Electronic supplementary data

Polylysine-based Fluorescent Probe for Sulfite Anion Detection in Aqueous Media via Analyte-induced Charge Generation and Complexation

Huafei Xie,[a] Fang Zeng,*[a] Changmin Yu,[a] Shuizhu Wu*[ab]

[a] Department of Polymer Science, College of Materials Science & Engineering, South China University of Technology, Guangzhou 510640 (P. R. China). E-mail: mcfzeng@scut.edu.cn; Tel: 86 20 22236363; Fax: 86 20 22236363.

[b] State Key Laboratory of Luminescent Materials & Devices, South China University of Technology, Guangzhou 510640 (P. R. China). E-mail: shzhwu@scut.edu.cn; Tel: 86 20 22236262; Fax: 86 20 22236262.

Contents:

Figure S1. ^{1}H NMR spectra of polylysine (A) and polylysine coupled with benzoic aldehyde groups (namely PL-CHO) (B).

Figure S2. ^{1}H NMR spectra of Compound **2** (A) and TPE-2N$^+$ (B).

Figure S3. (A) Mass spectrum of Compound **2** and (B) Mass spectrum of TPE-2N$^+$.

Figure S4. Fluorescence change of the probe solution in the absence of sulfite and upon addition of sulfite (PL-CHO: 1.0 mg/mL, TEP-2N$^+$: 20 μM in pH 7.0 HEPES buffered water). Photographs were taken under a hand-held 365 nm UV lamp.

Figure S5. The fluorescence intensity at 466 nm of the probe solution as a function of sulfite concentration in pH 7.0 HEPES buffered water. Sulfite concentration: 0 - 70 μM. (λexc = 330 nm).

Figure S6. (A) Reversible Schiff base formation in PL-CHO polymer solution. (B) Sulfite-induced generation of negative charges along the polymer chain.

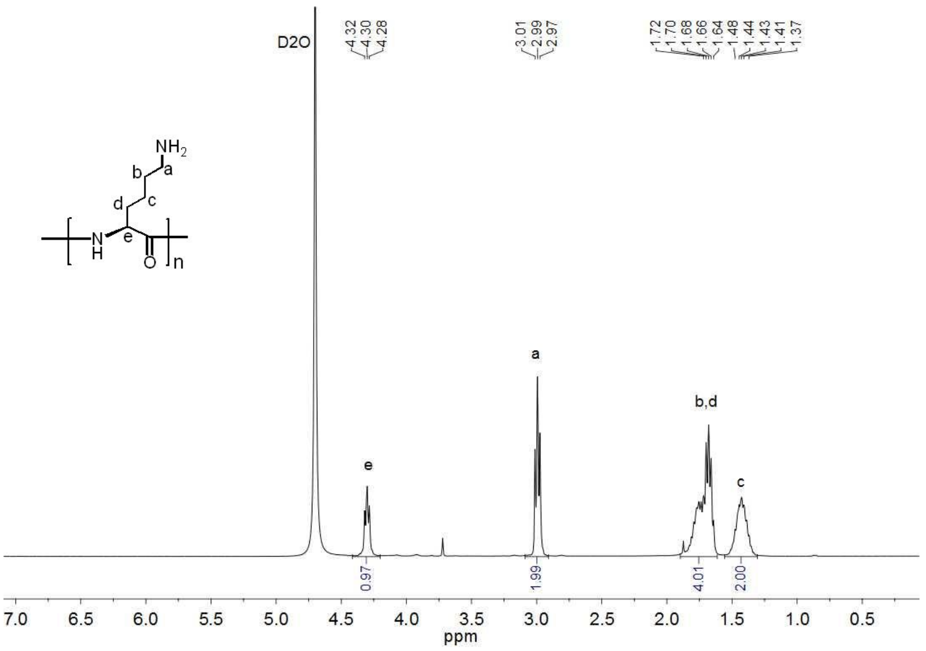
Figure S7. Diameter determined by DLS. A: the probe solution (PL-CHO: 1.0 mg/mL, TEP-2N$^+$: 20 μM in pH 7.0 HEPES buffered water) with the addition of varied amounts of sulfite anions; B – J: the probe solutions with the addition of different anions respectively.

Figure S8. Fluorescence spectra of the sensing system in the presence of some anions.

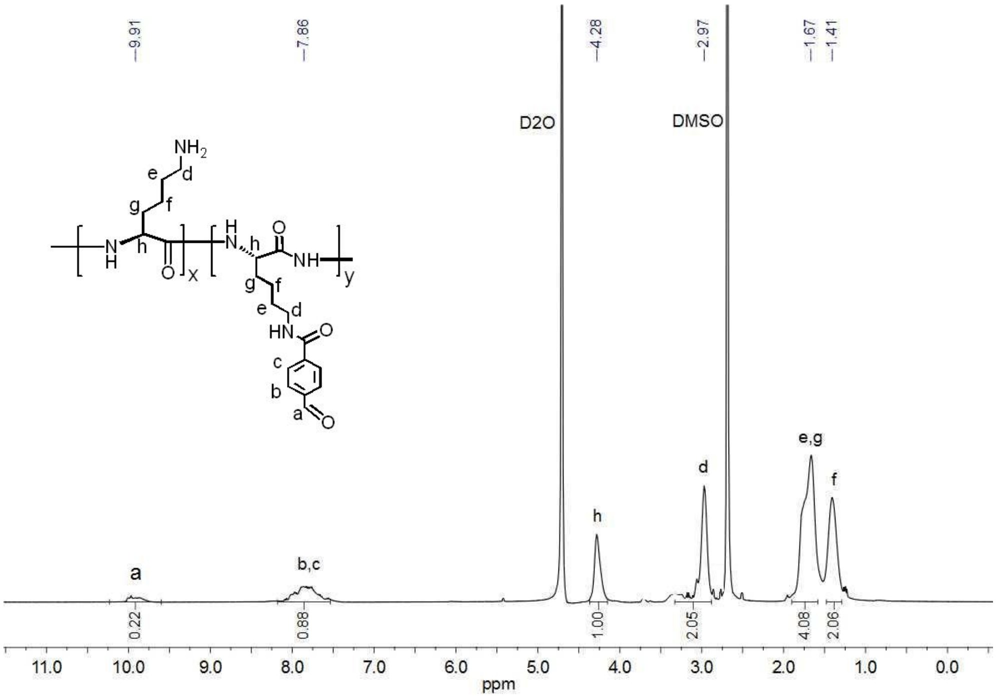
Tables S1. Determination of sulfite in beer.

