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**PROTOCOL**

# Absence of temperature effect on elution profiles on anionic and cationic ion-exchange resins from 4°C to 28°C

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**Rationale:** In labs devoted to the geochemistry of non-traditional isotopes, chemical elution is necessary to purify the element of interest. Elution is always performed in over-pressured and air-conditioned clean rooms. We took advantage of an air-conditioning failure in our lab during summer 2018 to study the effect of temperature on the characteristics of the elution profiles of ion-exchange resins.

**Methods:** We performed the ion-exchange separation of copper, iron and zinc on macroporous anionic AG MP-1 resin and that of calcium on cationic AG 50W-X12 resin, at 28°C, prior to the measurement of their isotopic ratios by mass spectrometry. We further performed these experiments in a clean hood in a cold room at 4°C. The elution curves were processed on biological standards, i.e. bovine liver (SRM-1577c), fetal bovine serum (FBS), bone meal (SRM-1486) and the seawater IAPSO standard.

**Results:** The elution profiles of major elements for each matrix, and those of copper, iron, zinc and calcium, were compared with those classically achieved at 20°C in air-conditioned conditions. The results show that the elution profiles preserve their characteristics whatever the temperature, suggesting that partitioning coefficients between resin and solution are thermo-independent in the range of temperature from 4°C to 28°C.

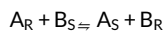
**Conclusions:** If generalized to other matrices, notably inorganic, and to other elements, notably the extreme case of the separation of Rare Earth Elements, the present results suggest that clean labs may not have to be air-conditioned. This would reduce installation and operating costs and have a positive effect on the environment, paving the way for the development of a “green geochemistry”.

## 1 | INTRODUCTION

The measurement of metal stable isotope ratios is of wide interest in many fields, from cosmochemistry (e.g.,<sup>1–3</sup>), endogenous geochemistry (e.g.,<sup>4–6</sup>), environmental geochemistry,<sup>7–9</sup> paleoenvironments,<sup>10–12</sup> to plant biology<sup>13–15</sup> and animal biology.<sup>16–18</sup> Recent advances have even been achieved in the field of medicine.<sup>19–21</sup> Whatever the application, a preliminary step consisting of the separation of the element of interest from the matrix is necessary prior to the mass spectrometric analyses of metal isotope ratios. This step is classically achieved by means of ion-exchange chromatography. The principle is

based on electrostatic interactions between a stationary phase displaying ionic functional groups with a mobile phase containing analyte ions with the opposite charge. The stationary phase can thus be anionic or cationic. Resins containing anionic or cationic functional groups were developed in the frame of the Manhattan project for the separation and purification of strategic metals to study their nuclear properties. Series of articles describing the protocol for eluting metals were first published in the 1950s by Spedding et al for cationic resins<sup>22</sup> and Kraus and Moore for anionic resins.<sup>23</sup> These protocols have evolved since then with the commercialization of new resins, and today methods even exist for the fully automated separation of

metals (e.g.,<sup>24,25</sup>). Whatever the type of the resin (anionic or cationic) and given the reversible exchange reaction between two ions A and B between a resin R and the solution S, the equilibrium is:



and applying the mass action law gives:

$$K_{A/B}^{\ominus} = \frac{|A|_S \times |B|_R}{|A|_R \times |B|_S}$$

where  $K_{A/B}^{\ominus}$  is the equilibrium constant and  $|A|$  and  $|B|$  the activity of A and B in the resin and the solution. As for all equilibrium constants,  $K_{A/B}^{\ominus}$  is related to the standard change of the Gibbs free energy of the reaction ( $\Delta G^{\ominus}$ ) in kJ:

$$\Delta G^{\ominus} = -RT \ln K_{A/B}^{\ominus}$$

where  $R$  is the universal gas constant and  $T$  the absolute temperature in °K. The value of  $K_{A/B}^{\ominus}$  varies as a function of temperature. Conventionally, ion-exchange chromatography is carried out in air-conditioned clean rooms kept at a constant temperature, usually set between 17°C and 20°C.

In a cleanroom, the temperature is regulated with an air-conditioning unit, which is often part of a heating, ventilating and air-conditioning (HVAC) system. HVAC thus provides both over-pressured and temperature-conditioned air in a single unit, but these can be regulated by two independent units. Cleanrooms are isolated from outdoor contaminations by venting filtered air that eventually goes through an additional hood air filter to protect the workplace.

