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Journal of Chromatography A, 789 (1997) 221–232

JOURNAL OF CHROMATOGRAPHY A

Review

Quantitation of sulfate and thiosulfate in clinical samples by ion chromatography

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Abstract

For assay of serum sulfate, quantitation by ion conductimetry after separation by anion-exchange chromatography is the method of choice. In comparison to classical barium precipitation methods, chromatographic methods demonstrate increased precision, specificity and sensitivity, and they may be superior to spectrophotometric methods that rely on organic cation precipitation of sulfate. The increased sensitivity and specificity, as well as the inherent capacity of chromatographic methods for simultaneous determination of other anions, has led to its increasing use in the determination of excreted sulfate in clinical profiles of urinary anion composition. Ion chromatography can also be used to quantitate free sulfate in other clinical samples, including cerebrospinal fluid, sweat, saliva, breast milk and human tissues. Finally, ion chromatography shows promise as a more precise and sensitive method for measurement of total acid-labile sulfoesters and thiosulfate.

Keywords: Reviews; Sulfate; Thiosulfate; Inorganic anions

Contents

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0021-9673/97/$17.00
PII S0021-9673(97)00821-2

1. Introduction

Sulfate and thiosulfate are divalent oxyanions that can be found in many biological fluids. While the use of ion chromatography to quantitate these species has been reviewed recently [1,2], there has been little attention paid to the unique aspects of this method as it is used in clinical laboratories. This review addresses pre-analytical, analytical and interpretive aspects of past studies and current procedures. Because our experience has been primarily with various post-column suppression instruments, we emphasize this configuration, but many of the issues raised apply to the other anion chromatographic techniques.

2. Sulfate

2.1. Introduction

In most clinical samples, sulfate is present in two forms. The preponderant form is the free inorganic sulfate anion (SO_4^{2-}), while a minor portion is covalently bound in ester or amide form (RSO_3^-) to a wide range of organic compounds. From the earliest days of clinical chemistry [3], the prevalent methods for sulfate determination have been based on quantitation of the barium sulfate precipitation reaction [4–6]. Although other assays for sulfate have been described [6], the only one that has been widely used in the clinical setting is the precipitation reaction with benzidine [7,8]. However, the reduced solubility of the benzidine sulfate complex, which makes this method inherently more sensitive than the barium reaction, is somewhat offset by the increased interference from other oxyanions, notably phosphate. More recently, increased recognition of the marked carcinogenicity of benzidine has seriously reduced its utilization, and it would be difficult to rationalize its re-introduction into the clinical laboratory, given the increasing safety requirements now being mandated by regulatory agencies. Turbidimetric assay of suspended barium sulfate microcrystals after addition of barium chloride and a barium sulfate ''seed'' remains a robust method in samples with high sulfate concentrations or stable matrix interferences [9,10]. In human serum, however, the range of sulfate concentrations is such that the assay becomes vulnerable to large and variable error rates at the lower limits of normal and pathological specimens [11]. Automation may reduce some of this variability [12,13], but the method is intrinsically limited in comparison to ion chromatography [14].

Shortly after the first descriptions of anion suppression chromatography in 1975 [15,16], Anderson [17] reported the potential of this technique for sulfate determination in clinical specimens. In 1981, we [18] validated the serum assay by comparison with a radiolabelled barium precipitation method we had already established (Fig. 1, right panel). In 1984, we argued that suppressed-mode ion chromatography and conductimetric quantitation should be the reference method for any other type of serum sulfate assay [14]. It should be noted that methods for reliable determination of serum sulfate by non-suppressed anion chromatography have been reported by Morris and Levy [19], Hoffman et al. [20] and Buchberger and Winsauer [21].

2.2. Serum sulfate

2.2.1. Pre-analytical considerations

Sulfate is ubiquitous in the laboratory environment and the accuracy of serum sulfate determinations by ion chromatography depends on careful attention to minimizing background interference. Among the contamination sources we have encountered more than once are: the water supply, the blood collection tubes, the heparin used for antiocoagulation [22], some reagent grade salts and acids, various filtration

