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PROPOSED MODEL FOR PEAK SPLITTING IN REVERSED-PHASE ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH COMPUTER PREDICTION OF ELUTED PEAK PROFILES

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SUMMARY

A simple retention model is advanced to explain the observation that a chromatographically pure solute can produce up to three discrete peaks under suitable mobile-phase conditions in reversed-phase, ion-pair high-performance liquid chromatography. This model is based on the proposal that peak splitting results from the interplay of two distinct retention mechanisms such as ion pairing or dynamic ion exchange. Transition between these two mechanisms is induced under certain mobile-phase conditions, for example the addition of suitable quantities of sodium sulphate to the eluent. The suggested model is used for the computer calculation of eluted peak profiles and these were found to closely resemble experimentally observed peak shapes.

INTRODUCTION

The retention mechanism which operates in ion-pair reversed-phase high-performance liquid chromatography (HPLC) is still a contentious issue, despite the fact that this technique has become well established and widely practised. This situation is undoubtedly due to the complexity of the chromatographic system involved, together with the difficulty of obtaining experimental data which unambiguously supports a single suggested mechanism.

In the simplest case, retention in ion-pair chromatography can be considered to result from the formation of neutral ion pairs in the mobile phase [1], with

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subsequent adsorption of these ion pairs onto the hydrophobic stationary phase. Alternatively the charged pairing ion can be envisaged to adsorb onto the stationary phase, resulting in the formation of a dynamic ion-exchange surface [2–4] which then can retain oppositely charged solutes by a conventional ion-exchange process.

The above two mechanisms can be considered to be extreme viewpoints and numerous alternative proposals have been made which embrace some elements of either or both of these mechanisms. These proposals include a combined desolvation and ion-exchange mechanism [5], an ion-interaction model [6] and a dynamic complex exchange model [7], all of which have been shown to be supported by experimental retention data. Knox and Hartwick [8] have pointed out that the mechanisms suggested for ion-pair chromatography involve kinetic processes and it is therefore inconclusive to use retention data (resulting from thermodynamic equilibria) to substantiate these proposed mechanisms. Kinetic effects in chromatography govern peak shape, therefore detailed study of peak shape in ion-pair chromatography may provide some insight into the mechanism operating.

In some earlier work [9, 10] in which we investigated certain selectivity effects arising from the addition of salts (such as sodium sulphate) to the mobile phase used for the separation of sympathomimetic amines by ion-pair chromatography, we observed severe peak distortion and peak splitting effects. These effects were observed for a large number of compounds and occurred for compounds which were proven to be chromatographically pure. We made the suggestion that peak splitting was the result of a composite interplay of two retention mechanisms, such as ion pairing and dynamic ion exchange, which were mutually competitive under certain conditions. This suggestion was based on considerable experimental data obtained by gas chromatography–mass spectrometry.

In this paper, we present a simple model to explain our experimental results, and this model is used for computer prediction of the distorted or split-peak profiles which we had observed.

THEORY

Development of a model

The experimental results obtained previously are summarized diagrammatically in Fig. 1, which shows that peak splitting could be induced by varying the concentration of either the added salt or the pairing ion in the mobile phase. The concentration range of salt or pairing ion over which peak splitting was observed was generally quite small and was strongly dependent on the nature of the solute used. In most cases, it is therefore quite unlikely that peak splitting would occur unless the chromatographer deliberately sought the appropriate conditions.

Analysis of eluted fractions corresponding to the three components of the split-peak profile in Fig. 1 revealed that the solute was present in each peak, but that ion pairs were present at appreciable concentrations only in the earliest eluting peak (A). Thus we have attributed this peak to an ion-pairing process and peak C was considered to result from an ion-exchange process. The

