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Separation selectivity of anionic metal complexes of *N,N'*-bis(hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid in ion and ion electrokinetic chromatography

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Abstract

The complexes of Fe(III), Co(III), Mn(III), Al(III), Cu(II), Ni(II), Cd(II) and Zn(II) with *N,N'*-bis(hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (HBED) were separated by ion exchange in different modes: ion chromatography (IC) and ion electrokinetic chromatography (IEKC). In column IC these complexes were separated on an IonPac AS4a anion-exchange column (Dionex, USA). Parameters of the background electrolyte that were examined in IEKC mode include polymer, competing ion concentration and pH. The use of poly(diallyldimethylammonium chloride) (PDADMACl) as a modifier in IEKC provides separation selectivity only slightly different from that observed in IC on the IonPac AS4a column. Optimal separation conditions were found to be: 0.1 mM HBED, 50 mM PDADMAOH, 10 mM Na₂B₄O₇, pH adjusted to 10 with acetic acid. The use of an aromatic ligand allowed a 10-fold decrease in detection limits of metal ions in comparison with previously studied EDTA. A separation efficiency up to 400 000 theoretical plates was demonstrated for IEKC. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The separation of metal ions by capillary electrophoresis (CE) has been of particular interest since the early 1990s. A main feature of the procedures applied for this purpose is the use of a complexation reaction for manipulating the separation selectivity or improving the detection characteristics [1]. All the ligands used can be divided into two groups, according to the thermodynamic stability of the complexes. Weakly complexing ligands, such as tartaric or 2-

hydroxyisobutyric acid, are usually applied for improving the selectivity of the separation with subsequent indirect absorption detection. The addition of ligands that form stable complexes to the sample solution or to the background electrolyte usually leads to the formation of anionic chelates. This strongly affects the separation selectivity and the approaches for its optimisation.

Since 1989, when 4-(2-pyridylazo)resorcinolato (PAR) chelates were separated for the first time by micellar electrokinetic chromatography (MEKC) [2], several mechanisms have been applied for the mobility differentiation in such systems. These include different types of reactions (interactions) of the

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species to be separated in the background electrolyte: acid–base [3], substitution of the water molecules in the coordination polyhedron of the central metal ion [4,5], ion-exchange [6] and ion-association interactions [7], and partitioning into the sodium dodecyl sulfate (SDS) micelles in the MEKC mode [8,9].

The main problem for multicomponent analysis of metal ions as their chelates is a choice of versatile chelating agent that combines high detection sensitivity and separation selectivity. Polyaminocarboxylates [EDTA or cyclohexane-1,2-diaminetetraacetic acid (CDTA)] form stable complexes with many metal ions, but show very poor separation selectivity due to a negligible difference in the charge-to-size ratio of separated species. The only interaction that solves this problem is ion exchange. Addition of cationic polymers to the carrier electrolyte reverses the electroosmotic flow (EOF), provides the conditions for fast determination of anionic species in a cathodic injection mode, and leads to different electrophoretic mobilities of anions depending on free/polymer-bound analyte ratio [10]. The best modifier – poly(diallyldimethylammonium chloride) – was chosen from the series of cationic polymers [11]. Since 1996, this approach has been expanded for the separation of PAR [6,11], EDTA, CDTA, Quin2 [10,12], and *N,N'*-bis(hydroxybenzyl)-ethylenediamine-*N,N'*-diacetic acid (HBED) [13] chelates. Very similar approaches for the optimisation of the separation and migration (retention) orders with common ion chromatography (IC) allowed us to designate this variant as ion electrokinetic chromatography (IEKC) [12].

The use of aromatic polyaminocarboxylates provides higher (in comparison with EDTA or CDTA) sensitivity of the direct spectrophotometric detection. Because of the high stability of the complexes and a low cost, HBED seems to be most convenient for determination of metal ions. Motomizu et al. [14] introduced this reagent for CE determination of Ca, Mg, and several transition metal cations. Recently, the IEKC mode was applied to a multicomponent separation of HBED complexes with 3+ cations [13].

