# STUDY OF THE CONTENT OF FOREIGN INORGANIC IONS IN SOLUTIONS OF BIOLOGICALLY ACTIVE SUBSTANCES BY METHOD OF ION CHROMATOGRAPHY

Ivankin A.N., Nekludov A.D., Karpo B.S.
The All-Russian Meat Research Institute, Talalikhina 26, 109316, Moscow, Russia

Food products contain cations of alkali, alkaline-earth metals and metals with variable valency, and many anions. Such elements are often identified by routine volumetric analysis or atom-absorption spectroscopy, but both require special sample preparation and the analysis itself is time-consuming [1].

Being mixed with water, food (including meat) products form solutions and suspensions containing inorganic ions, which penetrate into the products with water as well as with raw materials added during technological operations. Added ions may have toxic effects, that's why the content of ions should be limited in a number of cases.

Ion chromatography with conductometric detecting allows to determine the content of inorganic ions admixed in water solutions of biochemical extracts in relatively short time [2,3].

The aim of this work is to study analytical potentials of the ion chromatography method applied together with conductometric detecting for the determination of the content of free ions in meat products and wastes of the raw meat processing after extracting medicinal preparations of protein and polysaccharides nature. Hydrolyzates and some fractions of the blood as well as water-spirit extractions from fresh and extracted meat were the objects of the investigations. Ionic composition was determined by EPPENDORF-BIOTRONICS (FRG) portable chromatographer IC-2001 with the conductometric detector and columns for ions identification: BT V111-KA-P (with EK-V111-KA-P eluent for identification of $Li^+$, $Na^+$, $Nh_4^+$, $K^+$ ions and EK V111-KA-ER eluent for determination of $Mg^{2+}$ and $Ca^{2+}$ ions); BT 1X-KA (with EK 1X-KA eluent for determination of $Zn^{2+}$, $Co^{2+}$, $Fe^{2+}$, $Mn^{2+}$, $Cd^{2+}$ ions), and BT X-AN (with Bt-S-AG-P suppression column and eluent 3,5 mM $Na_2CO_3$ - 0,5 mM $NaHCO_3$ for determination of $F^-$, $Cl^-$, $NO_2^-$, $Br^-$, $NO_3^-$, $PO_4^{3-}$, $SO_4^{2-}$ anions). The solutions analyzed were pre-filtered through C18 cartridge and Millipore membrane with 0,22 mcm pores. Standard time of analysis was: 6 min. for the determination of alkali metals cations (0,5-5 mg/l concentration), 3 min. - for alkaline-earth metals cations (1-20 mg/l concentration), 30 min for heavy metals cations, and 15 min. - for anions (1-10 mg/l concentration). Chromatograms were calculated with the help of EPPENDORF-BIOTRONICS (FRG) automated computer program Winpeak V 3.24.

A graduating chromatogram of a standard set of anions as obtained with the use of a two-column version of chromatography is shown in Fig. It is seen that for standard solutions, containing 5-10 mg/l of anions $F^-$, $Cl^-$, $NO_2^-$, $Br^-$, $NO_3^-$, $PO_4^{3-}$, $SO_4^{2-}$, obtained by dissolving of corresponding salts in bidistilled water, there is a good resolution of separate peaks corresponding to individual anions.

Working chromatograms for natural extracts of pork or endocrine and enzymatic materials differed from standard chromatograms by the number of peaks corresponding to individual ions. Extracts for the analysis were prepared by means of multiple spirit-water extracting of 1 g of meat corresponding product at $20^0$ C followed by the removal of volatile solvents in rotor evaporator and solution of dry residue in 5 ml of citrate buffer with pH 2,2.

In all the analyzed samples there was a sufficiently reliable identification of the fluoride anion with the time of retention 1.4 min. (nitrates - 7.7 min., phosphates -9.7 min. and sulphates - 14.6 min). Because of the high content of chloride anions, the overlapping of peaks was sometimes observed in the field of 2.5-6.0 min. That corresponded to the time of retention for chloride and nitrite anions which could be identified by results of chromatography only in total. For the determination of nitrate and chloride anions separately, the sample of the extract was diluted 10-100 times, and the introduction of the specimen into the chromatograph was repeated for recording of the chromatogram of diluted solution. When the sample was 100 time diluted, the separation of nitrate and chloride peaks took place, but their integral intensity diminished, therefore some anions did not appear in repeatedly registered chromatogram of the diluted solution, e. g. fluoride and bromide anions.

As for the determination of different ions the conductometric detecting of their mobility was used, in some cases fields with negative base line were observed. The presence of some impurites in the analyzed solutions (in particular residues of organic solvents, e.g. ethanol used for extracting the meat) may be the cause of this phenomen. Those impurities changed the electric mobility of ions.

Thus, the analysis of anions, primarily nitrites and nitrates, the content of which should be limited from the considerations of safety, could be carried out simply enough.

To carry out the complete analysis of the ionic composition of biochemical extracts, it was necessary to have information about cations composition. Cations were analyzed in the same extracts in one-column chromatograph (columns: BT V111-KA-P or BT 1X-KA-P) selecting the concentration conditions of chromatography by dilutions. Table 1 shows the typical ionic composition analysis of blood hydrolyzates ABH-acid and ABH-enzymatic.

