

## Analysis of Local Viscosity in Alginate Gel via Bead-based Diffusometry

Ayako Koto,<sup>1,2</sup> Shota Nishimoto,<sup>3</sup> Hiroaki Sakamoto,<sup>4\*</sup> Han-Sheng Chuang,<sup>5,6</sup>  
Hideyuki Uematsu,<sup>7</sup> Shuichi Tanoue,<sup>7</sup> Eiichiro Takamura,<sup>4</sup> and Shin-ichiro Suye<sup>4</sup>

<sup>1</sup>Department of Advanced Interdisciplinary Science and Technology, Graduate School of Engineering,  
University of Fukui, 3-9-1 Bunkyo, Fukui 910-8505, Japan

<sup>2</sup>Advanced Technology Research Department, Institute of Surface Science Technology, Nicca Chemical Co., Ltd.,  
4-23-1, Bunkyo, Fukui 910-8670, Japan

<sup>3</sup>Department of Materials Science and Biotechnology, School of Engineering, University of Fukui,  
3-9-1 Bunkyo, Fukui 910-8505, Japan

<sup>4</sup>Department of Frontier Fiber Technology and Science, Graduate School of Engineering, University of Fukui,  
3-9-1 Bunkyo, Fukui 910-8505, Japan

<sup>5</sup>Department of Biomedical Engineering, National Cheng Kung University,  
1, University Road, Tainan 701, Taiwan (R.O.C)

<sup>6</sup>Medical Device Innovation Center, National Cheng Kung University,  
1, University Road, Tainan 701, Taiwan (R.O.C)

<sup>7</sup>Research Center for Fibers and Materials, University of Fukui, 3-9-1 Bunkyo, Fukui 910-8505, Japan

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In this study, we evaluated the microscopic viscosity of sol-gel films by observing the diffusion behavior (particle diffusivity) of fluorescent particles dispersed in colloids such as sols and gels. The dynamic viscosity was compared with the particle diffusivity for several alginate solutions of different concentrations, and it was found that the particle diffusivity and solution viscosity were correlated. Localized analysis of the inside of a gel thin film suggested that material phase changes can be evaluated by observing changes in particle diffusivity. Because the diffusion behavior of fluorescent particles was observed by microscopy, it was possible to obtain arbitrary local viscosity information from inside the material. Spatially and temporally continuous characterization of the inside of thin films made of polymeric materials can be performed by a very simple method.

### 1. Introduction

A polymer gel is a soft material comprising a polymer (solid) with a 3D network structure that is disrupted and diluted by a solvent (liquid) in which it is not soluble. Polymer gels obtained by crosslinking polymers behave as a solution at small spatial scales and as a solid at large spatial scales. Such gels are expected to be used as water-absorbing materials,<sup>(1–3)</sup> for example, in diapers and foodstuffs such as agar and jellies, as well as in the field of biomedicine as reservoirs of proteins and other bioactive substances.<sup>(4–6)</sup> In addition, polymeric gels composed

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\*Corresponding author: e-mail: [hi-saka@u-fukui.ac.jp](mailto:hi-saka@u-fukui.ac.jp)  
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of stimulus-responsive polymers, which dramatically change their properties in response to external stimuli, are used as functional materials.<sup>(7,8)</sup>

In recent years, gel thin-film materials prepared via sol-gel-based methods have become increasingly important as transducer layers for optical chemical sensors and biosensors.<sup>(9–13)</sup> Gel thin films can be formed using various film deposition methods, including nozzle, roll, and spin methods. However, in such cases, the polymer concentration in the gel film and its shape are nonuniform, degrading its performance. To obtain a high-quality homogeneous gel film, detailed microscale information (physical properties, shape, etc.) about the gel film is critical. Therefore, for polymeric gel and gel film materials, which are expected to be used in a wider range of fields in the future, further characterization of their intrinsic physical properties is becoming ever more important.

Texture profile analysis, dynamic viscoelasticity, and tensile and compression tests have been used to determine the mechanical properties of polymeric gels and gel thin films.<sup>(14–19)</sup> However, many of these methods do not allow distinctions to be made between local areas within the material, that is, localized measurements at the edges or in the interior of the material are not possible. Conventional measurement techniques can only provide average information on bulk materials or entire films and cannot provide local property information. In contrast, microscopy can be used to evaluate local properties by observing microscale dynamic changes inside the material. Single-particle tracking by microscopy has been used to evaluate diffusion distributions since the 1990s.<sup>(20,21)</sup> Recently, a study of correlation-based microscopy for 3D diffusion in a polymer film (Bartko *et al.*<sup>(22)</sup>) and a study on dynamic analysis of the diffusion coefficient including the spatial distribution in the curing of poly(dimethylsiloxene) (Iwao *et al.*<sup>(23)</sup>) have been reported.

