Journal of Colloid and Interface Science 368 (2012) 14–20

Contents lists available at SciVerse ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Diffusion of small molecules in a chitosan/water gel determined by proton localized NMR spectroscopy

Carlos García-Aparicio, Isabel Quijada-Garrido *, Leoncio Garrido

Instituto de Ciencia y Tecnología de Polímeros, Consejo Superior de Investigaciones Científicas (ICTP-CSIC), Juan de la Cierva 3, E-28006 Madrid, Spain

ARTICLE INFO

Article history:
Received 26 May 2011
Accepted 14 November 2011
Available online 23 November 2011

Keywords:
Chitosan
Proton localized NMR resonance
Proton pulsed field gradient NMR
Diffusion
Caffeine
Theophylline
Caprolactam

ABSTRACT

Proton localized NMR spectroscopy (MRS) has been applied to study the diffusion of three small molecules, caffeine, theophylline and caprolactam, in chitosan gels with different concentration of water. This technique allows the non-destructive monitorization of diffusant concentration as a function of time and location. Concentration profiles were compared with theoretical curves based on solutions of Fick's diffusion equation for the best fitting, with the appropriate boundary conditions. The measured concentration profiles show a good agreement with the Fickian law. Values of the diffusion coefficients D ranging from 6.1×10^{-6} to 3.4×10^{-6} cm^2 s^{-1} depending on chitosan concentration and type of diffusant molecule were determined. In addition, measurements of diffusion coefficients at equilibrium conditions with proton pulsed field gradient NMR methods supported the observed Fickian behavior and showed values of D in excellent agreement with those determined by proton MRS. All these facts demonstrate that proton MRS is an appropriate method for investigating diffusion process in complex systems, such as polymer gels.

1. Introduction

The design of viable drug release devices based on polymers requires detailed knowledge of transport and release of solutes in these systems. In this context, the measurement of diffusion coefficients represents one of the main approaches to better understand molecular transport [1,2]. Therefore, the development of a non-destructive method of analysis enabling the characterization of the diffusion process at the molecular level and revealing the mechanism of diffusion of solutes through polymeric systems such as gels, may be of interest.

In the last decades, many analytical methods for studying the diffusion process in polymers have been developed or applied. Thus, numerous reports describing methods for studying the migration of small molecules in polymeric systems are available [3–9]. Generally, diffusion coefficients in gels have been determined by measuring the concentration of solute (diffusant) absorbed or released to an external medium with the time of diffusion [10,11]. However, these methods do not offer information about the distribution of the diffusant inside the gel. There are only few techniques that allow performing diffusion measurements of a solute with adequate resolution, both spatially and temporally. Examples of these techniques include FTIR imaging [12], fluorescence spectroscopy [13], fluorescence recovery after photobleaching (FRAP) [14], electronic speckle pattern interferometry (ESPI) [15] and NMR imaging (MRI) [16–18]. At the same time, these methods avoid sampling and analyzing the medium in which the diffusant molecule is present. Recently, localized NMR spectroscopy (MRS, magnetic resonance spectroscopy) has been used to yield time-resolved information about the spatial distribution of several different species in complex systems [17,19–22].

The second most abundant occurring biopolymer in nature after cellulose is chitin, a natural polysaccharide that forms the base for the outer hard integuments of crustaceans, insects, and some other invertebrates and appears also in the cells of fungi and molds [23]. The partial *N*-deacetylation of chitin leads to chitosan, a copolymer of β-(1-4)-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose, which structure is shown in Fig. 1. This copolymer is currently of great interest for biomedical applications [24] because it is biodegradable, biocompatible, non-toxic and its chemical structure allows specific modifications to design polymers for selected applications.

In the field of drug delivery, the development of chitosan-based products for commercial applications depends primarily on the knowledge of the release kinetics in its various formulations. Considering the great interest of chitosan as matrix in drug delivery devices; in this report, the application of proton localized NMR spectroscopy to study the diffusion of three small molecules in gels of chitosan in water is proposed. The influence of the drug solubility, molecular weight and chitosan concentration has been investigated in order to explore the feasibility of the proposed

* Corresponding author. Fax: +34 91 564 48 53.
E-mail address: iquijada@ictp.csic.es (I. Quijada-Garrido).

0021-9797/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jcis.2011.11.028

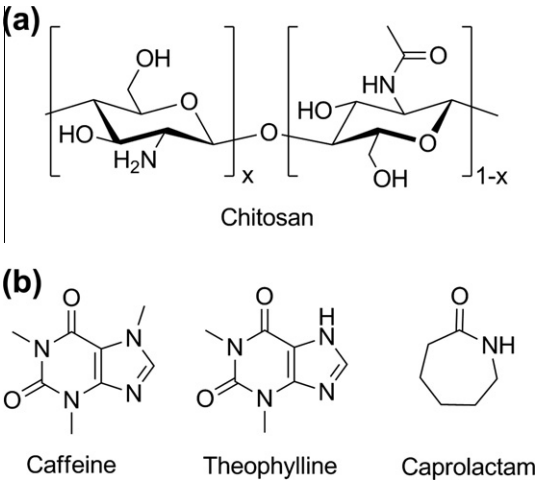

Fig. 1. Chemical structure of (a) chitosan and (b) the three diffusants: caffeine, theophylline and caprolactam.

methodology. Furthermore, proton pulsed field gradient NMR, a well established method for studying molecular diffusion in a variety of systems [25–27], is used to measure D and the results were compared with those obtained by the MRS method.