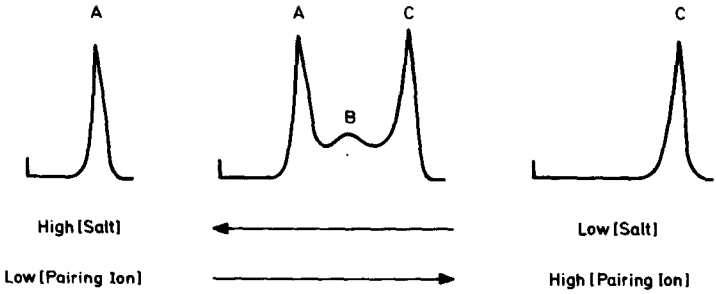


Fig. 1. Summary of experimental observations in ref. 9. Single peaks occur at the indicated conditions and peak splitting occurs at intermediate conditions. Peak A is considered to be the result of an ion-pairing mechanism, peak C is considered to result from dynamic ion exchange and peak B is attributed to an interplay of both mechanisms.

remaining peak (B) was generally quite small and was attributed to a composite interplay of the processes contributing to peaks A and C. Thus, under most conditions, a single retention mechanism operates, leading to formation of a single peak (A or C). Under exceptional conditions, particularly when a salt is added, peak splitting occurs owing to a transition in mechanism.

The simultaneous equilibria involved in the chromatographic retention process for a protonated base (B^+) in the presence of a pairing ion (A^-) can be represented as follows:

$$A_s^- + B^+ \rightleftharpoons (A_s^-B^+)_s \quad \text{(i)}$$
$$A_m^- + B^+ \rightleftharpoons (A_m^-B^+)_m \quad \text{(ii)}$$
$$(A_m^-B^+)_m \rightleftharpoons (A_m^-B^+)_s \quad \text{(iii)}$$

where subscripts m and s represent mobile and stationary phases, respectively.

In the case where a salt (for example sodium sulphate) is also added to the mobile phase, then the above equilibria would be influenced by competition between B^+ and sodium ions and to some extent, between A^- and sulphate ions. The data summarised in Fig. 1 supports the hypothesis that the dynamic ion-exchange process occurs mainly at low salt concentration while at high concentration the salt competes effectively with the protonated amines for the oppositely charged pairing ions which have adsorbed onto the C_{18} stationary phase. Under the latter conditions, ion-pair formation between the protonated amines and the pairing ions in the mobile phase is favoured. Thus, at low salt concentration the dominant species in solution is the protonated base (B^+), whereas at high salt concentration the neutral ion-paired species $(A_m^-B^+)_m$ is mainly involved. Between these two extremes, within the peak splitting range defined by the structure of the compound, interconversion between neutral $(A_m^-B^+)_m$ and charged species (B^+) can occur.

The model shown in Fig. 2 combines the essential requirements of the above hypothesis. The pairing ion A^- is present in both the mobile and stationary phases after the column has been equilibrated. In the absence of salt and at low salt concentrations, injected solute B^+ interacts with adsorbed pairing ion through an ion-exchange mechanism (process i). At high salt concentrations, competition from the salt cation diminishes the above process and the solute B^+ now interacts with pairing ions in the mobile phase. Neutral ion pairs are formed in the mobile phase (process ii) and these are then adsorbed onto the

stationary phase (process iii). The interconversion of the charged solute B^+ and the neutral ion pair (process ii) is assumed to be slower than the other processes.

Computer prediction of peak splitting patterns

In this section, the stochastic probability model given by Gidding's chromatographic theory [11, 12] was used. This theory, in its simplest form and within the context discussed here, gives the probability distribution for the relative times spent in the charged and ion-pair forms of the same amine compound when the reaction scheme is that denoted by process ii given in Fig. 2.

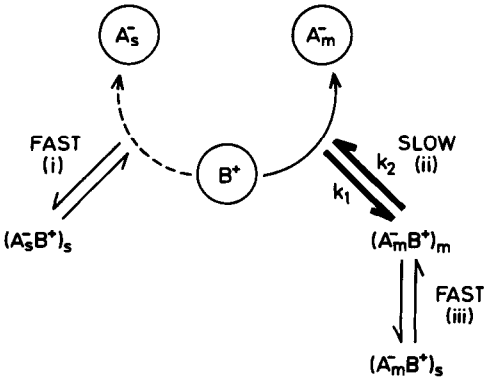


Fig. 2. Proposed model for peak splitting in reversed-phase ion-pair HPLC. B^+ is the protonated solute and A^- is the pairing ion. The subscripts m and s refer to the mobile and stationary phases, respectively. Three processes are identified in this scheme and they are denoted as i, ii and iii. The dashed curved arrow indicates the reaction pathway of B^+ in the dynamic ion-exchange mechanism, whereas the solid curved arrow is the pathway for the ion-pairing mechanism. The pathway followed is governed by the concentrations of salt and pairing ion in the mobile phase. k_1 and k_2 are the forward and reverse rate constants, respectively, for process ii.

