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On-column processes in ion chromatographic determination of nitrite and nitrate in heavy mineralised samples

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Abstract

The accuracy of the ion chromatographic determination of some anions in samples such as sea water, mineral waters, body fluids and samples digested by mineral acids, has been shown to depend strongly on the on-column co-processes caused by matrix components. For samples having other matrix components than the eluent, processes like the self-elution effect, the on-column change of the eluent, the on-column inter-eluent neutralisation, and the suppression of the detector response were already identified as being the cause for the change of retention behavior and detection sensitivity of nitrite, nitrate and chloride. This paper presents the results of the work focused on the ion chromatographic analysis of acidic samples and samples comprising the same matrix component as the eluent used. Hydronium cations in combination with porous stationary phase were found to induce intensive on-column inter-eluent neutralisation that caused a significant prolongation of retention times. The origin of nitrite peak-splitting that appeared when acidic samples were analysed on the column having porous stationary phase, was found to be the direct consequence of the presence of H_3O^+ cations in the sample. When the eluent anion was presented in the sample as the matrix component, a new effect called sample-induced micro-gradient elution was observed.

Keywords: Matrix effects; Nitrite; Nitrate; Inorganic anions

1. Introduction

Because of the effectiveness of ion chromatography (IC) a growing need for IC determination of some analytes in samples such as body fluids, different kinds of surface waters (sea water, mineral waters, brine), acidic or alkaline samples, samples containing organic solvents and others, appeared. Different means to overcoming the problems connected with complex matrix were investigated in the past. Alkaline (or acidic) samples were neutralised by using Donnan or electrodialysis [1–8], the problems caused by the organic solvents were solved by the introduction of solvent compatible stationary phases [9], most frequently encountered matrix components chloride and/or sulphate were removed by passing the sample through the pre-column containing Ag^+ or Ba^{2+} resin [10–17]. The matrix effects were reported to be minimised also by the use of ''heart-cut'' technique, applied by Killgore and Villaseñor [18]. Another technique, called on-column matrix elimination procedure, was proposed [19–22] to minimise matrix effects. The technique is based on

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the assumption that the matrix effect can successfully be eliminated by the replacement of the eluent component(s) by the matrix anion. Careful optimisation of the eluent concentration in order to obtain reasonable retention times and peak shapes is suggested by the authors.

According to the mechanism an interfering species influenced retention or detectability of some analytes, on-column co-processes caused by matrix components were divided into four groups, i.e., the self-elution effect, the on-column change of the eluent, the on-column inter-eluent neutralisation and the suppression of the detector response [8,23]. The self-elution effect was first mentioned by Jackson and Haddad [24]. The authors explained the influence of high concentration of the sulphate anion on the effects of the ion-exchange capacity of the concentrator column by the sulphate-induced self-elution effect and demonstrated that the dynamic capacity of a concentrator column depended inversely on the matrix anion concentration (sulphate) in the samples. Novič et al. [23] described self-elution effect in details and demonstrated that this phenomena resulted in the prolongation or shortening of the retention times when matrix component had smaller or higher affinity to the stationary phase than eluent component, respectively. It was also demonstrated that the prolongation of the retention times was a direct consequence of the phenomena called on-column change of the eluent. The on-column inter-eluent neutralisation was found to play an important role on the retention behavior of the analytes in the case when dissolved CO_2 was allowed to diffuse into porous stationary phase. During its re-diffusion into the mobile phase, carbonate/bicarbonate-based eluent is converted into bicarbonate-based eluent having low elution capacity what results in the prolongation of the retention times of all analytes present in the sample [23]. Co-elution of the matrix component with the analyte of interest results also in the reduction of the detection sensitivity of on-suppressor protonated analyte (nitrite co-eluted with chloride, [23]) or non-complete conversion of sodium chloride to hydrochloric acid due to physical blockage of the suppressor membrane by co-eluted CO_2 [8].

Two additional types of heavy mineralised samples can be encountered very often, i.e., samples containing matrix composed of the same anion(s) as the eluent, and the samples containing increased concentration of mineral acid(s). In the present paper the results of the analysis of such samples by IC are presented. The mechanism of the prolongation of the retention times of the analyte, when hydronium cations are present in the sample, are described. A mechanism for nitrite peak-splitting obtained during IC analysis of acidic samples is also proposed. The distortion of the analyte peaks, obtained during IC analysis of samples having a matrix component identical to that present in the eluent, is explained as the consequence of sample-induced micro-gradient elution.

