

## Even–odd alternation effect in the regularities of chromatographic retention

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### ABSTRACT

The influence of the structural factors due to difference in the configurations of molecules of homologues with even and odd carbon numbers on chromatographic retention is discussed. The relationship between retention and carbon number is expressed by a toothed (zig-zag) curve, *i.e.*, an alternation of retention values with transition from an even to an odd homologue is observed. Some data on the presence of the even–odd alternation effect in the regularities of changes in other physico-chemical properties of substances are given.

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### INTRODUCTION

When regularities in changes in the physico-chemical properties of substances belonging to homologous series are studied, account must be taken of the role of structural factors due to the peculiarities of the configurations of molecules with odd and even carbon numbers. This influence is manifested in a tooth-shaped (zig-zag) curve of the relationship between the physico-chemical characteristics and the carbon number of the molecule,  $z$ , *i.e.* there is an alternation of the values under consideration depending on  $z$  (whether it is odd or even). Recent studies have shown that a similar effect is manifested also in the regularities of changes in the characteristics of chromatographic retention within homologous series of solutes and stationary liquids. This paper reviews and discusses data available in the literature on this problem.

### ALTERNATION OF PHYSICO-CHEMICAL CHARACTERISTICS OF HOMOLOGUES

The influence of carbon number was first revealed during investigations of the regularities of changes in melting temperatures, first for the homologous series of  $n$ -alkanes<sup>1,2</sup> and later for the homologous series of fatty acids<sup>3</sup>. In both instances the relationship between the melting temperature,  $T_{\text{melt}}$ , and carbon number  $z$ , is different for even and odd homologues. An equation was suggested<sup>2</sup> relating  $T_{\text{melt}}$  to  $z$  and taking into account the alternation effect:

$$T_{\text{melt}} = 137.8 - \frac{2513}{5.141 + z - \varphi} \quad (1)$$

where  $\varphi = 1$  for *n*-alkanes with odd *z* and  $\varphi = 0$  for those with even *z*; 137.8 is the melting temperature of the limiting *n*-alkane,  $(\text{CH}_2)_\infty$ ; 2513 and 5.141 are empirical constants characteristic of the homologous series in question.

Calculations of  $T_{\text{melt}}$  according to this equation are in good agreement with the experimental values. Similar equations might be suggested for other homologous series.

The alternation effect for liquid crystals is displayed not only with respect to the melting temperatures but also the temperatures of other phase transitions: smectic–nematic and nematic–isotropic liquid<sup>4–8</sup>. For example, it is observed in the dependence of the clarification temperature on the carbon number in alkoxy side-chains in a series of symmetrical azoxy ethers such as *p,p'*-(dialkoxy)azoxybenzenes used as stationary phases in chromatography.

The influence of *z* on the values of dipole moments, heats of crystallization, refractive indices and the size of fatty acid crystalline cells of methyl and dimethyl ethers of dicarbon acids was studied by Liepin' and co-workers<sup>9,10</sup> on the basis of literature data<sup>3,11–14</sup> and their own experimental data. A tendency for alternation of these physical constants, due to difference in the configurations of molecules with even and odd *z*, was obtained.

#### ALTERNATION OF GAS CHROMATOGRAPHIC RETENTION WITHIN A HOMOLOGOUS SERIES OF SOLUTES

The influence of *z* on the nature of intermolecular interactions in solutions is only slightly displayed, which is why for a long time it was not possible to evaluate this slight effect even with the help of such a highly efficient method as chromatography and, consequently, to reveal the corresponding retention anomalies. However, it was shown in Kováts' first paper<sup>15</sup> that the retention index was determined by logarithmic interpolation on the basis of logarithms of retention volumes of *n*-alkanes belonging to an even-carbon series, however, later this was considered unnecessary, and interpolation was carried out on the basis of the retention values of neighbouring *n*-alkanes.

