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Practical aspects of suppressed ion chromatography for cations in human serum

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Abstract

In spite of the good accuracy and precision of ion chromatographic methods for the determination of mono- and divalent cations in human serum, the major drawback with these methods were problems with the membrane suppressor's performance. Here, we describe experiments undertaken to solve these problems. We address in particular the use of histidine–sulfuric acid eluents, sample purification with OnGuard-A cartridges and chromatographic "front-cut" for divalent cations. The latter two adaptations, resulting in removal of the anionic species from the sample, were successful in solving the observed suppressor problems. The eluent substitution, moreover, allowed us to switch from the chemical to the electric suppression mode. We believe that these adaptations will allow secure and robust determination of cations in human serum samples with ion chromatography.

Keywords: Mobile phase composition; Cations

1. Introduction

Ion chromatography has been successfully applied to the determination of various serum electrolytes [1–4]. Recently, we developed highly precise and accurate methods for the determination of total-Na^+, -K^+, -Ca^{2+} and -Mg^{2+} in human serum [5–7]. The methods for the monovalent ions used methanesulfonic acid eluents and electric suppression, while the methods for the divalent ions used DL-2,3-diaminopropionic acid monohydrochloride (DAP)–hydrochloric acid (HCl) eluents and chemical suppression. Originally, sample pretreatment was done by acidic dilution and filtration through 0.45 μm filters [5,6]. However, in the long term, we were faced with suppressor problems, such as increased back-pressure and decreased resolution and sensitivity. We initially assumed that the problems were caused by incomplete removal of proteins and/or organics from the sample. Therefore, sample pretreatment was changed to acidic dilution followed by heating at 70°C for 2 h and reversed-phase (RP) purification [7]. Nevertheless, the problems persisted, particularly in the methods for the analysis of Ca^{2+} and Mg^{2+}. We therefore considered substitution of the eluent with one that could be electrically suppressed. Such an eluent was found by combining sulfuric acid (H_2SO_4) with histidine [8]. Unfortunately, in spite of these modifications, the suppressor problems persisted. This drew our attention to the fact that anionic species might be responsible for the problems.

Here, we first describe some general advantages of substituting DAP-based eluents with histidine-based eluents in terms of method practicality, robustness, cost and environmental pollution. Additionally, we present results that verify the assumption that anionic

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species present in the human serum matrix cause the loss of suppressor performance.

2. Experimental

2.1. Instrumentation and materials

The basic chromatographic instrumentation consisted of a DX-100 ion chromatograph equipped with two IonPac CG10 columns, a suppressor in the chemical or electric suppression mode (type CSRS-I) and a conductimetric detector, all from Dionex (Sunnyvale, CA, USA). Ultrapure (18.2 MΩ electric resistivity) water was produced from an Elga apparatus (Bucks., UK). Integration of the chromatograms was performed with Dionex Peaknet software. For sample pretreatment, 0.45 μm filters were purchased from Millipore (Bedford, MA, USA) and OnGuard-RP and -A cartridges were from Dionex. A high-pressure in-line filter from Dionex was connected between the injection valve and the first CG10 column. H_2SO_4 (96%) was purchased from Fluka (Buchs, Switzerland), histidine was from Merck (Darmstadt, Germany) and acetonitrile "Super Purity Solvent" (CH_3CN) was from Romil Chemicals (Shepshed, UK). For further system descriptions, see [5,6].

2.2. Chromatographic conditions

The eluent used in combination with the two CG10 columns in the original Ca^{2+} (Mg^{2+}) method was a mixture of 4 m*M* (2 m*M*) DAP+40 m*M* HCl, at a flow-rate of 1 ml/min. Chemical suppression was performed with a 100 m*M* tetrabutylammonium hydroxide (TBAOH) solution in water. This eluent was replaced with 17 m*M* H_2SO_4+2 m*M* (0.7 m*M*) histidine+10% (v/v) CH_3CN at a flow-rate of 1 ml/min. The change of eluents allowed switching from the chemical to the electric suppression mode. "Front-cutting" was performed after 36 s (48 s), either manually or by connecting a second pump and switching valve.

2.3. Sample purification

The OnGuard-RP cartridges (OnGuard-A cartridges) were eluted with 5 ml of methanol and 10 ml of water (5 ml of water) before use, according to the manufacturer's instructions. The diluted samples were passed over both cartridges, with the OnGuard-A cartridges placed initially behind and in a second phase before the OnGuard-RP cartridges. The first 3 ml of eluate were discarded, the rest was collected for further analysis. An alternative sample purification method consisted of acidic sample dilution followed by filtration through 0.45 μm filters [5,6].

