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# Complementary analyses of fractal and dynamic water structures in protein–water mixtures and cheeses

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## HIGHLIGHTS

- ► Protein and cheese take fractal water structures of dispersion systems.
- ► The relaxation time of water increases with decreasing water content.
- ► The diffusion coefficient decreases with decreasing water content.
- ► Fractal analysis with the $\tau$–$\beta$ diagram characterizes various water structures.

## GRAPHICAL ABSTRACT
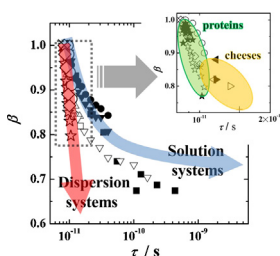


## ARTICLE INFO

## ABSTRACT

Water structures in protein–water mixtures and cheeses were examined by Time Domain Reflectometry (TDR) and Nuclear Magnetic Resonance (NMR). The relaxation time obtained from TDR increased and the diffusion coefficient obtained from NMR decreased with increasing protein concentration, even if those physical quantities and time scales of respective observation techniques were considerably different. The correlation of these dynamic behaviors obtained from TDR and NMR is also found for water structures in cheeses. Fractal analysis of protein solutions by TDR measurements suggested that water structures in both systems were same with usual dispersion systems much more than solution systems. The fractal analysis with the $\tau$–$\beta$ diagram using geometric characteristics of the whole diagram is effective to characterize various water structures.

## 1. Introduction

Water is a typical hydrogen-bonding liquid consisting of molecules having hydroxyl groups with both donor and acceptor site of hydrogen bonding. Water molecules exhibit Brownian movements, repeating breakup and recombination of hydrogen bonds. The breakup and recombination are considered as elementary processes of intermolecular correlations in the liquid structure, and interpret a model in which a concept of the dynamic cluster formed by 5–6 water molecules is often used today [1]. Generation and annihilation of such dynamic clusters of water molecules observed as a single relaxation process of Debye type by dielectric spectroscopy suggests why specific behaviors of the water are shown. The relaxation process observed for water indicates that the relaxation time $\tau$ is 8.27 ps at 25 °C [2–5]. The relaxation time is a characteristic time of rotational diffusion of molecules, while the presence of a small relaxation process reflecting more local dynamics has been reported in one or more decades higher frequency range of THz region. The elementary process of break up and recombination of hydrogen bonding may be closely related to this THz frequency process, the detailed discussion has not been completed yet.

A dynamic agglomeration structure of water molecules interacting with surrounding molecules identified by those dynamics

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behaviors is called water structure. In recent years, molecular mechanisms forming water structures has been extensively investigated in various polymer–water mixtures, and an importance to incorporate the handling of hydrogen bond network is pointed out in the interpretation of water structures. However, the dynamic range of molecular behaviors of water is extremely wide under various conditions, and those understanding have been delayed due to the dearth of enough wide time scale in conventional observation techniques. Then complementary experimental techniques of molecular dynamics in different time scales must be effective to discuss water structures in a wide time scale. The interpretation thus focused on the dynamic behavior of water molecules should be effectively employed in extensive area of applications as well as fundamental researches [6].

The relaxation time obtained for the relaxation process due to molecular dynamics of water increases and the distribution is broadened with increasing concentration in polymer–water mixtures. This behavior is called slow dynamics, and is an important concept to explain liquid structures, polymers, proteins, nucleic acids, and lipid molecules in biological systems, in which hierarchical structures of water molecules with solute molecules and dispersed particles appear. In order to characterize these hierarchical structures, fractal concept should be helpful because of the multi scaled treatment [7,8]. It is reasonably expected that the fractal concept can be utility to characterize various water structures.

The fractal expression of the relaxation process for water molecules obtained by dielectric spectroscopy has been developed to apply to various aqueous systems [9,10]. When exponential hyperbolic type dipolar correlation function corresponding to Cole–Cole equation [11] was compared with memory function, it was shown that exponent of Cole–Cole type and spatial–temporal fractal dimension are well corresponding. Cole–Cole equation is expressed as

$$\epsilon^* - \epsilon_\infty = \frac{\Delta\epsilon}{1 - (j\omega\tau)^\beta} \quad (0 < \beta \leq 1) \tag{1}$$

(1)$\epsilon^* - \epsilon_\infty = \frac{\Delta\epsilon}{1 - (j\omega\tau)^\beta}$ where $\varepsilon^*$ is the complex dielectric permittivity, $\varepsilon_\infty$ is the high frequency limit of the dielectric constant, $\Delta\varepsilon$ is the relaxation strength, $j$ is the imaginary unit, $\omega$ is the angular frequency, $\tau$ is the relaxation time, and $\beta$ is the relaxation time distribution parameter. The space fractal dimension to scale the volume division size of the relaxation structure unit is obtained from Eq. (1) with a relationship between, the relaxation time, and the exponential of Cole–Cole type relaxation time distribution through relaxation processes dependent on the water content.

