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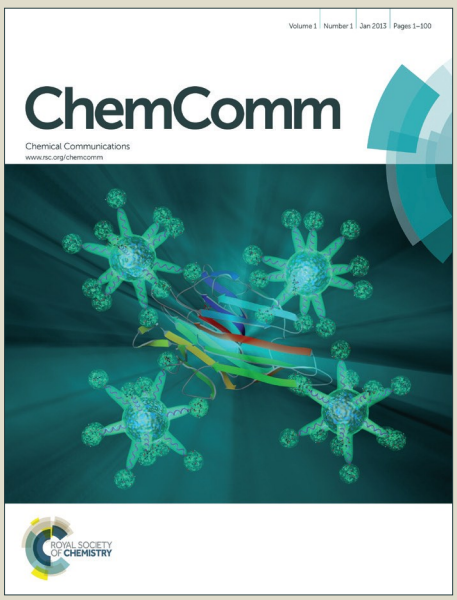

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Sulphate-selective optical microsensors: overcoming the hydration energy penalty†

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Novel membrane-free chemically modified polystyrene microspheres for the optical detection of sulphate in aqueous media are introduced. The working principle of this sensor is based on the surface mass-extraction equilibrium of the target species. This allows overcoming the strong hydration energy penalty, a typical problem for the detection of divalent anions. This optical sensor exhibits both enhanced sensitivity and selectivity, which allows the accurate detection of sulphate biological samples. To illustrate these features the determination of sulphate in urine is presented.

The sensitive and selective determination of divalent inorganic anions using chemical sensors still presents significant challenges, which are largely due to the strong hydration energy of these highly hydrophilic species.[1] A typical example is the determination of sulphate, an anion that plays an important role in biological systems and environmental pollution -among many other areas-. Physiological levels of sulphate are mostly due to the metabolism of certain proteins. In humans, inorganic sulphate generally originates from the catabolic biodegradation of the sulphur-containing amino acids (methionine and cysteine), although several organic and inorganic compounds present in food and beverages[2] are also a relevant source. Thus, monitoring sulphate levels in body fluids can be used as a marker for protein intake studies. Also, the determination of sulphate is used as diagnostic tool since abnormal levels of this anion in urine are indicators of renal failures or cardiovascular diseases[3]. Sulphate also plays an important role in the organoleptic properties of water and beverages and -in environmental monitoring- inorganic sulphate levels in soils and water are particularly relevant in problems such as the nuclear waste remediation.

Despite of this widespread interest, methods for the effective determination of sulphate in real samples are still very limited. Classical methods such as turbidimetry[4] or colorimetric strips[5] are still employed for environmental and clinical samples. Both techniques however, exhibit significant issues regarding sensitivity, precision and matrix interferences. The use of separation techniques such as ion-chromatography or capillary-electrophoresis help to overcome many analytical issues but at the expense of a more tedious sample handling, cost and simplicity.[6–8] In environmental monitoring, spectrophotometric techniques using barium chromate, microbial-based methods[9] and colorimetric sensing with positively charged gold nanoparticles[10] or indicator displacement agents[11] represent more sensitive approaches for analysis of real samples, although they are often complex and time-consuming.

Membrane-based sensors such as ion-selective electrodes (ISEs) and optodes have been used for decades as a powerful tool for research and diagnostics.[12] These devices, which make use of a polymeric membrane as a support for immobilizing the selective ionophore, the ion-exchanger and in the case of optodes also the chromoionophore, show many advantages in terms of simplicity, speed and robustness. Selectivity, however, is still a major issue, particularly in the case of anions, due to their significantly higher hydration energy when compared to cations.[13] As a result, membrane-based anion sensors typically display a selectivity pattern that closely follows the partition coefficients between the aqueous and the organic phases, commonly known as the Hofmeister series. Therefore, despite of the development of selective ionophores[14–16], the determination of highly hydrophilic divalent anions in complex matrices remains as a challenge. This is the case of the determination of sulfate in urine where both specific interferences due to other anions as well as matrix effects might be present.

Three years ago, Gyurcsányi et al. introduced membrane-free potentiometric sensors based on gold nanopores which exhibited considerable improvement in terms of selectivity.[17] Very recently, Bakker and co-workers elegantly introduced a new approach of a membrane-free optical sensor based on the use of surface functionalized polystyrene nano and microspheres for the detection of cations.[18–20] In these systems, the same components used in traditional optodes are immobilized on the hydrophobic surface of the beads, thus avoiding the need of a polymeric matrix support such as polyvinylchloride (PVC). Therefore, the response obtained does not follow the phase partition equilibrium typically found in this type of sensors, but rather a mass extraction equilibrium established between the surface of the beads and the aqueous media. As a consequence, by proper selection of the working conditions the system may work in an "exhaustive" mode