Chuang and coworkers reported the development of a biosensor based on the analysis of the diffusion behavior of small particles. The diffusion data obtained from the analysis technique were considered to be adequate for the sensing application. Hence, the development of diffusion measurement techniques is important as a means of facilitating sensor and biosensor development.<sup>(24–26)</sup> Microparticles within liquids undergo Brownian motion, moving in random directions. The displacement due to diffusion can be quantified in terms of the diffusion coefficient  $D$ . This coefficient can be defined in relation to the displacement resulting from the motion as follows:

$$\langle x^2 \rangle = 2D\Delta t, \quad (1)$$

where  $x$  is the displacement of the particles and  $\Delta t$  is the time interval. The diffusion coefficient  $D$  is expressed as a function of Boltzmann's constant  $k_B$ , temperature  $T$ , solvent viscosity  $\mu$ , and particle size  $d_p$  as

$$D = k_B T / (3\pi\mu d_p). \quad (2)$$

From Eq. (2), it is apparent that the diffusion coefficient decreases as the viscosity of the solvent increases. Therefore, the viscosity around the particles can be investigated by measuring

the diffusion coefficient of the particles added and dispersed in the gel thin film. The average of the squared distances between the start and end points of the motion of all particles observed in a certain time span (mean-square displacement [ $\text{pxl}^2$ ]) in a certain time width was calculated, and the slope of the plot of the mean-square displacement against the time width was defined as the diffusivity.

On the basis of the image analysis technique of Chuang and coworkers, in this study, the motion of particles is defined as a parameter called diffusivity, and a technique to evaluate gel viscosity using the diffusivity as an indicator is proposed. Our aim is to examine the possibility of evaluating the viscosity inside a material by computing the diffusivity from the displacement of particles due to Brownian motion (Fig. 1).

Alginate gel was used as the model polymer in this study. Alginate binds to monovalent metal ions to form a water-soluble gel and binds to metal ions with valences of more than one to form an insoluble gel. We analyzed the microscopic viscosity of alginate gels by tracking fluorescent particles dispersed in the gels and discuss the usefulness of diffusivity for the analysis of viscosity properties.

## 2. Materials and Methods

### 2.1 Materials

Sodium alginate 80-120, calcium chloride, sodium hydroxide, and disodium ethylenediaminetetraacetate ( $\text{EDTA-Na}_2$ ) were obtained from Wako Pure Chemicals. Fluoro-Max red aqueous fluorescent particles ( $2.0 \mu\text{m}$  diameter) were obtained from Thermo Fisher Scientific. The water used in the present study was first deionized and then passed through a Milli-Q water purification system.

### 2.2 Viscosity evaluation

The solution viscosity was measured using a modular compact rheometer (MCR302, Anton Paar GmbH). Aliquots of sodium alginate solutions (1.5 mL) of various concentrations from 0.5

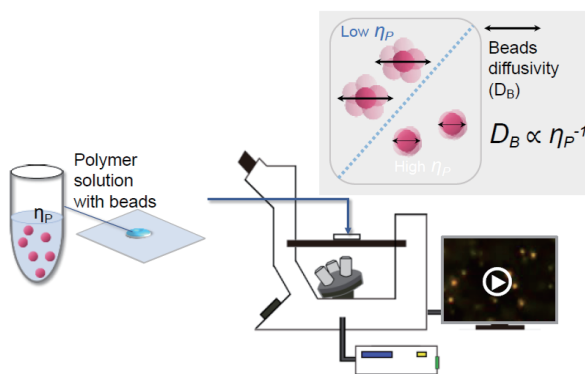


Fig. 1. (Color online) Schematic illustration of local viscosity analysis via bead-based diffusometric technique.

to 15 mg/mL were added dropwise onto flat plates. A cone plate with a diameter of 50 mm and a cone angle of  $1^\circ$  was used. The gap between the plate and the 50 mm cone plate was set at 0.099 mm, and the shear rate of the cone plate was varied from 0.1 to 1.000 /s.

### 2.3 Formation of gel films

A threefold dilution of 100  $\mu$ L of Fluoro-Max Red aqueous fluorescent particles in ultrapure water was added to a 5 mL sodium alginate solution (3 mg/mL). This mixture (100  $\mu$ L) was added dropwise onto a slide cover glass, immersed in a 0.3 mg/mL calcium chloride solution for 10 min, and then dried in a desiccator for 30 min to form a gel thin film.