Tables S2. Determination of sulfite in red wine.

Tables S3. Determination of sulfite in rain water.

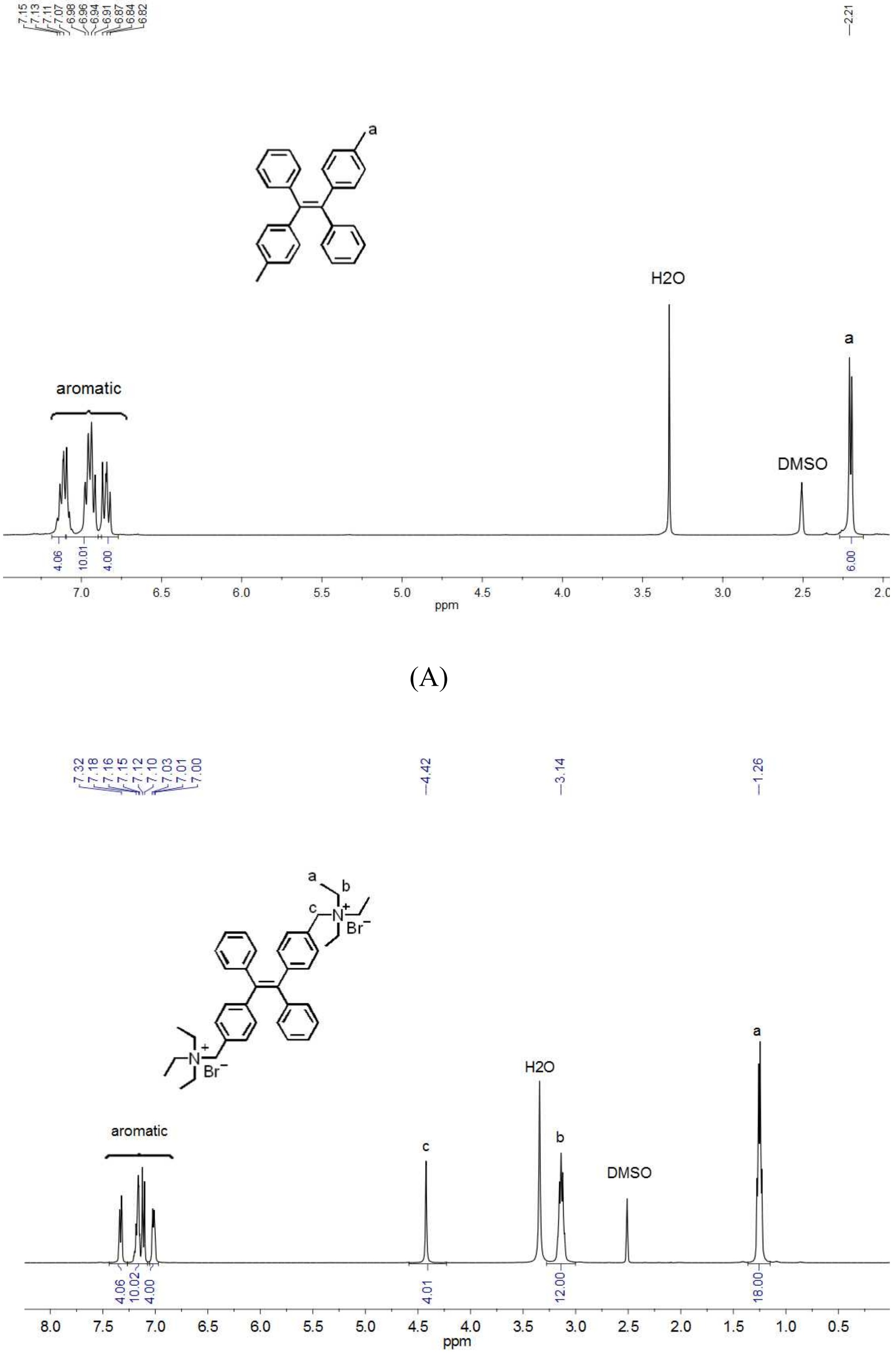


(A)



(B)

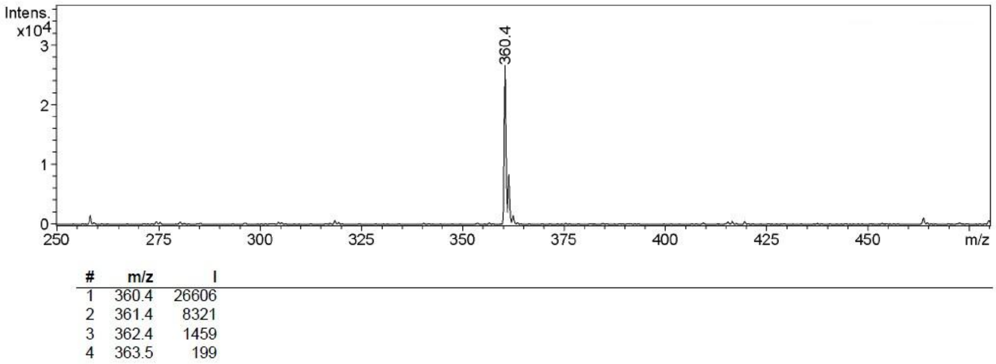
Figure S1. ^{1}H NMR spectra of polylysine (A) and polylysine coupled with benzoic aldehyde groups (namely PL-CHO) (B).