In July 2018, we experienced a HVAC breakdown at the cleanroom of the Laboratoire de Géologie de Lyon that lasted several weeks during a period of heat-wave. The temperature rapidly rose in the cleanroom and stabilized around 28°C. Most users then stopped working, but we took advantage of the situation to test the influence of the temperature on the  $K^{\ominus}$  values between metals and cationic and anionic resins. We executed the classical elution protocols and looked for changes in the elution profiles relative to those calibrated at 20°C. We then repeated the procedure at 4°C in a cold room. We performed the elution protocols at 28°C and 4°C for the separation of Cu, Fe, and Zn on AG MP-1 macroporous anionic resin following Maréchal et al<sup>26</sup> and for the separation of Ca on AG 50W-X12 cationic resin following Tacail et al.<sup>27</sup> We constructed the elution curves for standards that are routinely used in the lab and for which we have fairly good experience at 20°C, i.e. the SRM-1577c “bovine liver” international reference material and fetal bovine liver (FBS) for the separation of Cu, Fe and Zn, and the SRM-1486 “bone meal” and IAPSO seawater international reference materials for the separation of Mg and Ca. In all cases, we also monitored the elution profiles of the associated major elements.

## 2 | EXPERIMENTAL

### 2.1 | Reagents and digestion

Ultrapure water (resistivity 18.2 MΩ cm) was produced using a Millipore Synergy system (Merck, Darmstadt, Germany). Concentrated technical grade HCl and HNO<sub>3</sub> (Carlo Erba Reagents, Milan, Italy) were distilled at low temperature in PFA equipment (Savillex, Eden Prairie, MN, USA). Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%) Suprapur was purchased from Merck. Macroporous anion-exchange resin AG MP-1 (100–200 mesh) and cation-exchange resin AG 50W-X12 (200–400 mesh) were purchased from BioRad Laboratories (Hercules, CA, USA). About 160 mg of FBS (lot number O14 M3399; Sigma-Aldrich, St Louis, MO, USA), 50 mg of SRM-1577c from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), 200 μg of SRM-1486 (NIST) and 50 μL of reference seawater provided by The International Association for the Physical Sciences of the Oceans (IAPSO) were digested using 3 mL concentrated distilled HNO<sub>3</sub> for 24 h. The vials were heated at 110°C for 3 days, then allowed to cool to room temperature. About 0.5 mL of Suprapur H<sub>2</sub>O<sub>2</sub> (30%) was then added to the cooled down samples. After 2 h at room temperature, the vials were heated at 110°C on a hotplate for 2 days. The solutions were then evaporated to dryness and dissolved in 6 M HCl, evaporated again and taken up for chemical separation.

### 2.2 | Elutions on anionic resin

The FBS and SRM-1577c standards were redissolved in 3.2 mL and 4 mL of 7 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001%, respectively. Chemical separation was then implemented according to the method of Maréchal et al<sup>26</sup>: 1 mL of sample was loaded on a quartz column containing 1.6 mL of AG MP-1 resin previously rinsed with 0.5 N HNO<sub>3</sub> and conditioned with 7 mL 7 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001%. The matrix was eluted with 10 mL 7 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001%. Copper was eluted with 20 mL 7 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001%; Fe was eluted with 10 mL 2 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001%; and, finally, Zn was eluted with 10 mL 0.5 M HNO<sub>3</sub>. The eluted fraction was collected in a 2-mL aliquot, evaporated and taken up in 0.5 M HNO<sub>3</sub>. The elution of S, Mg, Na, K, P and Ca was monitored in addition to that of Cu, Fe and Zn.

### 2.3 | Elutions on cationic resin

The SRM-1486 and the IAPSO standards were redissolved in 200 μL 0.4 M HCl. Chemical separation was then implemented according to the method of Tacail et al<sup>27</sup>: 10 μL and 50 μL of sample for SRM-1486 and IAPSO were loaded, respectively, on a column containing 210 μL of AG 50W-X12 resin previously rinsed with 6 mL 6 M HCl and conditioned with 3 mL 0.4 M HCl. The matrix was eluted with 13 mL 0.4 M HCl. Mg was then eluted with 3.5 mL 1 M HCl and

finally Ca and Sr were eluted with 2 mL 6 M HCl. The eluted fraction was collected in a 1-mL aliquot, evaporated and taken up in 0.5 M HNO<sub>3</sub>. The elution of Mg, S, Na, P and Sr was monitored in addition to that of Ca.