### 2.4 Preparation of gel thin film by spin-coating

The cover glass was immersed in 20 mL of 10 wt% sodium hydroxide solution and then sonicated with 20 mL of ultrapure water for 10 min. Sodium alginate solution (100  $\mu$ L, 3 mg/mL) with added fluorescent particles was added dropwise onto the treated cover glass. The cover glass was spun at 300 rpm for 10 s to form a spin-coated film of alginate solution. The coated cover glass was immersed in 0.3 mg/mL calcium chloride solution for 10 min and then dried in a desiccator for 30 min to generate the gel thin film.

### 2.5 Analysis of diffusion behavior of fluorescent particles

Layers of colored Kraft tape and cellophane tape were applied to the glass slide to form a 120  $\mu$ m spacer. For solution samples, three 3.0  $\mu$ L drops of sodium alginate solution at various concentrations (0.5–15 mg/mL) containing fluorescent particles were added dropwise onto the glass slides, and then the slide cover glass was placed over the drops. For gel samples, a slide cover glass was used to cover another cover glass on which a gel containing fluorescent particles had been deposited. A fluorescence microscope was used to capture a 20 s,  $1016 \times 760$  pixel movie at 15 fps using a  $10\times$  objective and  $10\times$  eyepiece while exposing the particles to excitation light with a wavelength in the range of 470–500 nm.

The diffusion of the particles was analyzed using MATLAB code. The analysis program was provided by the Biofluid and Micro-Opto-Electro-Mechanical Systems Laboratory, National Cheng Kung University. The 20 s movie was divided frame-by-frame into still images using ImageJ. The displacements of the particles were analyzed in every frame (at intervals of 1/15 s) by comparing the coordinates of the particles. The diffusion of the fluorescent particles was calculated by averaging the obtained values across all particles.

## 3. Results and Discussion

### 3.1 Correlation between viscosity and diffusivity

Dynamic viscoelasticity evaluation using a rheometer is a common technique for accurately assessing the flow properties of fluids. Different concentrations of sodium alginate solutions

were used to verify the correlation between the bulk viscosity, as measured using a rheometer, and the degree of diffusion, obtained by image analysis techniques. The viscosity measurement results at various concentrations of sodium alginate and the observed diffusivity of fluorescent particles added to the sodium alginate solutions are shown in Figs. 2(a) and 2(b), respectively. In Fig. 2(a), it is apparent that the viscosity increases with the alginate concentration at concentrations above 0.5 mg/mL. This suggests that in dilute aqueous solutions of sodium alginate, no molecular interaction between alginate polymer chains is observed, but these interactions do occur in high-concentration solutions. However, from Fig. 2(b), it can be seen that the diffusion of the fluorescent particles in the sodium alginate solution decreases at concentrations of sodium alginate above 0.5 mg/mL. This could be because the lack of molecular interactions between different alginate polymers at concentrations below 0.5 mg/mL might allow the added fluorescent particles to move easily. However, at alginate concentrations above 0.5 mg/mL, the movement of the fluorescent particles is inhibited by the formation of a network of alginate ions, and hence the degree of diffusion decreases.

The diffusivity values are plotted versus the viscosity values measured at the corresponding alginate concentrations in Fig. 3. It can be seen that the diffusivity decreases as the viscosity increases. The increase in viscosity in the sodium alginate solution was a result of the stronger molecular interaction between polymer chains. Furthermore, the diffusion of the fluorescent particles was suppressed by the entanglement of the polymer chains. It was found that the degree of diffusion of the fluorescent particles calculated via image analysis correlated with the rheometric viscosity measurements.

### 3.2 Tracking the sol-gel transition by particle diffusion observation

When divalent cations are added to alginate solutions, the alginate ions form a 3D network structure, involving cross-linking via egg-box junctions.<sup>(27)</sup> Although these linkages are weak, they are reversible, and thus this behavior offers a high degree of flexibility in the control of physical properties. A cross-linked structure exists in the sodium alginate solution (sol), but the cross-linking is significantly altered by the addition of calcium ions, resulting in the formation of