The aim of this work was to study the parameters of the background electrolyte affecting the separation selectivity of metal–HBED complexes in IEKC with poly(diallyldimethylammonium chloride) as a modi-

fier. The same set of the metal ions to be separated, as for previous studies of EDTA and CDTA chelates [12], was chosen for these experiments. Comparison of the IC and IEKC separation modes was made as well.

2. Experimental

All the IEKC experiments were performed on a Bio-Focus 3000 (Bio-Rad) instrument. Fused-silica capillaries (GL Science, Japan) were 50 cm long (45.5 cm to detector) × 375 μm O.D. × 50 μm I.D. The negative connection of a power supply (–20 kV, cathodic injections) was used. Zones of metal–HBED complexes were detected in the capillary at 242 nm. Injections were produced by applying a pressure of 35–105 kPa × s. A chromatographic system equipped with a Shimadzu LC-10ATvp pump (Shimadzu, Japan), a Shimadzu SPD-10AVvp detector, and a Shimadzu SCL-10Avp control unit was used throughout. CLASS-VP software was used to control the HPLC system and for data handling. All the IC separations were performed on an IonPac AS4a (250 × 4 mm; Dionex, USA) column. A Rheodyne 7125 valve with a 100-μl loop provided sample injection into the chromatographic system. The eluent was delivered at a flow-rate of 1.0 ml/min. Direct detection at 242 nm was used for all the experiments. The absorption spectra of the ligand and metal chelate solutions were determined with a Shimadzu UV-2201 spectrophotometer.

Poly(diallyldimethylammonium chloride) (PDADMACI), as a 20% solution in water, and ligand were purchased from Aldrich (USA) and Dojindo (Japan), respectively. The background electrolytes were prepared with sodium tetraborate, sulfate and acetate (all reagent grade). For the concentrations see figures and the Results and Discussion section. They were filtered and degassed prior to use. Stock solutions of the ligand were prepared in 10 mM NaOH. Metal–HBED chelates were prepared by adding a desired amount of a metal-ion solution to 1 mM HBED at pH 9.2. The sample solutions of Co(II)– and Mn(II)–HBED complexes were stored several days at room temperature for complete formation of Co(III)– and Mn(III)–HBED chelates, respectively [13]. PDADMACI was converted into the –OH form

by the procedure described by Stathakis and Cassidy [15]. All the pH measurements were performed with a Horiba M-13 pH meter (Horiba, Japan). Deionised water (Elgastat UHQPC) was used throughout.

3. Results and discussion

Many metal ions form stable complexes with HBED (Table 1). The hard phenolate donor in the ligand structure provides high thermodynamic stability of the complexes with hard metal ions. Thus, FeHBED^- exists as singly charged anion up to pH 11 [16]. This makes it possible to use an alkaline carrier electrolyte, which can cause the formation of mixed hydroxo complexes and lead to peak broadening as in the case of FeEDTA^- . A borate buffer of pH 9.2 was selected to provide a difference in the charges between $\text{M}^{\text{II}}\text{HBED}^{2-}$ and $\text{M}^{\text{III}}\text{HBED}^-$. The choice of other parameters of the background electrolyte has been described elsewhere [10]. In these experiments, the following background electrolyte composition was used: 50 mM (of functional groups) PDADMACl, 5 mM sodium sulfate, 10 mM sodium acetate, 10 mM sodium tetraborate, pH 9.2. All parameters other than the one being examined were kept constant during these experiments.

Absorption spectra of these chelates have two maxima, at 242 and 293 nm. Because of its higher

molar absorptivity, the former was chosen for monitoring the analytes.

3.1. The influence of PDADMACl concentration

The concentration of a polymeric modifier is a crucial point for the optimisation of IEKC separation selectivity. Cationic macromolecules are strongly sorbed on the capillary wall, resulting in a positively charged surface and the anodic direction of EOF. This leads to the following migration order under the conditions of a negative polarity power supply (cathodic injection): doubly charged < singly charged anionic chelates < EOF. An increase in the modifier concentration in the carrier electrolyte gradually converts the system from an “electrophoretic mobility-controlled” into an “ion-exchange-controlled” state: doubly charged analytes migrate more slowly than singly charged ones (even after the EOF, as if they were positively charged species). Figs. 1 and 2 show the separation of a test mixture of HBED

Table 1
Stability constants of some metal–HBED chelates [16]