## 2.4 | Instrumentation and measurement protocols

Single-element standard solutions from SCP Science (Québec, Canada) were used for the quantification of Ca, S, Mg, Na, K, P, Fe, Cu, Zn and Sr. Scandium and In solutions from SCP Science were used for the internal standard calibration to correct for oxide interferences and instrumental drift. All measurements were performed at the Laboratoire de Géologie de Lyon. The concentrations of Cu, Fe and Zn were measured using inductively coupled plasma mass spectrometry (ICP-MS) (7500CX; Agilent, Santa Clara, CA, USA). The concentrations of Ca, S, Mg, Na, K, P and Sr were measured using inductively coupled plasma atomic emission spectrometry (ICP-AES) (iCAP 7000 Series instrument; Thermo Fisher Scientific, Waltham, MA, USA). The concentrations were calculated using calibration curves based on multi-elemental solutions. The multi-elemental solutions were also used to monitor and correct the instrumental drift over the analytical session. The validity and reproducibility of major and trace element concentrations are estimated to be better than 10% ( $\pm 2$  sd) based on repeated measurements of the international reference materials as unknown samples.

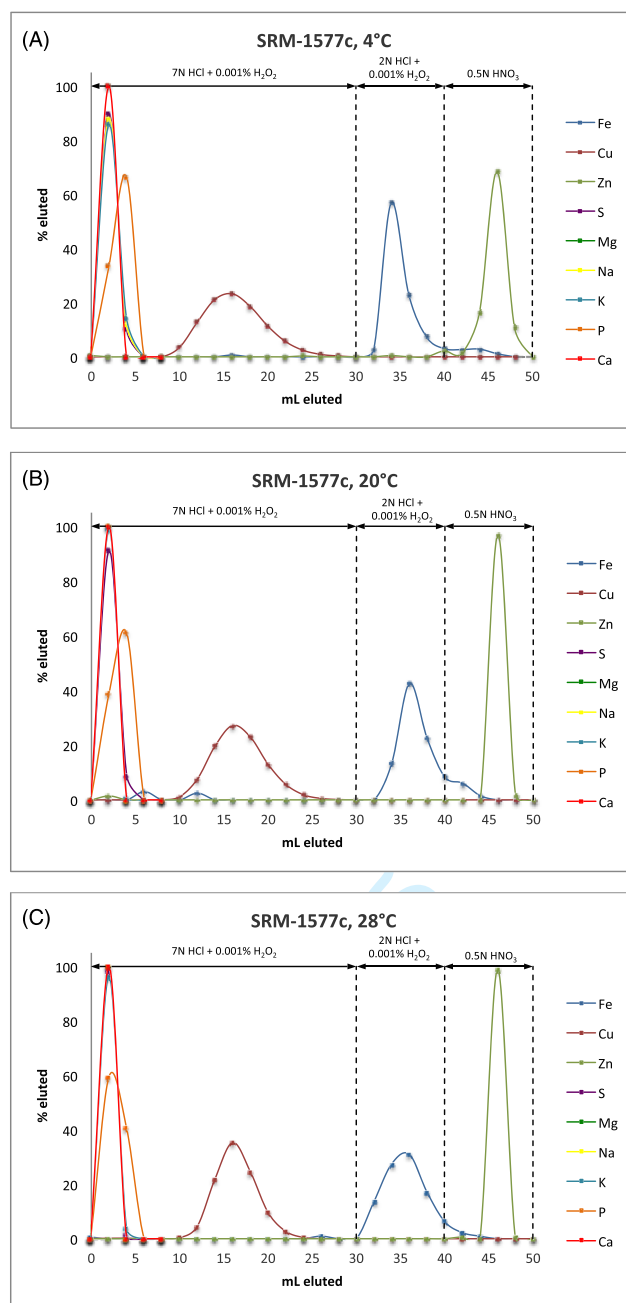
## 3 | RESULTS

### 3.1 | Elutions on anionic resin

The concentrations of Na, K, S, Ca, Mg and P, which are major elements in the matrices of the bovine liver SRM-1577c and the fetal bovine liver FBS standards, were determined along with the Fe, Cu and Zn concentrations. Elution curves for these two standards at 4°C, 20°C and 28°C are presented in Figures 1A–1C for SRM 1577c and in Figures 2A–2C for FBS. The first 10 mL of 7 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001% are used to discard elements present in the matrix: Na, K, S, Ca, Mg and P are totally discarded in this fraction, while Cu, Fe and Zn are still retained on the resin.