With further developments in chromatography, it was ascertained that the dependence between the retention values and the carbon numbers of the molecules of solutes for a homologous series, which had previously been postulated to be linear, often deviates from linearity<sup>16–20</sup>. These deviations may be caused either by anomalies in the properties of solutes or by the sorption properties of stationary phases. A detailed study of this problem led to the conclusion that such deviations from linearity were mainly connected with the peculiarities of the molecular structure of the first members of the homologous series and were due to irregularities in the increment for a repeated fragment of the molecule. These deviations are most vividly manifested when polar solutes and/or polar stationary phases are used. The correlation between the chromatographic retention and the carbon number of the molecule, *z*, can be represented by the equation

$$\log t'_{R_s} = a + bz + cz^2 + \dots \quad (2)$$

where  $t'_{R_z}$  is the adjusted retention time of a member of a homologous series with carbon number  $z$  and  $a$ ,  $b$  and  $c$  are constants.

In our work<sup>20</sup>, an effect was discovered, the essence of which is that the difference (ratio) of the retention values for a series of  $n$ -alkylbenzenes is minimal for the  $n$ -propylbenzene–ethylbenzene pair. Later this phenomenon was investigated by Soják *et al.*<sup>21</sup> and called “the propyl effect”.

The simultaneous manifestation of the two “anomalous” effects, the propyl and the even–odd effects, is supported, in particular, by the retention data for alkylbenzenes on columns of squalane, citroflex (tributylacetyl citrate) and 1,2,3-tris( $\beta$ -cyanoethoxy)propane<sup>22–25</sup>.

The role of carbon number as a factor affecting the retention of homologues was first revealed when the  $n$ -alkanes–polyethylene glycol system was investigated<sup>26</sup>. An experiment on a highly efficient open-tubular column showed the relationship between the retention and  $z$  to be a broken line, *i.e.*, alternation of the chromatographic retention values takes place with the transition from an even to an odd member of a homologous series. Thus, strictly, this relationship should be depicted in the form of two curves, one of which corresponds to even and the other to odd values of  $z$ .

Eqn. 2 taking into account the alternation effect may be written as<sup>27</sup>

$$\log t'_{R_z} = a_i + b_i z + c_i z^2 + d_i z^3 + D_i \varphi_z \quad (3)$$

where  $a_i$ ,  $b_i$ ,  $c_i$ ,  $d_i$  and  $D_i$  are constants and  $\varphi_z = +1$  for even  $z$  and  $\varphi_z = -1$  for odd  $z$ .

The alternation effect is slight, but it can be revealed when one considers the ratio of the adjusted values of the retention times,  $r = t'_{R_z}/t'_{R_{z-1}}$ , or the difference in the retention indices of neighbouring homologues, rather than the actual values of the retention time. When investigating the regularities of changes in retention with change in  $z$  for substances belonging to different homologous series, one should consider the ratio of the adjusted retention times or the difference in the retention indices of substances having the same carbon numbers, but belonging to two homologous series. The value of  $r$  is related to the difference in free enthalpies of evaporation from the stationary phase,  $\delta(\Delta G^0)_z$ , of two homologues with carbon numbers  $z$  and  $z - 1$  by the equation

$$\delta(\Delta G^0)_z = RT \ln (t'_{R_z}/t'_{R_{z-1}}) = RT \ln r \quad (4)$$

where  $R$  is the universal gas constant and  $T$  is the absolute column temperature. Hence the contribution of the carbon number can be represented in terms of energy units.

The distance between the lines on the plots for homologous series with even and odd  $z$  is characterized by the alternation criterion  $A_z$ <sup>9,10,27</sup>:

$$A_z = \log r_{z+1} - \log r_z = \log t'_{R_{z+1}} + \log t'_{R_{z-1}} - 2 \log t'_{R_z} \quad (5)$$

where  $r_z$  and  $r_{z+1}$  relate to the homologues with carbon numbers  $z - 1$ ,  $z$  and  $z + 1$ , respectively.  $A_z$  depends on  $z$  and changes with the transition to each subsequent homologue.

