pH/R_f DIAGRAMS OF NUCLEOTIDE BASES

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Khimiya Geterotsiklicheskikh Soedinenii, Vol. 4, No. 5, pp. 940–944, 1968

UDC 543.544.2:547.854.4'857.4.7

The chromatographic mobilities of uracil, cytosine, thymine, orotic acid, adenine, guanine, hypoxanthine, and xanthine as functions of the pH of buffer systems in the pH range from 0 to 12 have been studied by absorption chromatography on paper. Changes in R_f are observed comparatively rarely in the regions of the pK_a values.

Recently, communications have appeared in the literature [1–13] on paper chromatography devoted to so-called "pH chromatography", in which the R_f values

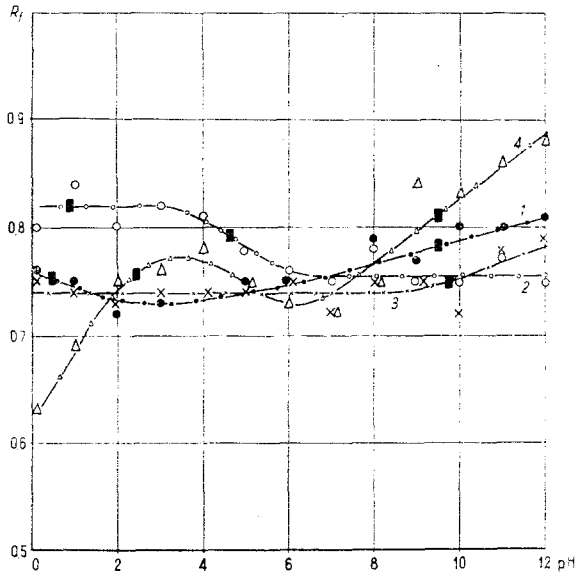

Fig. 1. pH/R_f diagrams in buffer systems: 1) uracil; 2) cytosine; 3) thymine; 4) orotic acid (the symbol ■ denotes the pK_a values of the bases).

of the compounds studied are investigated as functions of the pH of the system. Almost all these communications relate to chromatography on buffered paper, and theoretical calculations of this type of chromatography have been given [14–17].

The object of the present work was to study the "pH chromatography" or the pH/R_f diagram of the nucleotide bases by adsorption chromatography on paper. The chromatographic behavior of these substances has been studied by the method of paper chromatography in various systems fairly widely [18–20], but we have found no systematic study of the chromatographic behavior of the nucleotide bases as functions of the pH of the system in the literature.

To study the pH/R_f diagrams of the nucleotide bases we used as the chromatographic systems solutions of hydrochloric acid at pH values of 0 and 1 and buffer mixtures in the pH range from 2 to 12.

The nature of the curves that we obtained in the buffer solutions (Figs. 1 and 2) partly coincides with that of the curves obtained previously [14–17], i. e., in the regions of the pK_a values of the substances studied there is a change in their chromatographic mobility. However, in contrast to the curves obtained previously [14–17] a minimum mobility is found for the nucleotide bases in the case of the neutral forms and, conversely, the ionized forms have a high mobility. In comparing these results, it must be borne in mind that partition chromatography was used in the previous investigations [14–17].

It can be seen from the curves obtained that there is a difference between the pH/R_f diagrams of the purines and the pyrimidines. The curves of the purines (adenine, guanine, hypoxanthine, xanthine) and of orotic acid have well-defined inflections; the curves of uracil, cytosine, and thymine have gentler slopes. Both in the case of the purines and in the case of the pyrimidines, the pK_a values of the substances are close to the points of inflection.

In addition, we studied the pH/R_f diagrams of the nucleotide bases in the formic acid—water system (pH 0.48–2.35) (Figs. 3 and 4). Here the R_f values of the nucleotide bases decrease with a rise in the pH of the system, which is identical with the diagrams considered previously at low pH values. An exception is orotic acid, the mobility of which scarcely changes with a change in the pH in this system.

The dependence of the R_f values of some nucleotide bases on the temperature of chromatography in distilled water (Fig. 5) shows that the R_f values of adenine and guanine change more considerably with the temperature than the R_f values of cytosine, thymine, and uracil.

EXPERIMENTAL

Chromatographically pure samples of the nucleotide bases were used.

Chromatography was carried out by the ascending method in cylindrical glass chambers 50 cm high (internal diameter 14 cm) at 20 ± ± 1° C. Chromatographic paper of type FN 12 (Filtrak) was used, the dimensions of the sheets being 40 × 10 cm. The substances were dissolved in 10% aqueous ammonia and deposited in three portions to a total amount of approximately 0.05 mg. The distance between the points of deposition of the substances was 2 cm.