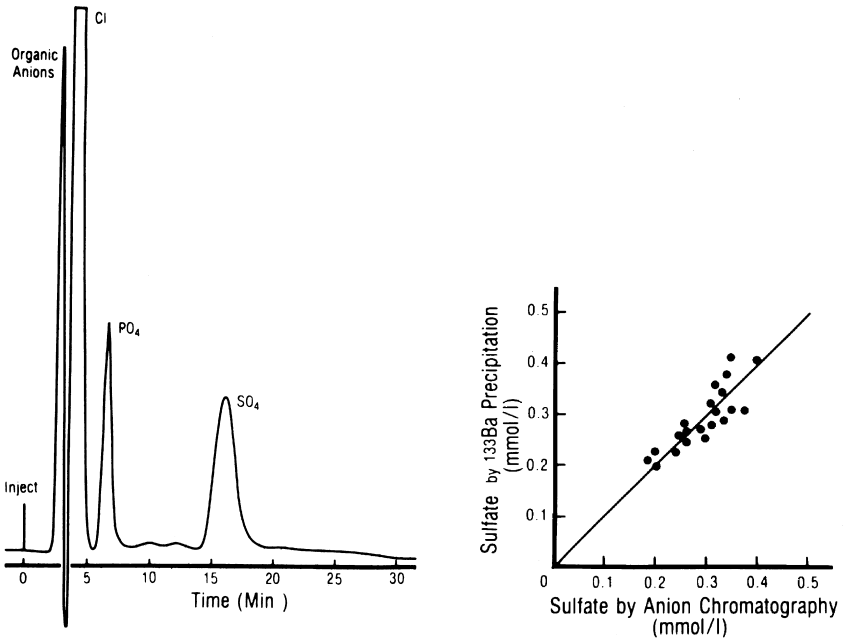

Fig. 1. Ion chromatography of plasma. The left panel shows the chromatogram for infant plasma analyzed on a first-generation system (D-10 Ion Analyzer, Dionex, Sunnyvale, CA, USA). The instrument was outfitted with an AG1 50×3 mm pre-column, an AS1 500×3 mm separator column and a 250×6 mm fixed-bed suppressor column, in series. The eluent (2.4 m*M* Na_2CO_3 and 3.0 m*M* $NaHCO_3$) flow-rate was 2.3 ml/min [59]. The right panel shows the line of identity and the good correlation ($r=0.87$, $p<0.001$) between values obtained with ion chromatography (abscissa) and those obtained with a barium precipitation method (ordinate) that relies on quantitation of the radiolabelled barium remaining in the supernatant [18,109].

devices, and plaster dust from nearby construction. We have also noted minor contamination from pipette tips, microfuge tubes and even tobacco smoke, on one occasion. In most instances, it is best to carry a blank through all of the assay steps, starting with the blood collection tubes. Our recommended practices include: (i) use of distilled, deionized water (ddH_2O), with periodic checks to ensure a background resistance of greater than 18 MΩ/cm; (ii) use of ultrapure reagents with documentation of low sulfate content; (iii) use of plastic disposable tubes that are essentially sulfate-free; (iv) analysis of serum rather than plasma, since most of the anticoagulants are potential sources of analytical problems and reference data are largely based on serum; and (v) use of disposable filtration devices that can be pre-washed with a sulfate-free solution, unless a significant volume of initial filtrate can be discarded. We found that 1 m*M* solutions of either ultrapure HCl or HNO_3 may be more effective as pre-wash solutions than ddH_2O in eliminating sulfate contamination, particularly with sulfate-containing filtration devices [23]. We suggest that the blank can be less than 10% of signal, if appropriate precautions are taken. A fasting morning sample is probably the preferred type of sample, since there is circadian variation [24] and variable increases associated with a protein meal [11,25].

For most serum analyses, serum deproteinization should be considered, since not all serum proteins will be cleared from the column with the standard eluents. If a small disposable pre-column is included in the configuration, it may be possible to dilute the sample and replace the pre-column at frequent intervals. Dilution of serum in 1 m*M* NaOH will help prevent any increase in pressure resulting from on-column protein precipitation, but it will not address the drift that tends to be seen in such systems because of loss of separation due to protein adherence to the column(s). Deproteinization by acid precipitation (e.g., trichloracetic acid) works well for most barium sulfate methods, but may be unsuited

for anion chromatography because the acid anion will saturate the conductivity detector and overwhelm the sample signal. Reiter et al. [26] have reported that perchloric acid can be used if the residual acid is precipitated with potassium carbonate. An alternative to acid precipitation is acetonitrile extraction if the column packing is compatible [19]; however, the residual protein in the water phase after extraction may be substantial [26]. Another strategy is to seek a filtration device that removes most higher-molecular-mass proteins and is relatively sulfate-free [20,27–29]. Sulfate is not significantly bound to serum proteins retained in the process of ultrafiltration, but the concentrating effect of protein exclusion may result in an increase of up to 7% in the sulfate concentration of the ultrafiltrate in comparison to unfiltered serum [23]. Hoffman et al. [20] suggest that efficiency of deproteinization can be checked with urine protein ''dipsticks'' found in most clinical chemistry laboratories.