Vigdergauz and Seomkin<sup>27</sup> succeeded in establishing the influence of  $z$  on the retention of  $n$ -alkanes, 1-bromoalkanes and 2-alkanones on a highly efficient column

with triethylene glycol dibutyrate, a stationary phase of moderate polarity. The investigation was carried out at 40 and 80°C. It was shown that the alternation effect increases in the series  $n$ -alkanes < 1-bromoalkanes < 2-alkanones and is clearly expressed by the relationship  $\delta(\Delta G^0)_z = f(z)$ . The toothed character of this relationship for 2-alkanones disappears with the transition to 1-bromoalkanes and then to  $n$ -alkanes. The relationship  $\delta(\Delta G^0)_z = f(z)$  for  $n$ -alkanes is an almost straight line. The influence of the temperature is only manifested in the shift of the points on the plot, the shape of the curve remaining the same.

It is also of interest that  $\delta(\Delta G^0)_z$  is smaller for 1-bromoalkanes with even than with odd carbon numbers, *i.e.*, there is a reverse regularity in the change in these values compared with  $n$ -alkanes. This effect can be accounted for by the role of the bromine atom in position 1, which makes the hydrocarbon chain longer, *i.e.*, the number of "chain-forming" atoms in 1-bromoalkanes with carbon number  $z$  should be considered to be equal to  $z + 1$ .

Fig. 1 illustrates the influence of carbon number on certain properties of  $n$ -alkanes<sup>27</sup>. The general shape of the dependence of the retention values on  $z$  is due to the regularities of changes in the saturated vapour pressures of the solutes (curve 1). The influence of  $z$  on the retention of  $n$ -alkanes (curves 2 and 3) is manifested more when polyethylene glycol 400 is used as the stationary phase than with triethylene glycol dibutyrate, which is a consequence of a more rigid molecular structure of the former. Curve 4 shows the dependence of the CH<sub>2</sub> group increment  $\Delta l/l_2$  on  $z$ , where  $\Delta l/l_2$  is the ratio of the difference in distances between the extreme carbon atoms for neighbouring homologues ( $\Delta l$ ) to the length of the C-C bond ( $l_2$ ). Curve 5 depicts the influence of  $z$  on melting temperature. The similar characteristics of curves 3 and 4 is

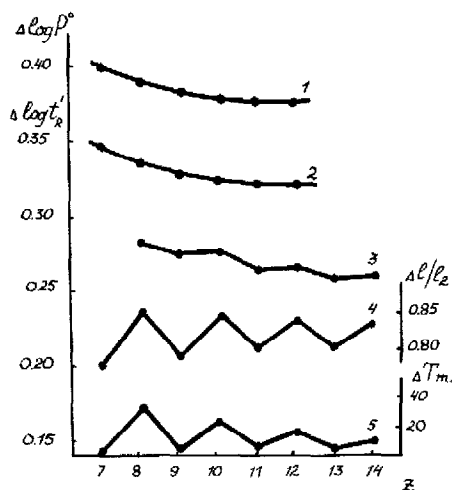


Fig. 1. Relationship between  $n$ -alkane properties and carbon number<sup>27</sup>. 1 = Homologous difference (difference for the neighbouring homologues) of logarithm of saturated vapour pressure,  $\Delta \log P^\circ$ , at 80°C; 2 and 3 = homologous difference of logarithms of adjusted retention times at 80°C on the columns (2) triethylene glycol dibutyrate and (3) polyethylene glycol 400; 4 = values of  $\Delta l/l_2$ , where  $\Delta l$  is the difference in distances between the extreme carbon atoms of the molecule for neighbouring homologues and  $l_2$  is the length of the C-C bond; 5 = homologous difference of melting temperature,  $\Delta T_{melt}$ .

evidence of a close relationship between the retention values and the molecular structure of the compounds in question.