For SRM-1577c, 96.5%, 98.8%, and 99.3% of Cu are eluted with the next 20 mL of 7 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001% at 4°C, 20°C and 28°C, respectively. Fe is further eluted at 92.7%, 86.9% and 95.5% with the next 10 mL of 2 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001% at 4°C, 20°C and 28°C, respectively. Finally, 96.6%, 98.6% and 99.4% of Zn are eluted with 10 mL 0.5 M HNO<sub>3</sub> at 4°C, 20°C and 28°C, respectively.

For FBS, 92.6%, 100% and 100% of Cu are eluted with the next 20 mL of 7 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001% at 4°C, 20°C and 28°C, respectively. Fe is further eluted at 84.6%, 93.1% and 83.6% with the next 10 mL of 2 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001% at 4°C, 20°C and

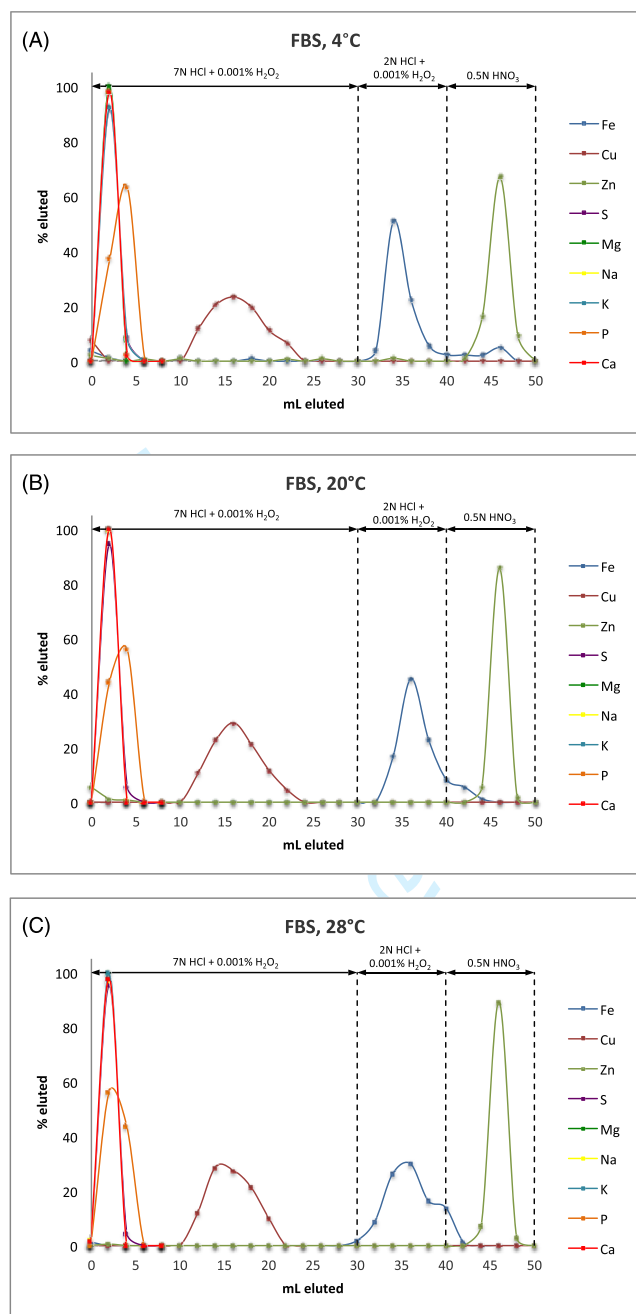


**FIGURE 1** Elution of matrix elements P, K, Na, Mg, S, Ca and Cu, Fe and Zn from the SRM-1577c bovine liver standard on the macroporous anionic AG MP-1 resin at A, 4°C; B, 20°C; and C, 28°C. Uncertainty on concentration measurement is  $\sim \pm 5\%$  [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

28°C, respectively. Finally, 93.1%, 92.4% and 99.5% of Zn are eluted with 10 mL 0.5 M HNO<sub>3</sub> at 4°C, 20°C and 28°C, respectively.