Cation	Log K_{ML}^{M}	Log $K_{\text{MHL}}^{\text{H}}$ ^a
H^+	$K_{\text{a1}} = 4.64$ $K_{\text{a2}} = 8.78$ $K_{\text{a3}} = 10.56$ $K_{\text{a4}} = 11.85$	
Mn^{2+}	14.78	7.66
Co^{2+}	19.89	7.77
Ni^{2+}	19.31	8.51
Cu^{2+}	21.38	8.63
Zn^{2+}	18.37	8.27
Cd^{2+}	17.52	8.11
Pb^{2+}	18.24	8.98
Al^{3+}	24.78 ^b	–
Fe^{3+}	39.68	–

^a $K_{\text{MHL}}^{\text{H}} = [\text{MHL}]/[\text{ML}][\text{H}^+]$.

^b Ref. [17].

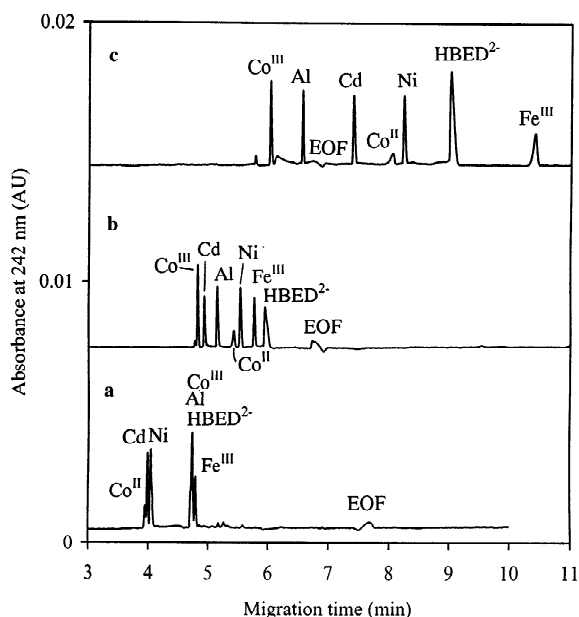


Fig. 1. The influence of PDADMACl concentration on the separation selectivity of metal–HBED chelates in IEKC. Background electrolyte: x mM PDADMACl, 5 mM Na_2SO_4 , 10 mM sodium acetate, 10 mM $\text{Na}_2\text{B}_4\text{O}_7$, pH 9.2; conditions: -20 kV; detection 242 nm, sample injection 35–105 kPa \times s; (a) 1 mM, (b) 10 mM, (c) 75 mM PDADMACl.

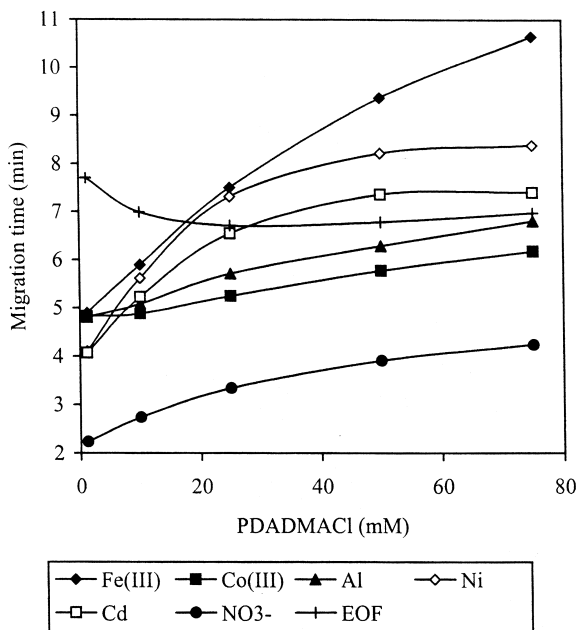


Fig. 2. The influence of PDADMACI concentration on the separation selectivity of metal–HBED chelates in IEKC. The NO_3^- anion was detected at 220 nm; other conditions as in Fig. 1.

chelates as a function of the PDADMACI concentration and a graphical representation of this function, respectively.

