Acoustic effects in chromatography

Slawomir Oszwaldowski[1], Tetsuo Okada*

Department of Chemistry, Tokyo Institute of Technology, Meguro-ku, Tokyo 152-8551, Japan

Abstract

An acoustic field effectively affects chromatographic retention in some separation modes and, thus, can be a novel factor controlling retention. After being transmitted into the column, ultrasound energy is mostly converted into heat as a result of absorption by stationary and mobile phases. Thus, ultrasound brings about temperature increases. However, actual temperature increases measured by thermosensors are smaller than those calculated from chromatographic retention changes. In addition, larger ultrasound effects are observed in chromatographic modes involving ionic interactions. These results possibly imply that ultrasound directly influences ionic interactions involved in retention mechanisms. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Acoustic effects; Ultrasound; Temperature effects; Polyethers

1. Introduction

Chromatographic retention is usually determined by the primary interaction of solutes with stationary phases under given conditions. In some instances, a secondary interaction taking place in the stationary and/or mobile phases modifies and sometimes drastically changes the retention of the solute. The chromatographic separation is thus usually optimized by adjusting the balance between these two interactions. Since both the primary and secondary interactions are basically chemical equilibria, such as partition, adsorption, ion-exchange, acid–base dissociation, complex formation, etc., chromatographic retention is in general controlled by changing factors, such as temperature, organic solvent concentration, pH, salt concentration, etc., which directly affect the thermo-

dynamics and kinetics of the retention equilibria. Effective involvement of other factors influencing chromatographic retention will allow further enhancements of chromatographic methodology and applicability.

External fields have recently received increasing attention because they are possible additional factors that are capable of controlling chromatographic equilibria without changing either the stationary or mobile phase compositions. The utilization of external fields in chromatography can be classified into two major categories: (1) external fields are indispensable for the separation (no separation occurs without external fields) and (2) solutes can be separated even without external fields, but their retention is effectively modified. Field flow fractionation (FFF) is a typical example of the former [1]. Some modes of FFF-like separation, namely magnetic chromatography [2] and optical chromatography [3], which have recently been proposed, should belong to this category. Other novel approaches have also been reported; for example, electrical [4–6] and thermal fields [7,8] have been

---

*Corresponding author. Tel.: +81-3-5734-2612; fax: +81-3-5734-2612.

E-mail address: tokada@chem.titech.ac.jp (T. Okada)

[1]Present address: Department of Analytical Chemistry, Warsaw University of Technology, ul.Noakowskiego 3, Warsaw, Poland.

successfully used to modify and enhance chromatographic separation and selectivity (the above category 2). Nagaoka et al. [4,5] and Deinhammer et al. [6] developed an electrically controlled porous carbon stationary phase, where the retention of solutes can be controlled by applied potential. It appears that electrical fields are very useful in the development of novel separation techniques because instrumentation is rather simple and electrochemical expertise is effectively applicable to the development of separation techniques as well as to the understanding of the phenomena involved. Temperature programming is another effective choice, especially when the retention interactions are sensitive to temperature changes [7,8].

In the present paper, we highlight ultrasound as a possible external field in chromatography. Ultrasound can bring about some effects, e.g. cavitation, ultrasonic vibration potential (Debye potential), volume relaxation, etc. [9]. The cavitation causes local increases in the pressure and temperature. The Debye potential possibly affects the electrochemical potential and the behaviors of ionic compounds. Enhanced mixing by ultrasound radiation was studied in ion-exchange experiments, and proved to be an alternative to mechanical stirring. Enhanced selectivity for Cl^- over NO_3^- was found in these studies [10]. In a preliminary paper [11], we reported ion-exchange selectivity enhancements, that is, the ion-exchange affinity of smaller ions is enhanced by ultrasound radiation. It was inferred that this effect is closely related to local temperature increases induced by ultrasound radiation. In the present paper, the relationship between chromatographic retention changes and ultrasound energy is verified through some chromatographic modes, and ultrasound is characterized as an external parameter in chromatography.

