Journal of Chromatography, 337 (1985) 259—266
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2371

DETERMINATION OF INORGANIC SULPHATE IN PLASMA BY REVERSED-PHASE CHROMATOGRAPHY USING ULTRAVIOLET DETECTION AND ITS APPLICATION TO PLASMA SAMPLES OF PATIENTS RECEIVING DIFFERENT TYPES OF HAEMODIALYSIS

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(First received May 4th, 1984; revised manuscript received September 11th, 1984)

SUMMARY

The determination of sulphate in plasma is described, making use of reversed-phase high-performance liquid chromatography with ultraviolet detection. The concentration of inorganic sulphate determined in plasma of twenty healthy volunteers was 0.307 ± 0.092 mmol/l (mean ± S.D.). In one stable chronic dialysis patient the kinetics of plasma sulphate removal were monitored during and after one single pass dialysis. In addition, plasma sulphate concentrations were determined in three stable chronic dialysis patients during a consecutive scheme of two single pass dialyses, five Redy® dialyses and three single pass dialyses. As expected, plasma sulphate accumulates in plasma to a high steady-state level under Redy dialysis, whereas during single pass dialysis sulphate is efficiently removed.

INTRODUCTION

The inorganic sulphate in the body is formed from sulphur-containing amino acids [1] and derived from resorption of inorganic sulphate from the gastro-

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intestinal tract [2]. The greater part of the inorganic sulphate is excreted by the kidneys, a minor part is used for sulphoconjugation reactions [3]. Mean serum or plasma sulphate values in healthy adults are found in the order of 0.3 mmol/l [4—14].

Up to now sulphate accumulation has only been mentioned in renal failure [1, 4, 15—17]. The exact clinical significance of elevated plasma sulphate concentrations is not known. Blum and Coe [18] mentioned metabolic acidosis in a diabetic patient with an elevated plasma sulphate level (2.6 mmol/l). This observation combined with studies of Hänze [19], who found a close inverse correlation between serum SO_4^{2-} and HCO_3^-, has led to the suggestion that SO_4^{2-} is involved in the development of metabolic acidosis in uraemia [17]. It is also suggested that elevated plasma sulphate levels could be involved in the pathogenesis of renal osteodystrophy [17].

During single pass dialysis many of the accumulated residual products, including sulphate, are efficiently removed [15]. The disadvantage of this dialysis technique, namely the use of ±180 l of dialysis solution, was overcome by the Redy® dialysis system. This system makes use of constantly regenerated dialysis solution (±6 l), which is filtered through a number of filter layers removing heavy metals and oxidizing agents, cations, anions, and organic compounds such as urea, creatinine and uric acid [20]. During this process also calcium, magnesium and potassium are largely removed so that these ions have to be constantly infused into the dialysate. On the other hand, it has been reported that sulphate accumulated in plasma when the original type of Redy cartridges were used [15]. For that reason we decided to undertake a study in order to find out whether such an accumulation also occurs in the more recently developed versions of the Redy system. For sulphate determination in plasma we worked out a reversed-phase high-performance liquid chromatographic (HPLC) method using ultraviolet (UV) detection.

MATERIALS AND METHODS

Materials

The anion-exchange resin AG1-X10 (100—200 mesh) was supplied by Bio-Rad Labs. (Richmond, CA, U.S.A.). All other chemicals were purchased from E. Merck (Darmstadt, F.R.G.) and were of analytical grade. Heparinized tubes (Venoject VT 100H) were from Terumo Europe (Belgium). Glass-distilled water was used throughout. The 10-cm analytical column (I.D. 4.6 mm) and 3-cm guard column RP-18 Spheri-5 (I.D. 4.6 mm) were from Brownlee Labs. (Santa Clara, CA, U.S.A.). For the single pass dialyses the Gambro AK-10 dialysis monitor (Lund, Sweden) was used. For the sorbent cartridge dialyses the Redy system, consisting of Sorbsystem SS-EA with cartridge type D 32/60 (Organon Technika, Turnhout, Belgium), was used.