(A)

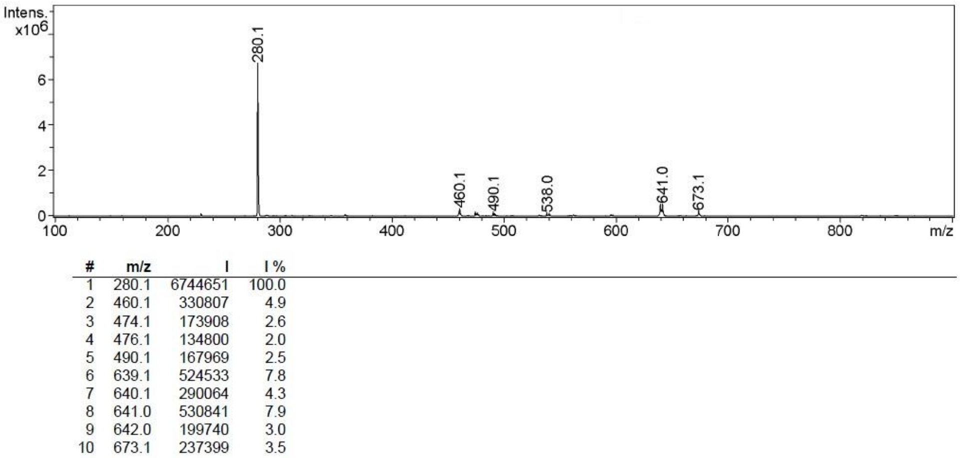
(B)

Figure S2. ^{1}H NMR spectra of Compound **2** (A) and TPE-2N$^+$ (B).



#	m/z	I
1	360.4	26606
2	361.4	8321
3	362.4	1459
4	363.5	199

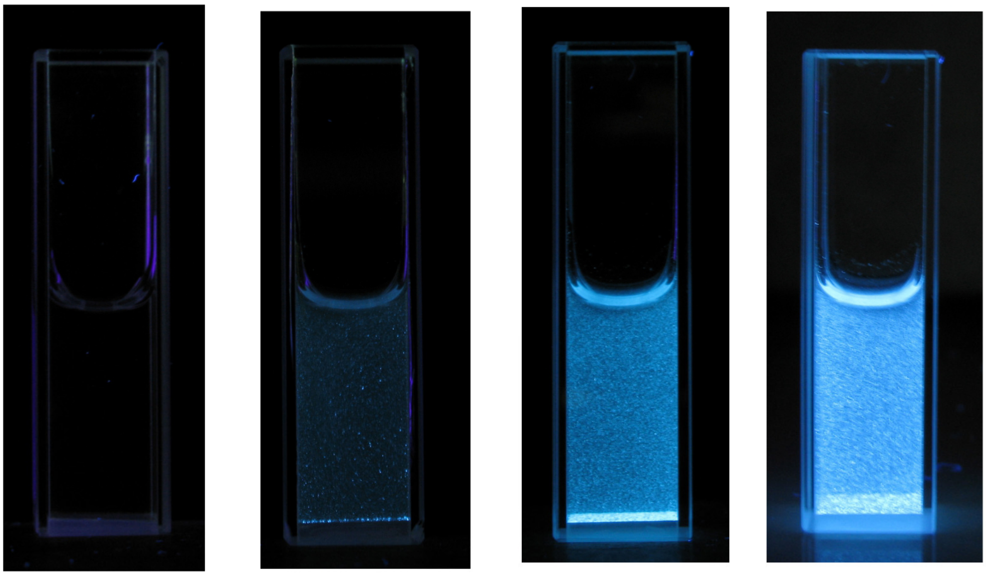
(A)



#	m/z	I	I %
1	280.1	6744651	100.0
2	460.1	330807	4.9
3	474.1	173908	2.6
4	476.1	134800	2.0
5	490.1	167969	2.5
6	639.1	524533	7.8
7	640.1	290064	4.3
8	641.0	530841	7.9
9	642.0	199740	3.0
10	673.1	237399	3.5

(B)

Figure S3. (A) Mass spectrum of Compound **2**, MS (ESI): m/z 360.4 $[M]^{+}$; (B) Mass spectrum of TPE-$2N^{+}$, MS (ESI): m/z 280.1 $[M-2Br]^{2+}$



0 μM 5 μM 50 μM 100 μM

Figure S4. Fluorescence change of the probe solution in the absence of sulfite and upon addition of sulfite (PL-CHO: 1.0 mg/mL, TEP-$2N^+$: 20 μM in pH 7.0 HEPES buffered water). Photographs were taken under a hand-held 365 nm UV lamp.

The method for determining the detection limit:

First the calibration curve was obtained from the plot of fluorescence intensity F, as a function of the analyte concentration (sulfite anion). The regression curve equation was then obtained for the lower concentration part.

The detection limit = 3 × S.D./ k

where k is the slope of the curve equation, and S.D. represents the standard deviation for the probe solution intensity in the absence of sulfite anion.

$F = 35.6 + 2.53E6 \times [S^{2-}]$ (R = 0.996)