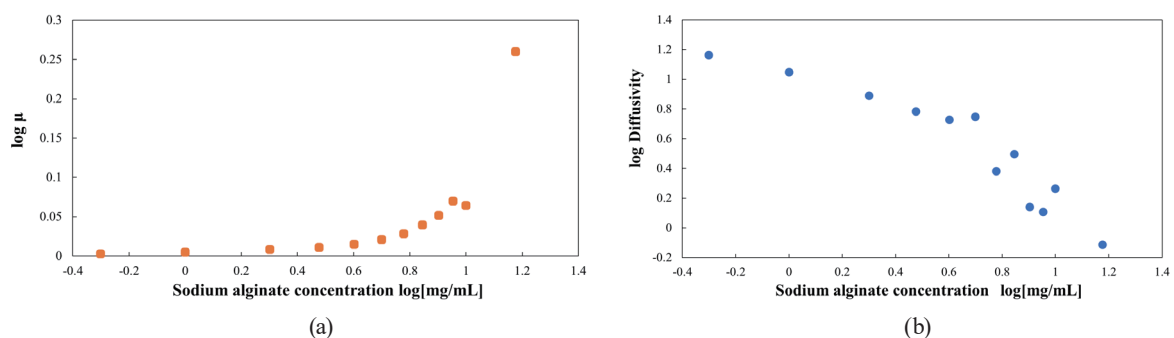


Fig. 2. (Color online) Plot of logarithmic values of (a) viscosity ( $\mu$ ) measured by rheometer and (b) diffusivity calculated from image analysis techniques versus sodium alginate concentration in the measurement solution.

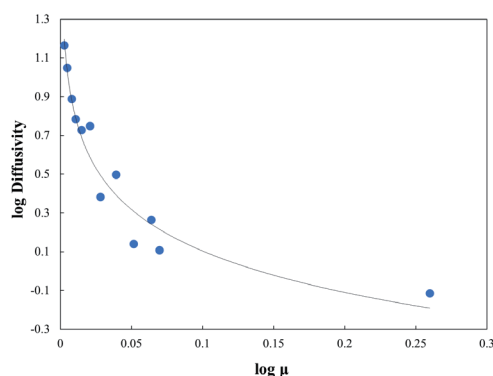


Fig. 3. (Color online) Correlation between diffusivity and viscosity.

a gel. The sol-gel transition does not require heating or cooling, which makes it ideal for the verification of our analytical technique.

We calculated the diffusivities of the fluorescent particles in a sodium alginate solution of 3 mg/mL, as well as in a gel 3D-cross-linked by adding calcium ions to the same solution. The fluorescent particles were dispersed in the solution and gel for the measurements. The particle diffusivities in the solution and gel were found to be 4.58 and 1.56, respectively. The diffusion of the particles was suppressed by the transition of the sol to the gel, resulting in reduced diffusivity. It is suggested that phase-transition phenomena in general can be evaluated using calculated diffusivities obtained via image analysis.

### 3.3 Analysis of local viscosity in alginate gel thin films

The characteristic feature of this analysis technique is that the fluorescent particles in the material are observed by microscopy. Therefore, it is possible to evaluate any part of the object of interest by selecting an appropriate focus and magnification for the microscope image acquisition.

The alginate film thickness was controlled during the coating process, for which an aqueous solution of sodium alginate containing fluorescent particles was used. Spin- and dip-coated glass substrates were immersed in a calcium chloride solution for 10 min to form two different types of gel. Each gel can be considered to be composed of three layers [Fig. 4(a)]. The particle diffusivity of each layer—lower, middle, and upper—was calculated [Fig. 4(b)].

The spin-coated gels displayed low diffusivity values for all the layers. The spin-coated gels were thin (12  $\mu\text{m}$ ), and for these gel samples, it can be assumed that gelation occurred uniformly over the entire film as very similar diffusivity values were obtained for all three layers.

However, for the three layers in the dip-coated gels, greater particle diffusion was observed in the lower layer. In the thicker dip-coated gel (223  $\mu\text{m}$ ), cross-linking progressed sufficiently in the upper part of the gel, which was in direct contact with the solution containing calcium ions, while the cross-linking rate was lower in the lower layer, where it was difficult for calcium ions to penetrate. In the middle layer, the error bars of the diffusivity were larger, which might reflect a rapid change in viscosity.