Patients and samples

A plasma pool was made by mixing plasma samples obtained from healthy persons as well as from patients. Plasma samples of ten healthy males and ten healthy females, aged 20—60 years, were used for the determination of the normal plasma sulphate concentration.

Patient A was a 69-year-old woman, patient B was a 52-year-old woman and patient C was a 44-year-old man, all stable, chronic dialysis patients, on single pass for many years. Patients A and C were dialysed on days 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, zero being the day of the first dialysis. On days 0, 3, 24, 28, 31 they were dialysed by single pass dialysis, on days 10, 14, 17, 21, 24 by the Redy dialysis system. Patient B was dialysed on days 0, 4, 7, 11, 14, 18, 21, 25, 28, 32, zero being the day of the first dialysis. On days 0, 4, 25, 28, 32 she was dialysed by single pass dialysis, on days 7, 11, 14, 18, 21 by the Redy dialysis system. All dialyses lasted 7 h; plasma samples were taken just before the start and immediately after the end of the dialysis. For monitoring the sulphate concentration during and after a single pass dialysis patient A was selected. The plasma samples were taken at 1 min before and 2, 4, 6, 7 h after the start of the dialysis. Seven hours after the start of the dialysis the patient was disconnected and again plasma samples were taken at 8, 9, 10 and 22.5 h (counted from the start of the dialysis).

Determination of inorganic sulphate in plasma

Sample preparation. The anion-exchange material was allowed to stand for 18 h on water before use. Columns with a height of 1 cm and an I.D. of 1 cm were prepared from this material. Before use the columns were washed successively with (a) 10 ml of 100 g/l ammonium acetate, (b) 5 ml of water, (c) 5 ml of 0.1 mol/l hydrochloric acid, and (d) 5 ml of water. This washing procedure was repeated once more. Then 200 μl of plasma or sodium sulphate standard solution were added to the column, which then was rinsed with 3 × 5 ml of water. The sulphate was eluted with 2 × 3 ml of 100 g/l ammonium acetate solution. The eluate was dried at 160°C under a stream of air. After disappearance of the solvent the sample was heated for another 30 min. Then 500 μl of water were added and heated to dryness at 160°C under a stream of air. After cooling to room temperature the samples were redissolved in 1000 μl of the HPLC mobile phase (see below). If necessary the samples were diluted further.

HPLC determinations. Determinations were carried out on a Waters HPLC pump Model M6000A (Waters Assoc., Milford, MA, U.S.A.) equipped with a reversed-phase guard column and a reversed-phase analytical column (see section on materials) and a Pye Unicam (Cambridge, U.K.) Model PU 4020 UV detector. The output filter of the UV detector was set at 2 sec and the range at 0.04 a.u.f.s. The electrical output was fed to a Philips dual-line recorder (Philips, Eindhoven, The Netherlands) Model PM 8252, which was set at 10 mV and 20 mV full scale and coupled to a Spectra Physics computing integrator (San Jose, CA, U.S.A.) Model SP 4100. The mobile phase was a solution of $5 \cdot 10^{-4}$ mol/l potassium hydrogen phthalate and 10^{-3} mol/l tetrabutylammonium bromide. The pH was adjusted to 5.9 with 1 mol/l sodium hydroxide. The flow-rate was 1.5 ml/min. A 100-μl volume of sample was automatically injected on the column with a WISP 710B autosampler (Waters Assoc.). The sulphate concentration was determined by comparing the integrated peak area with those obtained from standard sulphate solutions.