LOD = 3 × 3.08 / 2.53E6 = 3.6 μM

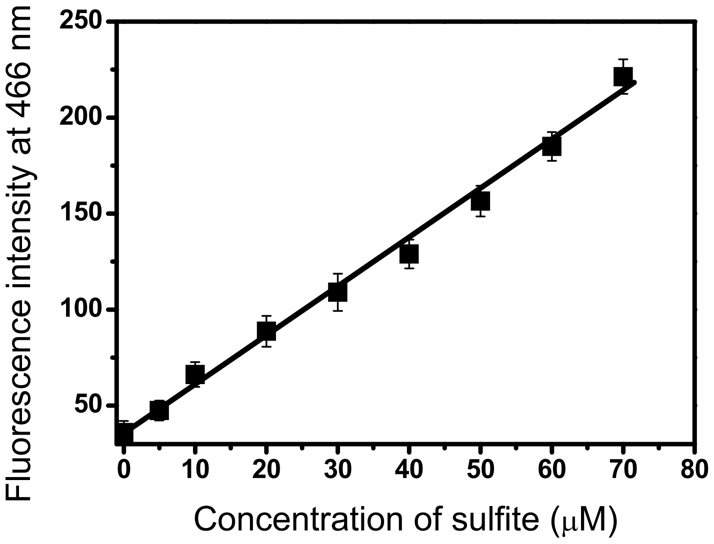
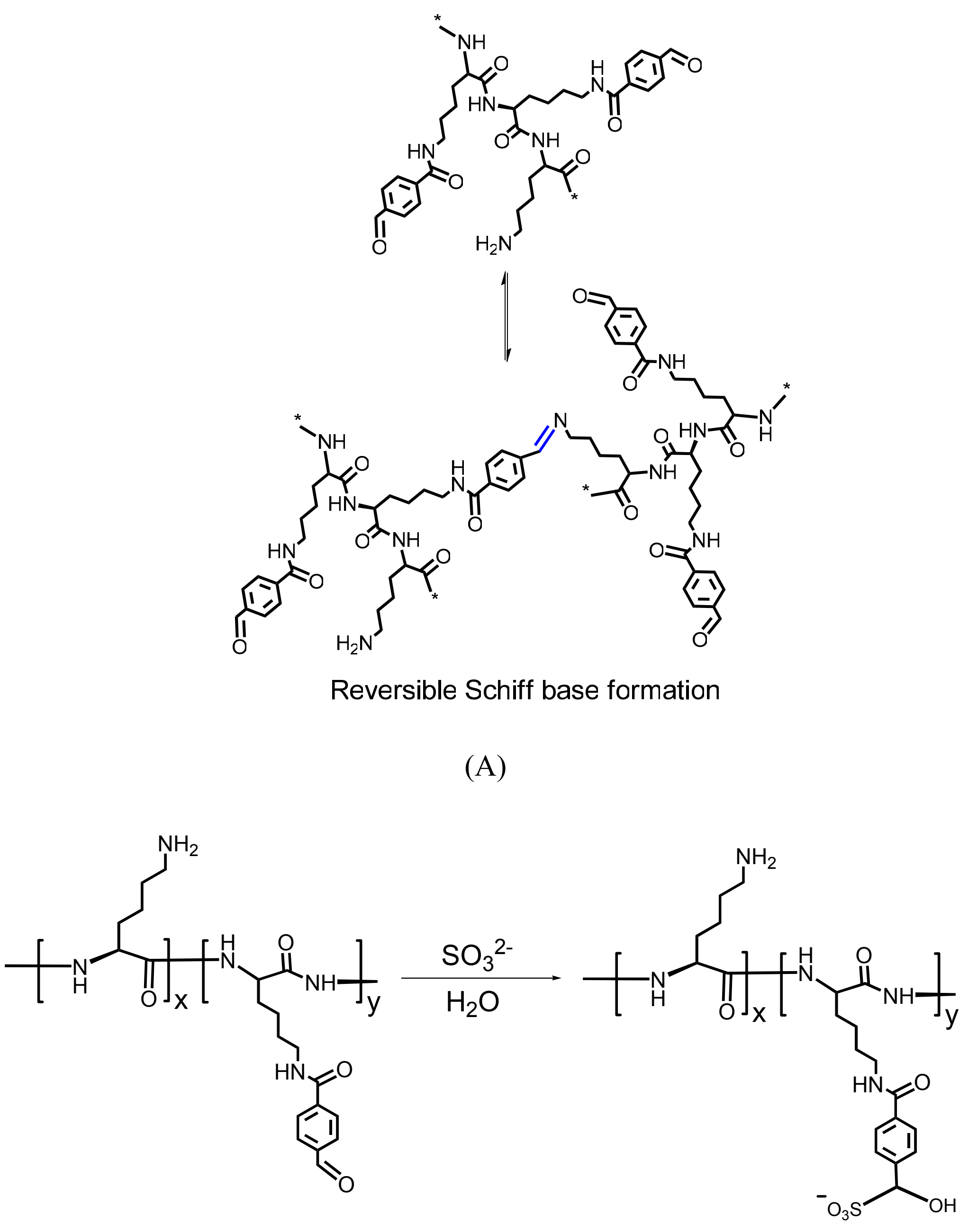


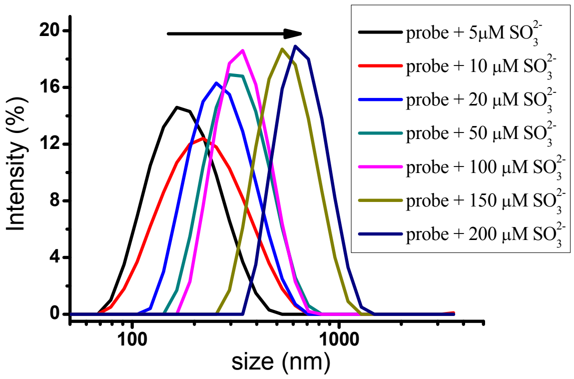
Figure S5. The fluorescence intensity at 466 nm of the probe solution as a function of sulfite concentration in pH 7.0 HEPES buffered water. Sulfite concentration: 0 - 70 μM. (λexc = 330 nm)



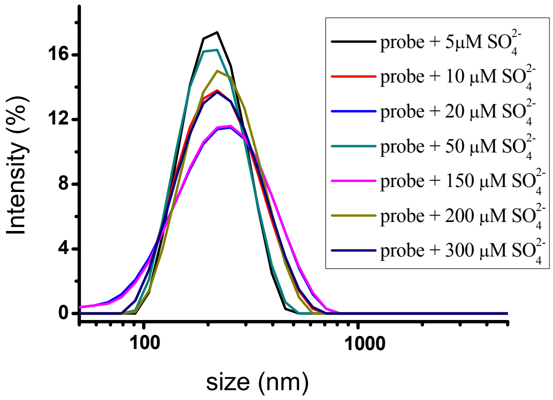
(A)

(B)

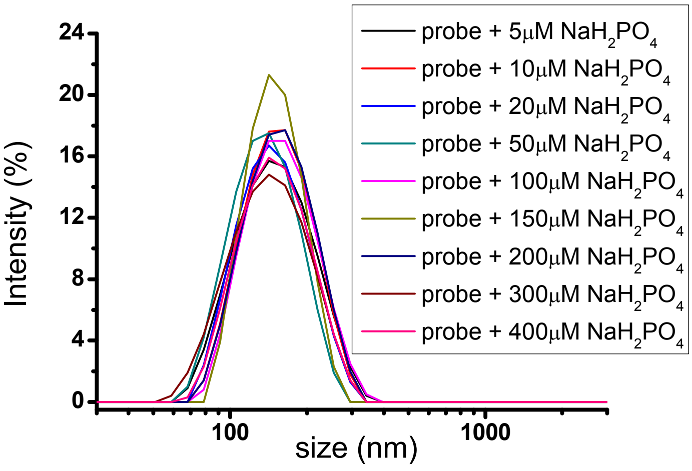
Figure S6. (A) Reversible Schiff base formation in PL-CHO polymer solution. (B) Sulfite-induced generation of negative charges along the polymer chain.