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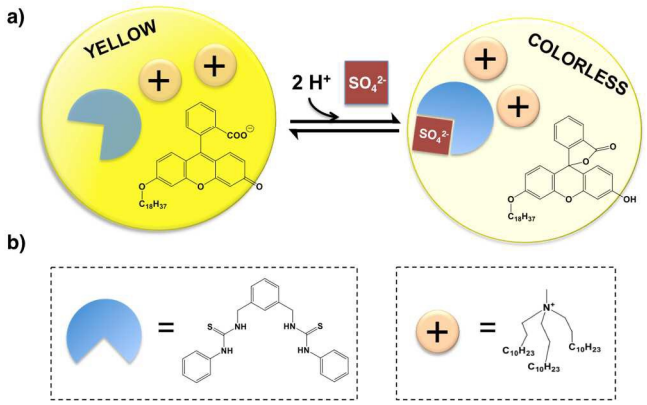

Fig. 1. (a) General mechanism of the sulphate sensor: the chromoionophore, the anion-exchanger and the sulphate ionophore are immobilized on the polystyrene beads. The carboxylate form of the chromoionophore, characteristic of the bright yellow color, and the lactone form, colorless, are represented. (b) Molecular structures of the sulphate ionophore and the anion-exchanger. For the sake of the simplicity, only one molecule of chromoionophore is represented (the whole species are presented in equation 2).

within a narrow range of concentrations, yielding a substantial increase in sensitivity. Based on these results, which suggest that hydration energy should have a much lower effect on the response of the sensor, we anticipated that such principle could be of significant benefit for sensing anions. In this work we present for the first time the detection of the highly hydrophilic divalent sulphate anion using polystyrene microspheres-based optodes which displays a dramatic enhancement of both, sensitivity and selectivity. The simple and accurate determination of sulphate in urine samples is presented to illustrate these features.

To build the sensor, the lipophilic ion-selective optode components: the chromoionophore fluorescein octadecyl ether (IndH), the anion-exchanger salt, i.e. tridodecylmethylammonium chloride (R^+Cl^-) and the bis-thiourea sulphate ionophore (L)[21], are immobilized by physical adsorption on the hydrophobic surface of polystyrene (PS) microspheres (0.8 µm diameter). Experimental steps to fabricate the sensors have been described elsewhere.[18] In short, a tetrahydrofuran (THF) solution containing the optode components was injected into a PS microspheres aqueous suspension (see experimental section in SI for details). Finally, the THF is removed from the suspension using a N_2 stream. A bright yellow color is immediately obtained, which accounts for the presence of the carboxylate form of the chromoionophore (Figure 1a).[22] This preferred form of the chromoionophore is in good agreement with a previous report based on cationic micelles where the carboxylate form was detected in presence of a lipophilic cationic surfactant cetyltrimethylammonium bromide (CTAB). Within this positively charged chemical surrounding, the pK_a of the fluorescein carboxyl moiety is 3.71.[23] The following experiments were then performed at pH 4.0 in acetic acid/magnesium acetate (HAc/MgAc) buffer solution to work under exhaustive sensing mode conditions.

First, a system labeled "blank sensor" containing all the components of the sensor with the exception of the ionophore was prepared. This system should display mostly unspecific anion-exchange properties. Second, the actual "sulphate sensor", which also incorporates the sulphate ionophore, was prepared in order to assess the influence of the charge and hydration energy of the anion together with the selectivity of the ionophore on the response (Table 1).

Successive additions of sulphate to the blank sensor (in HAc/MgAc buffer at pH 4.0) produced a drop of the absorbance in the visible range noticeable as the fading of the yellow color of the dispersion, which is measured as a decrease of intensity of the band with maximum absorbance at 459 nm (Figure 2a). This is an interesting observation, since the lack of a selective ionophore in the blank sensor suggests that the Coulombic interactions between the divalent sulphate and the anion-exchanger allow displacing the equilibrium present at the surface of the microspheres. Analogously to what was reported by Bakker et al[18], to hold the electroneutrality in the system, the extraction of SO_4^{2-} on the surface of the microsphere is followed by the transfer of two H^+ to two molecules of the chromoionophore, which forms the neutral lactone. Under these conditions, the chemical surrounding of the chromoionophore should be then comparable to the negatively charged micelles such as the sodium dodecyl sulfate (SDS) where the lactone form is almost colorless (Figure 1).[22] This proposed mechanism for the blank sensor can be expressed by the equation 1, where (PS) stands for polystyrene surface microspheres and (aq) for the aqueous media:

$$2\,R^+(\mathbf{PS}) + 2\,\mathrm{Ind}^-(\mathbf{PS}) + SO_4^{2-}(\mathbf{aq}) + 2H^+(\mathbf{aq}) \rightleftharpoons \rightleftharpoons (R^+)_2(SO_4^{2-})(\mathbf{PS}) + 2\,\mathrm{IndH}(\mathbf{PS}) \quad \textbf{(1)}$$