RESULTS AND DISCUSSION

At present, plasma sulphate determinations, in contrast to other anions (i.e. chloride, phosphate and bicarbonate), are not routinely performed in clinical chemical laboratories. Most of the determinations of plasma sulphate reported up to now, are based on the precipitation reaction $Ba^{2+} + SO_4^{2-} \rightarrow BaSO_4$. The $BaSO_4$ formed can be determined by gravimetry [4], nephelometry [5] or by turbidimetry [6]. Sulphate can also be determined indirectly by measuring the remaining Ba^{2+} concentration, after its addition in excess, employing flame photometry [7], radioisotope labelling [8—10] or atomic absorption spectrophotometry [11]. Another indirect method is by colorimetry [12, 21]. Recently, so-called ion chromatography, employing HPLC, has attracted attention as a very elegant method to measure a variety of ions. In general, commercially available instruments, so-called ion chromatographs, using conductimetric detection, are employed. This technique has

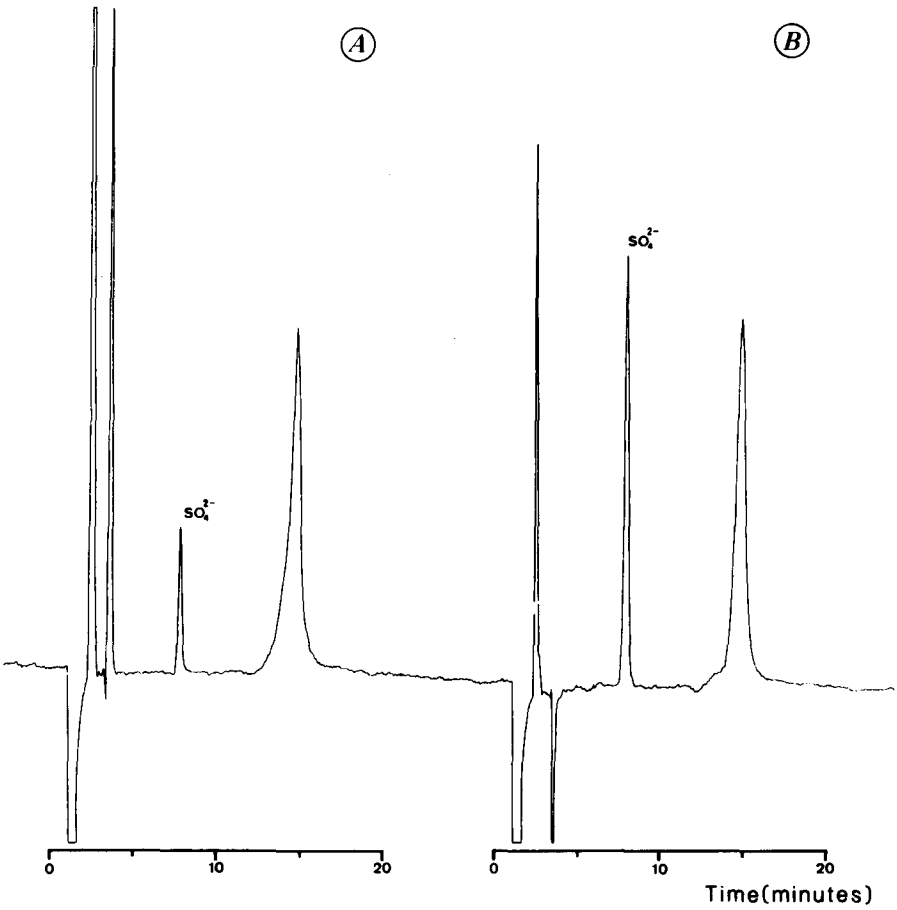

Fig. 1. Examples of chromatographic runs showing sulphate determinations of a normal plasma sample (A, sulphate amount on column 10 nmol) and a 1:1 diluted, elevated (B, sulphate amount on column 48 nmol), cleaned-up plasma sample. Ordinate represents decrease in absorption, a.u.f.s. = 0.04, recorder output 20 mV full scale.

been successfully applied in the determination of sulphate in plasma [13, 14], but most laboratories, including our own, are not in possession of this special instrumentation. However, anions can also be measured by HPLC with UV detection, as has been shown by Cochrane and Hillman [22]. It should be possible to adapt this method for the determination of plasma sulphate. As plasma and serum contain many interfering substances, considerable clean-up is needed before injecting the samples.