It is noteworthy that some proportion of Fe is eluted along with the Zn fraction: representing 6.7%, 7.7% and 3.1% of Fe for SRM-1577c at 4°C, 20°C and 28°C, respectively, and 9.2%, 6.9% and 15.2% for FBS at 4°C, 20°C and 28°C, respectively.





**FIGURE 2** Elution of matrix elements P, K, Na, Mg, S, Ca and Cu, Fe and Zn from the FBS standard on the macroporous anionic AG MP-1 resin at A, 4°C; B, 20°C; and C, 28°C. Uncertainty on concentration measurement is  $\sim \pm 5\%$  [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

### 3.2 | Elutions on cationic resin

The concentrations of P, Mg and Na, which are major elements in the matrix of the bone meal SRM-1486 standard, were determined along with Ca concentrations. Elution curves for the SRM-1486 standard at 4°C, 20°C and 28°C are presented in Figures 3A–3C. The concentrations of Na, S and Mg, which are major elements in the

matrix of the seawater IAPSO standard, were determined along with Ca and Sr concentrations. Elution curves for the IAPSO standard at 4°C, 20°C and 28°C are presented in Figures 4A–4C.

The first 13 mL of 0.4 M HCl are used to discard elements present in the matrix. The results show that Na, P and S are totally discarded. For both standards, Mg is completely eluted with 3.5 mL 1 M HCl at 4°C, 20°C and 28°C. For SRM-1486, Ca is completely eluted with 2.5 mL 6 M HCl at 4°C, 20°C and 28°C. For IAPSO, Ca and Sr are both completely eluted with 2.5 mL 6 M HCl at 4°C, 20°C and 28°C.