The influence of  $z$  on the incremental value of a functional group in molecules representing different homologous series has been investigated<sup>28</sup>.  $n$ -Alkanes, aldehydes and ketones were chosen as solutes. The investigation was performed on an open-tubular column with triethylene glycol dibutyrate as the stationary phase. The capacity factor  $k' = t'_R/t_0$ , where  $t_0$  is the hold-up time, served as a retention characteristic. The dependence of  $\log k'$  on  $z$  has a form of a broken line and can be represented by an equation similar to eqn. 3.

The even-odd alternation factor was shown to increase in the series  $n$ -alkanes < bromoalkanes < aldehydes < ketones, the influence of this effect being the strongest with the first members of the homologous series and decreasing with increase in the length of the alkyl chain.

The calculation of retention increments on the basis of literature data<sup>29</sup> shows that alternation of  $\Delta I$  values for the homologous series of methyl and chloromethyl ethers of  $n$ -C<sub>5</sub>-C<sub>12</sub> chlorocarbon acids on the open-tubular columns with the stationary phases SE-30 and Carbowax 20M has also been observed.

The alternation effect in the chromatographic retention of C<sub>6</sub>-C<sub>11</sub>  $n$ -alkanes is also manifested on packed columns with SKTFT-100 trifluoropropylmethylsilicone and pentaphenyl ether stationary phases<sup>30</sup>. The dependence  $r = f(z)$  possesses a toothed character. Thus, for SKTFT-100 stationary phase the value of  $r$  is in the range 1.40-1.58 (the evaluation error is 0.01-0.04) and the values of  $\Delta r = r_{z+1} - r_z$  is -0.11, 0.18, -0.14 and 0.14 for consecutive pairs of homologues.

The alternation effect becomes especially noticeable for polar solutes and polar stationary phases. In these instances alternation can be manifested even for packed columns of moderate efficiency. This phenomenon was studied, in particular, with fatty acids and their ethers<sup>9,10,31</sup>. The investigation was carried out at different temperatures on four stationary phases of different polarity, Apiezon L, Triton X-305, Carbowax 20M and diethylene glycol succinate (DEGS). A tendency for alternation of retention index ratios of the solutes in question was revealed on the columns with DEGS and Apiezon L. The alternation of these values was observed within the range  $z = 3-12$ , disappearing with further increase in the alkyl chain length.

The alternation effect of retention values of dimethyl esters of fatty dicarboxylic acids was also found on a column with the high-temperature liquid-crystal stationary phase (bis-*p*-ethoxybenzylideneamino)biphenyl<sup>32</sup>. The alternation is clearly expressed in the range C<sub>4</sub>-C<sub>8</sub> whereas with increase in the number of CH<sub>2</sub> groups in the molecule it decreases.

The alternation criterion  $A_z$  has been calculated in order to resolve the question of the alternation of the retention values  $t'_R$ .  $A_z$  was calculated at 190°C on two types of stationary phase, polar (DEGS) and non-polar (Apiezon L). It was found that the alternation of  $t'_R$  is observed only on the polar phase within the range  $z = 5-12$ . A similar calculation also demonstrated the alternation effect of the retention values for dimethyl esters of dicarboxylic acids (190°C, DEGS stationary phase) within the range  $z = 3-6$  and for free fatty acids (190°C, diethylene glycol adipate + H<sub>3</sub>PO<sub>4</sub> stationary phase) within the range  $z = 4-10$ . As an example, Fig. 2 illustrates the alternation of the chromatographic characteristics of free fatty acids.