Increasing the concentration of the polymeric modifier in the background electrolyte causes an increase in the migration times of complexes. Migration times of doubly charged chelates (Ni, Cd) increase faster than those of Al, Co(III) and NO_3^- in the PDADMACI concentration range of 0–30 mM. The same dependence for MEDTA^{2-} chelates has a maximum at 50 mM of the modifier [12] due to a simultaneous increase in the concentration of competing chloride anion. In the case of HBED, the migration times of MHBED^{2-} chelates increased monotonically within the whole range of polymer concentrations studied.

FeHBED^- migrates at the slowest rate at any polymer concentration. This deviation can be accounted for by its having the highest stability constant among all the studied complexes, the most compact complex structure, and, consequently, the highest ion-exchange affinity to polymer chains. Such a behaviour could also indicate the formation of the hydroxo complex Fe(OH)HBED^{2-} . However,

subsequent IC experiments did not confirm this suggestion.

3.2. The influence of competing ion

The same pH buffer and competing anion were chosen for IC experiments. Fig. 3 shows the effect of the sodium sulfate concentration on capacity coefficients of metal–HBED chelates and NO_3^- anion. This is a typical dependence for IC, where $\log k'$ can be expressed as:

$$\log k' = (a/b) \log Q - (a/b) \log E + \text{constant}$$

where a , b , Q , E and k' are the charges of analyte and competing anions, column capacity, eluent concentration and capacity coefficient, respectively. As can be seen from Fig. 3, the slopes of the dependences for doubly charged Ni and Cd chelates are higher by a factor of two than for FeHBED^- , CoHBED^- , AlHBED^- , NO_3^- . An eluent of 15 mM Na_2SO_4 , 10 mM, $\text{Na}_2\text{B}_4\text{O}_7$, pH 9.2 was used to provide the

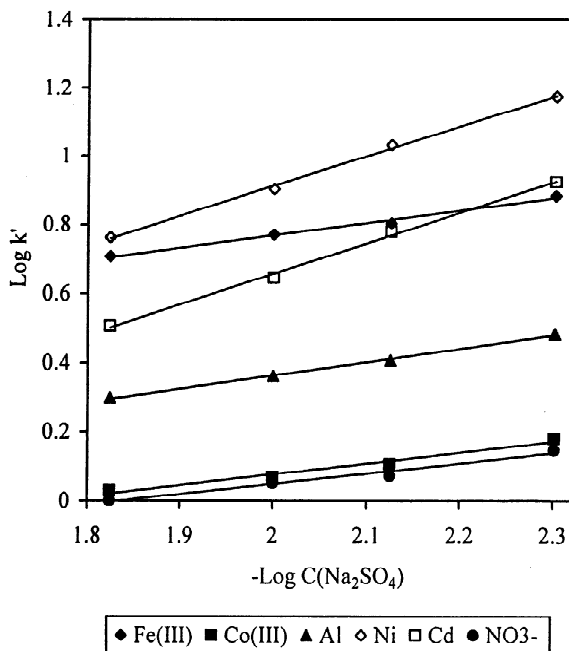


Fig. 3. The influence of sodium sulfate concentration on the separation selectivity of metal–HBED chelates in IC. Eluent: x mM Na_2SO_4 , 10 mM $\text{Na}_2\text{B}_4\text{O}_7$, pH 9.2; flow-rate 1 ml/min; sample volume 100 μl ; detection at 242 nm.

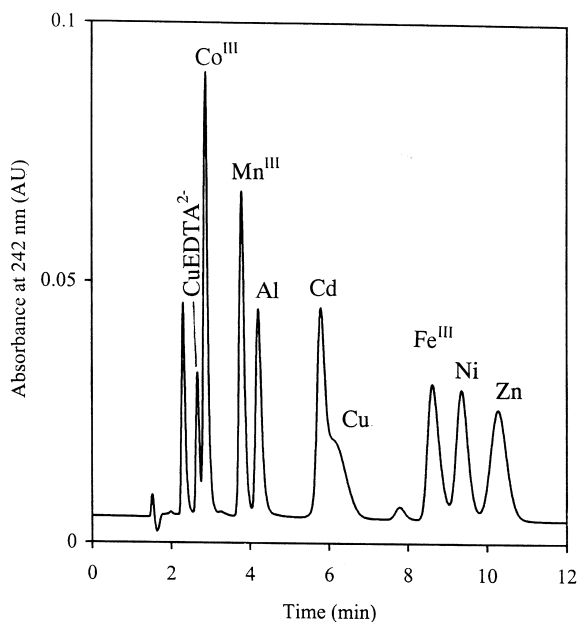


Fig. 4. IC separation of eight metal–HBED chelates. Eluent: 15 mM Na₂SO₄, 10 mM Na₂B₄O₇, pH 9.2; [MHBED]_T = 0.01 mM; other conditions as in Fig. 3.