The loading of the optode components in the blank sensor was first optimized to balance the maximum sensitivity and the best selectivity against chloride (one of the most abundant anions in biological fluids) and thiocyanate (a highly lipophilic anion, usually considered a typical interference) (Table T1, SI). Figure 2a shows the response of the optimized blank sensor upon additions of sulphate up to 70 µM. Figure 2b displays the

corresponding calibration curves for sulphate and for some selected anions present in biological fluids. These results show a selectivity pattern that clearly deviates from the expected trend predicted by the Hofmeister series. Even in the absence of any selective ionophore,[24] the response to sulphate competes with that of some strongly lipophilic anions such as salicylate or thiocyanate, suggesting that Coulombic interactions play an important role here. This behavior is well illustrated by equation 1 where a single sulphate displaces two chromoionophore molecules.

Thereafter, the sulphate sensor was tested. Bis-thiourea was used as ionophore (as reported by Bühlmann *et al.*) since it engages strong hydrogen bonds with sulphate in polar solvents.[21] With the use of this selective ionophore, the sensitivity and selectivity can be significantly enhanced, as shown in Table 1.

Table 1. Analytical parameters compared for the blank sensor and the sulphate sensor.

	Sensitivity (Abs/µM)	Linear Range (µM)	LOD (µM)	Abs SO_4^{2-} / Abs Cl^{-*}
Blank sensor	0.001	2 - 20	1.2	3.01
Sulphate sensor	0.025	1 - 40	0.06	4.29

**Values compared at 20 µM*

Figure 3a shows the response of the sulphate sensor upon addition of the divalent anion with the corresponding calibration curve. Similarly to the blank sensor, response to typical interferences and major anions present in biological fluids at this pH is presented in Figure 3b. The incorporation of the ionophore significantly improves the detection of sulphate in terms of sensitivity, selectivity and linear range (see Table 1).

It is worth noticing that upon the incorporation of the ionophore, a drastic enhancement of the initial absorbance (A_0) of the system is observed. For the blank sensor A_0 is 0.75, while for the sulfate sensor is 2.01 (compare Figures 2a and 3a). This change could be attributed to an acid-base displacement produced by the ionophore[11] or to an increase of the molar absorptivity of the chromoionophore under these conditions. However, a deeper study of the nature of this change is out of the scope of this communication.

The calibration plot for SO_4^{2-} covers a very narrow linear range of detection from 1 to 40 µM with almost 1 absorbance unit difference. The sulphate sensor exhibits a very high sensitivity and concentrations as low as 60 nM could be detected by this system, which is far below the required detection limit for both health and environmental issues. Although the response to other anions is also increased, no significant interference was detected within the linear range (Figure 3b, Table 1). Following the same reasoning, the mechanism of this sulphate sensor could be expressed as follows:

$$2\,R^+(\mathbf{PS}) + 2\,Ind^-(\mathbf{PS}) + L\,(\mathbf{PS}) + SO_4^{2-}(\mathbf{aq}) + 2H^+\,(\mathbf{aq}) \rightleftharpoons \rightleftharpoons 2\,R^+(\mathbf{PS}) + L(SO_4^{2-})(\mathbf{PS}) + 2\,IndH(\mathbf{PS}) \quad \mathbf{(2)}$$

Interestingly, the sulphate sensor reported here illustrates an anti-Hofmeister behaviour. This result is particularly relevant since traditional membrane-based ion-selective electrodes and optodes based using same components fail to afford suitable selectivity for complex matrices. For instance, lipophilic thiocyanate and nitrate produced strong interference in latter systems, while chloride yields similar response than sulphate.

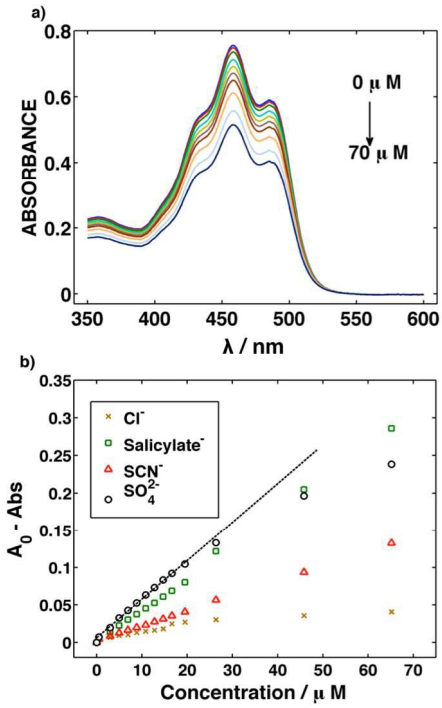

Fig. 2. (a) Spectra of the blank sensor in 10 mM HAc/MgAc at pH 4 upon additions of Na_2SO_4. (b) Calibration curves with the difference in absorbance at 459 nm (A_0- Abs) for SO_4^{2-} and other anions.

