Supporting information for

Rapid casein quantification in milk powder with aggregation induced emission character of tetraphenylethene derivative

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1. Materials and methods

1.1 Materials

BSPOTPE probe: the compound was synthesized according to previous reports. [S1]

Casein was purchased from Beijing Solarbio Science & Technology Co., Ltd. All other reagents including surfactants, sucrose, lactose and salts were analytical reagents grade. All the reagents were solved in 10 mM phosphate buffer solution (PBS) if there was no special instruction. The 4 full milk powder samples and 3 skim milk powder samples were purchased from supermarket. The information of these samples was listed in Table S1.

Table S1. The milk powder samples information

	Manufacturer (Brand)	Remarks	Company location
Full milk 1	Beingmate	For baby	China
Full milk 2	Nestlé	For baby	Switzerland
Full milk 3	Yili	Nutrition milk powder for female	China
Full milk 4	Yili	Nutrition milk powder for elderly	China
Skim milk 1	Dumex	For pregnant/prepare pregnant/lactating women	France
Skim milk 2	Yili	Skim milk	China
Skim milk 3	Wandashan	Skim milk	China

1.2 Methods

1.2.1 Standard curve

Casein stock solution was prepared with the concentration of 50 mg ml^{-1}. Some drops of 0.1 M NaOH solution were added to help solving, 0.1M acetic acid was applied for adjusting the pH of casein solution to pH 7.0. We diluted the casein stock solution with PBS buffer, the concentrations were from 10 μg ml^{-1} to 5000 μg ml^{-1}. The BSPOTPE were solved to 1 μM in distilled water. In following assays, we mixed 100 μl 1 μM BSPOTPE with 100 μl diluted casein solution (the final concentration of BSPOTPE was 0.5 μM). We recorded the fluorescence intensity with Tecan Infinite M200 Microplate reader. The excitation wavelength was 350 nm, and the emitting wavelength was 470 nm.

1.2.2 Specificity

For testifying the specificity of the casein assay, we tested the influence of different interferences. We diluted different reagents with distilled water to certain concentrations (w/w): Sodium dodecyl sulfate (SDS,1%), Triton (1%), Tween (1%), Cetyltriethylammnonium bromide (CTAB,1%), Sucrose(10%), Lactose (10%), NaCl (5%), KCl (5%), Casein hydrolysate (5%), PBS, 4-(2-Hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES), Melamine (0.3%), Urea (5%), Lecithin (5%), Casein (5%), the concentration here is weight percentage. The buffer solutions such as PBS and HEPES were normal concentration: PBS (10 mM), HEPES (10 mM). We mixed 100 μl 1 μM BSPOTPE with 100 μl interference solutions (the final concentration of BSPOTPE was 0.5 μM). We recorded the fluorescence intensity with Tecan Infinite M200 Microplate reader.

1.2.3 Casein quantification in milk samples

For overcoming the interference of fatty acid, we need an extra skimming step to de-fat full milkwhole milk powder in pre-treatment procedures, compared with skim milk powder.

1. Casein quantification of full milk powder: the steps were as follows: 1) 5 mg milk powder was dissolved in 1 ml distilled water and the concentration was 5000 μg ml^{-1}. 2) Milk solution was centrifuged for 10 minutes at 2000 rpm and the upper layer of fatty acid was peeled off [S2]. 3) Acetic acid (2 M) was added to adjust pH to 4.7, casein would be deposited. The solution was centrifuged for 10 min at 10000 rpm to get the precipitate. 4) The protein precipitate was rinsed with ethanol for 2 times to remove adhered fatty acid. 5) The precipitate was washed with distilled water for 2 times and resolved with PBS. A few drops of 0.1 M NaOH were added to help solving the casein precipitate and the final volume for sample solution was 1 ml. Finally, the pH was adjusted to pH 7.0 with 0.1M acetic acid.
2. Casein quantification of skim milk powder: 1) 5 mg milk powder was dissolved in 1 ml distilled water and the concentration was 5000 μg ml^{-1}. 2) 2 M acetic acid was added to adjust the pH to 4.7, the casein would be deposited and the solution was centrifuged for 10 min at 10000 rpm to get the precipitate. 3) The precipitate was washed with distilled water for 2 times and resolved with PBS., A few drops of 0.1 M NaOH were added to help solving the casein precipitate and the final volume for sample solution was 1 ml. Finally, the pH was adjusted to pH 7.0 with 0.1M acetic acid.

In BSPOTPE method, we mixed 100 μl 1 μM BSPOTPE with 100 μl prepared sample solution. For casein quantification in full milk, the solution prepared as previous instructions should be diluted to 10 times for final assay for minimizing the background. However, there is no need to adopt the diluting step for skim milk. The fluorescence intensity was recorded with Tecan Infinite M200 Microplate reader. The excitation wavelength was 350 nm and the emitting wavelength was 470 nm. Three parallel tests have been carried out at the same time.