2. Experimental

The chromatographic system was composed of a Shimadzu HPLC pump, model LC-10AT, a Tosoh UV–visible detector, model UV-8010, a Rheodyne injection valve equipped with a 100-μl sample loop, and a chart recorder. Ultrasound was generated in an ultrasonic bath [Sharp model UT-205, output power

200 W (max) and vibration frequency 35 kHz]. The flow-rate was 1.0 ml min^{-1}. The separation columns used were Wakosil 5C8 (particle size 5 μm, packed in a 50×4.6 mm I.D. PTFE column) and TSKgel IC-Cation-SW (particle size 5 μm, packed in a 50× 4.6 mm I.D. PTFE column). The separation columns were immersed in water thermostated at 25°C. Reagents used were of analytical grade.

The temperature inside the column was measured with a thermister or a thermocouple temperature sensor set at the column outlet. The acoustic pressure was measured with an ultrasound sensor (Nihon Tokyshu Togyou, model UTSP-60, sensitivity=− 202.3 dB).

3. Results and discussion

3.1. Acoustic pressure in ultrasonic bath

Acoustic pressures were measured to verify the relationship between the ultrasonic power and chromatographic retention. Acoustic pressure (p) is directly related to the intensity (I) of sound (energy per unit area) for plane waves by

$$I = \frac{p^2}{\rho c} \tag{1}$$

where ρ and c are the density and acoustic velocity in a given medium. Thus, sound energy is proportional to the square of the acoustic pressure.

The acoustic pressure in the ultrasonic bath used in the present experiments is basically independent of the position at a given horizontal cross-section, whereas it is appreciably changed at the vertical cross-sections. Fig. 1 shows the dependence of the acoustic pressure on the depth of water in the ultrasonic bath. The acoustic pressure is strongest at the bottom, and gradually decreases towards the surface ($p=0$). This is due to two effects: one is a decrease in acoustic energy caused the absorption by water, and the other is the reflection at the water/air interface. The latter is important in terms of the column material as well.

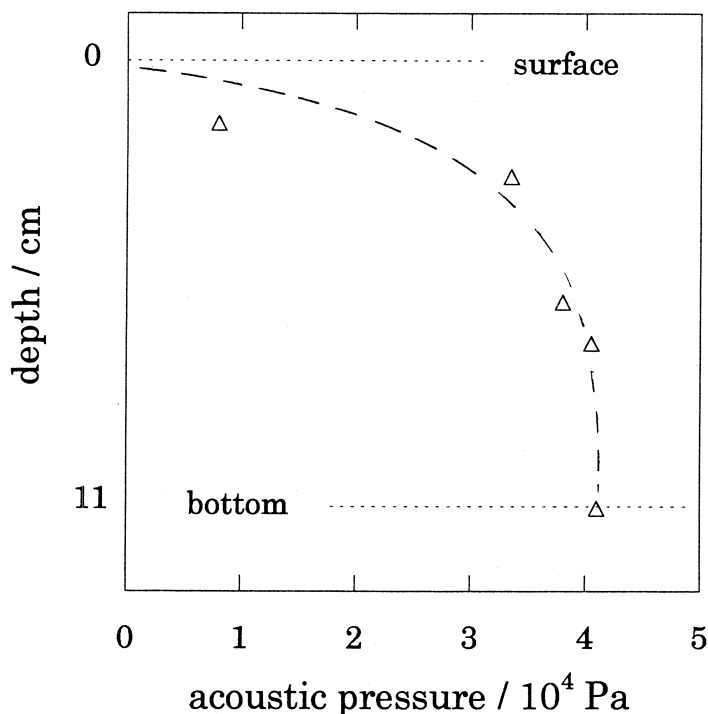When ultrasound is transmitted from medium 1 to medium 2, which differ in specific acoustic resist-

Fig. 1. Acoustic pressure in an ultrasonic bath filled with water thermostated at 25°C.

ance, molecular velocities in the transmitted (u_t) and the reflected (u_r) ultrasound field are given by [12]

$$u_t = \frac{2\rho_1 c_1 \cos\theta_2}{\rho_1 c_1 \cos\theta_2 + \rho_2 c_2 \cos\theta_1} u \tag{2}$$

$$u_r = \frac{\rho_1 c_1 \cos\theta_2 - \rho_2 c_2 \cos\theta_1}{\rho_1 c_1 \cos\theta_2 + \rho_2 c_2 \cos\theta_1} u \tag{3}$$

where u is the molecular velocity in an input ultrasound field, and θ_1 and θ_2 are angles of incidence and transmission, respectively. If $\theta_1 = \theta_2 = 0$, the above equations can be simplified as