An effective clean-up of plasma samples was achieved by passing them through an anion-exchange resin. After thoroughly washing with water, the retained anions, including sulphate, were then eluted as ammonium salts by concentrated ammonium acetate solution. After drying at 160°C, all volatile salts, such as ammonium acetate, ammonium bicarbonate and ammonium nitrate, including the greater part of the ammonium chloride, have disappeared. The "clean" plasma sample is, after redissolution in HPLC eluent, injected into the HPLC system. Examples of a normal and an elevated plasma sulphate level are shown in Fig. 1. Aqueous sodium sulphate standards were similarly worked up. A linear calibration curve was constructed in the range 0—2 mmol/l. Plasma samples with a sulphate concentration above 2 mmol/l were diluted with HPLC mobile phase.

The within-day variation and day-to-day variation in the pooled plasma and the corresponding mean values were determined, being 0.51 mmol/l, C.V. 4.8%, and 0.50 mmol/l, C.V. 5.3%, respectively. In addition, sulphate in varying amounts was added to aliquots of this pooled plasma. After determination of the sulphate concentration, recovery of the added sulphate was calculated. The results are listed in Table I.

After having established that plasma sulphate determinations were reproducible and that the recovery of added sulphate was correct, plasma sulphate levels of twenty healthy individuals were determined. They were normally distributed, the mean value ± S.D. being 0.307 ± 0.092 mmol/l. In Table II normal values of plasma or serum sulphate, as determined using different techniques, are shown and turn out to be in close agreement with those found by the present method.

The plasma sulphate was monitored in patient A during and after one single

TABLE I

RECOVERIES OF SULPHATE ADDED TO POOL PLASMA

Given are the measured amounts (μmol) of sulphate and the recovery percentages (in parentheses).

Amount of SO_4^{2-} added to 1 ml of pooled plasma (μmol)					
0	0.125	0.25	0.50	1.00	2.00
0.55	0.67 (96)	0.79 (96)	0.99 (88)	1.57 (102)	2.53 (99)
0.53			1.03 (100)	1.54 (101)	
0.52			0.98 (92)	1.49 (97)	
0.52			1.06 (108)	1.57 (105)	
0.50			0.98 (96)	1.45 (95)	
0.55			1.01 (92)	1.64 (109)	

TABLE II

NORMAL SERUM AND PLASMA SULPHATE LEVELS IN ADULTS

n = number of samples analysed.

Authors	Method	n	Concentration (mmol/l) (mean ± S.D.)
Kleeman et al. [12]	Colorimetry	15	0.296
Berglund and Sörbo [6]	Turbidimetry	10	0.33
Miller et al. [8]	γ-Spectroscopy	88	0.323 ± 0.085
Leskovar and Weidmann [7]	Flame photometry	50	0.315 ± 0.032
Cole et al. [9]	γ-Spectroscopy	19	0.297 ± 0.038
Cole and Scriver [10]	γ-Spectroscopy	10	0.33 ± 0.05
Michalk and Manz [11]	Atomic absorption spectrometry	12	0.290 ± 0.057
Cole and Scriver [13]	Ion chromatography	16	0.30 ± 0.05
De Jong and Burggraaf [14]	Ion chromatography	40	0.325 ± 0.053
This study	Reversed-phase chromatography	20	0.307 ± 0.092

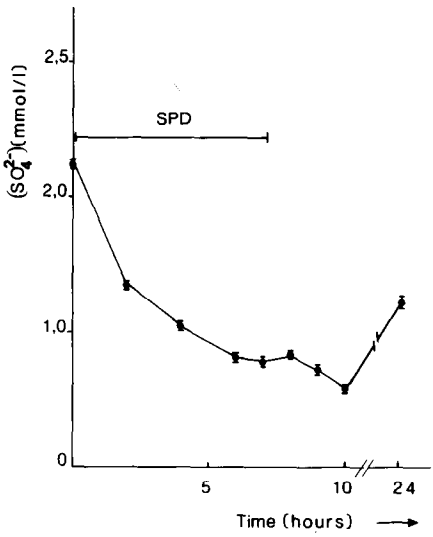

Fig. 2. The plasma sulphate concentration (± S.D.) in patient A during and after one single pass dialysis (SPD).

pass dialysis (see Fig. 2). The results clearly demonstrate a fall in plasma sulphate concentration during dialysis. During the last hour of this dialysis the sulphate seems to reach a steady-state level. After the end of the dialysis the sulphate concentration decreases slightly again, before giving the expected rise.

