltammetric behavior of MET at C/PE in this work was similar to that at the paste electrode prepared from molecular wires containing copper(II) and multiwalled carbon nanotubes in pH 7.2 Britton–Robinson buffer reported in our previous work [14]. The electrode process for MET can be described as:



The electrochemical oxidation of an imino-group in guanidino-group to a *N*-hydroxy imino-group produced the oxidation peak P_{a1}. Then most of the *N*-hydroxy imino-group fast hydrolyzed to a carbonyl imino-group, the reduction of the residuary *N*-hydroxy imino-group after hydrolyzing produced the reduction peak P_{c1}. Reversible redox reaction of the carbonyl imino-group produced the pair of the peak P_2.

The experiments showed that the peak current of the oxidation peak P_{a1} at 0.95 V was obviously increased and the peak potential was unchanged when Cu(II) ion was present. Cyclic voltammograms of MET in $NH_3{\cdot}H_2O$–NH_4Cl buffer (pH 8.9; 0.1 M) containing 2.0×10^{-4} M Cu(II) ion at C/PE was shown in Fig. 1 by curve b. A pair of reversible redox peaks of Cu(II)/Cu(0) couple appeared at about 0 V and overlaped the peaks P_{a2} and

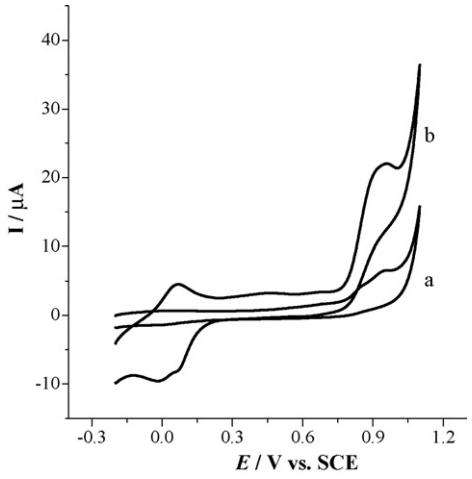


Fig. 1. Cyclic voltammograms of 9.0×10^{-4} M MET at C/PE in NH_3–NH_4Cl buffer (pH 8.9; 0.1 M) in the absence (curve a) and the presence (curve b) of 2.0×10^{-4} M Cu (II). Scan rate v, 0.1 V s^{-1}.

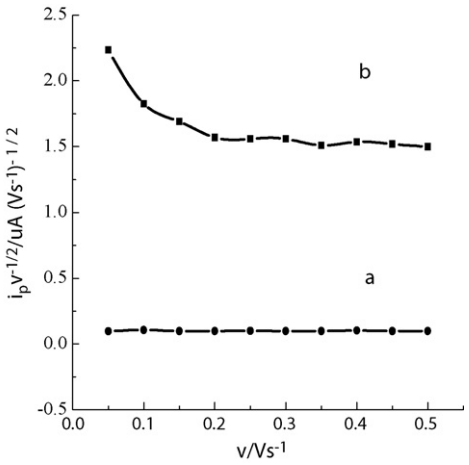


Fig. 2. Current function $i_P v^{-1/2}$ of 1.0×10^{-4} M MET in NH_3–NH_4Cl buffer (pH 8.9; 0.1 M) in the absence (curve a) and the presence (curve b) of 2.0×10^{-4} M Cu (II).

P_{c2} of MET. On anodic scan, the peak potential of the oxidation peak P_{a1} was unchanged at 0.95 V, but the peak current of the oxidation peak P_{a1} at 0.95 V was about 20 times higher than that in the absence of Cu(II) ion. Moreover, the peak current increased gradually with Cu(II) ion concentration increasing from 6.0×10^{-6} to 4.0×10^{-4} M. The ratio $i_{P_{a1,1}}/i_{P_{a1}}$ of the peak current $i_{P_{a1,1}}$ of the enhanced oxidation peak $P_{a1,1}$ to the peak current $i_{P_{a1}}$ of the corresponding oxidation peak P_{a1} had a linear relationship with the square root of Cu(II) ion concentration in the range mentioned above, $i_{P_{a1,1}}/i_{P_{a1}} = 0.4473 + 0.9012(C_{Cu(II)}/\mu M)^{1/2}$ ($r = 0.9901$, $n = 6$). The peak current of the enhanced oxidation peak $P_{a1,1}$ increased with potential scan rate v increasing. According to Randles–Sevcik equation, $i_{P_a} = 2.69 \times 10^5 n^{3/2} A C_R D_o^{1/2} v^{1/2}$, the current function $i_P v^{-1/2}$ was defined [15]. As shown in Fig. 3, the current function $i_{P_{a1,1}} v^{-1/2}$ of the enhanced oxidation peak $P_{a1,1}$ decreased sharply with the potential scan rate v increasing from 0.05 to 0.20 V s^{-1}, then leveled off in the range of 0.20–0.50 V s^{-1} (curve b). Differing from the case in the presence of Cu(II) ion, the current function $i_P v^{-1/2}$ of the corresponding oxidation peak remained nearly unchanged in the range of 0.05–0.50 V s^{-1} (curve a). The features indicated that the enhanced oxidation peak was a parallel catalytic one (Fig. 2).