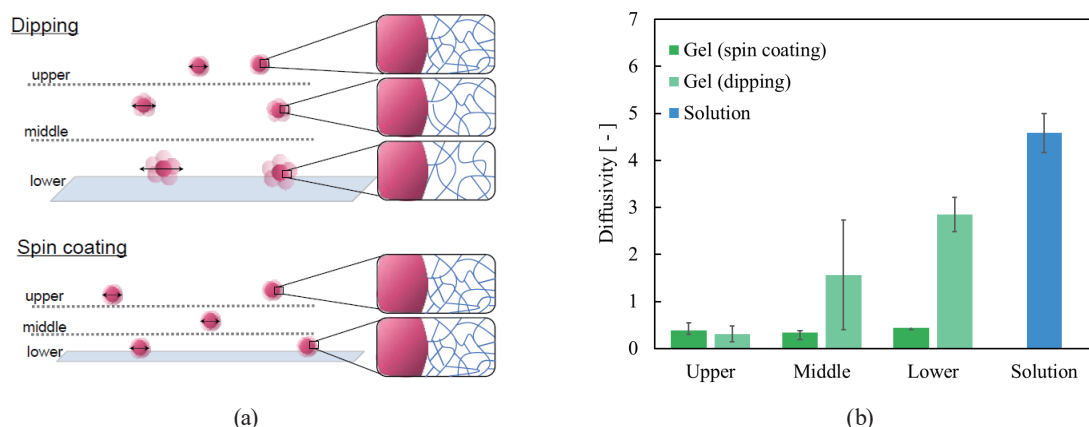


Fig. 4. (Color online) (a) Schematic cross sections of alginate films prepared by dip- and spin-coating showing their respective layers. (b) Diffusivity ( $n = 3$ ) of the particles in three different layers of alginate gels (spin-coating gel or dipping gel) or sodium alginate solution.

It was confirmed that this technique, which enables the position-selective analysis of diffusivity inside a substance, is capable of evaluating the local fine structure. Although a rheometer provides an accurate measurement of the bulk viscosity homogenized over the entire sample, our technique allows us to obtain information on the local structure within the sample.

### 3.4 Analysis of local structural collapse in alginate gel

Another advantage of using a microscope to observe the behavior of fluorescent particles is the possibility of observing partial structural changes in a material in real time. By focusing on the object of interest on a glass substrate for a certain period of time and analyzing the obtained images, structural changes in the material can be determined indirectly from changes in the behavior of the fluorescent particles.

Alginate gels cross-linked by calcium ions collapse when chelating agents such as EDTA are added. Therefore, to confirm that the viscosity change caused by the addition of a chelating agent can be measured by this technique, an alginate gel was prepared on a glass substrate and 0.5 M EDTA solution was added to the surface layer of the gel. The particle diffusivity values before and after the collapse of the gel were calculated. The diffusivity of the mid-layer region in the sodium alginate solution, that in the gel with calcium ions added to the solution, and that in the gel after the addition of EDTA solution were compared (Fig. 5).

A decrease in diffusion upon gelation and an increase in diffusion upon the addition of EDTA were observed, as expected. Gelation inhibited particle diffusion, but this was recovered by collapsing the cross-linked structure with EDTA. Furthermore, the error bars were calculated to be very large for the diffusivity of the alginate gel after the addition of EDTA. It is considered that partial survival of the cross-linked structure inside the collapsed gel is responsible for this variability in the diffusivity.



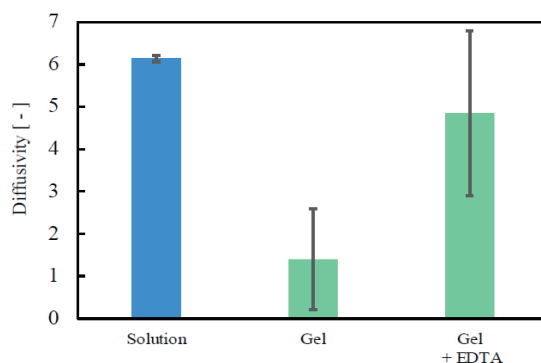


Fig. 5. (Color online) Diffusivity ( $n = 3$ ) of particles in sodium alginate solution, alginate gel, and alginate gel collapsed by addition of EDTA.

#### 4. Conclusions

In this study, we evaluated the microscopic viscosity of sol-gel films by observing the diffusivity of particles, and found that it is possible to capture spatially and temporally continuous viscosity changes inside a colloid by a very simple method.

The correlation between the viscosity of the sodium alginate solution and the diffusivity of the particles in the liquid revealed that changes in diffusivity can indirectly represent changes in viscosity. An analysis of particle diffusivity inside the alginate gels prepared under different conditions confirmed that the technique can be used to evaluate localized microscale viscosity.

From the analysis of alginate before and after the sol-gel transition, it was found that inhibition of the free motion of the particles because of the formation of a polymeric network was manifested as a decrease in the diffusion rate, and the recovery of the free movement of the particles by dissolution of the cross-linked structure was detected as an increase in the diffusion rate.

Fluorescent-particle diffusivity values obtained via image analysis can be used to evaluate the viscosity inside a material, and any local information within the gel can be selectively analyzed using a microscope. The technique proposed in this paper allows us to easily obtain local physical property information from inside the gel. It is expected that the proposed technique will enable more precise molecular design and be used as a tool for the creation of superior high-performance new materials.

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