$$u_t = \frac{2\rho_1 c_1}{\rho_1 c_1 + \rho_2 c_2} u \tag{4}$$

$$u_r = \frac{\rho_1 c_1 - \rho_2 c_2}{\rho_1 c_1 + \rho_2 c_2} u \tag{5}$$

Thus, ratios of the energy transmitted (τ) and reflected (R) to radiated ultrasound energy are given by

$$\tau = \frac{4\rho_1 c_1 \rho_2 c_2}{(\rho_1 c_1 + \rho_2 c_2)^2} \tag{6}$$

$$R = \left(\frac{\rho_1 c_1 - \rho_2 c_2}{\rho_1 c_1 + \rho_2 c_2}\right)^2 \tag{7}$$

Eqs. (6) and (7) clearly indicate that acoustic energy is better transmitted when the specific acoustic resistance ($= \rho c$) of medium 1 is similar to that of medium 2. The specific acoustic resistance of water at 20°C is $1.48 \cdot 10^6$ Pa s m^{-1}. In contrast, usual solid materials generally have larger specific acoustic resistances, e.g., $4.54 \cdot 10^7$ Pa s m^{-1} for steel, ca. 1.5×10^6 Pa s m^{-1} for glass and $2-3 \cdot 10^6$ Pa s m^{-1} for most plastics. Thus, the specific acoustic resistance of the column material should be similar to that of water to keep the ultrasound power as high as possible in the separation column. From the above values, plastic columns must be more suitable for the present purpose than steel columns. Although the ultrasound sensor used in this study is too large to directly measure acoustic pressure in the separation columns, the measurements in tubes with larger diameters indicated that acoustic pressure was lower in a steel tube ($1.2 \cdot 10^4$ Pa) than in a plastic tube ($1.9 \cdot 10^4$ Pa) by ca. 60% when the insides were filled

with water. From these considerations, PTFE columns were selected to verify ultrasound effects on chromatographic retention.

3.2. Ultrasound effects on chromatographic retention

In the previous preliminary paper [11], we reported that ultrasound affects ion-exchange chromatographic retention, and that the affinity of smaller ions towards an anion-exchange resin is enhanced whereas that of larger ions is lowered by ultrasonic radiation. We considered that this effect is basically due to the local temperature increases induced by the ultrasound radiation, because temperature increases and ultrasound radiation similarly affect solute retention. However, ultrasound effects were obviously larger than expected, based on the temperature increases measured inside the separation column, suggesting that ultrasound and temperature affect the retention in different ways. Since ion-exchange chromatographic retention is not very sensitive to temperature changes [13], this separation system is

not very suitable for studies on the effects of temperature. Thus, other chromatographic modes that are more sensitive to temperature changes should be better choices to quantitatively evaluate the effects of ultrasound on chromatographic retention. We selected the retention of polyoxyethylene (POE) on a cation-exchange resin for the present purpose for the following reasons: (1) Since POE of various chain lengths is available, systematic study is possible. (2) POE forms complexes with countercations in a cation-exchange resin, and these are retained according to their complexation ability [14,15], indicating that ionic interactions similar to ion-exchange are involved in the separation mechanisms. Thus, comparison with the effects of ultrasound on ion-exchange chromatographic retention is possible. (3) The retention of POE on cation-exchange resins in methanol has been well characterized, and has been proved to be an exothermic reaction with rather large apparent $-\Delta H^\circ$ values (see below) [16]. Thus, this separation mode is expected to be better for seeing the effects of ultrasound on solute retention.
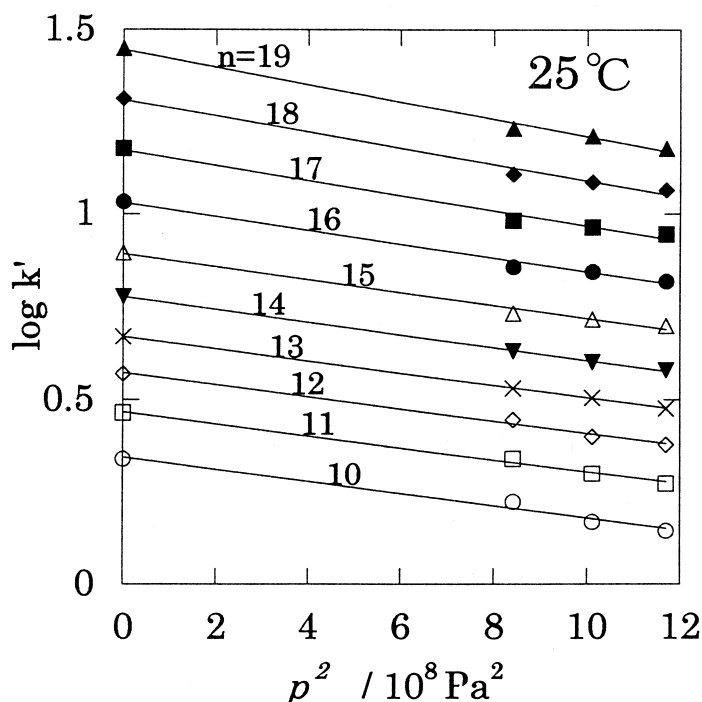
Fig. 2 shows the relation between log k' of POE