Sulfate is inert and therefore does not by itself present problems with storage or transport. However, a serum sample that is not refrigerated or frozen will eventually deteriorate and give rise to unpredictable matrix interference, even if it is subsequently deproteinized. It can be argued that samples that have been acid-deproteinized may eventually liberate inorganic sulfate from serum sulfoesters at room temperature, but the amounts would normally be insignificant. However, if the samples are derived from patients treated with drugs that undergo sulfoconjugation, this may not be so (M. Morris; personal communication). In such cases, deproteinization methods employing acetonitrile or ultrafiltration may be less problematic and have the added advantage of preserving the sample for transport over long distances at ambient temperatures.

2.2.2. Analytical

The initial description of suppression-mode anion chromatography specified a carbonate–bicarbonate buffer and a proprietary form of low-capacity anion-exchange resin [15,17]. Details of the basic instrument configuration can be found in many other sources [2,16]. For serum, this combination produces a low-conductivity carbonic acid background after suppression and accentuates the conductimetric signal of those few low-molecular-mass anions with very high acid dissociation constants. Chloride, which accounts for more than 90% of the non-protein fixed anionic charge in serum, is eluted early and its large conductimetric signal (>300-fold molar Cl^-/SO_4^{2-} ratio) does not interfere with the sulfate peak (Fig. 1: left panel). Because of the high pH of the carbonate–bicarbonate eluent, phosphate is present either as HPO_4^{2-} or PO_4^{3-}, and elutes in an intermediate position but its molar concentration is generally no more than 10-fold greater than sulfate and it does not interfere. Organic anions may co-elute with sulfate, if efforts are not undertaken to avoid this, but they generally have a very small conductimetric signal. Because of their high p*K* values, they contribute minimally to any sulfate signal. The small amounts of nitrate and bromide found in most serum samples elute near sulfate but do not normally interfere. Under appropriate conditions, they can be independently quantitated by ion chromatography [30].

Theoretically, the conductimetric signal becomes more linear with increasingly dilute sulfate solutions [16], in contrast to the loss of accuracy on dilution experienced with chemical precipitation methods. From a practical standpoint, though, serum dilutions that range between 1-in-5 and 1-in-50 appear to give the best results. At lower concentrations, background noise eventually limits accurate quantitation of the sulfate signal, while higher concentrations show increasing matrix interference. At concentrations between 1 and 25 μM, the conductimetric response is essentially linear, but it may be advisable to verify this if the ion chromatographic assay is being used to quantitate sulfate in pathologic specimens with either wide variation in sulfate concentrations or substantial deviations from the reference interval. An important example of this is the assay of sera in renal patients, with their substantial and variable increase in serum sulfate [11,31–35].

Although conductimetry is the preferred detection mode with suppression anion chromatography, serum sulfate has been quantitated using ultraviolet absorbance detection and a chromophoric eluent — for example, phthalate [36]. While precision is reduced [37], it obviates the necessity of having a conductimetric detector [36].

2.2.3. Interpretive

Although there is general agreement that the approximate physiological range of human serum

sulfate values extends from about 100 to 700 μ*M*, little is known about the extent to which the range of serum sulfate is regulated in the adult human. A comparison of fifteen studies (Table 1) indicates that the fasting serum sulfate measured by barium precipitation method is about 10% higher than that obtained with ion chromatography. In general, reported reference intervals have not been different between men and women, although there is some suggestion that levels in women may fluctuate with the menstrual cycle [11] and menopause [38]. There are significant age-related changes. In the elderly, the increased serum sulfate is probably related to physiological loss of renal glomerular filtration function [11]. In fact, any elevation of serum sulfate should be interpreted with caution if renal function has not been assessed. One exception is the elevation of serum sulfate in infants and young children, which may be related to altered renal handling to meet specific sulfate requirements associated with growth [39–41]. In late childhood and adolescence there may be a decline to concentrations less than those seen in adults [11,40]. Serum sulfate rises throughout pregnancy [11,42,43] as a result of increased renal reclamation [44]. This occurs in the face of net transfer to the growing fetus, as reflected in the increased serum sulfate observed in fetal or cord blood [42,45].