probe + 5μM SO_3^{2-}
probe + 10 μM SO_3^{2-}
probe + 20 μM SO_3^{2-}
probe + 50 μM SO_3^{2-}
probe + 100 μM SO_3^{2-}
probe + 150 μM SO_3^{2-}
probe + 200 μM SO_3^{2-}
Intensity (%)
0
4
8
12
16
20
size (nm)
100
1000

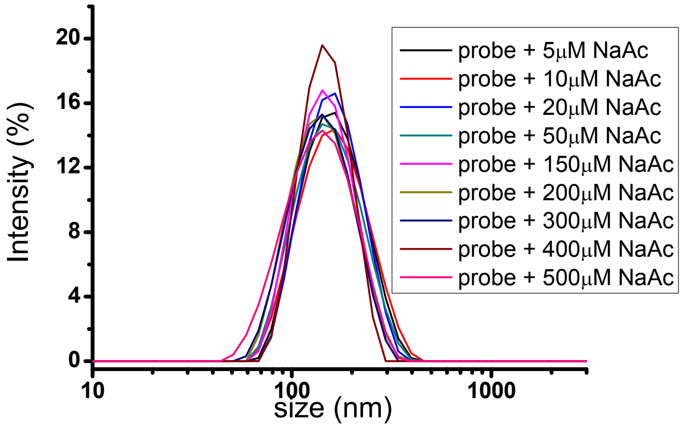
(A)


probe + 5μM SO_4^{2-}
probe + 10 μM SO_4^{2-}
probe + 20 μM SO_4^{2-}
probe + 50 μM SO_4^{2-}
probe + 150 μM SO_4^{2-}
probe + 200 μM SO_4^{2-}
probe + 300 μM SO_4^{2-}
Intensity (%)
0
4
8
12
16
size (nm)
100
1000

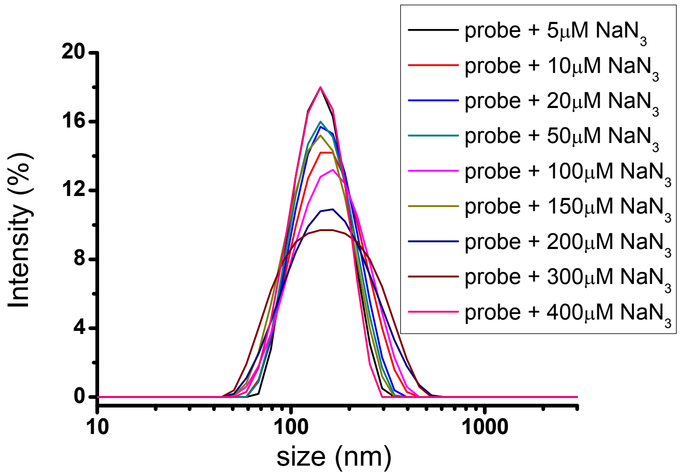
(B)


probe + 5μM NaH_2PO_4
probe + 10μM NaH_2PO_4
probe + 20μM NaH_2PO_4
probe + 50μM NaH_2PO_4
probe + 100μM NaH_2PO_4
probe + 150μM NaH_2PO_4
probe + 200μM NaH_2PO_4
probe + 300μM NaH_2PO_4
probe + 400μM NaH_2PO_4
Intensity (%)
0
4
8
12
16
20
24
size (nm)
100
1000

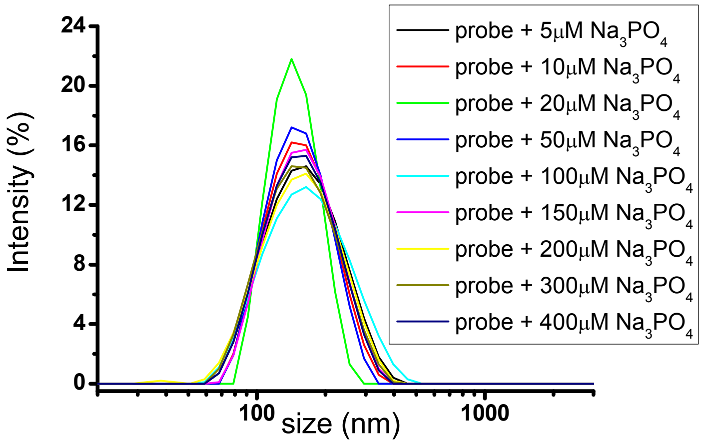
(C)


Intensity (%)
0
4
8
12
16
20
10
100
1000
size (nm)
probe + 5μM NaAc
probe + 10μM NaAc
probe + 20μM NaAc
probe + 50μM NaAc
probe + 150μM NaAc
probe + 200μM NaAc
probe + 300μM NaAc
probe + 400μM NaAc
probe + 500μM NaAc

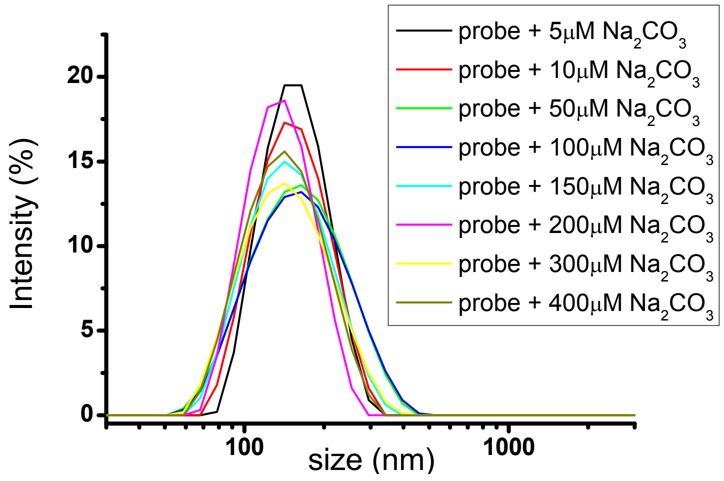
(D)


