Electronic Supplementary Material (ESI) for Analytical Methods

Supporting information

A water-soluble 1, 8-naphthalimide based aggregation induced emission for selective and sensitive recognition of casein

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1. Synchronous fluorescence spectra for system of 1-HSA

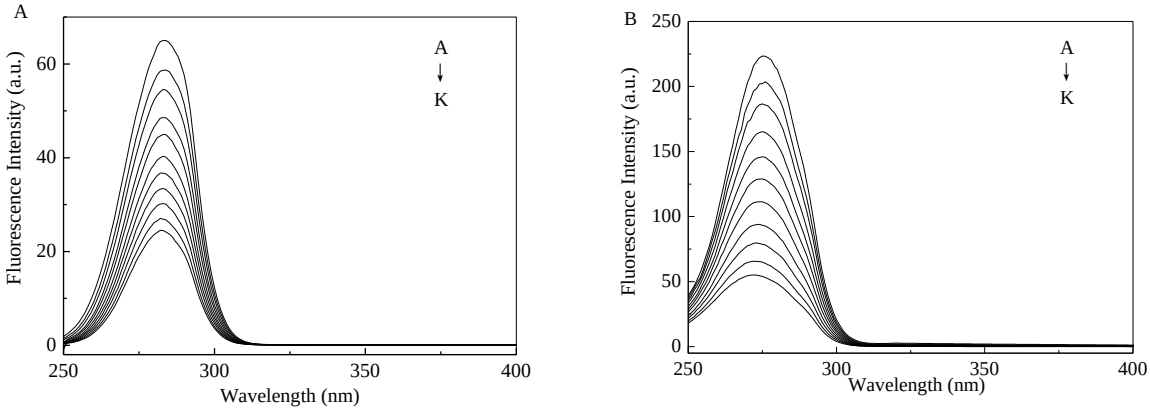

Figure S1 Synchronous fluorescence spectra for system of **1**-HSA

2. Stability

Table S1 showed the precision and accuracy of proposed method for detecting casein. The precisions were measured below 7.0 % in the relative recovery range between 97.7% and 101.3%. Our results demonstrated that the proposed method is precise and accurate, especially for the detection of casein at low levels. The effects of storage, freeze–thaw and heating–cooling on the stability of proposed method were investigated and the results were shown in Table S2. The proposed method exhibits stability for casein quantification under different conditions. The relative standard deviations are below 6.7 % for all tests. Long-term stability studies of casein in four experiment matrices showed appreciable stability over 3 days. Furthermore casein quantification in four experiment matrices is found to be stable at room temperature for a period of 6 h. All the results well meet the criterion for stability measurements.

Table S1 Summary of precision and accuracy of detecting casein by proposed method (n = 6 assays, six time replicates per assay)

Sample con. ($\mu g\ mL^{-1}$)	Added con. ($\mu g\ mL^{-1}$)	Found con. (mean ± SD, $\mu g\ mL^{-1}$)	Recovery (%)	RSD (%)
0	0.1	0.10	100.0	2.9
0.1	0.2	0.30	100.0	3.4

0.5	0.3	0.78 ± 0.01	97.52 ± 0.08	4.2
1	0.5	1.48 ± 0.02	98.67 ± 0.07	3.9
1.5	0.7	2.12 ± 0.05	97.78 ± 0.09	6.3
2	0.9	2.80 ± 0.07	96.55 ± 0.08	6.9
2.5	1.1	3.52 ± 0.25	97.78 ±0.04	4.2
3	1.3	4.25 ± 0.19	98.84 ± 0.11	7.0

Table S2 Summary of stability of proposed method under various storage conditions (n = 6)

Experiment matrix	Sample con. ($\mu g\ mL^{-1}$)	Added con. ($\mu g\ mL^{-1}$)	Found con. ($mg\ mL^{-1}$)	Recovery (%)	RSD (%)
Short-term stability	0	0.25	0.25	100.0	2.3
	0.1	0.5	0.60	100.0	2.6
	1	0.75	1.74	99.43	3.5
	3	1.0	3.98	99.5	4.1
Long-term stability	0	0.25	0.25	100.0	3.2
	0.1	0.5	0.60	100.0	3.7
	1	0.75	1.73	98.86	4.7
	3	1.0	3.91	97.75	4.8
Three freeze-thaw cycles	0	0.25	0.24	96.0	3.7
	0.1	0.5	0.59	98.33	5.2
	1	0.75	1.72	98.29	4.1
	3	1.0	3.92	98.0	6.0
Three heating-cooling cycles	0	0.25	0.24	96.0	3.7
	0.1	0.5	0.56	93.33	3.2
	1	0.75	1.65	94.29	7.1
	3	1.0	3.8	95.0	6.7

3. Casein quantification in milk powder samples

1) 50 mg milk powder was dissolved in 10 ml Tris-HCl buffer (pH 7.4).

2) Milk solution was centrifuged for 10 minutes at 4000 rpm and the upper layer of fatty acid was peeled off.

3) HCl (0.1 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 precipitate was washed with ethanol for 2 times to remove fatty acid, and then the precipitate was washed with buffer for 2 times. A few drops of 0.1 M NaOH were added to help solving the casein precipitate and the final volume for sample solution was 3 mL. Finally, the pH was adjusted to pH 7.0 with 0.1M HCl. 10 μL casein sample was added to a quartz cell with the **1** solution, and the fluorescence spectra were collected with the excitation wavelength at 430 nm and the emission wavelength at 550 nm.

Table S3. The milk powder samples information

Sample	Remarks	Manufacturer	Company
1	Full milk First stage	Beingmate	China
2	Full milk Second stage	Nestlé	Switzerland
3	Full milk Third stage	MeadJohnson	USA
4	Full milk for female	Yili	China
5	Full milk for elderly	Dumex	France
6	Full milk for pregnant	Wandashan	China