Mass Transfer in Mesoporous Microparticles Studied by Confocal Fluorescence Recovery after Photobleaching

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The intraparticle diffusion of a fluorescent dye in single microparticles in an aqueous solution was analyzed using fluorescence recovery after photobleaching, with a confocal fluorescence microscope. The fluorescence depth profile of single microparticles, and the fluorescence recovery at the particle center, were measured; further, the intraparticle diffusion coefficient was determined through simulations of three-dimensional diffusion in the respective microparticles. The intraparticle diffusion of coumarin 102 in octadecylsilyl silica gel was limited by the surface diffusion.

Keywords Porous material, diffusion, ODS silica gel, photobleaching, confocal fluorescence microscope

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Introduction

The mass-transfer processes of solutes in mesoporous material/solution systems are highly influential concerning the performance of liquid chromatography,1,2 solid-phase extraction,3 catalyst systems,4,5 and drug-delivery systems.6,7 These processes consist of diffusion through the pore solution (pore diffusion), diffusion along the pore walls (surface diffusion), adsorption/desorption and chemical reactions at the pore walls, and so on;1,6,7 further, quantitative measurements of intraparticle mass transfer rates are generally difficult to obtain. Pore and surface diffusion have been kinetically analyzed using sophisticated high-performance liquid chromatography,8,9 microspectroscopy for single microparticles,10-17 and so forth.18-20 We have reported on intraparticle diffusion in silica gel and octadecylsilyl (ODS) silica gel in aqueous solutions, using microspectroscopy for single microparticles.14-17 A single microparticle was injected into a solution, and the distribution or release rates of a solute between the microparticle and the surrounding solution phase were analyzed using microspectroscopic measurements of the single microparticle. In the silica-gel system, the mass transfer rate of rhodamine 6G (Rh6G) was limited by pore diffusion.21,22 In the ODS silica-gel system, on the other hand, the rate-determining step of the mass transfer processes of coumarin 102 (C102) was the diffusion between the microparticle surface and the bulk solution phase (external diffusion).23,24 However, the intraparticle diffusion of C102 in ODS silica gel was rapid, and could not be fully analyzed using the given technique. The application range of each method is limited; thus, further kinetic analysis methods will be necessary for a greater understanding of intraparticle mass transfer.

The diffusion of a fluorescent compound in biological cell systems has also been analyzed, using fluorescence recovery after photobleaching (FRAP), with a confocal fluorescence microscope.25,26 The distribution inequilibrium of the fluorescent compound could be rapidly obtained by laser irradiation of a micrometer-sized area; the diffusion rate was then measured based on the fluorescence recovery at the irradiated area. In the present study, the FRAP method was applied, to analyze the intraparticle diffusion rate of a fluorescent dye in silica gel and ODS silica gel systems. We here discuss the potential of this approach, and the intraparticle diffusion mechanism of C102 in ODS silica gel in an aqueous solution.

Experimental

Silica gel (Kanto Chemical Co., Silica Gel 60; spherical, particle radius (r), 20 - 50 μm; surface area (A), 700 m² g⁻¹; pore diameter (dp), 6.5 nm; pore volume, 1.15 cm³ g⁻¹), ODS silica gel (Wako Pure Chemical Industries, Wakosil 40C18; spherical, r, 10 - 30 μm; A, 330 m² g⁻¹; dp, 12 nm; pore volume, 1.0 cm³ g⁻¹), Rh6G (Aldrich, 99%), and C102 (Wako Pure Chemical Industries, JIS Special Grade) were used without further purification. Water was used after deionization and distillation (Yamato Scientific, Auto Still WG203). The silica gel was soaked in water overnight.23 Several silica-gel microparticles were injected into an aqueous Rh6G (1.0 × 10⁻⁶ mol dm⁻³) and HCl (pH 2) solution (4 cm³), using a microcapillary injection/manipulation system (Narishige, MN-151/IM-16), and stored for over 1 h for FRAP measurements. The ODS silica gel was soaked in an N,N-dimethylformamide (DMF, 50 vol %)-water solution, and stored for over a day. Several ODS silica-gel microparticles were injected into a DMF (0 - 5 vol %)-water solution containing C102 ((0.2 - 2.0)×10⁻⁶ mol dm⁻³) (4 cm³), and stored over 7 days for the FRAP measurements.25

The intraparticle diffusion in single microparticles was measured by the FRAP method, using a confocal fluorescence microscope system (Olympus, FV-1000D). The beam of a
405 nm-CW diode laser (50 mW) for C102 in ODS silica gel, and beams of 405 nm (50 mW) and 473 nm (25 mW)-CW diode lasers for Rh6G in silica gel, were irradiated onto the center of the respective microparticles, within a circular area (1.6 μm radius) at time (t) from 0 to 0.1 s (with the lasers in tornado scan mode). After photobleaching, the microparticles were irradiated with the beam of a CW diode laser of 405 nm (0.15 mW) for C102 or 473 nm (0.45 mW) for Rh6G, and the fluorescence of 425–525 nm for C102 or 485–585 nm for Rh6G was measured from t = 0.1 s. The fluorescence depth (longitudinal (Z axis) profile) was determined based on the fluorescence intensity of a lateral (XY axes) circular (1.6 μm radius) area from the lower to upper part, along the central axis, of each microparticle. All measurements were performed at room temperature (~298 K) using an objective lens of 60-fold magnification (Olympus, UPLSAPO 60XW, NA = 1.2) and a confocal aperture of 100 μm.