Intensity (%)
0
4
8
12
16
20
10
100
1000
size (nm)
probe + 5μM NaN_3
probe + 10μM NaN_3
probe + 20μM NaN_3
probe + 50μM NaN_3
probe + 100μM NaN_3
probe + 150μM NaN_3
probe + 200μM NaN_3
probe + 300μM NaN_3
probe + 400μM NaN_3

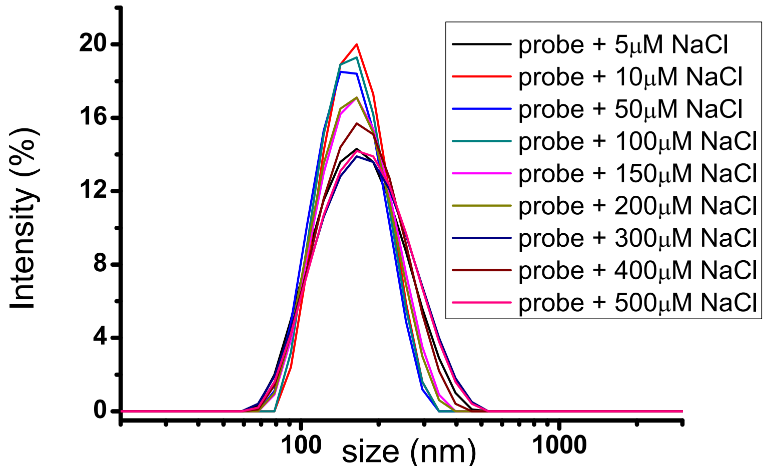
(E)


Intensity (%)
0
4
8
12
16
20
24
100
1000
size (nm)
probe + 5μM Na_3PO_4
probe + 10μM Na_3PO_4
probe + 20μM Na_3PO_4
probe + 50μM Na_3PO_4
probe + 100μM Na_3PO_4
probe + 150μM Na_3PO_4
probe + 200μM Na_3PO_4
probe + 300μM Na_3PO_4
probe + 400μM Na_3PO_4

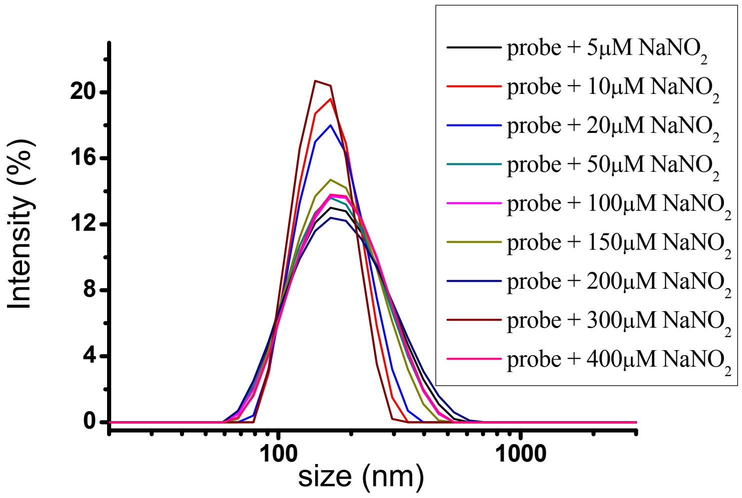
(F)


Intensity (%)
0
5
10
15
20
100
1000
size (nm)
probe + 5μM Na_2CO_3
probe + 10μM Na_2CO_3
probe + 50μM Na_2CO_3
probe + 100μM Na_2CO_3
probe + 150μM Na_2CO_3
probe + 200μM Na_2CO_3
probe + 300μM Na_2CO_3
probe + 400μM Na_2CO_3

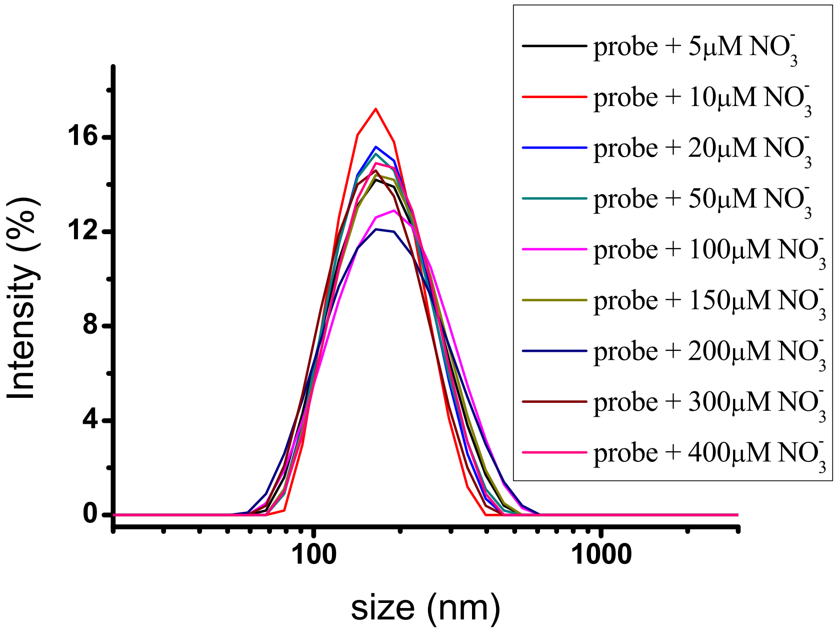
(G)


Intensity (%)
0
4
8
12
16
20
100
1000
size (nm)
probe + 5μM NaCl
probe + 10μM NaCl
probe + 50μM NaCl
probe + 100μM NaCl
probe + 150μM NaCl
probe + 200μM NaCl
probe + 300μM NaCl
probe + 400μM NaCl
probe + 500μM NaCl

(H)


Intensity (%)
0
4
8
12
16
20
100
1000
size (nm)
probe + 5μM $NaNO_2$
probe + 10μM $NaNO_2$
probe + 20μM $NaNO_2$
probe + 50μM $NaNO_2$
probe + 100μM $NaNO_2$
probe + 150μM $NaNO_2$
probe + 200μM $NaNO_2$
probe + 300μM $NaNO_2$
probe + 400μM $NaNO_2$

(I)



(J)

Figure S7. Diameter determined by DLS. A: the probe solution (PL-CHO: 1.0 mg/mL, TEP-2N$^+$: 20 μM in pH 7.0 HEPES buffered water) with the addition of varied amounts of sulfite anions; B – J: the probe solutions with the addition of different anions respectively.