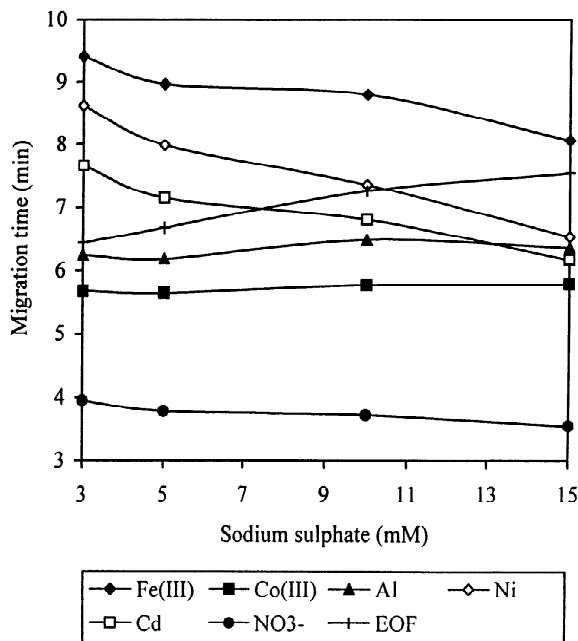


Fig. 5. The influence of competing ion concentration in IEKC separation mode. Background electrolyte: *x* mM Na₂SO₄, 50 mM PDADMACl, 10 mM sodium acetate, 10 mM Na₂B₄O₇, pH 9.2; other conditions as in Fig. 1.

separation of a test mixture of HBED complexes (Fig. 4). CuEDTA²⁻ was added to this sample to compare the retention of complexes with aliphatic and aromatic ligands.

In IEKC mode migration times of doubly charged complexes also decrease faster than for singly charged complexes (Fig. 5). Because of the decrease in EOF with increase of sodium sulfate concentration, migration times of singly charged complexes grow only slightly (except FeHBED⁻).

3.3. pH influence

The data in Table 1 show that the protonation of MHBED²⁻ chelates occurs within the pH range 7.5–9. Motomizu et al. [14] found a decrease in NiHBED²⁻, ZnHBED²⁻, CuHBED²⁻ electrophoretic mobilities with a shift in pH from 10 to 8. In the case of IEKC with a high polymer concentration, protonation leads to the weakening of polymer–analyte interactions and, therefore, to a decrease in the migration times (Fig. 6). The slopes of these dependences increase in the order: Zn < Ni < Cu,

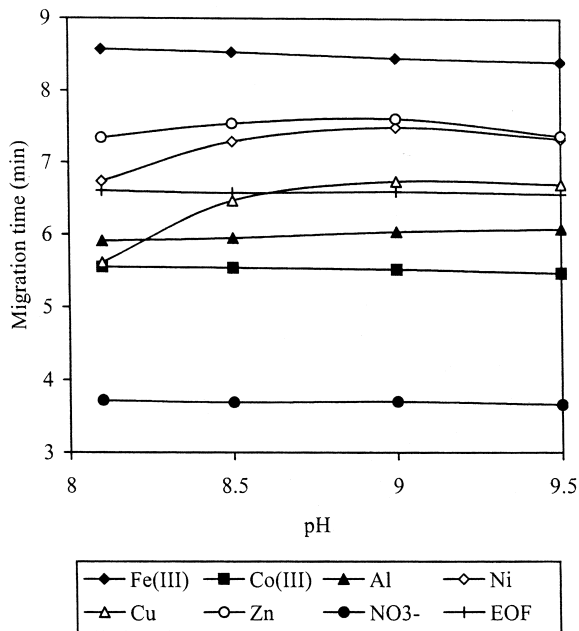


Fig. 6. The influence of pH on the separation selectivity in IEKC separation mode. Background electrolyte: 5 mM Na₂SO₄, 50 mM PDADMACl, 10 mM sodium acetate, 10 mM Na₂B₄O₇, pH adjusted with NaOH; other conditions as in Fig. 1.