The study of the chromatographic behaviour of free fatty acids also showed that

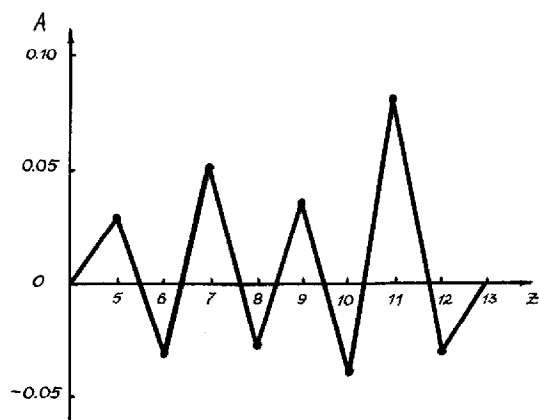


Fig. 2. Alternation of adjusted retention times of free fatty acids.

a decrease in the temperature of the column to 160°C results in the disappearance of the alternation effect; if the temperature is raised to 200°C, the effect is retained.

The alternation of the properties of a series of normal fatty acids is accounted for by the difference in molecular configurations of the even and odd homologues (Fig. 3). With monocarboxylic acids, if  $z$  is odd the methyl group is situated at one end of the molecule opposite the carboxyl group (B), and if  $z$  is even it is a methylene group (A). With dicarboxylic acids either the second carboxyl group or a methylene group is situated opposite the carboxyl group, depending on  $z$ . Therefore, if alternation is due to the configuration of the molecule, it is manifested more strongly with dicarboxylic acids and their derivatives. This is supported by experimental results<sup>9,10</sup>. Thus, if the alternation range of retention values is characterized by the difference  $\Delta A_z = |A_{z+1}| + |A_z|$ , it will be of the order 0.01–0.07 for free fatty acid methyl esters and 0.16 for the dimethyl esters of dicarboxylic acids. According to Liepin' and co-workers<sup>9,10</sup>, a considerable role in the alternation phenomenon can also be played by the polymorphism of fatty acids, as there exist three forms, the molecules of which differ in the angle of location of the carbon atoms.

The alternation effect was also revealed when the thermodynamics of the dissolution of fatty alcohols in cholesteryl myristate was subjected to chromatographic study<sup>33</sup>. This effect might be due to the fact that the introduction of molecules of

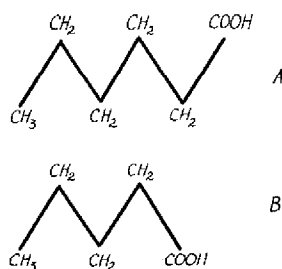


Fig. 3. Configuration of fatty acid molecules: (A) with even  $z$ ; (B) with odd  $z$ .

non-mesogenic additives into the mesophase structure results in different efficiencies of alignment between the molecules and separate parts of the mesophase, this alignment being mainly a face-to-face interaction between the molecules of a liquid crystal and non-mesogenic additive. In this case an increase in the length of the alcohol carbon chain will lead to a decrease in the smectogenic nature of the molecules of cholesteryl myristate.

The selectivity of *p,p'*-(methoxyethoxy)azoxybenzene (MEAB) as a stationary phase with respect to  $C_{10}$ – $C_{17}$  geometric and positional isomers of *n*-alkanes has been studied<sup>7,34</sup>. The dependence (Fig. 4) of the selectivity coefficient  $r$  on  $z$  is of a toothed character [ $r$  is the ratio of the adjusted retention times of *trans*-/*cis*-isomers at double bond position 2–8 (Fig. 4a, curves 2–8) and also the ratio of the adjusted retention times of the neighbouring *cis*-/*cis*- and *trans*-/*trans*-isomers (Fig. 4b)]. This confirmed the influence of  $z$  on the retention ratio of solutes having the same carbon number and belonging to different homologous series.