The interconversion reaction considered here is

$$A_m^- + B^+ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (A_m^-B^+)_m$$

where k_1 and k_2 are the rate constants for the forward and reverse reactions. For simplicity, the above reaction may be reduced to

$$B^+ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (A_m^-B^+)_m$$

This equilibrium represents the conversion of the charged form of B (i.e. B^+) into a neutral form $(A_m^-B^+)_m$ of the same compound. The original fraction of molecules in the protonated form, B^+, can be denoted by α, and the fraction in the neutral form, $(A_m^-B^+)_m$, can be denoted by β.

If x is the fraction of time a molecule spends in one form, then the probability that this fraction is in the range $x + dx$ is given by $P_i(x)$, where $i = 1, 2$, representing either of the two forms.

Starting with one form, each species may undergo one of three possible reaction paths: the molecule starts in one form and after at least one reaction cycle ends up in exactly the same form; the molecule starts in one form and ends up in the second form; or finally, starting in one form, the molecule is unaffected. To designate these possibilities, subscript j is used, and the probability function becomes $P_i^j(x)$, where $i = 1, 2$ and $j = 1, 2, 3$.

The probability functions for the protonated form ($i = 1$) are:

$$P_1^1(x)dx = a \exp[-a(1-x) - bx]\, I\sqrt{4abx(1-x)}\, dx \tag{1}$$

$$P_1^2(x)dx = \left[\frac{ab(1-x)}{x}\right]^{1/2} \exp[-a(1-x) - bx]\, I\sqrt{4abx(1-x)}\, dx \tag{2}$$

$$P_1^3(x)dx = \exp(-a)\, dx \tag{3}$$

and the probability functions for the neutral form ($i = 2$) are

$$P_2^1(x)dx = b \exp[-a(1-x) - bx]\, I\sqrt{4abx(1-x)}\, dx \tag{4}$$

$$P_2^2(x)dx = \left[\frac{abx}{1-x}\right]^{1/2} \exp[-a(1-x) - bx]\, I\sqrt{4abx(1-x)}\, dx \tag{5}$$

$$P_2^3(x)dx = \exp(-b)\, dx \tag{6}$$

where $a = k_1 t$, $b = k_2 t$, and I is the Bessel function, expressed here as

$$I = \frac{\exp 2[abx(1-x)]^{1/2}}{4\pi^{1/2}[abx(1-x)]^{3/4}}$$

The overall concentration profile of an eluted solute is proportional to the final probability density, $P(x)$, which is obtained by properly weighting the above expressions.

$$P(x) = \alpha[P_1^1(x) + P_1^2(x)] + \beta[P_2^1(x) + P_2^2(x)] \tag{7}$$

$$P(x = 0) = \alpha \exp(-a) \tag{8}$$

$$P(x = 1) = \beta \exp(-b) \tag{9}$$

To make the last two discrete functions continuous, which is convenient for graphical purposes, Gaussian distributions were used with $x = 0$ and $x = 1$, respectively, and a finite standard deviation of $\sigma = 0.1$. Therefore, for $x \leqslant 0$

$$P(x = 0) = \alpha e^{-a} \cdot f(x) \tag{10}$$

where

$$f(x) = \int_{-\infty}^{0} \frac{1}{2\pi\sigma^2} \exp -\tfrac{1}{2}\left[\frac{x-\mu}{\sigma}\right]^2 dx$$

$\sigma = 0.1$ and $\mu = 0$

for $x \geqslant 1$

$$P(x = 1) = \beta e^{-b} \cdot f(x) \tag{11}$$

where $f(x)$ and σ are the same as in eqn. 10 (except the limit of integration is from 0 to $+\infty$) and $\mu = 1$.

A computer program was written for the calculation of the probability density function of the two interconverting species (with different retention times) under a variety of initial conditions.