According to catalytic current theory in electrochemistry, the increase of the oxidation peak current of MET arouse from the reduction of the oxidation product *N*-hydroxyl imino-group of an imino-group in guanidino-group to reproduce the original imino-group by some substance as reducing agent. Evidently, Cu(II) ion cannot act as reducing agent because the low redox reaction potential of Cu (II)/Cu(0) couple. When ascorbic acid and H_2O_2 were added, respectively, the peak current of the catalytic oxidation peak $P_{a1,1}$ was nearly unchanged. When the chelating agent EDTA was added, the peak current obviously decreased with the concentration of EDTA increasing. Additionally, when organic solvent DMF was added to decrease H_2O content, the peak current also decreased with increasing the content of DMF. From these results, it was deduced that the catalytic oxidation current of MET in MET–Cu(II) system was caused by an unknown Cu(II)-containing species that may be a Cu(II)-oxo coordination compound of Cu(II) ion with active oxygen species such as superoxide anion and hydroxyl radical from H_2O oxidation [16–21]. Although the unknown Cu(II)-containing species could not be described clearly, it acted as reducing agent to reduce the oxidization product *N*-hydroxyl imino-group to the original imino-group, forming the catalyzed cycle, which led to a significant increase of the oxidation peak current of MET.

3.2. Voltammetric determination of MET

Carbon nanotubes (CNT) had an advantageous over graphite or carbon power in analytical sensitivity and selectivity when CNT used as an electrode material for a wide range of biologically significant species. Voltammetric behaviors of MET in $NH_3 \cdot H_2O$–NH_4Cl buffer (pH 8.9; 0.1 M) containing 2.0×10^{-4} M Cu(II) ion at C/PE and MWCNT/PE with the same surface area were the same, but the peak current of the catalytic oxidation peak $P_{a1,1}$ at MWCNT/PE was about three times higher than that at C/PE (Fig. 3), which was due to the large efficient surface area of MWCNT/PE from the surface effect of the used MWCNTs. With the more sensitive catalytic oxidation peak $P_{a1,1}$ at MWCNT/PE, a voltammetric method for the determination of MET is proposed.

Linear sweep voltammetry allowed more quickly analytical speed than different pulse and square-wave one. And because the catalytic oxidation peak $P_{a1,1}$ was very close to the background current from the oxidation of H_2O, second-order derivative linear sweep voltammogramm was recorded to improve resolving power. The medium conditions were optimized. The result showed that the peak current of the catalytic oxidation peak $P_{a1,1}$ in $NH_3 \cdot H_2O$–NH_4Cl buffer was more sensitive than that in HAc–NaAc, Britton–Robinson, phosphate, buffers. When pH was between 8.6 and 9.1, the peak current was maximum and almost constant. The peak potential $E_{P_{a1,1}}$ moved to negative direction with pH increasing from 8.0 to 10.1, and the regression equation obtained was $E_{P_{a1,1}}(V) =$

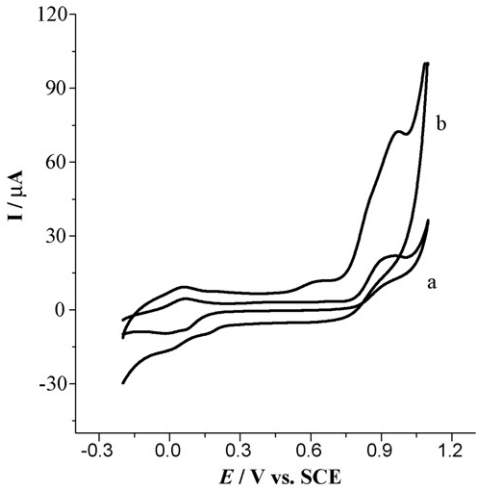


Fig. 3. Cyclic voltammograms of 9.0×10^{-4} M MET at C/PE (curve a) and MWCNT/PE (curve b) in NH_3–NH_4Cl buffer (pH 8.9; 0.1 M) containing 2.0×10^{-4} M Cu (II). Scan rate v, 0.1 V s^{-1}.