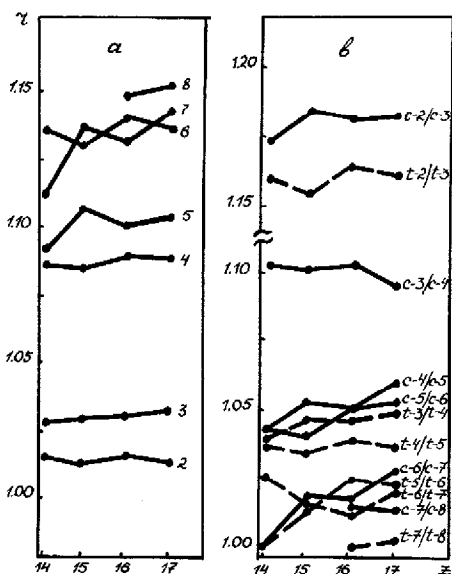


Fig. 4. Dependence of  $r$  on carbon numbers of  $C_{14}$ – $C_{17}$  *n*-alkane molecules on a column with MEAB at  $115^{\circ}\text{C}$ . (a) Separation of *trans*-/*cis*-isomers with the double bond in position 2–8; (b) separation of neighbouring *cis*-/*cis*- and *trans*-/*trans*-isomers.

#### ALTERNATION OF GAS CHROMATOGRAPHIC RETENTION VALUES ON COLUMNS WITH STATIONARY PHASES BELONGING TO THE SAME HOMOLOGOUS SERIES

The alternation effect is also manifested when the retention of the same solutes is studied on different stationary phases belonging to the same homologous series, in particular for the separation of close-boiling isomers on columns with liquid crystals of the nematic type, e.g., azoxy ethers, as stationary phases. The influence of  $z$  can then be considered from different viewpoints: one can count (i) the total number of carbon

atoms in a liquid crystal molecule or (ii) the number of carbon atoms in both side-chains. In addition, one can examine the influence of  $z$  in one side-chain. Thus, when *m*- and *p*-xylenes were separated with symmetrical azoxy ethers as stationary phases<sup>35-37</sup>, it was found that the dimethoxy derivative of azoxyanisole has a low selectivity, whereas the diethoxy derivative of azoxyphenetole has a much greater selectivity and consecutive homologues have nearly the same selectivity or a lower one (Fig. 5). However, if an asymmetric ether such as *p,p'*-(methoxyethoxy)azoxybenzene is used as a stationary phase the *meta-para* selectivity increases greatly and becomes higher than that with azoxyphenetole and its eutectic mixture with azoxyanisole<sup>38</sup>.

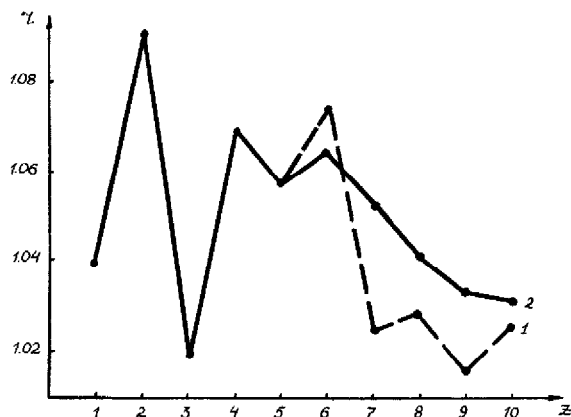


Fig. 5. Dependence of selectivity coefficient  $r$  on carbon number of the side alkoxy group of symmetric azoxy ethers for (1) smectic and (2) nematic mesophases.

The change in *meta-para* selectivity for a series of stationary phases prepared on the basis of symmetrical and asymmetric azoxy ethers has been investigated<sup>7,39</sup>. The selectivity coefficient  $r$  (in this instance the ratio of the adjusted retention times of *p*- and *m*-xylenes) was shown to experience alternation, which is due not only to the change in carbon number in the side-chains of azoxy compounds, but also to the dependence on the symmetry of the molecules. Note that, in general, asymmetric azoxy ethers possess a higher *meta-para* selectivity than the symmetrical compounds. MEAB has the highest *meta-para* selectivity in the series of asymmetric homologues.

An even-odd alternation effect in the retention of standard solutes (ethanol, methyl ethyl ketone, benzene and nitromethane) on liquid-crystal stationary phases belonging to the homologous series of nitroazoxy benzenes was revealed<sup>40,41</sup>. It should be mentioned that higher values of retention parameters are characteristic of odd- rather than even-carbon substituents.