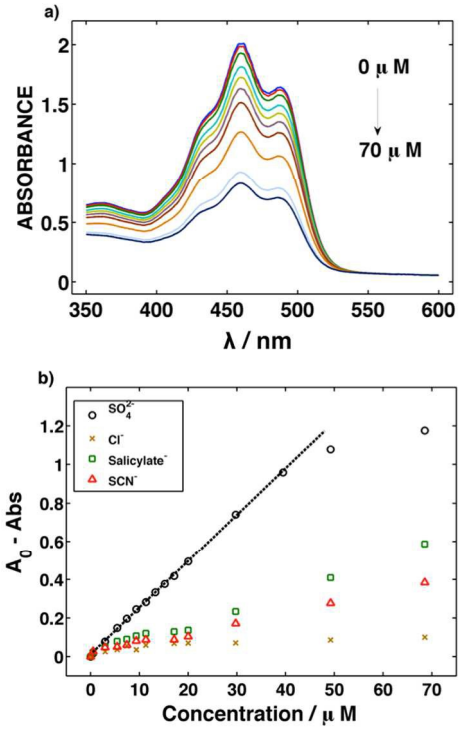

Fig. 3 (a) Spectra of the sulphate sensor in 10 mM HAc/MgAc at pH 4 upon additions of Na_2SO_4. (b) Calibration curves with the difference in absorbance at 459 nm (A_0- Abs) for SO_4^{2-} and other anions.

The dilemma of these membrane-based systems is that the partition between the organic and the aqueous phase leads to a selectivity that is largely dictated by the enthalpy of hydration of the anions. The absence of polymeric matrix, however, dramatically shifts the selectivity pattern, favoring the detection of highly hydrophilic divalent anions, such as sulphate. This fact has been confirmed in Fig S2, where a minimum amount of

15 times of Cl^- is needed to start having a significant interference from this anion. In addition, sulphate is one of the few divalent inorganic anions present at this pH in biological fluids.

The sensitivity and selectivity of the sulphate sensor encourage testing the ability of the sensor to measure sulphate in complex real samples (such as urine) since as previously mentioned monitoring this ion in clinical routine analysis is very difficult. In this work, determination of sulphate in five urine samples was performed. First, due to the physiological range of sulphate in urine (4-30 mM, Table T2, SI), samples had to be diluted to fit within the linear range of the technique. This is not a problem because of the low limits of detection achieved. Also, this dilution minimizes unspecific matrix effects. Direct measurement of the diluted samples was performed. All the values found felt within the expected physiological range.[6] Thereafter, a standard addition approach was followed by spiking the diluted samples with increasing amounts of sulphate. The slopes obtained by the standard addition approach did not significantly differed from the slope of the original calibration plot, evidencing that there is no significant matrix effects. Similarly, the estimated recovery of the added standards was always close to 100% (Table 2 and Table T2 SI). Finally, the differences between the standard addition and the direct calibration were less than 2%. Thus, the direct calibration should be enough.

Table 2. Detection of SO_4^{2-} in real urine sample.

Added (µM)	Predicted (µM)	Recovery (%)
3.9	4.0 ± 0.2	97 ± 4
4.8	4.9 ± 0.1	99 ± 3
5.8	5.8 ± 0.1	100 ± 3
6.8	6.8 ± 0.2	100 ± 4
7.6	7.8 ± 0.5	102 ± 7

Data shown are average of 3 measurements (n = 3)

In conclusion, an optical method to detect sulphate with modified polystyrene microspheres is detailed in this communication. The working principle is based on mass extraction equilibrium on the surface of the sphere, rather than on phase equilibrium. The modified spheres form a very stable suspension over time that displays a high sensitivity due to the bivalency of SO_4^{2-} within a narrow concentration range. Due to the enhanced selectivity achieved no significant interference from lipophilic anions was found. The enhanced sensitivity and selectivity allowed for the direct detection of sulphate in real urine samples with excellent recovery. The sensor developed here suggests a change of paradigm in the field of anion sensing where the Hofmeister behavior has been a strong limitation over the last decades in membrane-based sensors. Further work on sensing of other biologically relevant anions is currently underway in our laboratory.

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† Electronic Supplementary Information (ESI) available: Materials, experimental details and sensor are included. See DOI: 10.1039/c000000x/

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