The following buffer mixtures were used [21]: 1) for the pH range 2.0–5.0—citrate buffer; 2) for the pH range 6.0–8.0—phosphate-alkali buffer; 3) for the pH range 9.0–12.0—glycine buffer. The pH values of the chromatographic systems were measured with a LPU-01 pH-meter.

Before chromatography, the solvent system (200 ml) was poured into the chamber and allowed to stand for 2 hr with shaking from time to time. The chromatograms were run until the line of the front had travelled 30 cm (an average of 3 to 5 hr). Then the chromatograms were dried in the air and examined on the ultrachemiscope.

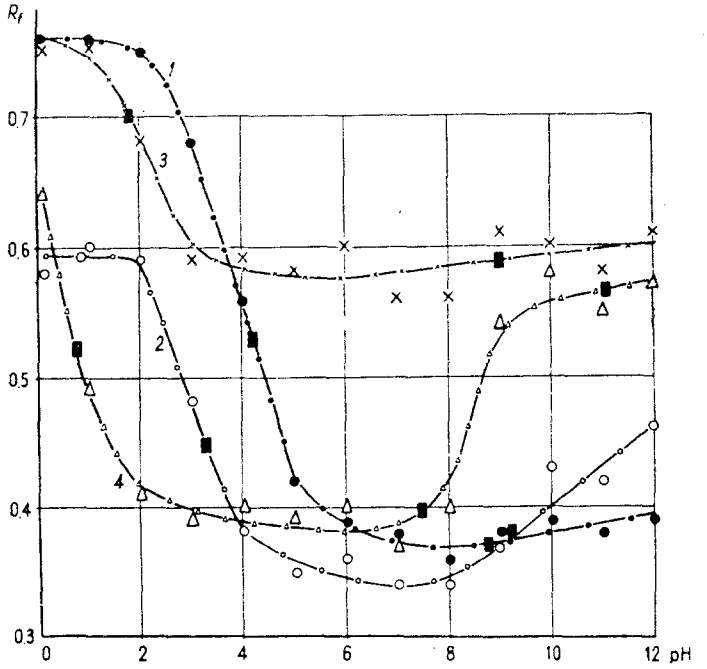

Fig. 2. pH/R_f diagrams in buffer systems: 1) adenine; 2) guanine; 3) hypoxanthine; 4) xanthine (the symbol ■ denotes the pK_a values of the bases).

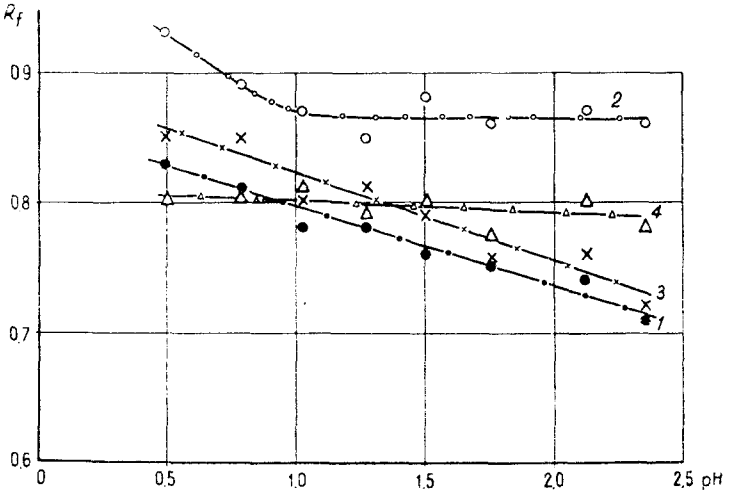

Fig. 3. pH/R_f diagrams in the formic acid—water system: 1) uracil; 2) cytosine; 3) thymine; 4) orotic acid.