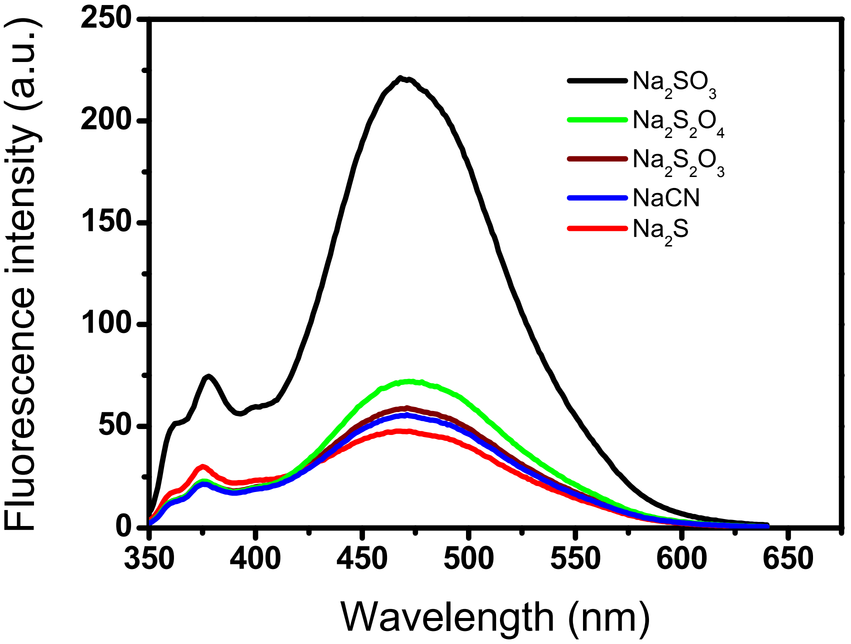


Figure S8. Fluorescence spectra of the sensing system(1.0 mg/mL PL-CHO and 20 µM TPE-2N$^+$ in pH 7.0 HEPES buffered water) in the presence of 70 µM of different anions respectively. (the fluorescence spectra were measured 1 min after the addition of the respective anions)

(1) Sulfide (S^{2-})

Sulfide (S^{2-}) exists only in strongly alkaline aqueous solutions. In aqueous solution, sulfide anion combines with protons to form hydrogen sulfide (HS^-). It usually takes dozens of minutes or hours for the reaction of hydrogen sulfide (HS−) with aldehyde to complete (for example, M. Boelens, L. M. Van der Linde, P. J. De Valois, H. M. Van Dort, H. J. Takken, J. Agr. Food Chem., 1974, 22, 1071-1076; Meyer B, Ward K, Koshlap K, Peter L "Second dissociation constant of hydrogen sulfide", Inorganic Chemistry, 1983, 22, 2345).

The dissolution process can be described as follows:

$$Na_2S + H_2O \rightarrow 2Na^+ + HS^- + OH^-$$

While the reaction between sulfite with aldehyde is completed within one minute. In this study, the fluorescence measurement was conducted one minute upon addition of sulfite into the sensing system. Within this timeframe sulfide anion has little effects on the sensing performance.

(2) Thiosulfate ($S_2O_3^{2-}$)

Thiosulfates ($S_2O_3^{2-}$, or hyposulfite) are stable only in neutral or alkaline solutions. As for the sensing of sulfite by the sensing system, no obvious interference by thiosulfate could be observed under the experimental conditions.

(3) Dithioniate ($S_2O_4^{2-}$)

The dithioniate anion ($S_2O_4^{2-}$, also known as hydroslfite) is an oxoanion of sulfur derived from thionous acid ($H_2S_2O_4^{2-}$). Sodium dithionite is stable when dry, but is slowly oxidized by air when in solution. In aqueous solution, sodium dithionite slowly decomposes into sodium thiosulfate and sodium bisulfite (Catherine E. Housecroft; Alan G. Sharpe (2008). "Chapter 16: The group 16 elements". Inorganic Chemistry, 3rd Edition. Pearson; Greenwood, Norman N.; Earnshaw, Alan (1997). Chemistry of the Elements (2nd ed.). Butterworth–Heinemann.).

$$2\ Na_2S_2O_4 + H_2O \rightarrow Na_2S_2O_3 + 2\ NaHSO_3$$

Under the experimental conditions herein, no obvious interference effect by dithioniate anion could be detected.

(4) Cyanide anion (CN^-)

A Cyanohydrin reaction is an organic chemical reaction by an aldehyde or ketone with a cyanide anion or a nitrile to form a cyanohydrin. This nucleophilic addition is a reversible reaction but with aliphatic carbonyl compounds equilibrium is in favor of the reaction products at 1 to 10% catalyst loading. The reaction of aldehyde with cyanide anion to form cyanohydrin usually requires an alkali catalyst (Cyanohydrin reaction of formaldehyde with potassium cyanide, Organic Syntheses Coll., Vol.3, p436; Vol.27, p41; Cyanohydrin reaction of formaldehyde to hydroxyacetonitrile or glycolonitrile with sodium cyanide in Organic Syntheses Coll. Vol.2, p387; Vol.13, p56). Under the experimental conditions herein, no interference effects by cyanide anion could be observed.

