Effect of Isomerization on Migratory Analysis

There is a simple explanation when three peaks appear in the analysis of a two-component system.

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It is customary to interpret schlieren patterns and also chromatographic and similar records in terms of one species for each peak. However, a substance existing in only two isomeric forms may show three peaks. This fact was established recently by three independent ways of numerically solving the basic equations describing the reactions, diffusion, and migrations involved (*1–3*). This anomaly may appear in any method of analysis or separation based on differential migration of the species whether it involves electrophoresis, centrifugation, chromatography, countercurrent extraction, or the like. Furthermore, the reaction involved need not be isomerization provided that it leads to a constant dynamic equilibrium during the migration and that it affects the rate of migration of the particles involved. Reactions with the solvent or with a uniformly distributed solute generally satisfy these requirements.

Since the numerical analysis referred to above does not provide any readily visualized physical explanation of this anomaly, it seems worthwhile to give a qualitative explanation of how the additional peak arises. This should also be useful in developing without mathematical encumbrances an appreciation of the conditions under which it may appear. For simplicity the following discussion is confined to electrophoresis but applies equally to other methods. We also neglect all nonidealities and so-called boundary anomalies.

Systems of Two Components

Electrophoresis. Let us first consider a system of two components, A and B, present in equal concentrations and forming a sharp boundary with pure solvent in a Tiselius type of apparatus. The corresponding concentration picture is shown at the top of Fig. 1, and the corresponding schematic schlieren pattern (which gives the concentration gradient) at the top of Fig. 2. If the two species have different electrophoretic mobilities, they will separate during electrophoresis. This is shown in the second line of the figures for the simplest case where the electrophoretic mobilities are equal and opposite. The left-hand diagram in each case shows the state if diffusion is slow, and the right-hand side if it is rapid.

Electrodiffusion. Now let us assume that A and B are two isomers in rapid equilibrium with each other so that the reaction

$$A \rightleftharpoons B$$

occurs continuously and rapidly and each particle of solute changes frequently but at random from the A state to the B state and back. The overall concentrations of A and B will remain equal under these conditions, and there will be no net electric transport of the solute, assuming again equal concentrations and equal and opposite mobilities. However, each individual particle of the solute will spend some time in the A state moving in one direction and some time in the B state moving in the opposite direction. If these two time intervals were exactly equal, the particle would return exactly to its starting point. Such *exact* equality is, however, as improbable as the exact matching of heads and tails in a large number of penny throws, because of the random nature of the occurrence of the reaction of the individual particle.

In general, therefore, a particle will spend a slightly larger amount of time in one of the two states, and as a result it will have moved either to the left or to the right of its starting point. The probability of such displacements decreases rapidly with their length, just as it does in diffusion. In fact, the random movement of a particle to the left and to the right as it changes from A to B and vice versa may be considered as an electrically induced sort of Brownian motion leading to a corresponding "electrodiffusion" (*4*) obeying essentially the same laws as diffusion (*5*). The corresponding electrodiffusion coefficient has been calculated (*6*) and shows that for high fields (10 volt/cm) and not too rapid reactions (half life of 1 second or more) this electrodiffusion can significantly exceed ordinary diffusion.

The resulting concentration patterns are shown in the lowest line of Fig. 1. The corresponding schlieren patterns appear in Fig. 2, showing a broad peak at the origin.

The third peak. Now, if the isomerizing reaction is not very rapid but has a lifetime of the same order as the length of the electrophoresis experiment, it may be considered natural that an intermediate situation will develop. Some of the schlieren peaks corresponding to the unreacted original A and B will persist, and the rest of the solute will begin to form the central broad peak. This central peak may or may not be visible, depending on the broadening produced by ordinary diffusion, as shown in the penultimate line of Fig. 2.

This is indeed the state of affairs revealed by the numerical computations. Thus (*2*) if the reaction lifetime is 1 minute, if the difference of the electrophoretic velocities of the two species is 10 μ/sec and if their diffusion coefficient 1.3×10^{-7} cm^2/sec, the two peaks will separate within half a second and the third one will appear between them after about 30 seconds. The three peaks will be visible for somewhat over 2 minutes before the two outer ones decrease to mere shoulders. If the diffusion coefficients become 10 times larger, the two peaks will separate after some 15 seconds, the dip between them will

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never exceed 10 percent of their height, and they will fuse again after 1½ minutes, just before the third peak becomes distinct. If the diffusion coefficient doubles again, the two peaks will no longer separate.