2. Experimental

2.1. Reagents and standard solutions

All the reagents used in this study (if not separately specified) were of analytical-reagent grade (Merck, Darmstadt, Germany). The synthetic samples containing only nitrite and nitrate in different matrices (water, sulphate, chloride) were analysed. The stock solutions of nitrite and nitrate (1.000 g l^{-1}) were prepared by dissolving appropriate amounts of $NaNO_2$ and $NaNO_3$ (dried at 103°C) in water. The stock carbonate–hydrogencarbonate eluent, chloride eluent and sulphate eluent were prepared by dissolving 1.908 g of Na_2CO_3 and 1.428 g of $NaHCO_3$, 2.925 g of NaCl or 7.100 g of Na_2SO_4 in 1 l of water, respectively. Working eluent solutions were prepared by appropriate on-line dilution by the quaternary pump incorporated into a Dionex (Sunnyvale, CA, USA) 4000i analytical system. The standard chloride and sulphate stock solutions (50.0 g l^{-1} and 25.0 g l^{-1}) were prepared by dissolving 82.42 g of NaCl and 36.98 g of Na_2SO_4 in 1 l of water. A series of standard nitrite and nitrate solutions was prepared by the appropriate dilution of standard stock solutions and addition of the appropriate amount of standard stock solution of chloride or sulphate. Amounts of 18 MΩ cm^{-1} water (Millipore, Bedford, MA, USA) were used for dilution in all cases.

The reagent for post-column derivatisation and detection of nitrite was prepared by dilution of 20 g

of sulphanilamide in 400 ml of water, subsequent addition of 50 ml of concentrated H_3PO_4 and 1 g of *N*-(1-naphthyl)ethylenediammonium dichloride and final dilution to 500 ml with water.

2.2. Chromatographic conditions

The Dionex 4000i system equipped with the Dionex IonPac AG4A-SC (50×4 mm) guard column and IonPac AS4A-SC (250×4 mm) separation column was used. The eluent conductivity was chemically suppressed on Dionex anion self-regenerating suppressor ASRS-1 (4 mm, current setting=1 or 2, external water mode). The sample-loop volume (if not specified otherwise) was 50 μl. The eluent and the H_3O^+ donor flow-rates were 2 ml min^{-1} and 3 ml min^{-1}, respectively. The chromatograms were obtained by using the conductivity detector (installed in 4000i apparatus) and the UV–Vis spectrophotometer Spectra-Physics SpectraSystem UV 2000 (Fremont, CA, USA) at 210 nm. In some experiments Dionex reagent delivery module RDM in combination with the Dionex membrane reactor were used for the derivatisation of nitrite. In this case the above mentioned spectrophotometer was used for the detection of nitrite at 543 nm.

The data for further evaluation were obtained by exporting the appropriate chromatograms into ASCII files. ASCII data files were further evaluated using Lotus 123R3 (Lotus Development Corporation, USA) and Microcal Origin (Microcal Software, USA) software packages.

3. Results and discussion

3.1. H_3O^+ influenced retention behaviour of nitrite and nitrate

As described in recent publications [8,23], matrix anions present in the sample at significantly higher concentration than the analyte anions, can cause shortening of the retention times due to the self-elution process or prolongation of retention times due to the combination of self-elution effect and on-column change of the eluent composition. It was demonstrated that self-elution was a predominant phenomena when matrix anion had significantly higher affinity to the stationary phase (sulphate) than the eluent component. In an opposite situation (eluent component has higher affinity to the stationary phase than the main matrix component, like chloride), the prolongation of the retention times is a consequence of the self-elution effect followed by the on-column change of the original eluent to the significantly weaker matrix component-based eluent.

During the IC analysis of acidic samples an increase of the retention times of nitrite and nitrate was observed compared to those obtained when matrix component was present in samples as a salt. Retention times were always prolonged regardless of the affinity of the main matrix anion to the stationary phase. In order to obtain experimental data for the appropriate explanation of mentioned phenomena, a series of experiments was carried out in which nitrite and nitrate were determined by IC in acidic media (HCl, CH_3COOH and H_2SO_4) and in samples containing the sodium salts of mentioned acids. Some results are presented in Fig. 1.