Fig. 2. Relations between log k' of POE and the square of acoustic pressure. n denotes the number of repeating oxyethylene units.

and p^2, which is proportional to the ultrasonic energy [see Eq. (1)]). The log k' values of POE linearly decrease with increasing ultrasonic energy, regardless of the chain length of POE, suggesting that ultrasound affects the retention mechanism of POE and reduces the retention. The temperature of the effluent was measured at the column outlet to determine whether this effect on POE retention was caused only by temperature increases or not. When the temperature outside the column was kept at 25°C, by vigorously circulating thermostated water, the temperature of aqueous effluents was 28.0°C, while that of methanolic effluents was 28.7°C. Temperature increases were estimated from measurements of the pressure drop along the column as well. With aqueous mobile phases, the pressure drop was decreased by 7% using ultrasonic radiation at 25.0°C. Since the viscosity of water at 25.0°C is $8.90 \cdot 10^{-4}$ N s m^{-2}

and the inlet pressure should be proportional to the viscosity, according to the Hagen–Poiseuille law, the viscosity of water inside the column is estimated at $8.30 \cdot 10^{-4}$ N s m^{-2}, which corresponds to the water viscosity at ca. 27°C [17]. Thus, an increase in the temperature induced by ultrasound radiation is ca. 2–3°C.

As previously reported [16], log k'–(1/T) plots are linear for all POE oligomers and, thus, apparent standard enthalpic changes of retention equilibria can be calculated from the slopes of the Van 't Hoff plots. The ΔH^0 values for the retention are plotted against POE chain lengths in Fig. 3, and range from 20 to 60 kJ mol^{-1} and increase with increasing chain length. Ultrasound effects on the retention of POE can be replaced by the temperature increase (ΔT), which can bring about the same effects as ultrasound radiation. ΔT can be an index to numerically express
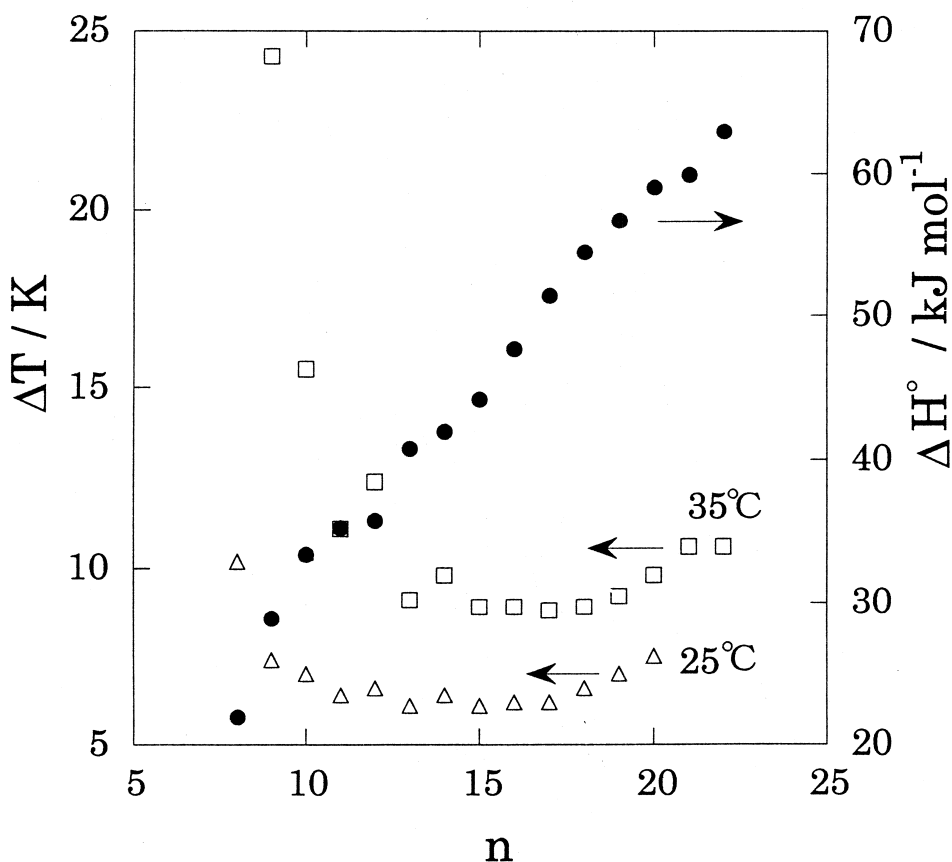

