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Polydiacetylene/triblock copolymer nanoblend applied as a sensor for micellar casein: A thermodynamic approach

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ABSTRACT

Polydiacetylene (PDA) and triblock copolymer nanoblends were synthesized to detect micellar casein (MC), the main milk protein and an indicator of milk quality. UV–Vis spectrum showed that MC induced blue-to-red transition in nanoblends. When nanoblends and MC were separated by dialysis membrane colorimetric response (CR) was similar, whereas a remarkable CR reduction was noticed after addition of dialyzed-MC, suggesting that small molecules present in MC (salts) caused PDA color change. Interaction enthalpy variation between nanoblends and MC showed an abrupt increase that coincided with MC concentration when colorimetric transition occurred. Copolymer hydrophobic/hydrophilic balance and presence of other molecules in the system affected nanoblends CR. MC salts were found to interact with nanoblends leading to color changes. MC concentration, MC salt release, copolymer hydrophobic/hydrophilic balance, and presence of other molecules in the system affected responses of the sensors. These results contribute to future applications of PDA/copolymer nanosensors to dairy models.

1. Introduction

Milk and milk products have been subjected to adulteration by fraudulent addition of cheaper ingredients (e.g. nitrate, urea, and melamine), presenting the dairy industry with a significant problem in many countries (Song et al., 2014). The official protein determination method for milk and dairy products is the Kjeldahl method (Finete, Gouvêa, Marques, & Netto, 2013). This analytical technique is based on the quantification of the nitrogen content; however, it is unable to distinguish between proteins and other nitrogen sources. Thus, the detection of casein, as present in milk, is an important tool for ensuring milk quality, and rapid casein content determination is of interest to the dairy industry (Sun, Liang, Fan, & Yang, 2013).

To date, many techniques have been developed to detect and quantify casein levels, including fourth derivative spectroscopy (Puhan & Luthi-Peng, 1999), liquid chromatography-mass spectroscopy (Dziuba, Nałe, & Minkiewicz, 2001; Ramírez-Palomino, Fernández-Romero, & Gómez-Hens, 2014), ELISA (Saenger et al., 2014), and electroluminescence probe immunofluorescence (Song et al., 2014). Although these techniques are sufficiently accurate, they are time-consuming, rely on complex procedures, and require expensive equipment.

Recently, there has been special interest in developing simple, low-cost, rapid, reliable, non-invasive, and non-destructive devices to evaluate real-time milk and dairy products' quality (Cavallo, Strumia, & Gomez, 2014; Kuswandi, Restyana, Abdullah, Heng, & Ahmad, 2012; Pires et al., 2011). Different sensors have been studied for this purpose (Beltrán, Althaus, Berruga, Molina, & Molina, 2014; Haugen, Rudi, Langsrud, & Bredholt, 2006; Magan, Pavlou, & Chrysanthakis, 2001; Villar et al., 2012). Polydiacetylene (PDA)

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has great potential to be used in colorimetric sensors because of its easy and low-cost processability and straightforward use, which results from the blue-to-red transition that is readily detectable even by the naked eye (Bhushan, Kundu, & Singh, 2014).

PDA consists of a conjugated backbone with alternating double and triple bonds, where the optical absorption occurs via π–π^* transitions. Generally, unperturbed PDA nanoaggregates exhibit a deep blue color (absorbance at ~640 nm), and a remarkable color transition to red (absorbance at ~540 nm) occurs in response to various environmental stimuli such as light, heat, solvents, and various biological molecules (Li, Yu, Jiang, Zou, & Zhang, 2012).

Diacetylene monomers can self-assemble to form different aggregates, including micelles, vesicles, nanotubes, lamellar structures, films, and nanocomposites (Delbecq & Kawai, 2013; Su, 2006). Their structures in solution and their stability depend mainly on local PDA interactions as well as on interactions between PDA and other molecules (Pattanatornchai, Charoenthai, Wacharasindhu, Sukwattanasinitt, & Traiphol, 2013).

PDA vesicles have been extensively used to detect different target molecules; however, they show some limitations due to their low stability to aggregation. In addition, colorimetric transitions are limited for some conditions and molecules, minimizing the colorimetric response. In order to overcome these disadvantages, novel PDA nanostructures should be designed. In previous studies, we have shown that PDA/triblock copolymer (TC) nanoblends are more stable and sensitive to color transitions than PDA vesicles (Ortega, 2013). The main limitation in the application of PDA as sensors for milk and dairy product analysis is related to the complexity of the matrices. Endogenous molecules (e.g. proteins) can interact with PDA, leading to false positives. Hence, investigation of the interactions between PDA and milk biomolecules is essential for developing efficient PDA sensors.

