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Application of chaotropic effect in reversed-phase liquid chromatography of structurally related phenothiazine and thioxanthene derivatives

J. Flieger*, R. Świeboda

Department of Inorganic and Analytical Chemistry, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland

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ABSTRACT

Chromatographic behavior of two main classes of neuroleptics, derivatives of phenothiazine and thioxanthene in RP systems modified by anionic additives: sodium perchlorate and sodium hexafluorophosphate possessing chaotropic properties, was examined. Influence of the method of pH lowering (by addition of acids: trifluoroacetic or perchloric or by adding the appropriate concentration of phosphate buffer) and the kind of organic modifier in the mobile phase (methanol, acetonitrile) were estimated. Stability of complexes created between protonated drugs and anions of added salts was evaluated by comparison of their desolvation parameters (K), limiting retention factors for unsolved molecules calculated on the basis of chromatographic data. Experimentally obtained parameters were used in QSAR studies. It appeared that chosen parameters reflect not only physico-chemical properties of analytes but also contain information about the strength of their antipsychotic activity. Multidimensional cluster analysis has been performed. On the basis of the results obtained, it could be concluded that chaotropic systems can generate useful parameters for further QSAR studies.

1. Introduction

The optimization in separation and retention control of charged analytes in reversed phase (RP) systems is generally connected with a variation of the mobile phase pH, owing to appropriate choice of buffer, variation of solvent strength through the changing of the type and concentration of organic solvent but the most beneficial for the separation selectivity is good selection of ion-pair reagent added to the eluent.

In the analysis of basic compounds anionic ion-pairing reagents such as sulfonic acids, alkyl sulfonates, bis-(2-ethylhexyl) orthophosphoric acid have been widely used [1,2]. However, their suitability is visible only for fully ionized solutes when pH of the mobile phase is smaller than pK_a −2 and the retention is determined by the creation of the ion-pair. Unfortunately, application of the highly hydrophobic additives is not very advantageous in column chromatography because of the difficulties in recovery of the column initial properties.

Recently, use of the inorganic salts (hexafluorophosphates, perchlorates, trifluoroacetates) as ion-interaction additives has gained in popularity [3–12]. These salts are located in the end of the Hofmeister series:

Cosmotropic ions Chaotropic ions

$$CO_3^{2-} < SO_4^{2-} < S_2O_3^{2-} < H_2PO_4^- < F^- < Cl^- < Br^- < NO_3^- < I^- < ClO_4^- < SCN^-$$

$$\xrightarrow{\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad}$$

increase of salting-in effect and decrease of surface tension

Salts used as ion-interaction reagents are known as chaotropic salts or order breaking (chao tropic) as they can damage the network of the hydrogen bonding between water molecules making hydrophobic interactions stronger. Enrichment of the mobile phase with chaotropic additives is beneficial in chromatography of protonated bases undergoing low retention on silica-based alkyl silane bonded stationary phases and giving wide peaks. Addition of chaotropic salts causes increase in their retention. Simultaneously, symmetry of peaks, efficiency and separation selectivity could be improved. For a fixed eluent pH and organic modifier concentration the dependence of retention factor (k) vs. concentration of added chaotropic salt is hyperbolic and reflects the Langmuir-type adsorption isotherm whereas the effect of the solvent composition in these systems could be described by linear solvent strength theory (LSST) [13]:

$$\ln k = \ln k_w - S\phi \qquad (1)$$

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* Corresponding author.
E-mail address: j.flieger@am.lublin.pl (J. Flieger).

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where k is retention factor, $\ln k_w$ is the extrapolated retention in pure water, ϕ is the volume fraction of organic modifier in aqueous mobile phase, S is the slope of a plot of $\ln k$ vs. ϕ.

However, good correlated linear dependences in the wide range of organic solvent content are obtained primarily for methanol creating monomolecular adsorbed layer. Deviations from linearity appear especially for acetonitrile forming a multilayer on the top of bonded phase where liophilic ions of added salts can be retained [14–17].

In contrast to ion-pair chromatography where solvent selectivity effects are rather small, changing of organic modifier in "chaotropic systems" provides a variation of system selectivity [18].

To describe this chromatographic method such terms as the "Hofmeister effect" or a "chaotropic effect" are adapted. The mechanism responsible for the observed increase in retention of bases upon addition of chaotropic additives still remains the object of great controversies. Contribution of either ion pair creation or dynamic ion exchange process is taken into account to explain obtained experimental results. Formation of ion-pairs has been independently confirmed owing to perturbation method proposed by Gritti and Guiochon [19] and capillary electrophoresis by Dai and Carr [5]. It should be stressed according to Dai's conclusions that at very low pH adsorbed anionic additives could generate also dynamic ion exchange mechanism.

This work concerns application of RP-HPLC with mobile phase modified with chaotropic salts for the separation of selected phenothiazine derivatives. These basic drugs are used as neuroleptic agents and belong to the typical or first generation antipsychotics. Their pharmacological action appears to be very complex. They block various classes of dopamine receptors (DA) in the brain. In spite of the fact that neuroleptics have many troublesome side effects, especially extra-pyramidal side effects- mainly Parkinson-like motor effects, they are of therapeutic and theoretical interest. Such compounds may aid not only in characterization of DA receptors but may also be used for estimation of pharmacological activity of new DA agonists. Fig. 1 depicts the structures of several phenothiazines studied.