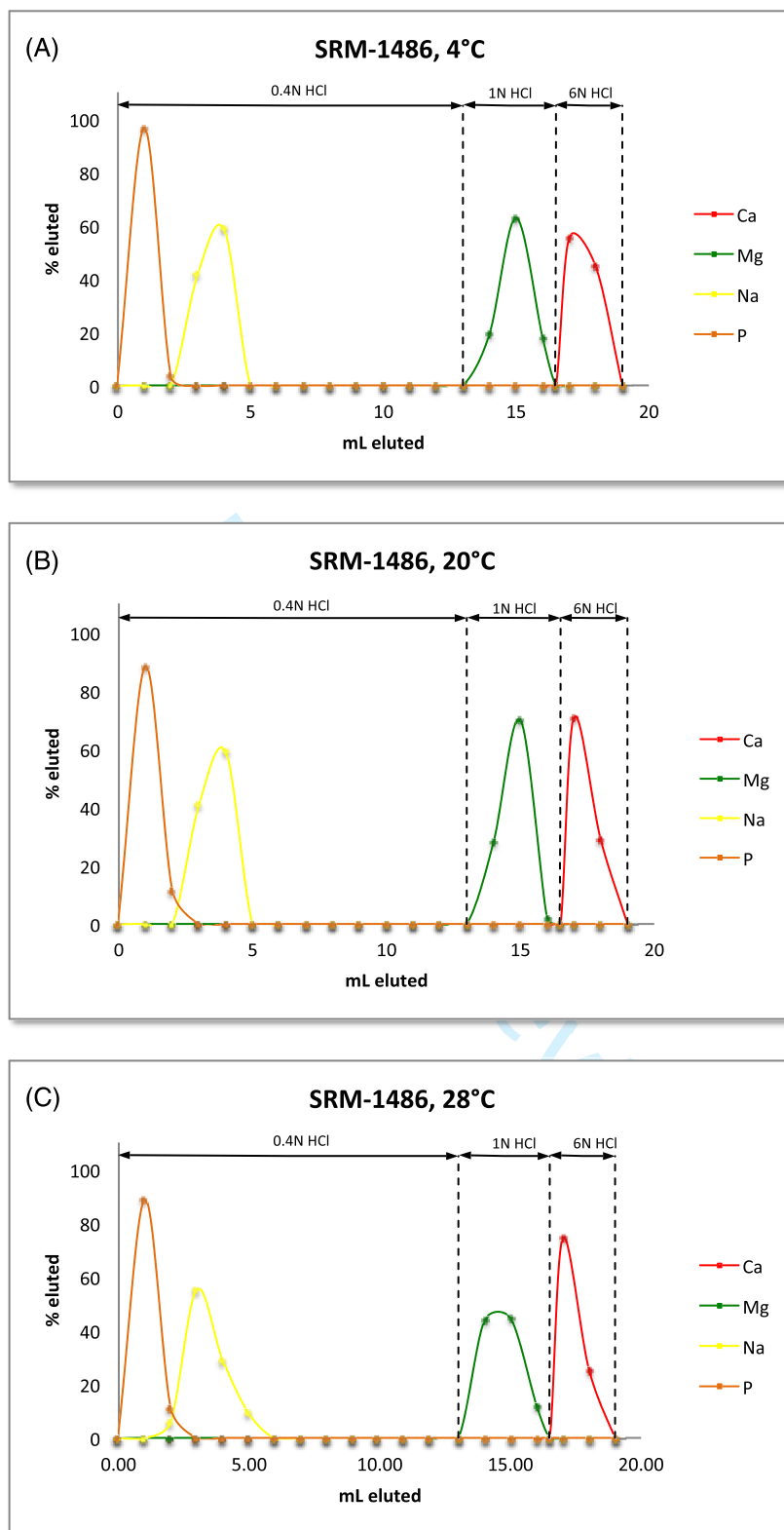
## 4 | DISCUSSION

The present results suggest some minor modifications to the protocol of Maréchal et al.<sup>26</sup> The elements of interest are mostly eluted as described by these authors for all temperatures. However, if all the matrix elements are always completely eluted with the first 6 mL 7 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001%, Cu begins to be eluted at the eighth mL of 7 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001%. To overcome any possible Cu loss, we suggest collecting the Cu fraction with 22 mL 7 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001% after discarding the first 8 mL of 7 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001% containing the matrix. Regarding Fe, a significant proportion is always retained on the resin and is eluted along with Zn in 0.5 M HNO<sub>3</sub>. To obtain a better Fe extraction yield, the initial 10 mL volume of 2 M HCl + H<sub>2</sub>O<sub>2</sub> 0.001% should be extended to 15 mL.

Overall, the results show that the elution profiles for major and trace elements in various matrices, either on cationic or anionic resins, remain identical whatever the temperature between 4°C and 28°C. This suggests that the thermo-dependence of the partitioning coefficients  $K^{\ominus}$  for most the elements and cationic and anionic resins is not high enough in this range of temperature to be measured.

For a given element, the  $K^{\ominus}$  values are generally higher in anionic than in cationic resins. A comparison of  $K^{\ominus}$  values for cationic and anionic resins is given as an example in Table 1. Over a suite of elements including Cu, Zn, Cd, Fe, Ga, In, Th and U, only Th exhibits  $K^{\ominus}$  values higher in the AG MP-50 cationic resin than in the AG MP-1 and AG 1-X8 anionic resins. Some  $K^{\ominus}$  values for anionic resins are so high that this kind of resin is sometimes said to be a “stick non-stick” resin. In these conditions, it is tempting to posit that anionic resins do not exhibit any apparent sensitivity to temperature, but the results of the study of Kraus and Raridon show that this is not the case.<sup>30</sup> Using the pre-loaded column technique, i.e. the resin is equilibrated with the ion whose adsorption is to be studied prior to being uniformly loaded in the column, these authors showed that the  $K^{\ominus}$  values for Zn and Ga decreased with temperature increasing from 25°C to 150°C. These authors also published results for cationic resins showing that  $K^{\ominus}$  values increase (Be, La, Co, Zn, Eu<sup>3+</sup>) or decrease (Na, K, Ba, Rb, Cs) with increasing temperature from 2°C to 145°C.<sup>31</sup> The thermo-dependence of the  $K^{\ominus}$  values was therefore established, but no generalized thermodynamic laws have been proposed between the various ion-exchange reactions. Some elements, such as Ba, even exhibit a curved thermo-dependence relationship.<sup>31</sup> The absence of a