Fig. 3. Relationship between ΔT and n (open symbols) and between $-\Delta H^\circ$ and n (solid symbols).

the ultrasound effects on the retention of POE, and facilitates a comparison of them with simple temperature effects, as shown in Fig. 3. If the ultrasound effects were explained only by temperature increases, ΔT should be equal to the temperature increments stated above (i.e., 2–3°C). The ΔT values calculated from POE retention data are typically 7°C at 25.0°C, and 9°C at 35.0°C; these are obviously larger than the temperature increases measured by temperature sensors. If the temperature increase is totally responsible for the reduction in retention, ΔT values should correlate with $\Delta H°$ values. However, ultrasound-induced reductions in retention exhibit a minimum at $n = 15$, whereas the $-\Delta H°$ increases with increasing number of oxyethylene units (n).

Temperature effects were tested for reversed-phase chromatography as well. The ΔT values were 4.2°C at 25.0°C and 3.8°C at 35.0°C for phenol, and 4.9°C at 25.0°C and 6.8°C at 35.0°C for toluene. These values are still slightly larger than bulk temperature increases, but are obviously smaller than for POE retention, as stated above. Although a detailed mechanism of ultrasound interaction with chromatographic retention has not been elucidated, these experiments suggest that the effects of ultrasound are closely related to the chromatographic retention mechanisms involved. In reversed-phase chromatography, ultrasound effects can almost be explained by the temperature increases. In contrast, when ionic interactions are involved in the separation mechanisms, the effect is amplified considerably; even in the retention of POE on cation-exchange resins, POE forms complexes with countercations, and the resulting complex interacts with cation-exchange sites (sulfonate groups in this instance).

Anions and cations have different sizes in solution and, thus, differ in their mobilities. Ultrasound radiation allows them to distribute in solution in a slightly different manner and produces ultrasonic vibration (Debye) potential, which possibly affects the ionic interactions. The ionic interaction taking place on the charged surface is governed by electrostatic potential, which is usually determined by the concentration and nature of salts, the nature of the solvent, and the charge density of the surface, according to classical electrostatic theory [18–20]. Ultrasound changes the surface's electrostatic potential and affects the ionic interaction governing the

chromatographic retention of ions. Thus, the effects of ultrasound should be related to the separation mechanisms, and are highlighted when ionic interactions are involved.

Fig. 4 shows an application of ultrasound to the chromatographic separation of POE oligomers. The retention of POE oligomers on a K^+-form cation-
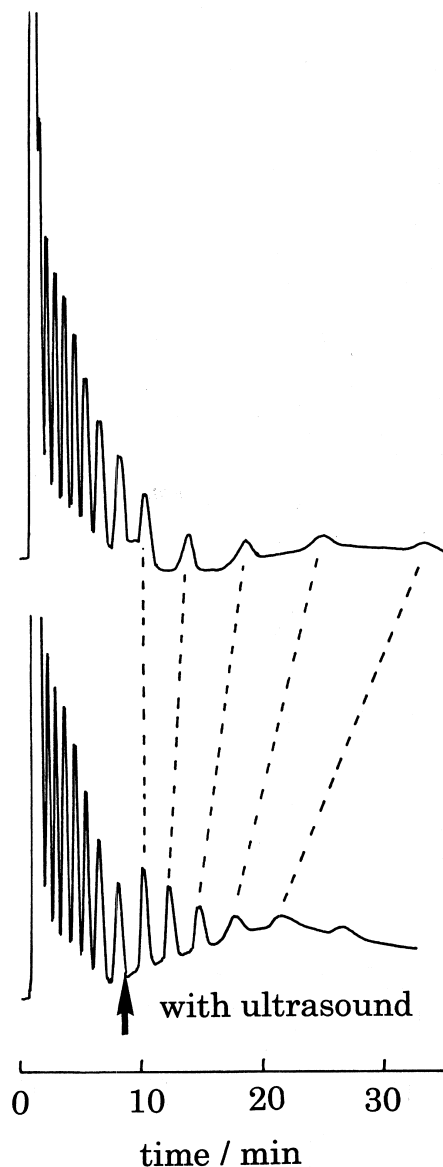