Fig. 1A and B clearly show that not only the anion present in the sample as a matrix component, but also the co-ions, e.g., hydronium cations, play an important role with regard to retention times of nitrite and nitrate.

The calculated capacity factor for nitrite in the presence of chloride as 100 m*M* NaCl increases from 1.2 to 2.1, whereas the increase to 3.1 is observed in the case of 100 m*M* HCl. The capacity factor for nitrate increases only slightly in the presence of 100 m*M* NaCl (from 2.83 to 2.95), whereas it increases significantly in the presence of 100 m*M* HCl (from 2.83 to 3.8) (Fig. 1A). This signifies that H_3O^+ cations interfere with nitrate stronger than chloride anions, while in the case of nitrite they almost double the effect caused by chloride only.

The acetate anions possess about a three-times smaller selectivity constant than chloride anions and do not have strong self-elution capacity [25]. Therefore, when present as a sodium salt, even in a high concentration (100 m*M*), only a slight prolongation of retention time of nitrite from 2.1 to 2.3 min can be observed. On the contrary, when acetic acid is present as an interfering species, the retention times of both analytes increase substantially (about 18% for nitrate and almost 60% for nitrite, Fig. 1B).

Longer retention times for nitrite and nitrate in

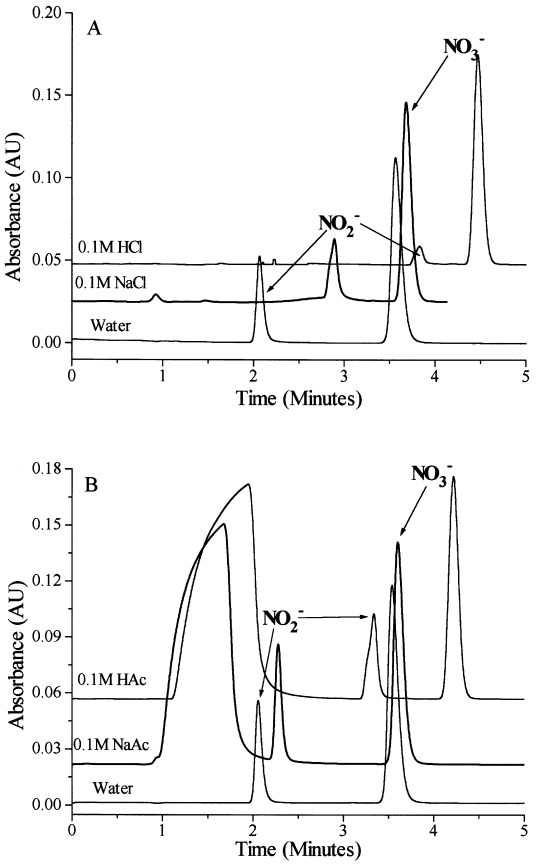

Fig. 1. The influence of the chloride anions present in the sample as NaCl or HCl (A), and acetate anions (Ac) present in the sample as CH_3COONa or CH_3COOH (B), respectively, on the retention behavior of nitrite (2 mg l^{-1}) and nitrate (5 mg l^{-1}). The concentrations of the individual interfering compound marked in (A) and (B). Chromatographic conditions as cited in Section 2.2, UV–Vis spectrophotometer was installed after the suppressor column.

HCl and CH_3COOH than in their sodium salts, respectively, can be explained by on-column neutralisation/conversion of the eluent. Upon entering the analytical column, H_3O^+ from the sample plug converts CO_3^{2-} groups fixed on the stationary phase and the carbonate anions in the mobile phase to CO_2 and water. Excess of released CO_2 diffuses into porous stationary phase. During the re-diffusion of CO_2 back into the mobile phase, the on-column inter-eluent neutralization takes place, converting carbonate–hydrogencarbonate-based eluent to bicarbonate-based eluent. The retained nitrite and nitrate are therefore firstly under the influence of week bicarbonate-based eluent, later they are under the influence of chloride–hydrogencarbonate-based eluent, and finally they are eluted by the original eluent.

Accordingly to the proposed explanation a similar effect was observed when comparing the chromatograms of Na_2SO_4 and H_2SO_4. The results are shown in Fig. 2.