A discussion of serum sulfate changes in response to disease is beyond the scope of this review, but some relevant clinical conditions (with pertinent references) are given in Table 2.

2.3. Urine sulfate

Because sulfate is found in millimolar amounts in urine, a variety of barium precipitation methods give accurate clinical quantitation [12,46,47]; previous reviews [11,48] will direct the reader to earlier literature summarizing other methods. Ion chromatography has been used in the determination of urinary sulfate determination for two reasons. First, urinary sulfate itself has been increasingly regarded as a clinically important factor in the pathobiology of renal stone formation [49]; second, other anions that contribute more directly to urinary stone formation, particularly oxalate, can also be measured by ion chromatography [50–53] — in some cases simul-

Table 1
Range of serum sulfate concentrations assayed by different methods

Investigator (Ref.)	Serum sulfate (μ*M*)	Method (Ref.)
Kleeman et al. [8]	296[1]	Benzidine precipitation and spectrophotometry
Berglund and Sorbo [5]	330	Barium turbidimetry
Michalk and Manz [107]	291±57	Ba^{2+} precipitation and atomic absorption spectrophotometry
Morris and Levy [43]	333±38	Barium turbidimetry [5]
Morris and Levy [108]	410±43	Barium turbidimetry [5]
Miller et al. [109]	323±85 (88)	Radiolabelled barium assay
Cole et al. [18]	297±38 (19)	Modified radiolabelled barium assay [109]
Cole and Scriver [40]	330±50 (10)	Modified radiolabelled barium assay [18]
Soliman et al. [110]	400±90 (93)	Barium precipitation with [^{35}S]sulfate
Cole and Scriver [59]	300±50 (16)	Suppression ion chromatography and conductimetry
Cole and Landry [23]	298±37 (22)	Suppression ion chromatography and condictimetry [59]
de Jong and Burggraf [30]	325±53 (40)	Suppression ion chromatography and conductimetry
Reiter et al. [26]	302±57 (41)	Suppression ion chromatography and conductimetry
Koopman et al. [36]	307±92 (20)	Ion chromatography and UV detection
Benincosa et al. [38]	290±60 (22)	Ion chromatography and conductimetry [19]
Overall mean[2]	322±10 (15)	

[1] Values are mean±S.D. (if sufficient information available), with the number of subjects tested in parentheses.
[2] Overall mean is an unweighted average (±S.E.) for all fifteen studies. There was significantly less variation amongst the ion chromatography methods ($p=0.0056$; *F*-test comparison of variances), and the average concentration for the IC methods (304±5 μ*M*, $n=6$) was less than that for the precipitation assays (334±43 μ*M*, $n=9$) but the difference was not significant.

Table 2
Clinical conditions associated with altered serum sulfate

Increased serum sulfate	Decreased serum sulfate
Renal glomerular dysfunction [11]	Hypothyroidism [11]
Renal failure [32,34,112]	Diabetic ketoacidosis [118]
Hyperthyroidism [11]	Rheumatoid arthritis [119,120]
Hypertension	Fanconi syndrome [121]
Essential [113]	Acetaminophen ingestion [108,122]
Pregnancy-induced [44]	Ingestion of drugs undergoing sulfation [119] as well as other agents [123]
Total parenteral nutrition	
HSO_3^- or SO_4^{2-} solutions [114]	
Elemental sulfur ingestion [115,116]	
Magnesium sulfate treatment [117]	
Calcitriol treatment [111]	

taneously [28,54]. Efficient analysis of urinary sulfate is usually achieved with 1:100 dilutions of sample and there is little matrix interference. In adults and children, we find values [55] that are essentially the same as those determined by barium sulfate turbidimetry [9]. However, it should be noted that urinary sulfate concentrations normally vary much more widely than those for serum sulfate, largely because the kidney acts as a homeostatic regulator of the serum pool [56]. Thus, it may be necessary to analyze some samples at different dilutions. Samples from infants tend to contain less sulfate and can be more precisely analyzed at 1:30 or lower dilutions [39]. For specimens that have been refrigerated or frozen, it may be advisable to warm the samples to body temperature (37°C), mix them well, allow them to cool to room temperature, then centrifuge them to remove urinary crystals, cellular debris and other precipitates that might otherwise shorten the life of the column. Finally, it may be useful to pass the sample through a solid-phase C_{18} cartridge to remove lipophilic matrix components that also shorten column life, but this could necessitate contamination checks and further recovery studies for certain types of pathological urine specimens.