In this study, we performed TDR measurements on protein–water mixtures and cheeses in order to investigate water structures. NMR measurements on diffusion process of water molecules are also performed to compare the results with the relaxation parameter obtained from TDR measurements. It is expected that molecular dynamics related with the rotational and the translational diffusion, respectively, by TDR and NMR measurements with different characteristic time scales of observation are compared. Fractal analysis using the $\tau$–$\beta$ diagram obtained from TDR measurements is also examined to discuss hierarchical structures of water and proteins. We focus on the space fractal dimension as physical quantity to characterize water structures of water mixtures.

## 2. Experimental

### 2.1. Sample preparations

Albumin from bovine serum (BSA), Ovalbumin (OVA), and Lysozyme from Chicken Egg-White (CEWL) were purchased from Sigma–Aldrich. Each protein was mixed with distilled water (Millipore, ultrapure water with resistivity higher than 18.2 MΩ cm). Each protein concentration was adjusted to 5, 10, 20, 30, and 40 wt%.

Various kinds of cheese available in market were purchased. Prepared in the present work were mozzarella cheese (KRAFT), camembert cheese (MEGMILK SNOW BRAND Co., Ltd.), cream cheese (Bel Japon K.K.), processed cheese (MEGMILK SNOW BRAND Co., Ltd.), gouda cheese (TOKYO DAIRY CO., LTD), and red cheddar cheese (TOKYO DAIRY CO., LTD).

Samples were kept in the refrigerator before measurements, and the sample temperature was kept at $25.0 \pm 0.2\,°C$ during both TDR and NMR measurements.

### 2.2. Time domain reflectometry measurements and fractal analysis

Digitizing Oscilloscope Mainframe (HP 54120B) and its Four Channel Test Set (HP 54124A) was employed as the TDR system [12–16] to make dielectric measurements especially for a relaxation process due to molecular dynamics of free water in the frequency range from 0.1 to 30 GHz. Reflected signal including information of the dielectric behavior of the sample responding to the incident step pulse was observed.

The complex permittivity of the sample can be expressed by the basic equation of TDR method [12–14] as

$$\epsilon^* = \frac{c'}{j\omega\gamma d} \frac{v_0 - r}{v_0 + r} X \cot X \tag{2}$$

where $c'$ is the speed of propagation in a coaxial line, $v_0$ and $r$ are the Laplace transforms of the incident and reflected pulse waveforms, $X \cot X$ is propagation and multiple reflections in the sample and coaxial line geometry, and $\gamma d$ is a value of corrective coefficient. Since accuracy would be better off using the difference method [12–14].

50 Ω semi-rigid coaxial cable (SUHNER) with outer diameter $d = 2.20$ mm and electric length $\gamma d = 0.165$ mm is used for electrodes of the TDR measurement.

The $\tau$–$\beta$ diagram shows the characteristic behavior of slow dynamics for each material, and some tendencies were obtained for the type of polymers.

Ryabov et al. expressed the relationship between the Cole–Cole parameter $\beta$ and the relaxation time $\tau$ in the fractal structure expression [9]

$$\beta = \frac{d_G}{2} \frac{\ln(\tau\omega_s)}{\ln(\tau/\tau_0)} \tag{3}$$

where $\tau_0$ is the cutoff time of the scaling in time domain, $d_G$ is the fractal dimension of the point set where relaxing units are interacting with the statistical reservoir,

$$\omega_s = \frac{2d_E G^{2/d_G} D_s}{R_0^2} \tag{4}$$

is the characteristic frequency of the self-diffusion process where $d_E$ is the Euclidean dimension, $D_s$ is the self-diffusion coefficient, $R_0$ is the cutoff size of the scaling in the space, and $G$ is a geometrical coefficient approximately equal to unity.

### 2.3. Frequency sweep dielectric measurements

Frequency sweep dielectric measurements were carried out using a Vector Network Analyzer (Agilent N5230C) with a dielectric kit probe (Agilent 85070E) in the frequency range from 0.5 to 50 GHz.

VNA measurements offers the complex reflection coefficient $\Gamma^*(\omega)$, from which the complex dielectric constant $\varepsilon^*(\omega)$ is obtained