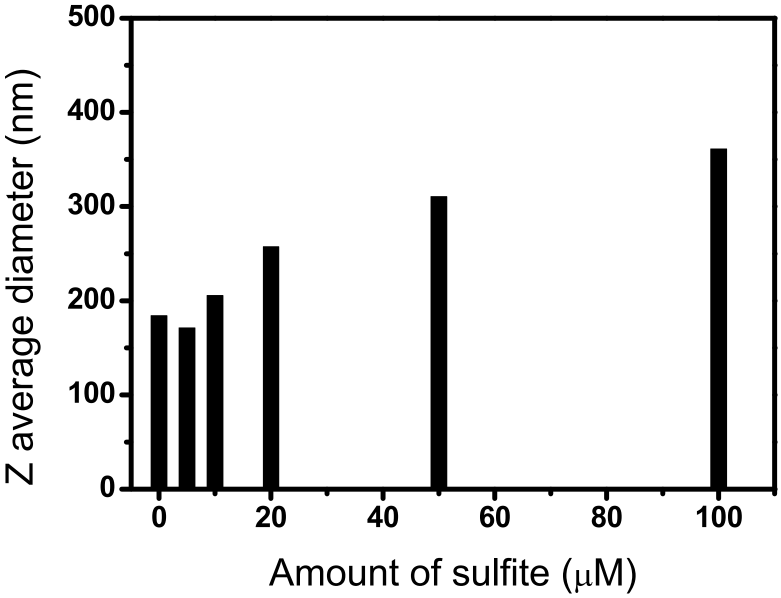


Figure S9. Size of the aggregates formed in the sensor solution upon addition of different amounts of sulfite anions (determined by DLS).

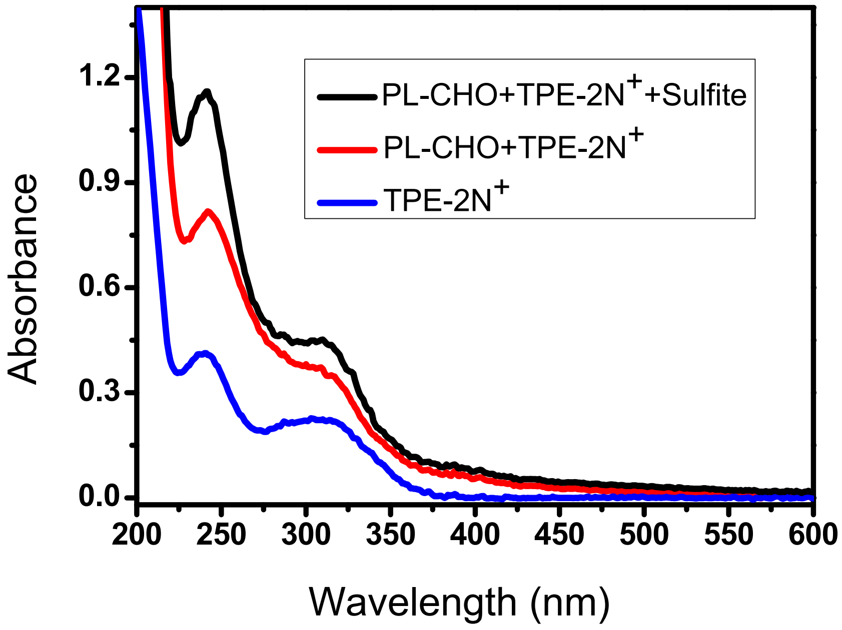


Figure S10. Absorption spectra for TPE-2N$^+$, TPE-2N$^+$ / PL-CHO, and TPE-2N$^+$ / PL-CHO / Sulfite.

Table S1. Determination of sulfite in beer.

Determined sulfite (μM)	Added sulfite (μM)	Combined sulfite (μM)	Measured (μM)	Recovery (%)
8.16 [(a)] [7.75 [(b)]]	-	-	-	-
	1	9.16	9.19	100.3
	5	13.16	12.87	97.80
	10	18.16	18.57	102.2
	20	28.16	27.70	98.40
	30	38.16	37.11	97.30
	40	48.16	49.39	102.50
	50	58.16	59.25	101.90
	60	68.16	68.29	100.20
	70	78.16	78.15	99.90

Note: (a) The final concentration of beer in the analytical solutions is 10-fold diluted. The determined sulfite level in the diluted beer sample without spiking sulfite is 8.16 μM, which means the sulfite concentration in undiluted beer sample is ca. 81.6 μM (or 5.22 mg/L expressed as sulfur dioxide, or 10.28 mg/L expressed as sodium sulfite).

(b) Determined according to routine titration method with the deviation of less than 10%.

Table S2. Determination of sulfite in red wine.

Determined sulfite (μM)	Added sulfite (μM)	Combined sulfite (μM)	Measured (μM)	Recovery (%)
5.7 [(a)] [5.3 [(b)]]	-	-	-	-
	1	6.7	6.5	97.5
	5	10.7	10.6	98.7
	10	15.7	15.3	97.8
	20	25.7	25.6	99.6
	30	35.7	36.6	103.0
	40	45.7	44.5	97.5
	50	55.7	57.6	103
	60	65.7	64.6	98.4
	70	75.7	74.9	98.9

Note: (a) The final concentration of red wine in the test solution is 10-fold diluted. The determined sulfite level in the diluted red wine sample without spiking sulfite is 5.7 μM, ca. 57 μM (or 3.65 mg/L expressed as sulfur dioxide, or 7.18 mg/L expressed as sodium sulfite).

(b) Determined according to routine titration method with the deviation of less than 10%.

Table S3. Determination of sulfite in rainwater.

Determined sulfite (μM)	Added sulfite (μM)	Combined sulfite (μM)	Measured (μM)	Recovery (%)
6.4 (a)	-	-	-	-
	1	7.4	7.3	98.8
	5	11.4	11.7	102.9
	10	16.4	16.3	99.2
	20	26.4	25.9	98.1
	30	36.4	36.1	99.3
	40	46.4	45.5	97.9
	50	56.4	55.0	97.5
	60	66.4	65.7	98.9
	70	76.4	76.1	99.7

Note: (a) The final concentration of rainwater in the test solution is 3-fold diluted. The determined sulfite level in the diluted rainwater sample without spiking sulfite is 6.4 μM, which means the sulfite concentration in undiluted rainwater is ca. 19.2 μM.