For preservation and transport of urine samples, clinical laboratories have often relied on the addition of a few drops of 6 *M* HCl to prevent crystal formation and inhibit bacterial overgrowth. Although acid hydrolysis of sulfoesters is slow at ambient temperatures, this practice could be a concern. More importantly, because some forms of HCl may contain significant amounts of sulfate, we have recommended a commercially available boric acid-based preservative (b.a. tablets, Oti Specialties, Santa Monica CA, USA). The borate produces only a small non-interfering conductimetric peak, the tablets introduce no detectable sulfate contamination, and the pH shift is small. Toluene and other less toxic organics have been also recommended as preservatives but we would avoid them because of their potential adverse effects on the columns.

Urine samples pose specific problems in reporting and interpretation. It is rare that the concentration itself is clinically useful, since the concentration of most urinary metabolites (including sulfate) varies with varying urinary volume, which itself is a renal homeostatic response to water and electrolyte changes. In some cases, it may be sufficient to report the daily excretion, but this necessitates obtaining (and verifying?) complete 24-h urine collections. As Lundquist et al. [9] have reported, it also fails to correct for gender differences. Creatinine may be used as a denominator [39], so that excretion is reported in mol sulfate per mol creatinine and in that way untimed single urine samples can be compared. However, this practice introduces another analytical variable which may complicate clinical interpretation. In instances where renal handling of sulfate is being evaluated in individuals of different age and gender, body weight is often a critical covariate [44,57,58]. In undertaking urinary determinations, it would be helpful for analytical personnel to consult

with clinical staff about sampling protocols and the supplying of the essential clinical information, such as age, weight or gender.

2.4. Sulfate in other fluids

2.4.1. Cerebrospinal fluid

The matrix of cerebrospinal fluid (CSF) differs significantly from serum or plasma in that there is 200-fold less protein and the normal sample is transparent and colourless. Although anion chromatography can be performed on samples without deproteinization [59], it would be wise to check the protein content of any turbid or coloured samples. While the chloride content can be predicted on the basis of Gibbs–Donnan equilibrium between the circulation and the CSF spaces, sulfate concentrations are much lower, in keeping with an active transport process [59,60]. We observed a mean CSF concentration of 160 ± 90 μM in nine infants and children [59], in comparison to a mean of 135 ± 66 μM ($n=25$) that we found using a barium method [60]. Using unsuppressed ion chromatography [19], Morris et al. [61] observed significantly lower values (89 ± 31 μM, $p=0.006$, Student t-test, correcting for unequal variances) in seventeen adults who were 22 to 72 years old.

2.4.2. Sweat

In clinical studies, sweat specimens are routinely collected for the diagnosis of cystic fibrosis, but the sample volume is usually on the order of 100 μl or so, even with stimulation of sweat secretion by pilocarpine iontophoresis. Using conventional collection apparatus, we found that uncontaminated samples required careful pretreatment, particularly the absorbent disks used to collect the sweat, but we [62] were able to demonstrate substantially lower levels in normal sweat (72 ± 4 μM) than in serum (Fig. 2). More efficient collection of uncontaminated samples can be achieved if a plastic collection device (Macroduct, Wescor Instruments, Logan, UT, USA) is used [62], and it appears that the risk of contamination is reduced.

2.4.3. Saliva

To our knowledge, the only published assay of sulfate in saliva by ion chromatography is a study we reported [23] in the context of examining the anion composition of secreted fluids in patients with cystic fibrosis (Fig. 2). Saliva is particularly rich in macromolecular polyanions which promote column degradation, so saliva must be pretreated. Using prewashed, sulfate-free ultrafiltration membranes, we found a salivary sulfate concentration of 72 ± 4 μM in seventeen adult fasting volunteers. de Vries et al. [63] found a mean salivary sulfate of 310 ± 10 μM ($n=20$) using a benzidine method but did not indicate how the samples were processed. The discrepancy may arise from non-specific benzidine trapping with soluble salivary polyanions or interference by the abundant sulfated macromolecules in the salivary matrix.