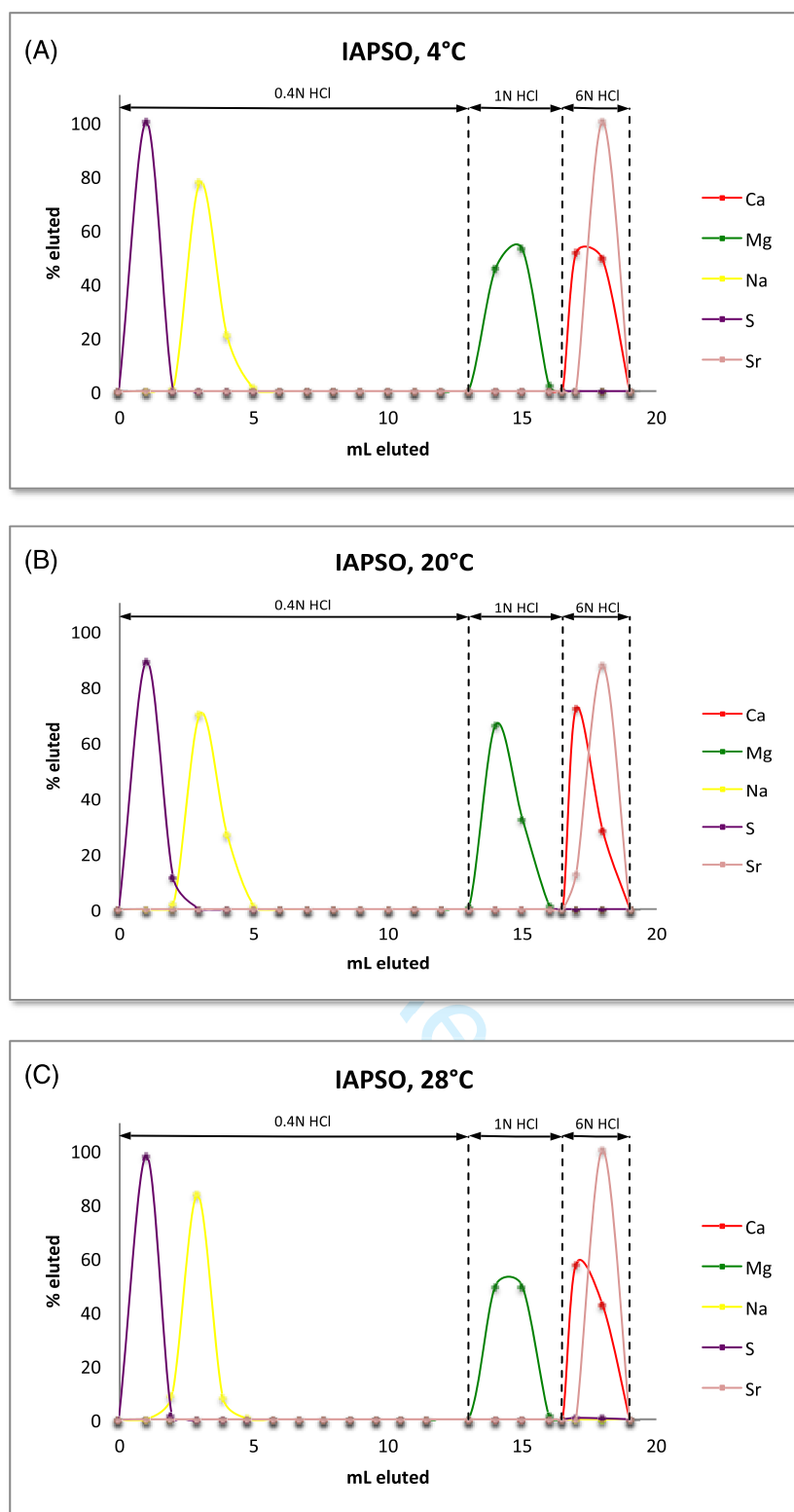
**FIGURE 3** Elution of matrix elements P, Na and Mg and Ca from the SRM-1486 bone meal standard on the cationic AG 50W-X12 resin at A, 4°C; B, 20°C; and C, 28°C. Uncertainty on concentration measurement is  $\sim \pm 5\%$  [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



clear correlation between temperature and ion-exchange equilibria on cationic resins using the pre-loaded column technique is reinforced by results obtained using batch experiments.<sup>32–34</sup>

Theoretically, the varying temperature should have an effect not only on the value of the equilibrium constant, but also on the kinetic rate constant, i.e. with higher temperature, to a certain limit,

increasing kinetics and therefore the separation factor. Note that the kinetics is also affected by the degree of crosslinking of the resin, with higher crosslinking decreasing kinetics. There have been fewer studies aimed at manipulating temperatures to improve separation factors on cationic resins than those dedicated to evaluating the thermo-dependence of ion-exchange equilibria. The separation of Rare Earth