$1.50 - 0.068\,\mathrm{pH}(r = 0.994)$. As mentioned above, the peak current increased with Cu(II) ion concentration increasing from 6.0×10^{-6} to 4.0×10^{-4} M. But the excessive Cu(II) ion concentration made it difficult to renewal the electrode surface. Therefore, $NH_3{\cdot}H_2O$–NH_4Cl (pH 8.9; 0.1 M) buffer containing 2.0×10^{-4} M Cu(II) ion was used as supporting electrolyte in this work.

4. Evaluation of the proposed method

4.1. Calibration plot for MET

Under the optimum conditions mentioned above, second-order derivative linear sweep voltammogramm was recorded by use of linear sweep voltammetry. The second-order derivative peak current $i''_{P_{a1,1}}$ of the catalytic oxidation peak $P_{a1,1}$ was measured. The peak current increased linearly with MET concentrations in the range of 2.0×10^{-7} to 1.0×10^{-5} M. Four calibration curves were obtained independently, the linear regression equation was $i''_{P_{a1,1}}(\mu A\,s^{-2}) = 0.01 \pm 0.036 + 0.016 \pm 0.032C(\mu M)(r = 0.998)$. The detection limit was 6.7×10^{-8} M and the quantitation limit was 2.1×10^{-7} M, which was calculated from the calibration curves as $3s_1/m$ and $10s_1/m$, respectively, where s_1 was the standard deviation of the intercept and m was the slope. The detection limit was improved one order in magnitude in comparison with that reported in our previous work [14].

4.2. Precision, accuracy and repeatability

In order to evaluate the precision, accuracy and repeatability of the proposed method, three concentration levels of MET were measured by five independent measurements over 1 day (Intra-day assay) and for 3 days over a period of 1 week (Inter-day assay), respectively. The results and statistical parameters were summarized in Table 1. At the same time, 5.0×10^{-6} M MET was determined eight times in succession by use of the same MWCNT/PE and the relative standard deviation was 4.1%. When the same determination was performed by using six MWCNT/PEs with the same surface area, the relative standard deviation was 3.7%. These results revealed that the precision, accuracy and repeatability of the proposed method were good.

Table 1
Mean recovery (R%), precision (R.S.D.%) and accuracy (R.E.%) for assaying three concentration levels of MET by the proposed method, $n = 5$

$C_{MET}/10^{-6}$ mol L^{-1}	0.8	2.0	7.0
Intra-day			
R%	98.8	103	103.1
R.E.%	−1.3	2.8	3.1
R.S.D.%	4.1	4.2	3.9
Inter-day			
R%	102.5	96	97.9
R.E.%	2.5	−3.8	−2.1
R.S.D.%	3.4	4.5	4.3

4.3. Robustness

The experimental conditions of the proposed method were loose, and allowed small variations. When the pH was in the range of 8.6–9.1 and the concentration of Cu(II) ion was in the range of 1.8×10^{-6} to 2.2×10^{-6} M, the obtained mean recoveries by assaying 2.0×10^{-6} M MET were not significantly affected. Thus, the proposed method was considered robust.

4.4. Interference study

For possible analytical application of the method, the effects of some common interferences on the determination of 8.0×10^{-6} M MET was examined. The tolerable limit was defined as the interferences concentration, and the peak current gave an error less than ±5.0%. The result showed that when a 300-fold sucrose and glucose, a 100-fold L-serine, ascorbic acid, dopamine and a 80-fold urea were present in the synthetic mixture, the average recovery in the determination of MET was 101.3%. From the results, the selectivity of the method was acceptable.

4.5. Analytical application

The proposed method was applied to the determination of MET in metformin hydrochloride tablets. The obtained result by the proposed method and official UV spectrophotometric method (233 nm) [22] was 98.8 ± 3.5 and 99.3 ± 2.9 ($R\% \pm$ R.S.D.), respectively. At the same time, F-test and t-test were conducted and the calculated values were 3.78 and 1.71, which were less than the theoretical ones (obtained at 95% confidence limit, $n_1 = 5, n_2 = 5$) of 5.05 and 2.07, respectively. These indicated that there were no significant differences between the proposed and UV spectrophotometric methods. Recovery experiments were carried out by use of standard addition method for metformin hydrochloride tablets, the average recovery was 101.8%. These results indicated that the proposed method were available.