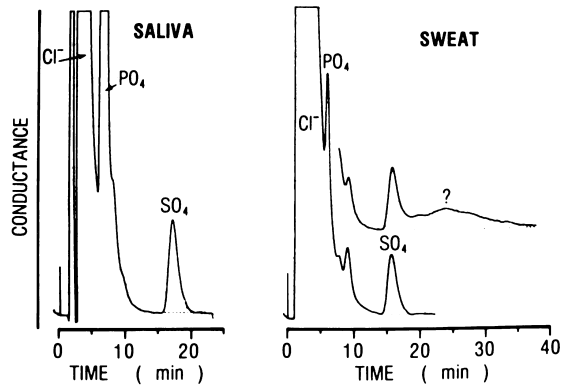

Fig. 2. Chromatographic profiles for saliva and sweat [23]. Chromatographic conditions were the same as those described in Fig. 1, except that an additional AG1 guard column preceded a second AG1 guard column in series, improving the separation but lengthening the elution time. Note that the separation of sulfate from major anions is essentially not different from that for serum, although the concentrations of other anions vary considerably. Unidentified, late-eluting anions (probably organic, indicated by ?) were found to limit the precision of analysis in some sweat samples.

2.4.4. Amniotic fluid

Clinically, amniotic fluid may be obtained from the amniotic sac at the end of the first trimester of pregnancy. Until the end of the second trimester, its composition resembles that of fetal serum, since the largest source is exudate through the fetal dermis. In the last trimester, the fluid increasingly resembles fetal urine as the fetal skin becomes impermeable

and maturation of renal function leads to excretion of concentrated fetal urine into the amniotic sac. No special treatment of the amniotic fluid sample appears to be required. As might be expected, the concentrations rise from levels close to that of maternal serum in the second trimester (reference range: 160–580 μM) to levels more typical of urine in the third trimester (reference range: 550–890 μM) [64].

2.4.5. Tissue sulfate

Under most circumstances, intracellular fluids are not routinely sampled in the course of clinical medicine. However, it is now clear that inherited deficiencies of sulfate transporters [65] mediating exchange between intra- and extracellular spaces, cause severe congenital anomalies of bone and cartilage [66,67]. This is in contrast to earlier work that suggested sulfate was excluded from intracellular spaces [68,69]. The fact that radiolabelled sulfate is excreted in the urine before equilibrating with intracellular spaces, only confirms that some large intracellular pools such as that of skeletal muscle [70] are indeed low in sulfate, while others, such as cartilage, liver and kidney, maintain high levels of intracellular sulfate for sulfoester synthesis. Although this can be determined qualitatively by tracer experiments, direct assay of sulfate allows monitoring of changes in specific activity with endogenous pools. For tissue determination, the barium method may be adequate [11,71,72], but it is difficult to show that other co-precipitants are not confounding the results and recovery does vary. In this context, we found that ion chromatography has the advantage of small sample requirement, increased sensitivity and lack of interference from other anions [59]. In human placental tissue, we used ion chromatography to validate [^{35}S]sulfate kinetics [37], as have other investigators using human epithelial cell culture systems [73,74].

2.4.6. Dialysate fluid

Ion chromatography has been used to assay the free sulfate content of dialysate fluids in the study of end-stage renal failure patients being treated by peritoneal dialysis [58] and hemodialysis [29]. In both therapeutic manoeuvres, the removal of accumulated sulfate is sufficient to lower serum sulfate but levels do not return to normal [29,32,36,58,75–78]. For peritoneal dialysate [58], we found that the protein content was sufficient to warrant deproteinization (Cole et al., unpublished observation), while Marangella et al. [29] reported that they also subjected their hemodialysates to ultrafiltration.

2.4.7. Breast milk

Breast milk is a complex colloidal suspension that undergoes substantial changes in composition during the lactation process. Initial milk fluids (colostrum) are much higher in solute and protein while later or ''mature'' milk fluids have the highest lipid content. For sulfate determination by ion chromatography, it is necessary to obtain an aqueous infranatant by centrifugation prior to deproteinization. We were unable to detect any sequestration of free inorganic sulfate in the upper lipid layer [79]. We found less free sulfate in mature milk (29 ± 6 μM) than in colostrum (66 ± 21 μM) [80], but both types of human milk have much lower sulfate concentrations than found in most other body fluids.

2.5. Organosulfate determination

A variety of ways have been developed for determining the sulfate content of various macromolecules with the anion in ester or amide linkage (RSO_3). In most instances, acid hydrolysis is followed by sulfate assay [3,63,81,82], and the difference between hydrolyzed and unhydrolyzed samples is assumed to be a reasonable estimate of all organosulfates. That this assumption could ever be formally tested is unlikely, because there are dozens of sulfoester classes and thousands of individual sulfoester compounds. Nevertheless, acid hydrolysis can be shown to cleave most conjugated sulfoester species [57,63,83–85]. With both the barium precipitation and ion chromatographic methods, attention must be paid to the purity and proportion of the acid used in the hydrolysis step.