**FIGURE 4** Elution of matrix elements P, Na and Mg and Ca from the IAPSO seawater standard on the cationic AG 50W-X12 resin at A, 4°C; B, 20°C; and C, 28°C. Uncertainty on concentration measurement is  $\sim \pm 5\%$  [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Elements (REEs) has long been a focus of ion chromatography development, and the effects of temperature on the REE separation factors have been tested on several occasions. Glass<sup>35</sup> tested the influence of eluting Eu, Cm, Am and Pm on AG 50W-X12 at 87°C instead at room temperature, and found that the elution peaks appeared more rapidly and were less separated, suggesting that

temperature improves kinetics and has a negative effect on the separation factor. Conversely, Strelow and Gricius<sup>36</sup> found a strong positive effect on the separation factor between La and Th on AG 50W-X8 at 50°C compared with room temperature, implying that the temperature effect on the separation factor is element-dependent.

**TABLE 1** Comparison of  $K^{\ominus}$  values for some elements on cationic and anionic resins in various HCl molarity

	Cationic resin AG MP-50 <sup>a</sup>			Anionic resin AG MP-1 <sup>b</sup>			Anionic resin AG 1-X8 <sup>b</sup>		
	1 M	3 M	5 M	1 M	3 M	5 M	1 M	3 M	5 M
Cu(II)	27.7	1.9	<1		13.2	27.2		4	13.1
Zn	19.7	1.8	<1	412	830	416	554		276
Cd	2.1	<1	<1	986	989	473	626		269
Fe(III)	87	2.2	0.7		19.6	158	35.6		340
Ga	110	5	1.9		18.6	1020	24.3		1780
In	1.4	<1	<1		17.4	29.2	20.1		19.4
Th	104	1810	285		4.1	4.5	1.7		1
U(VI)	68	12.1	5.2		33.2	234	21.1		111

<sup>a</sup>Values from Strelow.<sup>28</sup><sup>b</sup>Values from van der Walt et al.<sup>29</sup>

Here, we found no effect of the temperature on the elution profiles of ten elements for four different types of matrices (i.e. serum, bone, liver and seawater). Our results are therefore divergent from the conclusions reached from the above literature references. Some allowances must be made that could reconcile these two apparently contradictory outcomes. First, our results are representative of a very narrow range of temperature, i.e. from 4°C to up 28°C, while those mentioned above can reach 200°C, with the first temperature point above room temperature, usually at 45–50°C. Second, our results were obtained on real samples while those mentioned above were obtained using synthetic solutions. In the former case, competition of the elements for adsorption on the functional groups is certainly important, and possibly attenuates any potential temperature effects.

The installation and the maintenance of HVAC in a clean lab represent a significant cost, but the impact on the environment also has to be considered. Nowadays the clean lab impact on the environment is a critical issue that must be taken into account, and reducing this impact is the goal of green chemistry. Originally, the principles of green chemistry were established through twelve rules by Anastas.<sup>37</sup> More recently, Gałuszka et al.<sup>38</sup> extended the use of twelve hallmarks to green analytical chemistry. In all cases, the methodological developments of green chemistry involve not only reducing the samples, reagents and waste volumes production, but also minimizing the use of energy consumption, while keeping analytical performances unchanged. This represents the sixth of the twelfth principles of green chemistry proposed by Anastas "Design for Energy Efficiency. Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure".<sup>37</sup> Because our study shows that elution curves for 12 major and trace elements are not affected by temperature in a range compatible with natural seasonal variations, this suggests that air conditioning might not be necessary in clean labs used to perform ion-exchange chromatographic experiments. It does mean that air-conditioning is

not important for comfortable working conditions. The present results should be challenged by further studies on other matrices and elements.

## 5 | CONCLUSIONS

For the ten monitored elements (S, Mg, Na, K, P, Ca, Cu, Fe, Zn and Sr), our results show no sizeable change in the elution pattern on both cationic and anionic resins as a function of the temperature. This suggests that partitioning coefficients between resin and solution are thermo-independent in the range of temperature from 4°C to 28°C, typical of seasonal variations. To complete the present study elution profiles of other elements and on other matrices should be performed, but the present results already suggest that clean labs may not need to be air-conditioned which represents, to our knowledge, the first proposition for the development of green geochemistry.

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