Chemical Engineering Science 158 (2017) 349–358

Contents lists available at ScienceDirect

Chemical Engineering Science

journal homepage: www.elsevier.com/locate/ces

Spatial quantification of hydrogels swelling using wide-field fluorescence microscopy

Weiji Liu[a], Xiao Dong Chen[a,b,*], Ruben Mercadé-Prieto[b,*]

[a] Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, Fujian 361005, PR China

[b] Suzhou Key Laboratory of Green Chemical Engineering, School of Chemical and Environmental Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou City, Jiangsu 215123, PR China

ARTICLE INFO

Keywords:
Whey protein hydrogels
Swelling
Fluorescence
Rhodamine B

ABSTRACT

Wide-field fluorescence microscopy (WFM) is used to spatially quantify the protein content of large hydrogels during swelling. Whey protein gels made at different protein concentrations, labelled with rhodamine B isothiocyanate (RITC), were used as a model system. Labelling and swelling measurement conditions were optimized. Dynamic swelling experiments at different pH agree very well with the expected fluorescence decrease for isotropic gels using overall volumetric data, despite the existence of internal gradients. Deviations are observed at large swelling degrees, provably due to protein-dye leakage, and at high protein concentrations. This simple and ubiquitous technique is used to spatially quantify the swelling of protein hydrogels in 2D at different swelling times, highlighting the existence of a variety of swelling profiles inside the gels with time.

1. Introduction

Hydrogels formed by polymers, polysaccharides or proteins have been extensively studied due to their many useful applications (Peak et al., 2013; Wu and Gong, 2011), for example as biomaterials for drug delivery and tissue engineering (Deng et al., 2010; Vashist et al., 2013), or in foods (Shewan and Stokes, 2014). However, the formation of hydrogels can be undesired as in the case of pasteurization in the dairy industry (Jimenez et al., 2013), which is mainly caused by the heat induced aggregation and gelation of whey proteins, of which β-lactoglobulin (β-Lg) is the most important (Xin et al., 2002). Consequently, cleaning processes are applied industrially to remove daily these unwanted fouling deposits using alkali-based solutions (Alvarez et al., 2010).

One of the initial mechanistic steps during the removal of proteinaceous deposits with alkali is the swelling of such deposits (Visser and Jeurnink, 1997). It has been studied the qualitative reasons why extensive swelling can be beneficial (or not, depending on the protein (Li et al., 2015)) for the dissolution rate (Mercadé Prieto et al., 2006; Saikhwan et al., 2010). However, any attempt at quantification will fail as we cannot measure the local swelling degree (or the protein concentration) of a hydrogel, particularly close to the interface (Mercadé-Prieto et al., 2008). In this manuscript, we try to establish a technique that could be used for such purpose, aiming particularly at fouling and cleaning problems.

Extensive work has been conducted to understand the swelling of hydrogels in general, experimentally and theoretically (Blanco et al., 2013; Ganji et al., 2010). Most studies consider stable polymeric hydrogels, i.e. they are not easily chemically degraded. Overall swelling data can be obtained from classic gravimetric or volumetric measurements, but if the swelling degree distribution inside the gel is desired, new techniques are needed. Key among these is magnetic resonance imaging (MRI) with its ability to quantify water in hydrogels (Goycoolea et al., 2011). MRI has been used to study qualitatively the swelling of whey protein gels (Oztop et al., 2010), but no quantitative study has been reported to date. An obvious and cheaper alternative to MRI is fluorescence microscopy using suitable fluorescent dyes to stain the solid matrix. Fluorescence microscopy has been extensively used in protein hydrogels for qualitative purposes (Coskun et al., 2015), as in the swelling of whey protein microgels using confocal laser scanning microscopy (CLSM) (Sağlam et al., 2013). However, we are not aware of the use of fluorescence microscopy to quantify the swelling of macroscopic (protein) hydrogels. This is not surprising, despite the ubiquity of fluorescence microscopy, due to the uncertainties when using the fluorescence intensity for quantification. For example, the fluorescence intensity in a hydrogel can change due to different scattering conditions (Yadavalli et al., 2005), photobleaching (Diaspro et al., 2006), non-uniform illumination (Zwier et al., 2004),