The earliest studies of sulfoesters refer to the conjugated fraction as ''ethereal sulfate'' on the basis of its extractability into diethyl ether [3], but this fractionation probably identifies only the arylsulfates and excludes most, if not all, sulfates bound to simple or complex carbohydrates. Many decades ago, considerable effort was expended in developing

accurate methods to determine the serum ethereal sulfate pool, and such studies suggested that this fraction comprises 5 to 15% of the total serum sulfate [86–88]. Urinary RSO_3 has been more extensively documented, particularly in relation to free sulfate, because of its potential for indicating the net activity of the sulfoesterification pathway in different clinical circumstances. Using the turbidimetric barium precipitation method [5], Lundquist et al. [9] reported the first major effort to define the clinical characteristics of sulfoester excretion. Daily urinary excretion in ten males was not different from ten females and constituted 6 to 8% of the free sulfate fractions. Protein deprivation leads to a marked decrease in free sulfate excretion [89], whereas fasting causes only a moderate decrease and sulfoester excretion remains unchanged [47]. Two independent studies [90,91] have shown an absolute and a relative increase in sulfoester excretion in patients with cancer.

To our knowledge, neither serum or urine RSO_3 has been quantitated by ion chromatographic methods. However, we [79,80] have reported acid hydrolysis conditions for ion chromatographic assay of free and conjugated sulfate fractions of human breast milk.

3. Thiosulfate

Thiosulfate ($S_2O_3^{2-}$) is a sulfate analog with a thiosulfur substituent and is found in human serum and urine. Although its clinical biochemistry is more obscure, most thiosulfate probably arises from reactions of endogenous, reactive thiosulfur groups (such as thiocysteine) with sulfite. The enzyme, rhodanese (thiosulfate-sulfurtransferase, EC 2.8.1.1), catalyzes this synthesis, although its principal function is probably as a reservoir of thiosulfur for a variety of other thiol exchange and thiolation reactions [92]. The strongest evidence for the clinical significance of this pathway is found in an inborn error of metabolism, sulfite oxidase deficiency [93]. Infants born with this condition show signs of serious prenatal neurologic damage and often die in early childhood. Although sulfite spontaneously oxidizes to sulfate in physiological media, sulfite is a highly reactive metabolite of mitochondrial sulfur amino acid oxidation [94,95] that is immediately scavenged by the perimitochrondrial sulfite oxidase enzyme as newly synthesized sulfite exits the organelle [96].

In individuals homozygous for sulfite oxidase deficiency, sulfite and its intermediates accumulate in large amounts, but a more stable product, also found in excess, is thiosulfate [55,93,97]. Moreover, normal individuals who consume large amounts of dietary sulfite (a major component of beer, wines and a preservative found in some processed food) may also show increased thiosulfate excretion [98,99].

Togawa et al. [100] used anion-exchange chromatography and a thiol-specific fluorescence detection system to determine serum thiosulfate and found it to be present in sub-micromolar quantities (606 ± 66 nM, $n = 5$). Whether it can be quantitated by ion chromatography has not been determined. Other studies have shown that the rubber stoppers found in the common Vacutainer blood-collection tubes may be significant sources of contamination [101].

For many years, urinary determination of thiosulfate has been based on cyanolysis of thiosulfate and colorimetric determination of the thiocyanate product [99,102,103]. However, differences in thiosulfate excretion have been reported with high-performance liquid chromatography (HPLC) methods that use electrochemical detection [104]. We developed an ion chromatographic method that obviates some of the difficulties associated with electrochemical and colorimetric assays [105]. To successfully detect micromolar amounts of thiosulfate using standard columns and conductimetric technology, we resorted to a column switching technique that avoids swamping of the separation capacities of the analytical column. Reviewed by Villaseñor [106], this ‘‘heart-cut technique’’ relies on partial separation of anions by the first column and final separation of other anions —including, in this case, thiosulfate— on a second analytical column. The initial urinary fraction containing millimolar quantities of urinary chloride, phosphate, and sulfate, and the final fraction, containing large unidentified polyanions that might persist on the column, are diverted to waste (Fig. 3). If there is a disadvantage to this sort of column configuration, it lies largely in the additional equipment required and the necessity for high-quality valve-switching configurations on the instrument.