The distribution coefficient and Langmuir isotherm parameters of C102 in the ODS silica gel system were determined on the basis of absorption microspectroscopy for single microparticles, and conventional absorption spectroscopy for the aqueous solution phase.¹⁵

Results and Discussion

FRAP in a single mesoporous microparticle

The fluorescence depth profiles of Rh6G in a single silica gel microparticle were measured at the center of the microparticle before and after photobleaching (t = 0.4 s) (Fig. 1). For kinetic analysis of intraparticle diffusion, the coordinate value of the microparticle center was defined as the origin in polar coordinates. The Rh6G was adsorbed into the silica gel microparticle interior before photobleaching. The adsorbed Rh6G molecules were efficiently bleached by high-power laser irradiation (distribution inequilibrium), and the fluorescence intensity was recovered with t. The shape of the bleached Rh6G distribution at t = 0.4 s was analogous to the Gaussian shape. The bleached Rh6G distribution depends on the photochemical reactivity of the fluorescent dye, the refractive indices of the microparticle and the solution, and so forth. Therefore, the intraparticle diffusion of Rh6G in a single silica-gel microparticle was analyzed in terms of both the fluorescence depth profile at t = 0.4 s (Fig. 1) and the t course of the fluorescence intensity (I0) at the microparticle center (Fig. 2).

The t dependence of the fluorescent dye concentration in the microparticle (C(r, θ, t)) was simulated using Eqs. (1) and (2).⁶,⁷,¹⁴ The intraparticle diffusion rate of the dye may be changed by microscopic inhomogeneous distribution of d, averaged d = 6.5 nm for silica gel), the pore shape, and the distance between adsorption sites near to the adsorbed dye. Nonetheless, the intraparticle diffusion rate was assumed to be analyzable using a single diffusion coefficient. The initial and boundary conditions are given by C(r, θ, t), with A = 6 s⁻¹ and B = 0.03 μm⁻².

$$\frac{\partial C(r, \theta, t)}{\partial t} = D \left[ \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C(r, \theta, t)}{\partial r} \right) + \frac{1}{r^2} \frac{\partial^2 C(r, \theta, t)}{\partial \theta^2} \right]$$

The solid (D = 3 × 10⁻⁹ cm² s⁻¹) curves represent the simulations using Eqs. (1) and (2), with A = 6 s⁻¹ and B = 0.03 μm⁻².

$$\Delta \approx \frac{\pi}{18}$$

The calculated fluorescence depth profile was corrected by the spatial resolution of the confocal fluorescence microscope (spread of fluorescence from a position under the present experimental conditions, exp(-0.807Z²)).²² The corrected
fluorescence depth profile and $I_f$ were compared with the observed data. The $D$ value for the best-fitted curves in the silica-gel system was $(5.0 \pm 0.5) \times 10^{-9} \text{cm}^2 \text{s}^{-1}$ (Figs. 1 and 2), which agreed very well with that determined by the distribution rate measurements for Rh6G from water into single silica-gel microparticles.\(^{15,22}\) The fluorescence-recovery curve in a biological cell system has frequently been analyzed in terms of two-dimensional diffusion.\(^{26}\) When the fluorescence-recovery curve in the present study was analyzed using the two-dimensional diffusion model, the estimated diffusion coefficient was $15 \times 10^{-9} \text{cm}^2 \text{s}^{-1}$, three times greater than that determined by using Eqs. (1) and (2). Thus, for the silica gel system, three-dimensional diffusion analysis is indispensable; and the intraparticle diffusion in mesoporous microparticles was here successfully analyzed on the basis of the FRAP and three-dimensional diffusion analysis.

**Pore and surface diffusion in ODS silica gel**

The intraparticle diffusion of C102 in the ODS silica gel/water (0 vol% DMF) system was analyzed using the above FRAP and three-dimensional diffusion analysis, as shown in Figs. 3 and 4. As an additional boundary condition of the simulations in the C102-ODS silica gel system, $\partial C_{r0}/\partial r$, $\partial C_{r0}/\partial t$, and $\partial C_{r0}/\partial \theta$ were assumed to be equal to 0 because the external diffusion of C102 was much slower than the intraparticle diffusion. The $D$ value for C102 in the ODS silica gel/water system ($2.5 \times 10^{-11} \text{cm}^2 \text{s}^{-1}$) was much higher than that for Rh6G in the silica gel system ($5 \times 10^{-10} \text{cm}^2 \text{s}^{-1}$). As reported previously, in release-rate measurements for Rh6G from water into single silica-gel microparticles, the rate-determining step of the intraparticle diffusion was pore diffusion.\(^{23,24}\) This conclusion is consistent with the results of the present study.