3. Results and discussion

3.1. Substitution of DAP–HCl eluents with histidine–H_2SO_4 eluents

Switching from the chemical to the electric suppression mode urged us to leave the DAP-based eluent because DAP is only available as a HCl-salt and electric suppression of HCl-containing eluents is not possible, due to the formation of Cl_2 gas, which irreversibly damages the micromembrane suppressor. In its place, we selected histidine, which is commercially available as a free base and has been used before for ion chromatographic analysis of divalent ions [8]. In addition, it has comparable physicochemical properties to DAP (the pK_a values of DAP are 1.39, 6.60 and 9.08 and the pK_a values of histidine are 1.78, 5.97 and 8.97). As a consequence, both compounds are present at the eluent pH in an equilibrium of two different ionic species (see Fig. 1), which allows one to adjust the elution power of the eluent. Furthermore, in both cases, the product of the suppression reaction is the zwitterionic form, which has no conductance (see Fig. 1). Following these considerations, the eluent that we finally chose for Ca^{2+} analysis on the two CG10 columns was 17 m*M* H_2SO_4+2 m*M* histidine+10% (v/v) CH_3CN (for Mg^{2+}, the eluent contained 0.7 m*M* histidine). The addition of CH_3CN was advantageous for column stability (see discussion below). These eluents gave very similar chromatographic performances in terms of retention time, baseline stability and resolution to those of the original eluents based on DAP–HCl (see Fig. 2, for Ca^{2+}). Our original hope, however, of solving the encountered suppressor problems with these eluents could not be ful-

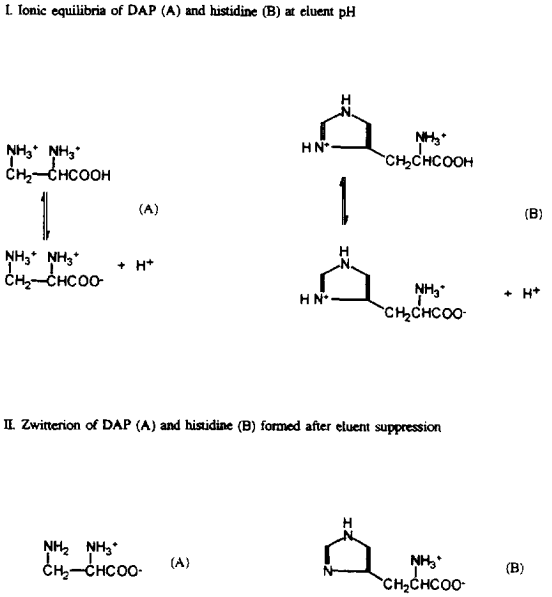

Fig. 1. Ionic species of DAP (A) and histidine (B) present in the eluent (I), and after suppression (II).

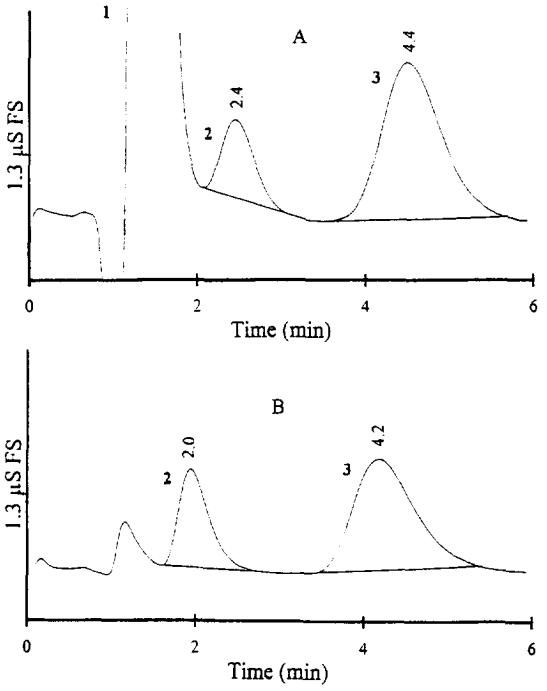

Fig. 2. Comparison of chromatography for Ca^{2+} analysis with DAP–HCl eluent and chemical suppression (A) versus histidine–H_2SO_4-based eluent, electric suppression and system "front-cut" (B). Operating parameters for each method are listed in the text. Peak identification: 1=monovalent cations, 2=Mg^{2+} and 3=Ca^{2+}

filled. Nevertheless, we found that the histidine-based eluents had significant practical advantages over the DAP-based eluents. These were (i) electric suppression was possible, thereby allowing the suppression processes for both mono- and divalent cations to be standardized; (ii) increased practicality and robustness of the system; (iii) environmental pollution was reduced as TBAOH, used for eluent suppression in the chemical suppression mode, could be omitted and (iv) working-costs were greatly reduced (see Table 1).