* Corresponding authors.
E-mail addresses: xdcxmu@gmail.com (X. Dong Chen), ruben@suda.edu.cn (R. Mercadé-Prieto).

http://dx.doi.org/10.1016/j.ces.2016.10.014
Received 20 July 2016; Received in revised form 13 September 2016; Accepted 14 October 2016
Available online 18 October 2016
0009-2509/ © 2016 Elsevier Ltd. All rights reserved.

dye leakage, etc (Waters, 2009).

The aim of this work is to explore the use of fluorescence microscopy for the quantification of the protein content in hydrogels undergoing swelling, and eventually dissolution. Following our previous swelling studies (Li et al., 2015), we have considered macroscopic hydrogels (disks of 5–9 mm in diameter) in order that they can be easily weighted for calibration purposes. The large size of these gels when swollen and the extensive monitoring time used in this study discouraged the use of CLSM. Instead, a cheaper wide-field fluorescence microscopy (WFM) was used here, with the additional benefit that if quantification is shown to work for WFM, it could be easy to extend it for CLSM if needed.

2. Experimental procedure

2.1. Preparation of RITC labelled whey protein solution

Whey protein isolate (WPI) was purchased from Davisco Foods International Inc. (Le Sueur, MN, USA), with a protein content of 91 wt % as given by the manufacturer, of which ~ 65% is β-lactoglobulin (β-Lg) and ~ 27% α-lactalbumin (α-La). The selection of the protein labelling dye is critical for quantification. Dyes that photobleach easily, such as fluorescein isothiocyanate (FITC), were shown not to be suitable in preliminary studies. On the other hand, very stable dyes are commercially available but at a high price. Cost was found an important parameter in the selection of the dye because we intend to stain large amounts of proteins in future fouling and cleaning studies. We chose Rhodamine B isothiocyanate (RITC) as a compromise between the price and its desirable photostability in the pH range considered (Fikry et al., 2009). WPI solutions of 2 wt% were well homogenized and then mixed with 2% (v/v) RITC (Exciton, USA) stock solutions (~20 mM in ethanol). The pH was then adjusted to 9 by adding ~0.5 N NaOH. Staining solutions were left overnight at room temperature under mild stirring and in the dark. The WPI-RITC solutions were then dialyzed using a 6–8 kDa MWCO membrane (Spectra Laboratories), against 0.02% (w/v) sodium azide solutions, in order to remove the unreacted RITC. The dialysis solution was renewed twice a day. The concentrations of WPI and RITC inside and outside the membrane were measured in the initial trials when renewing the dialysis solution using a spectrophotometer (SpectraMax M5, Molecular Devices) at 280 nm and 555 nm, respectively. The dialysis process was normally stopped after around 65 h.

2.2. Heat-induced WPI-RITC hydrogel

The WPI concentration after dialysis was ~1.5 wt%, too low to form hydrogels. WPI powder was added to the dialyzed solution to achieve protein concentrations of 12–25 wt%, as desired. After the addition of protein powder, the pH was ~7.1. Heat-induced WPI-RITC hydrogels were formed by heating the WPI-RITC solutions inside straight centrifuge tubes (QSP, 508-GRD-Q, 9 mm i.d. and 40 mm high) for 30 min at 80 °C ± 0.1 °C in a water bath as reported in previous swelling studies (Mercadé-Prieto et al., 2016). Smaller gels were considered (made in smaller tubes, QSP, 431-Q, 6 mm i.d. and 20 mm high) in order that the gel diameter could still be determined with the same microscope at high swelling degrees. The WPI gels formed were transparent and thereby stranded-like (Langton et al., 1992). Transparent stranded gels were only considered in this initial study to minimize the effect of the sample scattering on the fluorescence intensity.

