Short Communications

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Frictional effects in the migration of mixtures of hyaluronic acid and serum albumin

Earlier interpretations of the anomalous appearance of boundaries in the electrophoresis or sedimentation of mixtures were usually in terms of the formation of stable complexes between components. However, GILBERT[1-5] has shown that the boundary system can differ from simple expectation even when kinetically unstable complexes are formed, some of the following effects being expected.

1. In the descending limb in electrophoresis, and in sedimentation, the slower boundary usually represents a pure component, eventually resolving from a faster boundary. The faster boundary, however, is a reaction boundary representing all components; it may develop multimodality but will never resolve completely. In an ascending limb, the situation is reversed.

2. Ascending and descending boundaries are always non-enantiographic and may contain different numbers of "peaks".

3. The sizes of the boundaries do not measure the amounts of different species in an equilibrium mixture and apparent concentrations estimated from them do not obey a mass-action law.

4. Hypersharpening of boundaries may occur in the migration of mixtures even when absent from that of the separate components.

These criteria can be used to distinguish the formation of kinetically stable from kinetically unstable complexes. In favourable cases[5] the theory allows the boundary pattern to be interpreted quantitatively.

It is possible that boundary phenomena of this type may result from frictional interactions between migrating components, rather than from formation of mobile complexes. Indeed, the GILBERT AND JENKINS[5] treatment is equivalent to the use of composition-dependent constituent mobilities[6] and their equations can be rewritten in these terms. They consider constituent mobilities as determined by complex formation, but any other law which makes each constituent mobility vary monotonically with composition between the value for the pure component and some limiting value will predict boundaries of the same type, with the difference that one would not then expect the values of a_0 and b_0 (the apparent concentrations of free A and B in the undisturbed solution) to be consistent with the mass law.

GRAMLING, NIEDERMEYER, HOLLEY AND PIGMAN[7] have described an additional "Pi" boundary which appears between the hyaluronic acid and "albumin" boundaries in the ascending limb of U-tube electrophoresis experiments. They seem to interpret this boundary as representing a stable complex between hyaluronic acid and albumin, by identifying each peak in the pattern with a component. It seems more likely, however, that the formation of this boundary is a consequence of the formation of an unstable complex according to the theory of GILBERT AND JENKINS (their Case ii

or Case ivc-ivd). The following facts are consistent with this rather than with the formation of a stable complex.

1. It seems (PLATT *et al.*[8]) that the extra boundary is formed only in the ascending limb. No details are given about the appearance of the descending boundary complex.

2. A leading boundary, characteristic of hyaluronic acid, is always observed in the ascending limb even at the highest concentration of albumin and even when the "Pi" boundary has reached its greatest relative size.

3. The "Pi" boundary is never completely resolved from the "albumin" boundary. Its mobility measured from the diagrams of GRAMLING *et al.*[7] is extremely constant, independent of the composition of the system studied, whereas the mobility of the "albumin" boundary varies considerably with composition and is always greater than that of albumin alone (Table I. 1).

TABLE I

Relative mobilities of boundaries in the experiments of GRAMLING *et al.*[7] and calculated values for the ratios of free concentrations of albumin (a_0) to hyaluronic acid (b_0) as a function of total concentrations of albumin (A) and hyaluronic acid (B). All mobilities are relative to $v_B = 1$. Concentrations are in arbitrary units.

1

*Expt.**	v_A	v_B	v_0	v'	A	B	A/B
Fig. 1E	0.74	1	0.880	0.944	306	132	2.32
Fig. 2A	0.74	1	0.843	0.948	480	153	3.14
Fig. 2B	0.74	1	0.864	0.940	360	153	2.35
Fig. 2C	0.74	1	0.875	0.944	280	146	1.92
Fig. 3B	0.74	1	0.839	0.949	76	89	0.85

2

Expt.	A/B	$v_c = 0.955$			$v_c = 0.965$			$v_c = 0.935$		
		a_0/k	b_0/k	a_0/b_0	a_0/k	b_0/k	a_0/b_0	a_0/k	b_0/k	a_0/b_0
Fig. 1E	2.32	4.6	7.3	0.64	2.3	4.5	0.53	1.15	4.9	0.23
Fig. 2A	3.14	10.2	7.7	1.33	4.5	3.8	1.19	2.10	3.8	0.55
Fig. 2B	2.35	4.1	5.2	0.78	2.4	3.5	0.68	1.21	4.0	0.30
Fig. 2C	1.92	4.4	6.6	0.66	2.5	4.3	0.59	1.24	4.8	0.26
Fig. 3B	0.85	10.4	7.4	1.41	5.0	3.9	1.30	2.17	3.9	0.56

* These refer to figere numbers of GRAMLING *et al.*[7].

These facts are consistent with characteristics (1), (2) and (3) of a system in mobile equilibrium, as listed above, but cannot be used to distinguish between Cases ii and ivc–ivd. However, the latter implies that the mobility of the complex equals that of the faster component, which appears unlikely in the situation under discussion.

In view of the argument given above, it is also possible, however, that the phenomenon may be the result of frictional interaction between the migrating components rather than to mobile complex formation. The following evidence supports this view.

1. LAURENT AND PIETRUSZKIEWICZ[9] have shown that there is strong interaction between a variety of particles (including serum albumin) and hyaluronic acid in sedimentation and that this obeys a common law. It would be unlikely that the

proteins studied would fit the same equations as such particles as polystyrene latex if the behaviour of the former were also modified by complex formation.

2. GRAMLING *et al.*[7] have shown that with partly depolymerised hyaluronic acid the "Pi" boundary does not appear. This would be expected strongly to affect frictional interactions, but not necessarily complex formation.

3. GILBERT AND JENKINS' Equations 17, 18 and 19 can be used to calculate from the relative boundary velocities in the experiments of GRAMLING *et al.*[7] the values of $a_{0/k}$ and $b_{0/k}$ and the ratio a_0/b_0 of the concentrations of free albumin (A) and hyaluronic acid (B) in the undisturbed region of the solution. To do this, the relative values of the mobilities v_B, v_A, v_0 (mobility of "albumin" in the presence of hyaluronic acid) and v' (the mobility of the "Pi" boundary) were measured from the figures of GRAMLING *et al.* The value of v_c is not obtainable directly from the data, but must lie between v_B and v' (see GILBERT AND JENKINS, Case ii); calculation has been performed for three values of v_c. The results are given in Table I.2. From this it is seen that though the values of $a_{0/k}$ and $b_{0/k}$ are affected by the value taken for v_c their relative changes with total composition are little affected. The value of $b_{0/k}$ (which should be proportional to b_0, k being an equilibrium constant) does not vary significantly with A at constant B, whereas the assumed mass-action relationship would require it to do so. Also the ratio a_0/b_0 should vary more rapidly than the ratio A/B (since $a_0/b_0 = (A - c_0)/(B - c_0)$) but does not do so. If data were available on the descending limb, the method of LONGSWORTH[6] could be used to test the constancy of k. This would be a more direct approach as it would eliminate the need to use v' and v_0 as a basis for calculation.

4. LAURENT AND OGSTON[10] have shown that the osmotic pressures of mixtures of hyaluronic acid and albumin are always in excess of the values expected from the osmotic pressures of the separate components at the same concentrations. The formation of an association complex should have the opposite effect. It would be opposed also to the partitions of albumin observed by OGSTON AND PHELPS[11].

We conclude that the boundary phenomena observed by GRAMLING *et al.*[7] are unlikely to be due to complex-formation in any chemical sense, whether the complex is a stable or an unstable one, but are to be interpreted as due to mutual frictional effects.

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