3.2. Investigation of the cause of suppressor poisoning

3.2.1. Experiments with the OnGuard-A column

We investigated the use of OnGuard-A cartridges for additional sample clean-up because they are designed for removal of anions and sample neutralization. Placing the OnGuard-A cartridges behind the OnGuard-RP cartridges avoided the decrease of suppressor performance. However, accuracy was severely affected. Interestingly, recovery for one sample was still nearly 100% with the additional OnGuard-A cartridge, indicating that the recovery problem is very much related to the particular matrix of each serum sample.

Placing the OnGuard-A cartridges before the OnGuard-RP cartridges resulted in complete removal of the divalent cations (Note: the package insert of the OnGuard-A columns mentions recoveries of >98% for mono- and divalent cations). The reason for this might be a precipitation of the divalent cations because of the neutralization of the sample

Table 1
Comparison of total analysis reagent costs of one working day (10 h) for Ca^{2+} measurement, comparing DAP- (A) and histidine (B)-based eluents

Compound	A	B
$HCl–H_2SO_4$	2.55 ml=US$ 0.28	525 µl=US$ 0.02
DAP–histidine	0.3374 g=US$ 5.74	0.3103 g=US$ 0.29
CH_3CN	–	60 ml=US$ 1.55
TBAOH–H_2O	153.6 g=US$ 30.87	–
Total cost	US$ 36.89	US$ 1.86

through the OnGuard-A purification step. Thus, while the OnGuard-A cartridge purification process improved suppressor performance, the approach was plagued by poor recovery and, hence, other strategies were investigated.

3.2.2. Effects of "front-cut"

We first realized "front-cutting" by interrupting the flow after the first CG10 column (36 s for Ca^{2+} and 48 s for Mg^{2+}). While this had a positive effect on suppressor performance, it created "ghost peaks" in the chromatogram, which made quantification impossible. Therefore, we installed a second pump and an additional switching valve, which maintained eluent flow through the suppressor during "front-cutting" (see Fig. 3 for plumbing scheme). Because the "front-cut" was done for 36 and 48 s, respectively, it was possible to activate the switching valve in connection with the injection valve and software adaptation was not necessary. Additionally, the chromatography was not affected by the "front-cut" (see Fig. 2, for Ca^{2+} analysis).

With the last described system, we were successful in maintaining suppressor performance. In addition, it allowed us to apply the originally selected sample preparation that was based on simple acidic dilution and filtration [5,6]. Nevertheless, one additional modification was necessary, i.e. the addition of 10% CH_3CN to the eluent. This was done because after 200 injections under "front-cutting" conditions, a decrease of response due to column contamination was observed. The addition of 10% CH_3CN to the eluent resulted in trouble-free operation of the modified system for more than 800 injections: the retention time only decreased from 4.2 to 4.08 min, the response was stable and the peak asymmetry factor only increased from 1.69 to 1.73.

4. Conclusion

The substitution of DAP–HCl eluents with histidine–H_2SO_4 eluents had no adverse effect on retention time, baseline stability or chromatographic resolution and allowed electric suppression of the eluent. In this way, we could use the same suppression mode for analysis of both mono- and divalent cations, which increased the practicality and robustness of the system. Finally, working costs and environmental loading were considerably reduced.

Furthermore, we demonstrated that the observed suppressor problems were most probably caused by anionic species present in the serum matrix. Removal of anions by application of OnGuard-A columns was not possible because it affected method accuracy unpredictably. Conversely, removal of anions by "front-cut" had no adverse effect on accuracy.

In conclusion, we believe that histidine–H_2SO_4 eluents with electric suppression and application of "front-cut" allows secure and robust measurement of human serum samples with ion chromatography.

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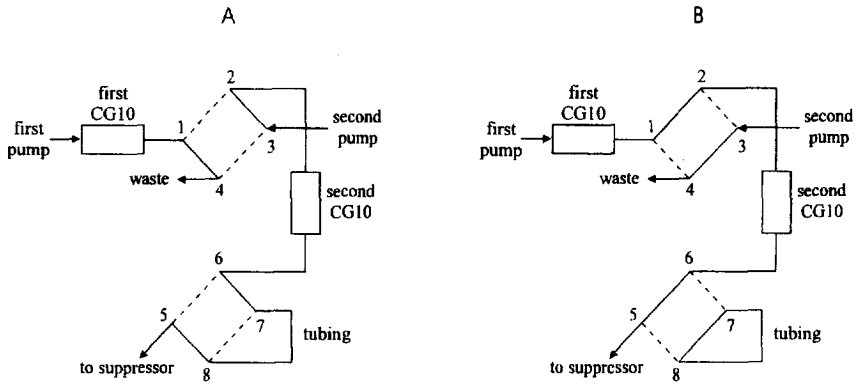

Fig. 3. Plumbing scheme for "front-cut". A=front to the waste; B= divalent cations to the second CG10 column.

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