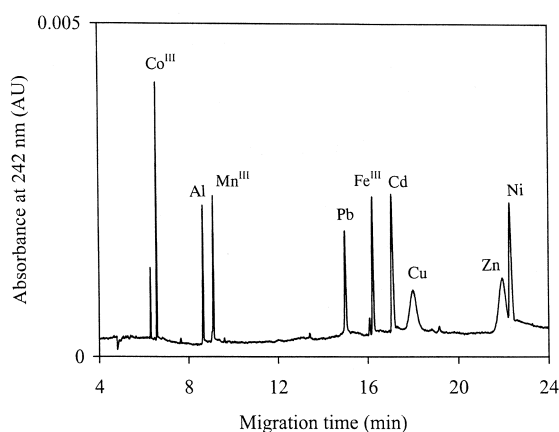


Fig. 7. IEKC separation of nine metal–HBED chelates using PDADMA polymer in acetate form. Background electrolyte: 0.1 mM HBED, 50 mM PDADMAOH, 10 mM $\text{Na}_2\text{B}_4\text{O}_7$, pH adjusted to 10 with acetic acid; sample injection 42 kPa \times s; $[\text{MHBED}]_{\text{T}}=0.05$ mM; $[\text{HBED}]_{\text{T}}=1$ mM; other conditions as in Fig. 1.

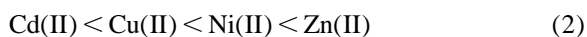
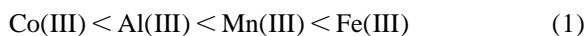
which agrees well with the row of $\log K_{\text{MHL}}^{\text{H}}$: Zn (8.27) < Ni (8.51) < Cu (8.63). Thus, pH of the background electrolyte (eluent) can also be used for changing the ion-exchange separation selectivity of metal–HBED chelates.

3.4. Comparison of IC and IEKC separation modes

Increasing the polymer concentration was found to be very important for the optimisation of the separation selectivity. However, some of the chelates studied (MnHBED^- and AlHBED^-) migrate almost together at any PDADMACl concentration. Because of lower ion-exchange affinity of acetate competing ion, the acetate form of the polymeric modifier was used to achieve baseline resolution of these analytes (Fig. 7).

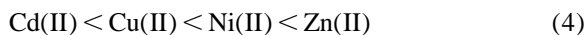
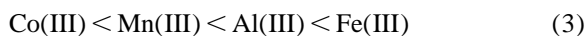
The separation selectivities of IC and IEKC

separation modes were compared using a background electrolyte (eluent) of pH 9.2. Migration times of the chelates in IEKC increase in the following order:



It should be noted, that Ni and Zn change their migration order when the pH is shifted from 9.2 to 10 (Fig. 7).

The same sequence was found for doubly charged HBED chelates in IC mode, and an only slightly different one for singly charged:



The lowest number of theoretical plates was calculated for Zn and Cu complexes for both IC (Fig. 4) and IEKC (Fig. 7). This phenomenon can be accounted for by the lower coordination requirements of these metal ions [16]. This leads to partially uncoordinated donor groups and the appearance of additional interactions in the separation system, which decrease the separation efficiency.

The separation efficiency, ion-exchange capacity of the systems and detection limits of metal ions for both modes are summarised in Table 2. The use of an aromatic ligand allows about a 10-fold decrease in the limits of detection in comparison with EDTA [12]. IEKC has evident advantages, including a wider set of the parameters, which can be used for the optimisation of the separation selectivity. However, higher detection sensitivity in IC mode should be noted.

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Table 2
The comparison of IC and IEKC

Separation mode	Separation efficiency (theoretical plates)	Capacity of the separation system ($\mu\text{equiv.}$)	Detection limits (mol/l)
IC	1500–4000	20	$1 \cdot 10^{-7}$ – $n \cdot 10^{-8}$
IEKC	20 000–400 000	0.05	$n \cdot 10^{-6}$

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