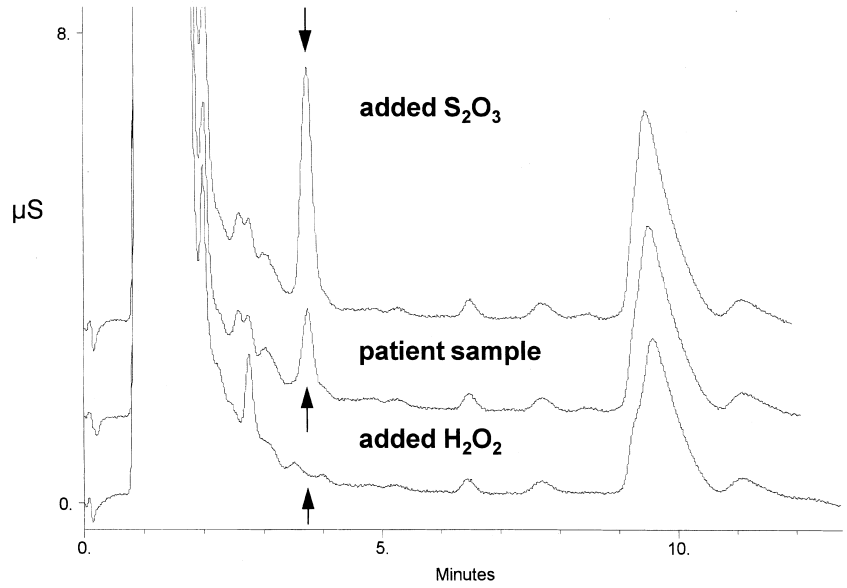

Fig. 3. Ion chromatograms of urine with separation and conductimetric detection of thiosulfate [105]. Urine samples were analyzed on a DX-500 Ion Chromatograph [Dionex (Canada), Oakville, Canada). The configuration included a GP-40 gradient pump connected alternatively to the 50×4 mm Ionpac AG4A-SC guard column and thence to the 250×4 mm Ionpac AS4A-SC analytical column (both from Dionex), or to the analytical column only. A WCP-1 auxiliary pump was plumbed so that its eluent stream passed alternatively through the guard column only or was directed to waste. The eluent from the analytical column was directed to the ASRSI anion self-regenerating suppressor column, and thence to an ED40 conductivity detector. The mobile phase contained 10 m*M* Na_2CO_3 and 10 m*M* $NaHCO_3$ and the flow-rate was set at 1 ml/min for the auxiliary pump and 2 ml/min for the primary pump. Column-switches were set for 0.1 and 1.5 min after injection (25 µl volume). This ''heart-cut'' method [106] reduces matrix interference and substantially reduces baseline noise. In all three tracings, the thiosulfate peak elutes at 3.8 min (arrow). The top tracing shows the effect of adding 50 µ*M* thiosulfate standard to a urine sample (middle tracing). The bottom tracing shows the effect of adding hydrogen peroxide to the same urine sample. The thiosulfate peak is lost with peroxide oxidation to sulfate, but the increase in the sulfate peak (elution time of ~2.8 min) reflects oxidation of other reduced sulfur species as well.

We found a widely variable thiosulfate excretion in normal children and adults. Because many of the concerns about normalizing the urine concentration are essentially no different for thiosulfate than for sulfate (discussed in Section 2), we have suggested that it may be appropriate—at least in the context of detecting sulfite oxidase deficiency—to express thiosulfate excretion as a ratio of urinary sulfate excretion [55]. Our values more closely match those found by Kågedal et al. [104] than those reported by investigators using the chemical method, and we speculate that background interference, perhaps from endogenous thiocyanates or other thiosulfur compounds, generates a modest but significant positive bias in the results based on colorimetric methods [105]. We found thiosulfate to be stable in urines maintained for weeks at 4°C and for months at −20°C, and there is no significant background interference in normal samples.

Acknowledgements

D.E.C.C. wishes to credit Prof. Charles R. Scriver (McGill University, Montreal, Canada) for first kindling his interest in sulfate metabolism and supporting our earliest ventures into the ion chromatographic assay of sulfate in biological fluids. Neither author has a commercial interest in the Dionex Corporation, but we wish to acknowledge the advice and technical support we have received over many years from various corporate employees in Canada and the USA. We also thank Dr. M. Morris (State University of New York at Buffalo, Amherst,

NY, USA) for her critical review of the manuscript. This work was supported in part by grants from the PSI Foundation and the Heart and Stroke Foundation of Ontario.

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