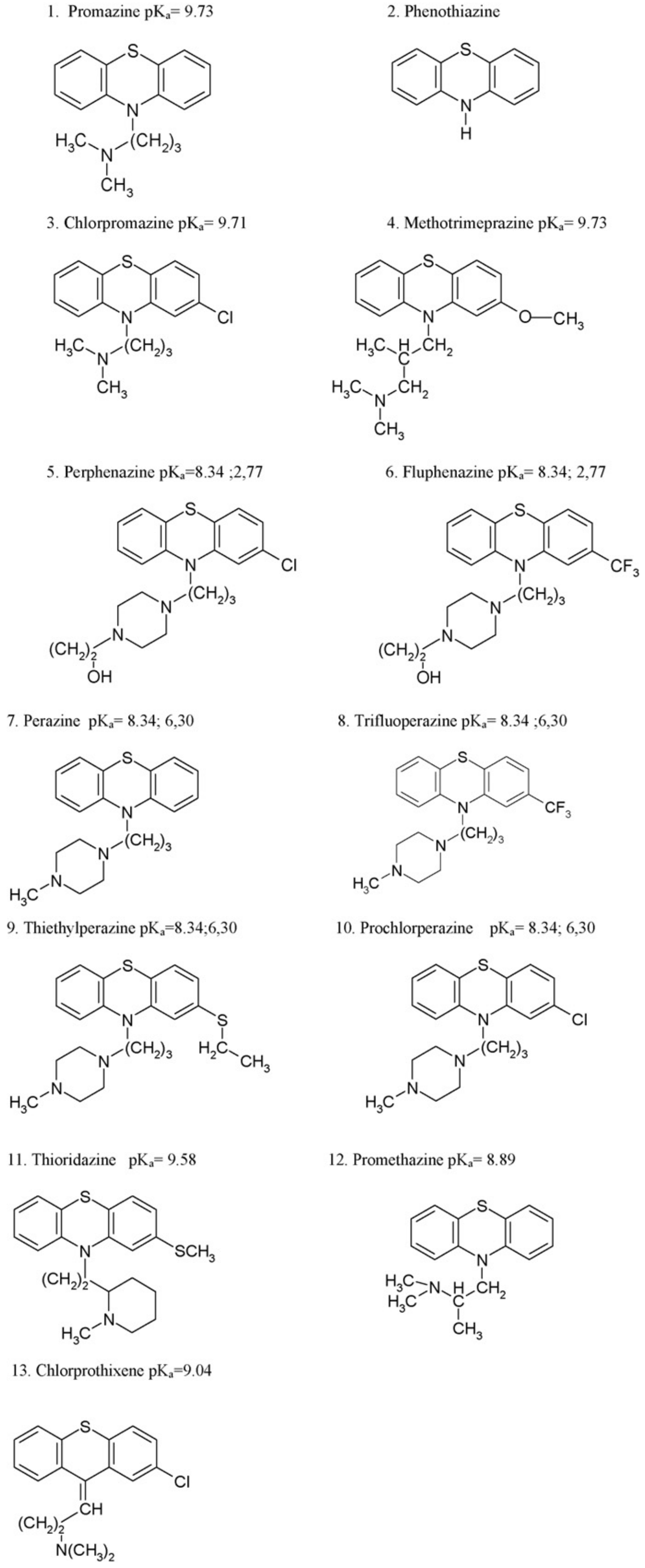
So far phenothiazines have been separated by HPLC [20–22] and CE [23–25] Some of these methods concern analysis of phenothiazine enantiomers, because ramification of an aliphatic side chain attached at the 10-position of the phenothiazine ring causes chirality of the molecules.

Presented study focuses on the retention behavior of structurally related phenothiazines as a function of type and concentration of two chaotropic mobile phase additives: $NaClO_4$ and $NaPF_6$. Appropriate choice of the method of solutes ionization gave opportunity to establish such parameters as: the desolvation parameters, the limiting retention factors for solvated and unsolved molecules. Regression analysis of the relationships: $\ln k$ vs. ϕ gave the $\ln k_w$ and S values.

2. Experimental

2.1. Materials and reagents

Phenothiazines were obtained from Sigma (St. Louis, MO, USA). Sodium perchlorate and sodium hexafluorophosphate were obtained from Sigma–Aldrich (St. Louis, MO, USA). Perchloric acid (70%) and trifluoroacetic acid (99%) were purchased from Merck (Darmstadt, Germany). HPLC grade methanol (MeOH) and acetonitrile (ACN) were purchased from Merck. HPLC water was obtained from Barnstead deionising system (Dubuque, IA, USA). The buffer solutions were prepared from reagent-grade orthophosphoric acid (80 mL) distilled and deionizer water (+0.5 mL) and 85%



1. Promazine pK_a= 9.73

2. Phenothiazine

3. Chlorpromazine pK_a= 9.71

4. Methotrimeprazine pK_a= 9.73

5. Perphenazine pK_a=8.34 ;2,77

6. Fluphenazine pK_a= 8.34; 2,77

7. Perazine pK_a= 8.34; 6,30

8. Trifluoperazine pK_a= 8.34 ;6,30

9. Thiethylperazine pK_a=8.34;6,30

10. Prochlorperazine pK_a= 8.34; 6,30

11. Thioridazine pK_a= 9.58

12. Promethazine pK_a= 8.89

13. Chlorprothixene pK_a=9.04

Fig. 1. The structural formulas of the investigated compounds and pK_a values predicted by the use of Pallas program version 3.1.1.2.