In Buiret method: the Biuret reagents were prepared as the formula in reference (Chinese GB/T 21676-2008; NY/T 1678-2008). We mixed 100 μl Biuret reagents with 100 μl sample solution. We recorded the absorbance value with Tecan Infinite M200 Microplate reader. The results have been read at 540 nm. Three parallel tests were carried out at the same time. All the reagents were prepared at room temperature.

As for the result evaluation in both BSPOTPE and Biuret method, according to the national standard(Chinese GB/T 21676-2008; NY/T 1678-2008), absolute difference of the two independent test results obtained under repeatable conditions shall not exceed 10 % of the arithmetic mean, otherwise the result would be disposed.

1.3 Imaging.

All the photos in the figures were captured by the digital camera. The optical and fluorescence images were collected with Laser Scanning Confocal Microscope (Carl Zeiss LSM 710), the excitation wavelength was 405 nm.

2. The role of casein protein structure in aggregation induced emission

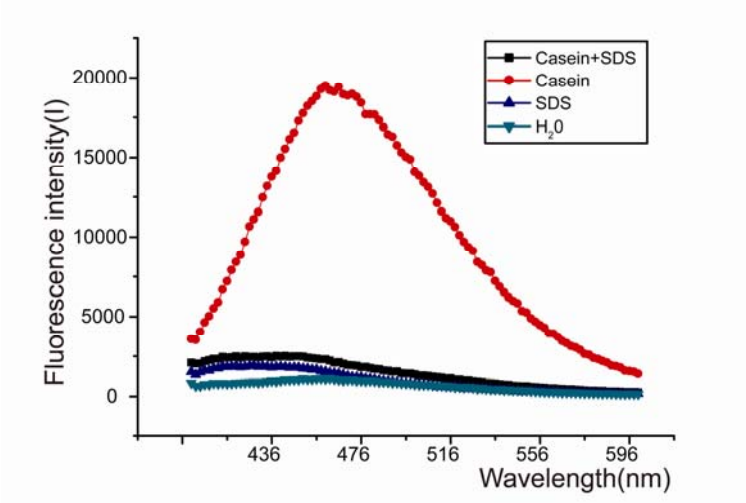

Figure S1. The role of protein structure in aggregation induced emission based casein assay [S3]. The fluorescence intensity spectrum of casein solution (500 μg ml^{-1}) with or without SDS (0.05% w/v) at the present of BSPOTPE (1 μM in distilled water). The SDS and distilled water would neither induce the fluorescence. The excitation wavelength was 350 nm.

3. The interference effect of different substances on BSPOTPE

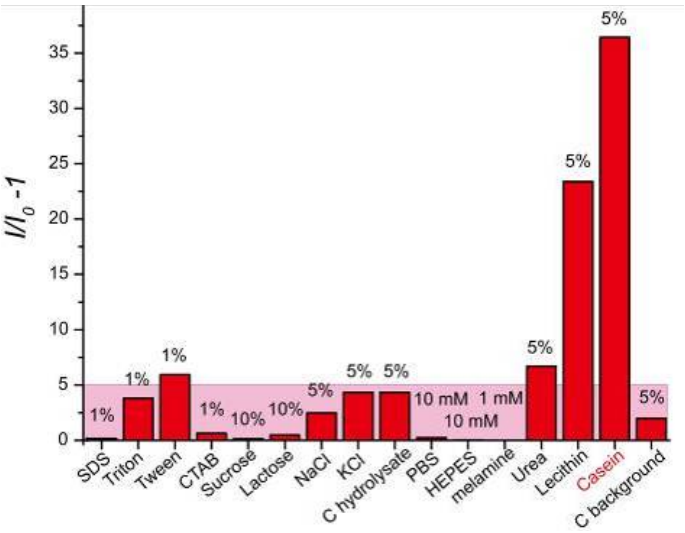

Figure S2. The interference effect. The concentration of BSPOTPE is 1 μM in distilled water and the excitation wavelength was 350 nm. We have tested the interferences of surfactants (SDS, Triton, Tween and CTAB), carbonate (sucrose, lactose), salts (NaCl, KCl), amino acid (casein hydrolysate), buffers (PBS, HEPES), nitrogenous substances (melamine, urea) and fat (lecithin), compared with casein. The concentration was on top of each column for the substances.

S1 H. Tong, Y. N. Hong, Y. Q. Dong, M. Haussler, Z. Li, J. W. Y. Lam, Y. P. Dong, H. H. Y. Sung, I. D. Williams, B. Z. Tang, *J. Phys. Chem. B*, 2007, **111**, 11817.

S2 J. H. Choi, Y. T. Kim, J. H. Lee, Analyst, 2010, **135**, 2445–2450.

S3 C. X. Yuan, X. T. Tao, L. Wang, J.X. Yang and M. H. Jiang, *J. Phys. Chem. C*, 2009, **113**, 6809–6814.