EXPERIMENTAL

The liquid chromatograph, gas chromatograph and mass spectrometer used to obtain experimental data have been described previously [9], together with the procedures used. Computer simulation of peak splitting patterns was carried out using a Finnigan (Sunnyvale, CA) Model 6100 interactive data system, consisting of an Alpha LSI 100 Series computer (Irvine, CA, U.S.A.) and a Zeta Research (Lafayette, CA, U.S.A.) X—Y plotter. The program for calculation of the probability density function was written in Finnigan BASIC language [13] and copies are available from the authors on request.

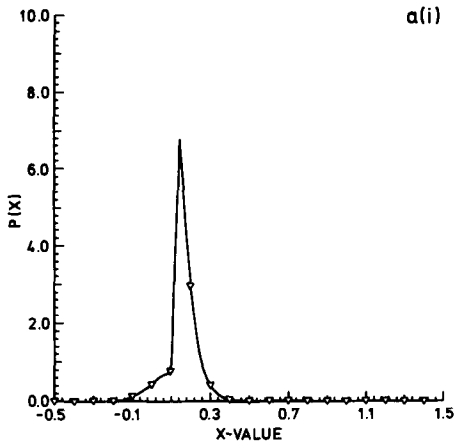
RESULTS AND DISCUSSION

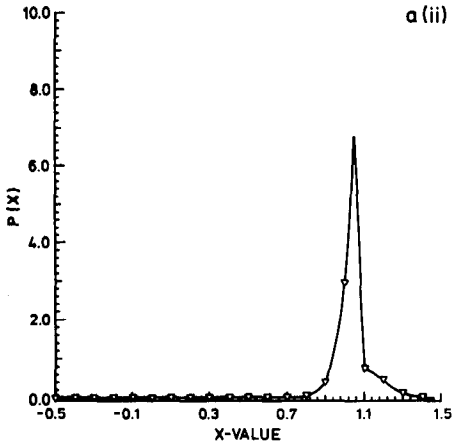
The parameters used to define the initial conditions in the solution of eqn. 7 above were a, b, α and β. Variation in a and b results from changes either in the rate constants (k_1 or k_2) or in the elapsed time, t. When t is constant, a and b are essentially the rate constants. With the rate constants fixed, an increase or decrease in t represents the calculation of a given chromatogram at respectively large or small time intervals. The rate constants (and hence a and b) are dependent on the nature of the solute. The parameters α and β define the fraction of solute molecules present in the charged (B^+) or neutral [$(A_m^-B^+)_m$] forms, respectively. Changes in α and β are considered to result directly from changes in the concentration of salt or pairing ion (A^-) added to the mobile

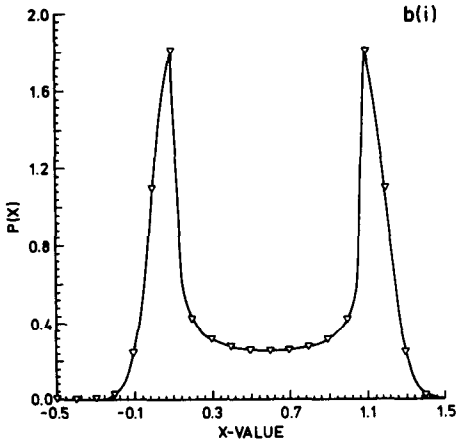
TABLE I

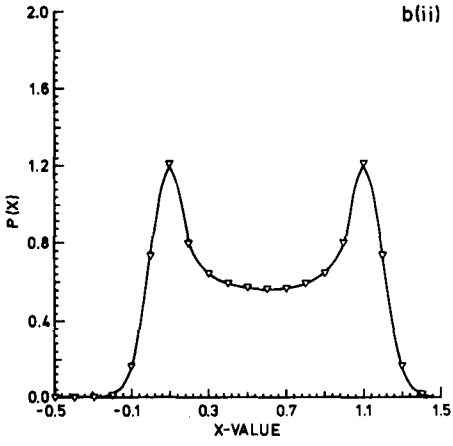
VALUES OF α, β, a AND b USED FOR CALCULATION OF THE PROBABILITY DENSITY FUNCTIONS (eqn. 7) DISPLAYED IN Fig. 3