5. Conclusion

The catalytic oxidation peak of MET in the presence of Cu(II) ion was studied, which was attributed to that an unknown Cu(II)-containing species as reducing agent reduced the oxidization product *N*-hydroxyl imino-group of MET to the original imino-group, forming the catalyzed cycle. In addition, a more sensitive voltammetric method for the determination of MET using MWCNT/PE was proposed and could be applied to analysis MET in pharmaceutical formulations.

Acknowledgements

The authors gratefully acknowledge the National Natural Science Foundation of China (Grant No. 20475043) for financial support, and Shandong Keyuan Pharmaceutical Co. Ltd., China for supplying raw material of metformin hydrochloride.

References

[1] A. Scheen, P. Lefebvre, Drugs 55 (1998) 225–236.
[2] L.J. Dominguez, A.J. Davidoff, P. Srinivas, P. Standley, M.F. Walsh, J.R. Sowers, Endocrinology 137 (1996) 113–121.
[3] N. Musi, M.F. Hirshman, J. Nygren, M. Svanfeldt, P. Bavenholm, O. Rooyackers, G. Zhou, J.M. Williamson, O. Ljunqvist, S. Efendfic, D.E. Moller, A. Thorell, L.J. Goodyear, Diabetes 51 (2002) 2074–2081.
[4] G. Zhou, R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fujii, N. Musi, M.F. Hirshman, L.J. Goodyear, D.E. Moller, J. Clin. Invest. 108 (2001) 1167–1174.
[5] M. Zou, S.S. Kirkpatrick, B.J. Davis, J.S. Nelson, W.G. Wiles, U. Schlattner, D. Neumann, M. Brownlee, M. Freeman, M.H. Goldman, J. Biol. Chem. 279 (2004) 43940–43951.
[6] L. Ratnakumari, I.A. Qureshi, R.F. Butterwoth, B. Marescau, P.P. De Deyn, Neurosci. Lett. 215 (1996) 153–156.
[7] B. Viossat, A. Tomas, D. Nguyen-Huy, Acta Cryst. C 51 (1995) 213–215.
[8] P. Lemoine, M. Chiadmi, V. Bissery, A. Tomas, B. Viossat, Acta Cryst. C52 (1996) 1430–1436.
[9] F. Bentefrit, G. Morgant, B. Viossat, S. Leonce, N. Guilband, A. Pierre, G. Atassi, D. Nguyen-Huy, J. Inorg. Biochem. 68 (1997) 53–59.
[10] D. Sweeneya, M.L. Raymera, T.D. Lockwood, Biochem. Pharm. 66 (2003) 663–677.
[11] L.C.Y. Woo, V.G. Yuen, K.H. Thompson, J.H. McNeill, C. Orvig, J. Inorg. Biochem. 76 (1999) 251–257.
[12] K.H. Thompson, C. Orvig, J. Inorg. Biochem. 100 (2006) 1925–1935.
[13] J.J. Davis, J.R. Coles, H. Allen, O. Hill, J. Electroanal. Chem. 440 (1997) 279–282.
[14] X.J. Tian, J.F. Song, Anal. Bioanal. Chem. 386 (2006) 2081–2086.
[15] R.S. Nicholson, I. Shain, Anal. Chem. 36 (1964) 706–723.
[16] I. Shinobu, Curr. Opin. Chem. Biol. 10 (2006) 115–122.
[17] L.M. Mirica, X. Ottenwaelder, T.D.P. Stack, Chem. Rev. 104 (2004) 1013–1045.
[18] K.D. Karlin, N. Wei, B. Jug, S. Kaderli, P. Niklaus, A.D. Zuberbühler, J. Am. Chem. Soc. 115 (1993) 9506–9514.
[19] A. Wada, M. Harata, K. Hasegawa, K. Jitsukawa, H. Masuda, M. Mukai, T. Kitagawa, H. Einaga, Angew. Chem. Int. Ed. Engl. 37 (1998) 798–799.
[20] S. Yamaguchi, A. Kumagai, S. Nagatomo, K.T. Teizo, Y. Funahashi, T. Ozawa, K. Jitsukawa, H. Masuda, Bull. Chem. Soc. Jpn. 78 (2005) 116–124.
[21] T. Fujii, A. Naito, S. Yamaguchi, A. Wada, Y. Funahashi, K. Jitsukawa, S. Nagatomo, T. Kitagawa, H. Masuda, Chem. Commun. (2003) 2700–2701.
[22] China Pharmacopoeia Committee, Chinese Pharmacopoeia, Chemical Industry Press, Beijing, 2005, part 2, p. 458.