Casein is the main protein in cow milk, accounting for around 80% of the total milk protein content. Casein self-assembles to form nanoaggregates (casein micelles) with diameters of 50–300 nm. Casein micelles consist of different casein fractions (α_{s1}, α_{s2}, β, and κ-casein) and salts (calcium phosphate, mainly) (Choi, Horne, & Lucey, 2011). Micellar casein (MC) is known to interact with different macromolecules such as chitosan (Ausar et al., 2001), pectin (Cucheval, Al-Ghobashy, Hemar, Otter, & Williams, 2009), carrageenan (Wang et al., 2014), and TCs (Kessler, Menéndez-Aguirre, Hinrichs, Stubenrauch, & Weiss, 2014).

In this context, we propose to investigate the interactions between MC and PDA/TC nanoblends in the presence and absence of the receptor molecule k-carrageenan (k-CAR). The determination of the interaction parameters is important for the development of an efficient nanosensor for casein-containing foods. To achieve this goal, we developed a novel nanosensor (PDA/TC nanoblend) for the detection of different MC concentrations. In addition, we evaluated the specific MC/nanosensor interactions through UV–Vis, microcalorimetry, fluorescence, and electrophoretic mobility techniques.

2. Material and methods

2.1. Materials

MC (90 wt% pure), obtained by ultrafiltration, was kindly provided by the Reference Center of Membrane Technique Applied to Dairy Industry (Viçosa, Brazil). All reagents were of analytical grade and were used as received without further purification. 10,12-Pentacosadiynoic acid (PCDA, 97 wt%) was purchased from Fluka (Milwaukee, USA). Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) ($(EO)_n$-$(PO)_m$-$(EO)_n$) TCs (L64 and F68) with average molar masses (M_m) of 2900 and 8400 g mol^{-1}, respectively, were used. These TCs, acquired from Aldrich (St. Louis, USA), had the following nominal compositions: L64 = $(EO)_{13}(PO)_{30}(EO)_{13}$ and F68 = $(EO)_{80}(PO)_{30}(EO)_{80}$. k-CAR was purchased from Sigma (St. Louis, USA). Sodium azide, used to avoid casein spoilage, was provided by Vetec (Duque de Caxias, Brazil). Millipore (Billerica, USA) water was used in all experiments ($R \geqslant 18.2$ MΩ cm^{-1}).

2.2. Nanoblend production

PDA/L64 or F68 nanoblends were prepared by dissolving the TC in water at concentration of 1.0% (w/w). PCDA (1 mM) was dissolved in the TC solution, and the mixture was sonicated for 10 min until a clear solution was obtained. This solution was then filtered through a 0.45 μm PVDF filter (Millipore). The system was kept overnight at 4 °C to orientate the PCDA monomers and promote the polymerization reaction. Photopolymerization was carried out by exposing the solutions to UV light (254 nm) for 10 min, generating blue PDA/TC nanoblends. To evaluate the effect of different receptor molecules, nanoblends containing k-CAR were also prepared. The receptor (0.5 wt%) was added to the copolymer solutions at the initial stage, prior to PCDA addition.

2.3. Colorimetric response (CR)

To investigate the interaction between MC and PDA/TC nanoblends, MC suspensions (0.5 wt%) were added to the nanoblend suspensions to obtain mixtures with different concentrations up to 2.76×10^{-9} mol L^{-1}. The mixtures were then stirred for 30 s and maintained at 25 °C for 12 h until the color change equilibrium was attained. The spectra were obtained between 350 and 900 nm (Shimadzu UV-2550) at 25 °C. To quantify the blue-to-red transition, the CR (%) was calculated using Eq. (1). The CR is a semi-quantitative parameter representing the percentage of PDA molecules that undergo blue-to-red transitions (Charych, Nagy, Spevak, & Bednarski, 1993).

$$\mathrm{CR}\ (\%) = \left(\frac{\left(\frac{A_{650}}{A_{650}+A_{540}}\right)_b - \left(\frac{A_{540}}{A_{650}+A_{540}}\right)_a}{\left(\frac{A_{650}}{A_{650}+A_{540}}\right)_b} \right) \times 100 \qquad (1)$$

where A is the absorbance of blue ($\lambda \sim 650$ nm) and red components ($\lambda \sim 540$ nm), determined by UV–Vis spectroscopy. The terms 'blue' and 'red' are related to material appearance, and the indices 'b' and 'a' represent the absorbances before and after MC exposure, respectively.

To verify the contributions of casein micelle, casein fractions, and salts found in the micelle to the nanoblend colorimetric transitions, the CR experiment was repeated using a dialysis system. In the first experiment, PCDA/L64 nanoblends were added to a dialysis bag, which was allowed to interact with an MC suspension at concentrations previously used. The systems were maintained at 25 °C for 12 h, and the nanoblends were then collected from the dialysis bag for CR determination. In the second experiment, the CR was determined using dialysed MC (D-MC), which was maintained inside the dialysis bag at 25 °C and washed daily for one week. This step was necessary to ensure that casein micelle and fractions were kept within the dialysis membrane. For both experiments, dialysis bags with a molecular weight cut-off of 3.5 kDa were used.

2.4. Light scattering and electrokinetic measurements

Size and zeta potential of nanostructures were measured at 25 °C with Zetasizer Nano ZS90 (Malvern). These parameters were determined for the MC suspensions before and after the dialysis