#### THE EVEN-ODD ALTERNATION EFFECT IN LIQUID CHROMATOGRAPHY

The influence of  $z$  on sorption properties is also related to the corresponding regularities in the compositions of crude oils. Thus, the correlation of odd and even *n*-alkanes in present-day deposits and crude oils is shown to be different<sup>42-45</sup>.



Molecules with an odd carbon number predominate in deposits, whereas in crude oils the prevalence of molecules with an odd  $z$  is slight, if any. To characterize this relationship, an "oddness index" was suggested<sup>46</sup>, which is the ratio of the number of  $n$ -alkane molecules with an odd  $z$  ("odd" molecules, O) to that with an even  $z$  ("even" molecules, E). The O/E ratios in present-day deposits and crude oils are 2–5.5 and 0.9–1.2, respectively. The oddness index is used by many workers as an oil characteristic which makes it possible to evaluate the origins of crude oils. Another oil characteristic for identification purposes is the ratio of the contents of two alkanes with an isoprenoid structure in it, *i.e.*, pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethylhexadecane)<sup>47,48</sup>. The prevalence of even and odd alkanes in crude oils (normal and branched) has long been related to the organic origin of petroleum in literature, whereas the fact that migration of crude oils through rocks, which are active solids, may lead to changes in oil composition was completely ignored<sup>49</sup>. To confirm this fact, one can use the results of studies<sup>50,51</sup> in which the alternation effect in the sorption properties of  $n$ -alkanes was investigated by liquid chromatography. It was shown that if a mixture of  $n$ -alkanes passes through a column of silica gel impregnated with hexane, the first fraction eluted is enriched with even-carbon hydrocarbons.

Fig. 6 depicts the change in the ratios of the concentration of the neighbouring  $n$ -alkanes with carbon numbers  $z$  and  $z - 1$  when the initial (1) and final (2) fractions of the eluate were compared with the original mixture (0). The enrichment coefficient of the initial fraction of the eluate with the even-carbon alkanes was 1.24 for the chosen conditions.

It has been shown<sup>51</sup> that the filtration of crude oil through rock leads to the changes in the ratios not only of even/odd  $n$ -alkanes, but also of pristane/phytane, as compared with their ratios in the initial oil. A liquid chromatographic study was performed<sup>52</sup> close to conditions of fluid migration in nature, with activated silica gel impregnated with  $n$ -hexane as a porous medium, and the even-odd alternation effect

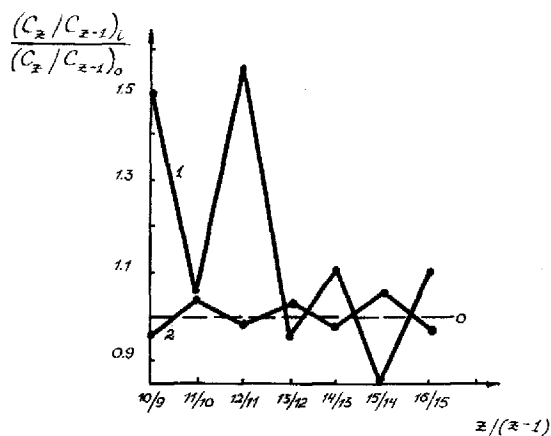


Fig. 6. Change in concentration ratios of the neighbouring  $n$ -alkanes with carbon numbers  $z$  and  $z - 1$ , when the initial (1) and final (2) eluate fractions are compared with the original mixture (0)<sup>49</sup>.

was also revealed. The content of pristane in the initial fractions increased in comparison with that of phytane. In subsequent tests, this sorption effect gradually decreased and the filtrate composition became close to the original.

## CONCLUSION

The even-odd alternation effect is manifested in the regularities of changes in the sorption properties of substances and, consequently, it must be taken into account when developing analytical methods and investigating related phenomena.

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