**Table 1.** Ionic composition of blood hydrolyzates [4] (mg/l in 1% solution):

| Samples | $Li^+$ | $Na^+$ | $NH_4^+$ | $K^+$ | $Mg^{2+}$ | $Ca^{2+}$ | $Zn^{2+}$ | $Co^{2+}$ | $Fe^{2+}$ | $Mn^{2+}$ | $Cd^{2+}$ | $F^-$ | $Cl^-$ | $NO_2^-$ | $Br^-$ | $NO_3^-$ | $PO_4^{3-}$ | $SO_4^{2-}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ABH-A | 0.03 | 72.1 | 75.8 | 9.4 | 160.0 | 327.0 | 0.3 | - | 20.5 | 9.35 | 9.11 | 4.1 | 77.0 | 200.0 | traces | | 50.0 | 450.0 |
| ABH-E | 0.01 | 275.8 | 45.3 | 171.5 | 16.2 | 5.9 | - | 0.01 | - | 0.05 | 0.05 | 5.0 | 40.2 | 350.0 | traces | | 45.0 | 240.0 |

To compare, Table 2 shows the basic ionic composition (mg/l) of blood proteins obtained according to [4]. According to the data obtained it is clear that, when the samples were precipitated from the water phase, admixtured salts (sodium chloride) prevailed in their composition.

Table 2. Ionic composition of some blood protein fractions

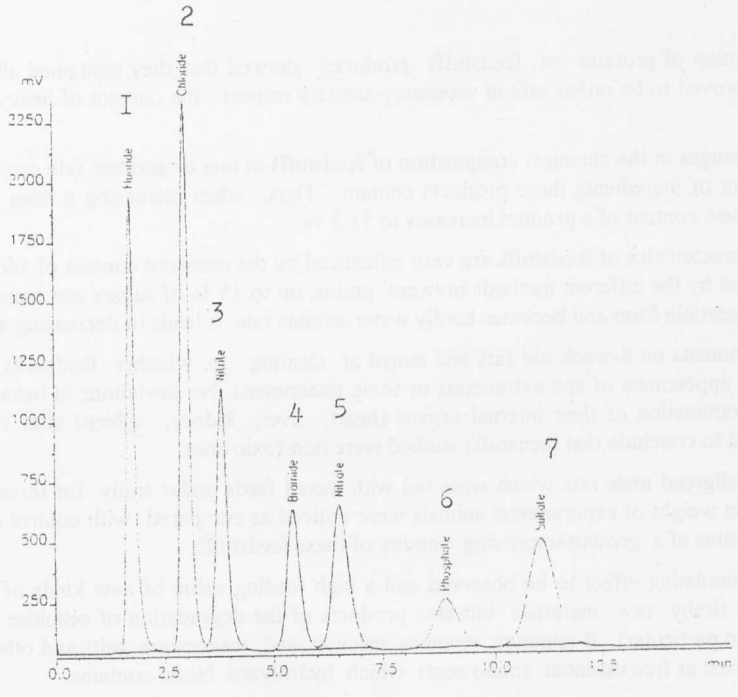
| Samples | $Na^+$ | $NH_4^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{+2}$ | $Cl^-$ | $NO_3^-$ | $PO_4^{3-}$ | $SO_4^{2-}$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 565.0 | 33.9 | 126.4 | 16.0 | 24.2 | 328.0 | 19.9 | 62.7 | 22.5 |
| 2 | 22.5 | 15.7 | 2.8 | 30.8 | 22.0 | 219.7 | 64.8 | 192.9 | 62.2 |
| 3 | 494.1 | 5.2 | 40.8 | 16.1 | 1.0 | 1076.3 | traces | 76.2 | 9.5 |
| Water | 15.8 | 0.6 | 4.2 | 0.7 | 0.6 | 0.5 | 1.9 | 2.1 | 2.0 |

Notes: Samples 1, 2, 3 are 1% aqueous solution:; sample 1 is the whole blood proteins isolated from the solution by the use of chromatography on CMC (carboxymethyl cellulose) without washing with pH 7 and then dried lyophilically [5]; sample 2 is washed blood proteins got after fractional precipitation from the semi-suspension [5]; sample 3 is blood proteins dried from the solution with pH 7 after hemin precipitation on CMC.

Study of the ionic composition of blood hydrolyzates and endocrine and enzymatic materials wastes showed that the content of basic ions in hydrolyzate samples was 0.01-200 mg/l in 1% solution. The nature of admixtured inorganic ions and their ratio depended on the preliminary conditions of processing of animal raw materials used for obtaining hydrolyzates.

**References**


1. Krylova N.N., Liaskovskaya Yu.N. Physico-chemical methods of the study of animal food products. M.: Pischevaya Promyshlennost, 1965. 316 p.
2. Zolotov N.A. Ion chromatography. M.: MGU. 1983. 263 p.
3. Schwedt G., Schlosser W. // Fresenius Z. Anal. Chem. 1985. V. 321, p. 136-140.
4. Nekludov A.D., Ivankin A.N., Karpo B.S. et al. //Myasnaya Promyshlennost 1996, No. 3, p. 11-12
5. Yevstafieva Ye.A., Mikheeva L.M., Nekludov A.D. // Myasnaya Promyshlennost 1993, No. 6, p.34


Pic.1. Analysis of standard anion mixture (two-column model; column BT X AN-S with suppression column BT S AG-P, eluent 3.5 mM $Na_2CO_3$-0.5 mM $NaHCO_3$ 1.4 ml/min., 50 atm, sample volume - 100 mcl), mg/l.: 1- fluoride, 2 - chloride, 3 - nitrite, 4 - bromide, 5 - nitrate, 6 - phosphate, 7 - sulfate.