In the three patients (A, B, C) the plasma sulphate was measured before and after a number of consecutive dialyses: first, two single pass dialyses; secondly, five Redy dialyses, and finally, three single pass dialyses (see Fig. 3). It is clear that in all three patients sulphate accumulation occurs during Redy dialysis, as already found by others using older types of cartridges [15]. The plasma sulphate level finally seems to reach a maximum value during the Redy dialysis;

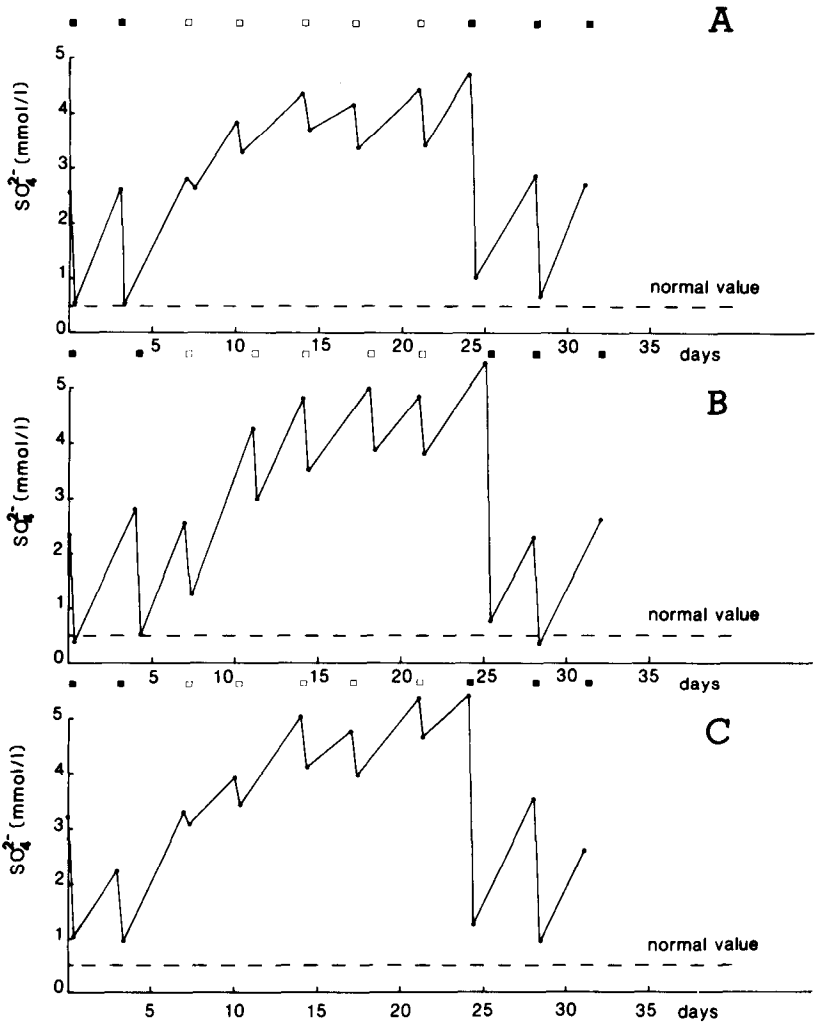

Fig. 3. The plasma sulphate concentration in patients A, B and C during a scheme of consecutive two single pass dialyses, five Redy dialyses and three single pass dialyses. ■, Single pass dialysis; □, Redy dialysis.

after subsequent single pass dialysis the excess sulphate is again efficiently removed.

From these relatively simple experiments it can be concluded, that the present method can be successfully applied in studies concerning the removal of plasma sulphate in patients subjected to different kinds of dialysis.

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