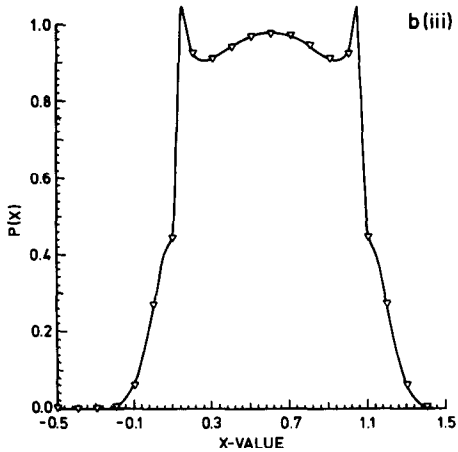
α	β	a	b	Fig. 3
0.5	0.5	1	30	a (i)
0.5	0.5	30	1	a (ii)
0.5	0.5	0.1	0.1	b (i)
0.5	0.5	0.5	0.5	b (ii)
0.5	0.5	1.5	1.5	b (iii)
0.5	0.5	4.0	4.0	b (iv)
0.35	0.65	0.5	0.05	c (i)
0.75	0.25	0.5	0.05	c (ii)
0.10	0.90	0.5	0.05	d (i)
0.10	0.90	0.005	0.005	d (ii)
0.35	0.65	1.5	1.5	e (i)
0.95	0.05	1.5	1.5	e (ii)
0.50	0.50	1	0.001	f (i)
0.50	0.50	5	0.005	f (ii)











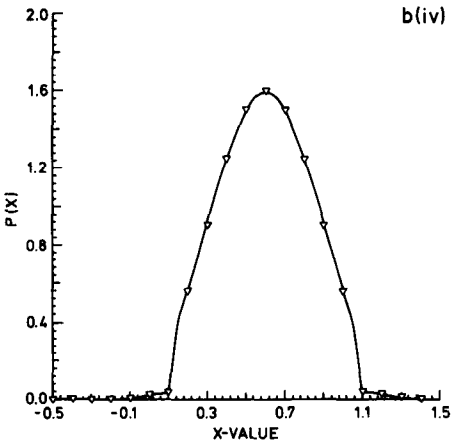


Fig. 3.

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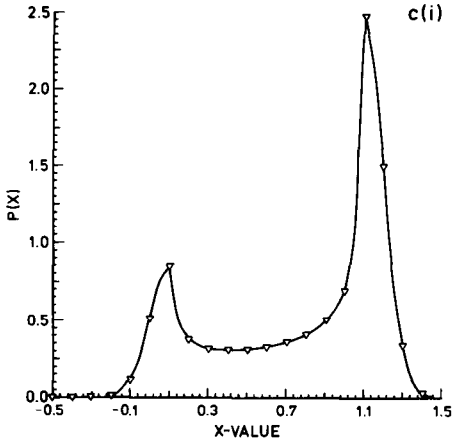
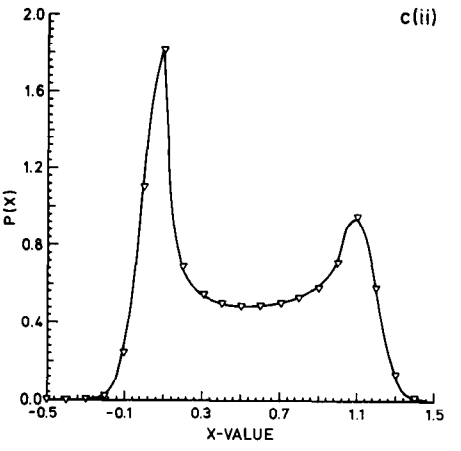