2. Background

2.1. Proton localized NMR spectroscopy

MRS can be considered a sister technique of MRI [28]. Similar to other NMR techniques, MRI is also based on the general resonance condition $\omega_0 = \gamma B_0$, being ω_0 the Larmor frequency, γ the gyromagnetic ratio of the nucleus being observed and B_0 the external magnetic field. However, instead of providing spectral information, the resonance condition in MRI is used to encode spatially the NMR signal with the overlay of external magnetic field gradients. Current methods used for spatial localization of MRS signals derive from techniques similar to those used in MRI, i.e. radiofrequency (rf) pulse selective excitation with gradient pulses used for the spatial definition of a given volume [29–31]. Localization techniques are based on the number of voxels from which the spectra are obtained in each measure. In single voxel techniques (also called single voxel spectroscopy or SVS), the spectrum is acquired from a single small volume of sample which is defined by applying three orthogonal magnetic field gradients and selective excitation for the location of the volume element of interest. Thus, the volume to be analyzed is defined by the successive application of three selective radiofrequency pulses in the presence of the corresponding magnetic field gradient pulses along three orthogonal directions of space. The combination of rf and field gradient pulses determine three orthogonal planes whose intersection corresponds to the volume under study.

Generally, due to the fact that molecules under study are found at lower concentrations than water in the sample, their direct detection results very difficult. Thus, the suppression of the water signal is essential to observe the compounds of interest. Solvent-suppression techniques have been combined with localization schemes to produce spatially localized solvent-suppressed spectra (i.e. chemical shift selective saturation CHESS [32] and variable press powers and optimized relaxation delays VAPOR [33].

2.2. Determination of the diffusion coefficient

Diffusion coefficients were determined by comparing the experimental concentration profiles with profiles calculated with Fick's laws of diffusion [34]. General solutions of the diffusion equation, given the concentration vs. time and position, can be obtained for a variety of initial and boundary conditions, provided that the diffusion coefficient is constant. One example is the diffusion of a compound in a semi-infinite medium ($x > 0$), where the interface is maintained at constant concentration (C_0) and the initial concentration is zero in the medium, i.e. a slice or reservoir containing the substance to diffuse and whose concentration remains constant throughout the experiment, in contact with a semi-infinite medium that initially does not contain this substance. This slice or reservoir is called "source."

The initial and boundary conditions will be:

$$t = 0 \quad x > 0 \quad C = 0$$
$$t > 0 \quad x = 0 \quad C = C_0$$

As a source, a deposit of substance with an amount above that of the maximum solubility in the medium could be used. At the interface, between the source and the solution, it is assumed that equilibrium is established and, consequently, the concentration of the diffusant remains constant and equal to the solubility of the substance C_0 at the selected temperature.

The solution of Fick's second law for these conditions is given in the following equation:

$$C = C_0\left(1 - erf\frac{x}{2\sqrt{Dt}}\right) \tag{1}$$

where C is the concentration of the diffusant in any location x at a time t, and D is the diffusion coefficient.

3. Materials and methods

3.1. Materials

Chitosan was supplied by Aziende Chimica e Farmaceutica (ACEF) Spa Fiorenzuola D'Arda (Piacenza), Italy. Chitosan was obtained by deacetylation of chitin from shells of marine animals, with a minimum degree of deacetylation of 90.0% (manufacturer's specification). Caprolactam ($C_6H_{11}NO$, $M_w = 113.15$ g mol^{-1}) supplied by Fluka (Steinheim, Germany), with purity $\geqslant$98%, caffeine ($C_8H_{10}N_4O_2$, $M_w = 194.19$ g mol^{-1}) and anhydrous theophylline ($C_7H_8N_4O_2$, $M_w = 180.16$ g mol^{-1}) were obtained from Aldrich (Steinheim, Germany) and both with purity $\geqslant$99%. These compounds were selected as model substances, since they are relatively soluble; they are used in the pharmaceutical industry (caffeine and theophylline) and exhibit a good ^{1}H NMR signal. Glacial acetic acid was from Aldrich with purity of $\geqslant$99.7%. The water used was Milli.Q from the water purification facility (millipore Milli-U10).

3.2. Preparation of gels

Gels with concentrations of chitosan of 2, 4 and 5 wt.% were prepared as follows: the desired amount of dry chitosan powder was added to 5 mL of water. Under constant stirring, glacial acetic acid was added (30 μL per 100 mg of chitosan) and was left stirring 24 h to form a transparent gel. The gels were transferred to glass tubes for the NMR diffusion measurements.

3.3. Magnetic resonance imaging (MRI) and localized NMR spectroscopy (MRS)

The MRI/MRS measurements were performed on a Bruker Avance™ 400 spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a superconducting magnet of 9.4 T (Larmor