The retention times of nitrite and nitrate in 100 m*M* Na_2SO_4 are shortened compared to retention times of the same anions in water because of the strong self-elution capacity of sulphate (curve II, Fig. 2). The self-elution effect of sulphate acts also in the case of H_2SO_4, however, additional on-column inter-eluent neutralisation appears. Because both effects act in the opposite directions, a chromatogram was obtained (curve III, Fig. 3) in which almost identical retention times for nitrite and nitrate can be observed compared to the retention times (for the same analytes) obtained when samples prepared in pure water were analysed (curve I, Fig. 2).

A closer look at the chromatogram of a sample containing nitrite and nitrate in 100 m*M* H_2SO_4 (curve III, Fig. 2) revealed another interesting phenomenon, namely the nitrite peak seemed to be split in two peaks. In order to prove that both sub-peaks actually originate from nitrite, a series of chromatograms of the sample containing increased concen-

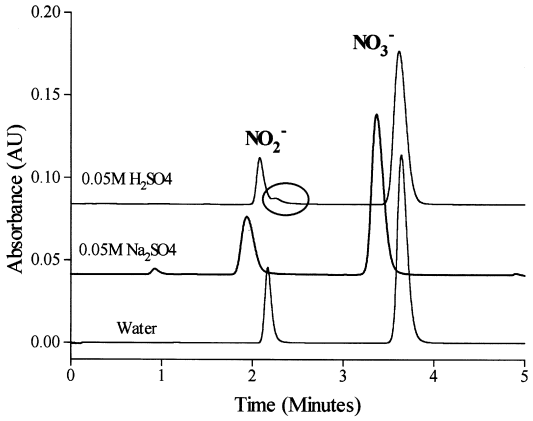

Fig. 2. Chromatograms of the samples containing 2 mg l^{-1} nitrite, 5 mg l^{-1} nitrate in water, (I) in 0.05 *M* Na_2SO_4 (II), and in 0.05 *M* H_2SO_4 (III). Deformed nitrite peak is marked by the circle. Other experimental conditions as in Fig. 1.

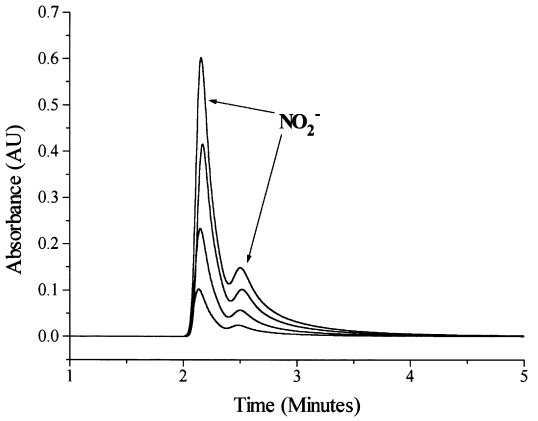

Fig. 3. Chromatograms of sample (NO_2^- content 2, 4, 7 and 10 mg l^{-1}, respectively in 100 m*M* H_2SO_4) recorded after post-column derivatisation of nitrite by *N*-(1-naphthyl)ethylenediammonium dichloride. Colored compound was monitored spectrophotometrically at 543 nm, other experimental conditions as in Fig. 1.

trations of nitrite in 100 m*M* H_2SO_4 was recorded (Fig. 3).

As a detector, a spectrophotometer measuring the absorbance of post-column-derivatised nitrite with *N*-(1-naphthyl)ethylenediammonium dichloride at 543 nm was used. The experiment undoubtedly confirmed that both peaks in Fig. 3 were caused by nitrite anions. The splitting of the nitrite peak can be explained by the protonation of nitrite anion and by the formation of non-dissociated nitrous acid HNO_2 ($pK_a = 3.29$). When acidic sample plug enters the analytical column, non-dissociated HNO_2 diffuses into the porous stationary phase, while the rest of NO_2^- are displaced down the column because of the self-elution effect of sulphate anions. Later, HNO_2 diffuses back into the basic eluent converting to NO_2^- ions. Re- diffusion is time-lagged and, therefore, a significant broadening of the nitrite baseline width can be observed (Fig. 3).