c(i)
P(X)
X-VALUE


c(ii)
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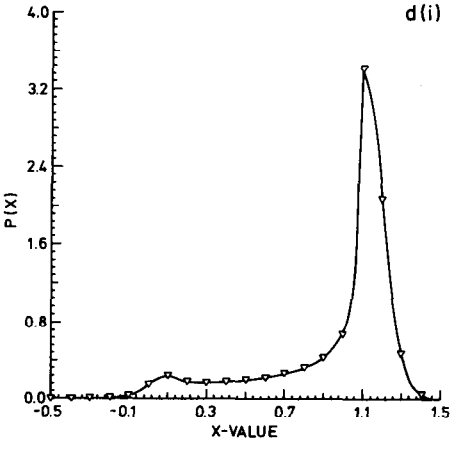
d(i)
P(X)
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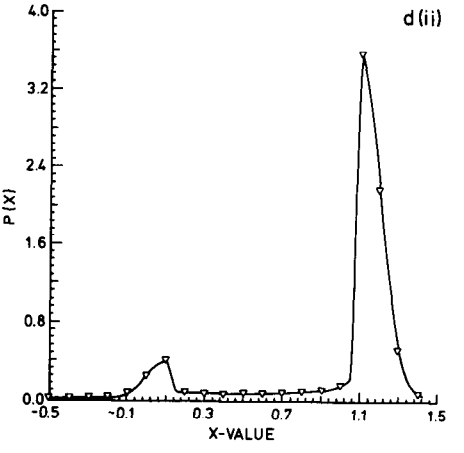
d(ii)
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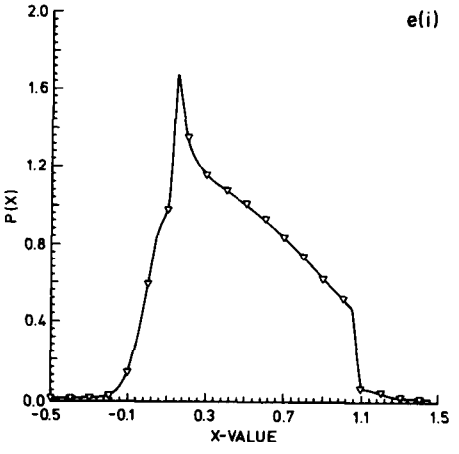
e(i)
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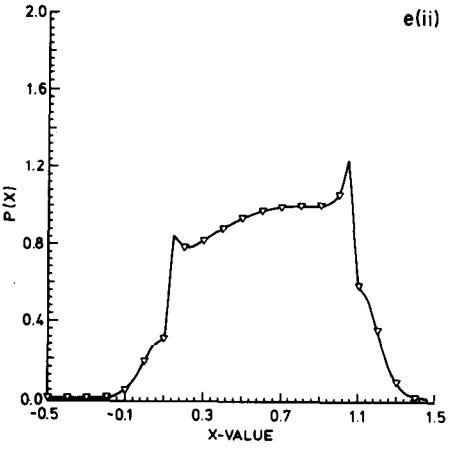
e(ii)
P(X)
X-VALUE

Fig. 3.

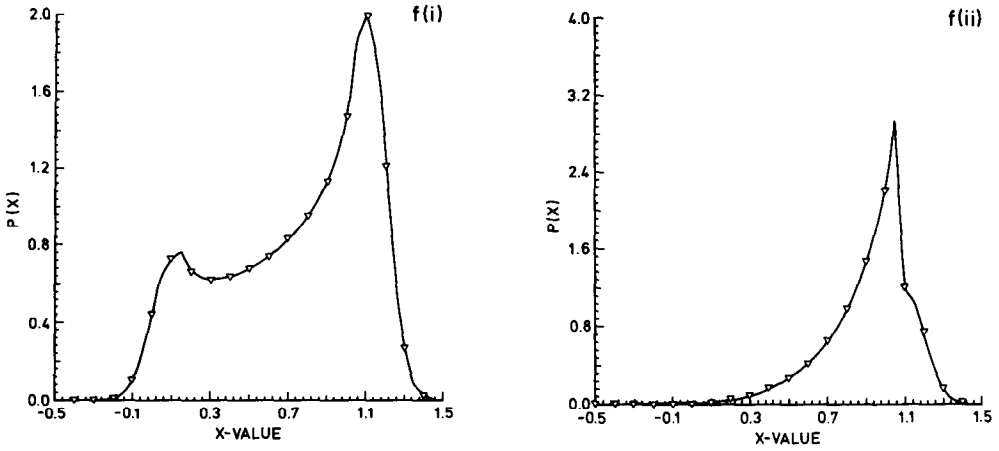

Fig. 3. Calculated probability density functions (eqn. 7) for the initial conditions indicated in Table I.

phase. Thus, selection of appropriate values of the parameters a, b, α and β can be used to simulate changes in mobile-phase composition or changes in forward and reverse rate constants for the interconversion reaction between the charged and neutral forms of the solute.