The intraparticle diffusion of C102 in ODS silica gel was analyzed based on the pore and surface diffusion model.\(^{4,21,28-30}\) In this model, $D$ is given by

$$D = D_s H/\tau_\theta (1 + R) + D_a R/\tau_a (1 + R),$$

where $D_s$ is the surface diffusion coefficient. $D_a$ was reported to be $6 \times 10^{-6} \text{cm}^2 \text{s}^{-1}$.\(^{31}\) $H$ is the hindrance parameter, dependent on the molecular diameter of C102 (1.2 nm) and $D_a$ (12 nm). Using the Renkin equation: $H = [1 - (a/D_a)^2/(1 - 2.10(a/D_a) + 2.09(a/D_a) - 0.95(a/D_a)^2)]$,\(^{32}\) $H$ was calculated to be 0.69. $\tau_\theta$ and $\tau_a$ are the tortuosity of the pore and surface diffusion, respectively; and were reported to be 1.5 – 2.3 for silica gel.\(^{20}\) $R$ was determined using $R = C_{eq}(\tau_\theta C_{eq})$,\(^{29}\) where $C_{eq}$ is the C102 concentration in the aqueous solution at the distribution equilibrium and the porosity of ODS silica gel (0.69),\(^{11}\) respectively. The first and second terms on the right side of Eq. (3) correspond to the pore and surface diffusion terms, respectively.

According to Eq. (3), $D$ is a function of $R$. In order to confirm the $R$ dependence of $D$, $R$ was varied by the addition of small amounts of DMF to the aqueous solution. A decrease in the relative permittivity of the solution and/or an increase in the microscopic polarity of the adsorption sites by distribution of DMF in the ODS phase will proceed by the DMF addition. Acually, $R$ of C102 as a poorly water-soluble organic compound decreased with the increasing DMF concentration. As summarized in Table 1, $R$ varied with the varying DMF concentration (0 – 5 vol% DMF), while the maximum fluorescence wavelength of C102 (466 – 470 nm) was almost independent of the DMF concentration. In the present system, the surface-diffusion term in Eq. (3) is approximated to $D_s H/\tau_\theta$, because $R >> 1$. Therefore, the slope and intercept of the $D$ vs. $(1 + R)^2$ plot correspond to $D_s H/\tau_\theta$ and $D_s/\tau_\theta$, respectively. For 0 – 5 vol% DMF, the $D$ value was independent of $R$, namely, $(1 + R)^{-3}$, indicating that the contribution of pore diffusion to $D$ is negligibly small. In the Rh6G-silica gel/water system, $D$ was directly proportional to $(1 + R)^{-1}$ without the intercept, so that the rate-determining step of the intraparticle diffusion is pore diffusion.\(^{21,22}\) In the ODS silica-gel system, furthermore, the pore diffusion term is theoretically calculated to be $3 \times 10^{-11} – 3 \times 10^{-10} \text{cm}^2 \text{s}^{-1}$, using Eq. (3). Thus, $D$ is expected...
to be governed by the surface diffusion of C102, and \( D_s \) was determined to be \((2 - 7) \times 10^4 \text{ cm}^2 \text{ s}^{-1}\) using \( \tau_s = 1.5 - 2.3 \)\(^2\). The area occupied by an adsorbed C102 molecule (\( S \)) in the ODS silica gel system was calculated to be 1.8 \( \times 10^{-17} \text{ m}^2\), using \( S = \frac{A}{[N_\text{C102}] (1 - \varepsilon_p)} \) in the absence of DMF, where \( \rho_c \) and \( N_\rho \) are the particle density (2.2 g cm\(^{-3}\))\(^{26}\) and the Avogadro constant, respectively. \( C_{\text{sat}} \) is the saturated amount of C102 adsorbed in the microparticle and was determined to be \((2.0 \pm 0.4) \times 10^{-2} \text{ mol dm}^{-3}\) from the Langmuir isotherm analysis for \( C_{\text{sat}} \) and the three-dimensional diffusion simulations.

estimated to be 4.8 nm. Meanwhile, the length of an octadecyl group is 2.3 nm,\(^{32}\) and \( a \) for C102 is 1.2 nm. Therefore, C102 will efficiently diffuse along the pore walls covered by the octadecyl groups, without the desorption of C102 into the pore solution (surface diffusion).\(^{26,33,34}\)

Conclusions

The intraparticle diffusion of a fluorescent dye in mesoporous microparticles in solution was elucidated by the FRAP method and the three-dimensional diffusion simulations. \( D \) values less than \( 10^4 \text{ cm}^2 \text{ s}^{-1}\) could be determined by the present technique. The intraparticle diffusion of C102 in ODS silica gel was analyzed on the basis of the pore and surface diffusion model, and was governed by surface diffusion, in contrast to the intraparticle diffusion of Rh6G in silica gel (pore diffusion). The present approach has significant potential for analyzing chemical and physical processes in mesoporous particles.

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