3.2. Sample-induced micro-gradient elution

The on-column matrix elimination, suggested as an alternative for the determination of some analytes in samples containing high concentrations of chloride or sulfate [19–22] was applied also in our work. However, using the proposed procedure it was found that matrix effects of chloride or sulphate anions present at the concentrations higher than approx. 1 g l^{-1} could not be eliminated by the mentioned technique effectively (nitrite and nitrate peaks tend to broaden at the baseline and become asymmetric). It should be emphasized, that the experiments in this work were carried out with unbuffered eluents in combination with the analytical column having the total capacity of approx. 23 μequiv. (AS4A-SC, Dionex). The method was however unsuitable for effective matrix elimination. A systematic study with chloride-based and sulphate-based samples using chloride-based and sulphate-based eluents, respectively, was carried out in order to find an appropriate explanation of the observed phenomena. The results of such an experiment with chloride-based eluent are presented in Fig. 4.

The chromatograms shown in Fig. 4 were obtained by using the eluent with fixed concentration (0.5 g l^{-1} NaCl). Two general tendencies are apparent in Fig. 4.: (1), with increased concentration of chloride in samples, nitrite and nitrate peaks tend to broaden and (2), nitrite and nitrate peaks, respectively, end at the same retention times regardless on the chloride concentration in samples.

Apparently, both phenomena are connected to the eluent composition and to the main matrix component in the sample. A closer look at both com-

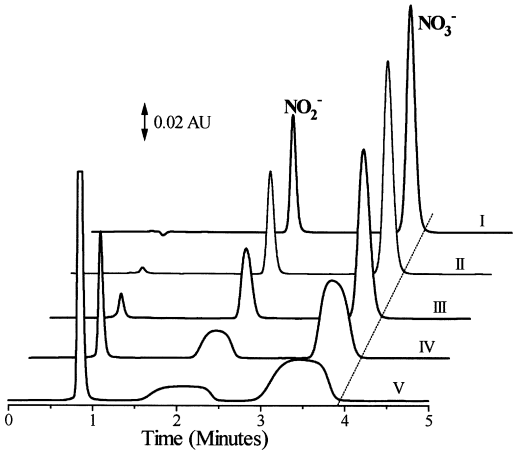

Fig. 4. The chromatograms of the samples containing 2 mg l^{-1} of nitrite and 5 mg l^{-1} of nitrate in samples with chloride concentrations 0.0 g l^{-1} (I), 1.0 g l^{-1} (II), 2.5 g l^{-1} (III), 7.5 g l^{-1} (IV) and 15.0 g l^{-1} (V). The eluent was 14 m*M* NaCl. Nitrate peak-ends are marked with a dotted line.

positions revealed that they were equal, the only difference was the concentration of active species in eluent compared to those in the sample. This actually means that when such a sample plug enters the analytical column, a micro-gradient of eluent is established. According to Eq. (1), capacity factor k' for both analytes is significantly lower at the segments covered by the sample plug if supposed that all the other parameters remain unchanged.

$$k' = \frac{(\mathrm{A})}{[\mathrm{A}]} \cdot \frac{V_{\mathrm{st}}}{V_{\mathrm{mb}}} = K_{\mathrm{E}}^{\mathrm{A}} \cdot \frac{(\mathrm{E})}{[\mathrm{E}]} \cdot \alpha, \qquad \alpha = \frac{V_{\mathrm{st}}}{V_{\mathrm{mb}}} \tag{1}$$

Round parentheses and square parentheses in Eq. (1) denote the concentrations on the stationary phase and in the solution, respectively, A is the analyte, E is the eluent and V_{st} and V_{mb} denote the volume of the stationary and the liquid phase, respectively.

According to Eq. (1), increased eluent concentration in the sample plug decreases the k' values for the analytes which results in the greater retention of the analytes on a greater number of the theoretical plates of the analytical column used (until the individual analyte is not quantitatively excluded from the sample plug) which can be observed finally as the broadening of the analyte peaks. Regardless of the value of k' with the increased matrix concentration, a certain amount of the analyte is always retained at the column inlet. This portion of the analyte is consequently eluted at the longest retention time, regardless on the concentration of the matrix component, which can be observed on the chromatograms as the constant retention time of peak-ends.