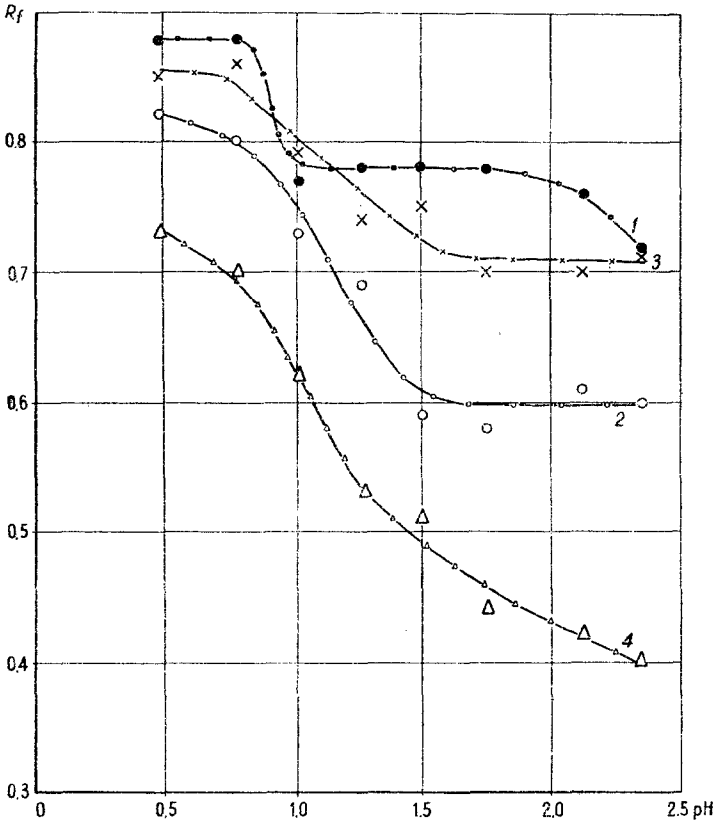

Fig. 4. pH/R_f diagrams in the formic acid—water system: 1) adenine; 2) guanine; 3) hypoxanthine; 4) xanthine.

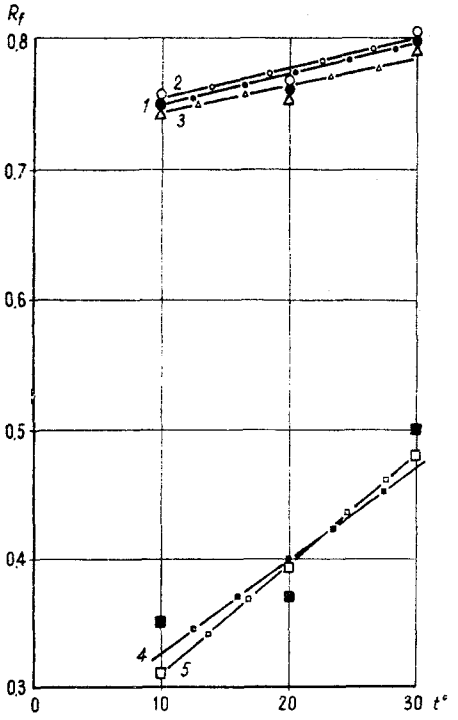

Fig. 5. R_f values as a function of the temperature of chromatography: 1) 1) uracil; 2) cytosine; 3) thymine; 4) adenine; 5) guanine.

To construct the pH/R_f diagrams the arithmetic mean values of the R_f from 5 repeated experiments were used. The mean error was ±0.03 of the R_f values.

In the study of the dependence of the R_f values on the temperature, the chromatographic chamber was placed in a thermostatted vessel.

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29 June 1967

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INFLUENCE OF π-ELECTRONIC CHARGES ON THE CHEMICAL SHIFTS OF THE meso-PROTONS IN β-ETHOXYCARBONYL-SUBSTITUTED PORPHYRINS

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Khimiya Geterotsiklicheskikh Soedinenii, Vol. 4, No. 5, pp. 944–946, 1968

UDC 547.749:541.67

A calculation has been made of the influence of the electric field of π-electronic charges on the chemical shifts of the meso-protons in β-ethoxycarbonyl-substituted porphyrins. A comparison of the calculated and experimental figures shows that the change in the chemical shifts taking place with the introduction of ethoxycarbonyl substituents into the β-position is due mainly to the changes in the π-electronic charges.

It is known that the presence of an electric field effects the screening constant of an atom [1]. The source of the electric field acting on an atom in a molecule is the charges on the individual atoms. In conjugated molecules, considerable changes in the distribution of charges take place when electro-negative substituents of the type of formyl and ethoxycarbonyl groups and groups similar to them are introduced [2]. These changes appear fairly clearly in the proton resonance spectra [3].

We have considered the influence of the electric fields of the π-electronic charges on the chemical shifts of the meso-protons in β-ethoxycarbonyl-substituted porphyrins: 2-ethoxycarbonyl-1, 4, 5, 8-tetramethylporphin (**II**), 2, 3-diethoxycarbonyl-1, 4, 5, 8-tetramethylporphin (**IIIa**), 2, 7-diethoxycarbonyl-1, 4, 5, 8-tetramethylporphin (**IIIc**), 2, 3, 6-triethoxycarbonyl-1, 4, 5, 8-tetramethylporphin (**IV**), and 2, 3, 6, 7-tetraethoxycarbonyl-1, 4, 5, 8-tetramethylporphin (**V**) in the form of dications.

The spectra of 0.03 M solutions of these compounds in deuteriochloroform with the addition of deuteriotrifluoroacetic acid were recorded on a JNM-C-60 spectrometer at 25° C. Hexamethyldisiloxane was used as internal standard.

In order to isolate the effects of the ethoxycarbonyl substituents, the calculation of the chemical shifts was made from the signals of the meso-protons of 1, 4, 5, 8-tetramethylporphin (I) ($\tau \approx -90$ ppm).

The distribution of the π-electronic charges for compounds I–V in the form of dications calculated by