with ultrasound

Fig. 4. Step ultrasonic elution of POE. Upper, isocratic elution of POE. Lower, step ultrasonic elution; ultrasonic power was switched on at the arrow.

exchange resin almost exponentially increases with increasing n. Gradient elution must be employed to reduce the total analysis times for highly polydispersed samples. In Fig. 4, the isocratic separation of POE is compared with that obtained using step ultrasonic radiation; at the arrow in the figure, ultrasound power was switched on. Since ultrasound does not change the properties of the solution, a refractive index and a conductivity detector, which are sensitive to bulk solution properties, can also be used.

Thus, ultrasound can be a potentially effective external field in chromatography, especially when ionic interactions are involved in the separation mechanism. However, there still remain numerous problems with this method. From a practical point of view, the acoustic power obtained with the present instrument is too weak to provide drastic retention changes. Ultrasound is absorbed before reaching the stationary phase surfaces and influencing the retention mechanism. However, high acoustic power increases the temperature and, thus, it becomes more difficult to distinguish ultrasonic effects from temperature effects. Hence, we have not been able to completely elucidate the effects of ultrasound on chromatographic retention, but believe that these effects would possibly facilitate an understanding of separation mechanisms.

Acknowledgements

References

[1] K.D. Caldwell, Anal. Chem. 60 (1988) 959.
[2] T. Nomizu, H. Nakashima, M. Sato, T. Tanaka, H. Kawaguchi, Anal. Sci. 12 (1996) 829.
[3] T. Kaneta, Y. Ishidzu, N. Mishima, T. Imasaka, Anal. Chem. 69 (1997) 2701.
[4] T. Nagaoka, M. Fujimoto, H. Nakao, K. Kakuno, J. Yano, K. Ogura, J. Electroanal. Chem. Interfacial Electrochem. 350 (1993) 337.
[5] T. Nagaoka, N. Nakao, K. Tabuse, J. Yano, K. Ogura, J. Electroanal. Chem. Interfacial Electrochem. 371 (1994) 283.
[6] R.S. Deinhammer, E.-Y. Ting, M.D. Porter, Anal. Chem. 67 (1995) 237.
[7] H. Kanazawa, K. Yamamoto, Y. Kashiwase, Y. Matsushima, A. Kikuchi, Y. Sakurai, T. Okano, Anal. Chem. 69 (1997) 823.
[8] T. Okada, Anal. Chem. 63 (1991) 1043.
[9] H. Nomura, F. Kawaizumi, S. Koda, Ekitai oyobi Yoeki no Onpa Bussei, Nagoyadaigaku Shuppankai, Nagoya, 1994.
[10] K.L. Cheng, Z. Wang, Mikrochim. Acta II (1982) 399.
[11] T. Okada, J. Chromatogr. A 793 (1998) 365.
[12] J. Igarashi, Onkyo to Shindo, Kyoritsu, Tokyo, 1968.
[13] N.E. Fortier, J.S. Fritz, Talanta 34 (1987) 415.
[14] T. Okada, Anal. Chem. 62 (1990) 327.
[15] T. Okada, Macromolecules 23 (1990) 4216.
[16] T. Okada, J. Chem. Soc. Faraday Trans. 87 (1991) 3027.
[17] J.A. Dean (Ed.), Lange's Handbook of Chemistry, 13th ed, McGraw-Hill, New York, 1985.
[18] T. Okada, J. Phys. Chem. B 101 (1997) 7814.
[19] T. Okada, J. Phys. Chem. B 102 (1998) 3053.
[20] T. Okada, Anal. Chem. 70 (1998) 1692.