Table I lists the values used for a, b, α and β, whilst the corresponding probability density functions are shown in Fig. 3. These results indicate that the peak profile depends both on the initial proportions of the two species and on the magnitudes of a and b in the probability function.

When the initial proportions of the two species are equal, and a and b are equal or greater than 1, then only one peak results if a is grossly greater than b or vice versa. This is shown by Fig. 3a(i) and 3a(ii), where the two peaks are attributed to the charged and neutral species, respectively. These two peaks have been assigned slightly different retention times in accordance with experimentally observed behaviour [9]. In the symmetrical cases, where $\alpha = \beta$, and $a = b$, the principal consequence of increasing a and b is the reduction of peaks at either extreme and the filling in of the region between them [Fig. 3b(i)—3b(iv)]. When α and β are unequal and a and b are less than 1, the dominant peak of the resultant doublet depends on the initial proportions of the species B^+ and $A_m^- B^+$. This is shown by Fig. 3c(i) and 3c(ii) as well as 3d(i) and 3d(ii). In the latter cases the minor peak can easily be mistaken for an impurity peak. When a and b increase to more than 1, severe distortion of peaks is noted, as shown in Fig. 3e(i) and 3e(ii). Equal ratios of a:b do not guarantee the same peak splitting pattern; this is shown by Fig. 3f(i) and 3f(ii). Here, $\alpha = \beta$ and a:b ratios are equal, but the patterns obtained are dramatically different. Clearly, the absolute magnitude of a and b play a major part in determining the eventual splitting pattern.

The peak splitting patterns shown in Fig. 3 closely resemble those obtained experimentally [9], and identical matching can be achieved by careful adjustment of the input values of a, b, α and β. Whilst it is clear that further study is required for rigorous interpretation of the physical roles of the above parameters, the results obtained here strongly support the contention that the

observed peak splitting patterns are the result of an interconversion process. In the case studied, this interconversion is essentially regulated by mobile-phase concentrations of salt and pairing ion, and also by the nature of the solute used.

CONCLUSIONS

A simple model wherein changes in mobile-phase composition or solute type can produce peak splitting has been suggested in this paper. The model attributes peak splitting to an interconversion process between changed solute ions and neutral ion pairs in the mobile phase. When this model was used for the prediction of eluted peak profiles under a variety of mobile-phase conditions, the predicted profiles agreed closely with experimental results.

REFERENCES

1 D.P. Wittmer, N.O. Neussle and W.G. Haney, Anal. Chem., 47 (1975) 1422.
2 P.T. Kissinger, Anal. Chem., 49 (1977) 883.
3 J.H. Knox and J. Jurand, J. Chromatogr., 186 (1979) 763.
4 J.C. Kraak, K.M. Jonker and J.F.K. Huber, J. Chromatogr., 142 (1977) 671.
5 C.T. Hung and R.B. Taylor, J. Chromatogr., 202 (1980) 333.
6 B.A. Bidlingmeyer, S.N. Deming, W.P. Price, Jr., B. Sachok and M. Petrusek, J. Chromatogr., 186 (1979) 419.
7 W.R. Melander, K. Kalghatgi and Cs. Horváth, J. Chromatogr., 201 (1980) 201.
8 J.H. Knox and R.A. Hartwick, J. Chromatogr., 204 (1981) 3.
9 G.K.C. Low, A.M. Duffield and P.R. Haddad, Chromatographia, 15 (1982) 289.
10 G.K.C. Low, P.R. Haddad and A.M. Duffield, J. Chromatogr., 261 (1983) 345.
11 R.A. Keller and J.C. Giddings, J. Chromatogr., 3 (1960) 205.
12 J.C. Giddings and H. Eyring, J. Phys. Chem., 59 (1955) 416.
13 Finnigan BASIC Reference Manual, Finnigan Corporation, Finnigan, CA, 1977. Catalog No. U6100-90200.