The same set of experiments was repeated also by using sulphate-based eluent for IC analysis of samples having sulphate as the matrix component. The results are presented in Fig. 5. It can be observed immediately from the Fig. 5 that nitrite and nitrate peak ends have a constant retention time, regardless of the sulphate content in the sample.

It can therefore be concluded that suggested mechanism called sample-induced micro-gradient elution is valid for double-charged matrix components as well as for single-charged ones. But what is rather different than in the case of chloride interferences are peak shapes. Significant peak broadening and asymmetry was achieved at very high sulphate concentration (above 30-times higher than in the

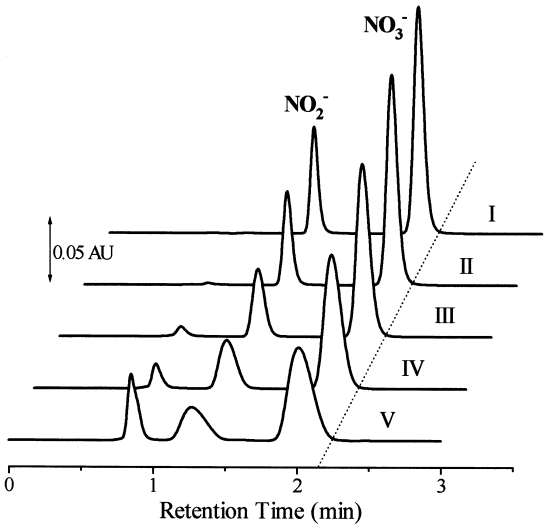

Fig. 5. The chromatograms of the samples containing 2 mg l^{-1} of nitrite and 5 mg l^{-1} of nitrate in samples with sulphate 0.0 g l^{-1} (I), 1.0 g l^{-1} (II), 5.0 g l^{-1} (III), 15.0 g l^{-1} (IV) and 50.0 g l^{-1} (V). The eluent was 5.2 m*M* Na_2SO_4. Nitrate peak-ends are marked with a dotted line.

eluent). This phenomena can be explained by rearranging Eq. (1). Taking into account the differences in analytes and sulphate charges, Eq. (1) should be rewritten as follows:

$$k' = \frac{(\mathrm{A})}{[\mathrm{A}]} \cdot \alpha = \left(K_{\mathrm{E}}^{\mathrm{A}}\right)^{1/2} \cdot \left(\frac{(\mathrm{E})}{[\mathrm{E}]}\right) \cdot \alpha \tag{2}$$

From Eq. (2) it is clearly evident that with increased eluent concentration, the capacity factor k' decreases much slower (by square root) than in the case of a single-charged eluent anion. Therefore, the analyte peaks retain their peak shape even at very high sample-to-eluent concentration ratio.

4. Conclusions

In the present paper effects of strong mineral acids (HCl and H_2SO_4) and a weak acid (CH_3COOH) in comparison to effects of their corresponding salts on the retention behaviour of nitrite and nitrate were studied. It has been shown that, apart of the pure self-elution of sulphate and self-elution in combination with on-column change of the eluent of chloride and acetate, the H_3O^+ ions additionally affect the

retention behavior of the analytes due to the on-column inter-eluent neutralisation. Additionally, a on-column nitrite peak-splitting was observed. This phenomena was explained by taking into account the protonation of nitrite in the sample plug. Because of the protonation, charge-free nitrous acid diffuse through the Donnan membrane (anion-exchange sites) into the porous stationary phase. The diffused nitrite fraction is therefore retained resulting in peak splitting and in significant broadening at the baseline.

The other interesting effect of sample matrix, i.e., the sample-induced micro-gradient elution, was observed when samples containing the same matrix anion as eluent were analysed. It was shown that this phenomenon tends to broad analyte peak, retaining the retention time of the analyte peak end at the same time (at the constant eluent concentration), regardless of the concentration of the matrix component. Peak distortion increased by increasing the concentration difference of the anion in the matrix and in the eluent. Therefore, the sample-induced gradient elution can be expected when the matrix component is the same as that of the eluent and its concentration in the sample higher than in the eluent. Peak broadening can be diminished by the increase of eluent concentration. The maximum eluent concentration depends both on the capacity of the analytical column and the minimum acceptable resolution of the analytes and therefore, has to be optimised for each individual case.

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