The final concentration of RITC in the gels was adjusted to ~0.02 mM in order to be within the linear range for the WFM. Control gels were also made with only WPI using the same method described above. All gels were stored at 4 °C; they were allowed to equilibrate to room temperature for at least one hour before experiments started.

2.3. Wide-field Fluorescence Microscope (WFM)

Fluorescence imaging was performed with a wide-field fluorescence microscope (MacroZoom Z16, Leica) equipped with a suitable filter (set 41007 from Chroma) and a 1.0x Planapochromatic objective (magnification 7.13x–115x). The 8-bit images were captured with a monochrome CCD camera (ICX285ALCCD, ToupTek, 1360×1024 pixels, chip size 6.45 μm, maximum exposure time 4 min), using the manufacturer's acquisition software. RITC was excited at a peak wavelength of 530 nm (520–550 nm band) with a LED light (LED4D067, ThorLabs) at maximum power, ~15 mW according to the manufacturer.

Uneven illumination, particularly significant at low magnifications, can be detrimental for quantitative measurements. Image corrections were applied to reduce the uneven illumination using Matlab® (Fig. S1). Homogeneous Rhodamine B (RhB) solutions were used as reference.

2.4. WFM calibration of Rhodamine B dyes

In order to quantify the swelling degree with WFM, it must be first determined the range where the fluorescence intensity increases linearly with the dye concentration. First, it was determined using a fluorescence spectrophotometer (SpectraMax M5, Molecular Devices, bandwidth 7 nm) at Ex/Em =555/580 nm with a 1 cm path-length quartz fluorescence cuvette. Then it was determined using the WFM. Briefly, 2 ml dye solutions were placed in a small petri dish (solution depth ~2.6 mm). The fluorescence intensity of solutions at different dye concentrations was recorded at different exposure times and magnifications.

The photostability of the dyes used was tested in the WFM after continuous irradiation at 530 nm for 40 min at the maximum power of the LED, while collecting fluorescence images at 10 s intervals.

CLSM is typically preferred compared to WFM due to its capability to discard out-of-focus fluorescence using a pinhole, thus providing sharper images. However, in the present application this is not a limitation. In a disk-shaped hydrogel undergoing swelling some water/protein gradients are expected on the thickness dimension. However, current swelling validation data is obtained from the overall volume or weight, therefore z-stacks of the hydrogel would be required to estimate the mean intensity using CLSM for comparison; this being very time and equipment consuming. On the other hand, in WFM all the fluorescence at different heights can be collected in one image if there is little scattering.

WFM was therefore used to provide directly the thickness integrated fluorescence in fairly thick disks of 2–3 mm. Experiments were performed to test the validity and the practical limits of this assertion in the microscope used. Two kinds of experiments were conducted: (a) a RhB solution of known concentration was added to a 10 mm quartz cuvette at increasing volumes, while the fluorescence intensity was acquired at different magnifications and exposure times; (b) an initial RhB solution was diluted with water. In experiments (a) the volume increases but the dye concentration is constant, whereas in (b) the concentration decreases with the added volume.

2.5. Swelling quantification using WFM

The swelling of protein hydrogels at different NaOH concentrations was studied as reported previously (Li et al., 2015; Mercadé-Prieto et al., 2015). Gels were cut into ~2 mm thickness (initial diameter for the larger gels 8.5 ± 0.5 mm, and 5.1 ± 0.2 mm for smaller ones), and weighted on an analytical balance (Sartorius CPA225D). Gels were placed inside containers with ~230 ml solution at different pH using 0.5 N NaOH; solutions were renewed several times a day. The gel diameter was measured at the lowest magnification of the microscope (i.e. 7.13×), and was calculated as the Feret diameter since gels were

Download English Version:

https://daneshyari.com/en/article/6467883

Download Persian Version:

https://daneshyari.com/article/6467883

Daneshyari.com