Proof

The problem. The above discussion leads one naturally to expect the appearance of the third peak, but it should be noted that we have based the plausibility of the formation of the single symmetrical peak of electrodiffusion on the fact that every particle undergoes a very large number of reactions. This is clearly not the case at times of the same order as the lifetime of the reaction. Yet it is only during this early period that the three peaks can coexist.

It may be worthwhile, therefore, to prove that the central peak, when not obscured by diffusion, forms even in the very earliest stages. We will see, however, that its formation still depends on the repeated occurrence of the $A \rightleftharpoons B$ reaction.

Schlierens and concentrations. To simplify our proof let us first note that Fig. 2 not only shows the schlieren patterns corresponding to the concentrations of Fig. 1, but also represents directly the *concentration* changes expected if our A and B solutes had originally the concentration shown in the top line—that is, were all concentrated in a very narrow region such as could be formed in paper electrophoresis. In the absence of reaction the two species would have completely separated, as shown in the second line, and in the presence of reaction they would have given the concentrations shown on the lower two lines.

This ability to represent the behavior of concentrations is quite a general property of schlieren patterns and can be proven rigorously (*2*). We shall re-

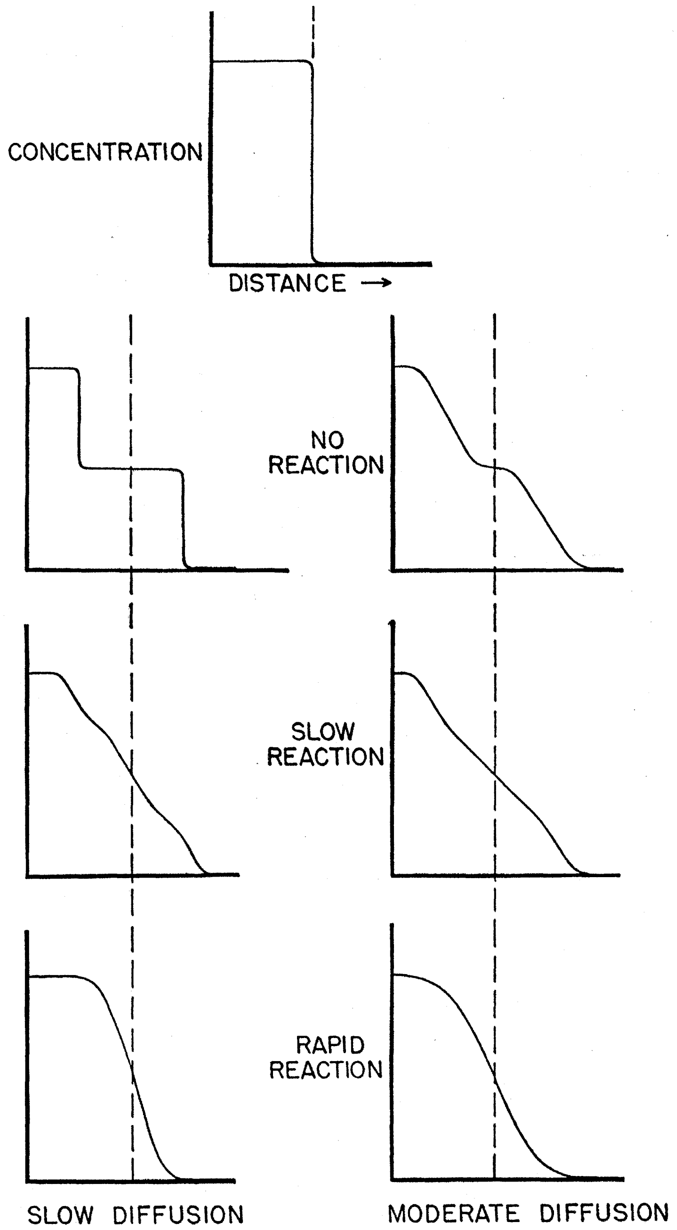

Fig. 1. Changes from the initial distribution (top) after migration as the rate of the isomerization reaction increases (from top to bottom) and as diffusion becomes more rapid (from left to right).

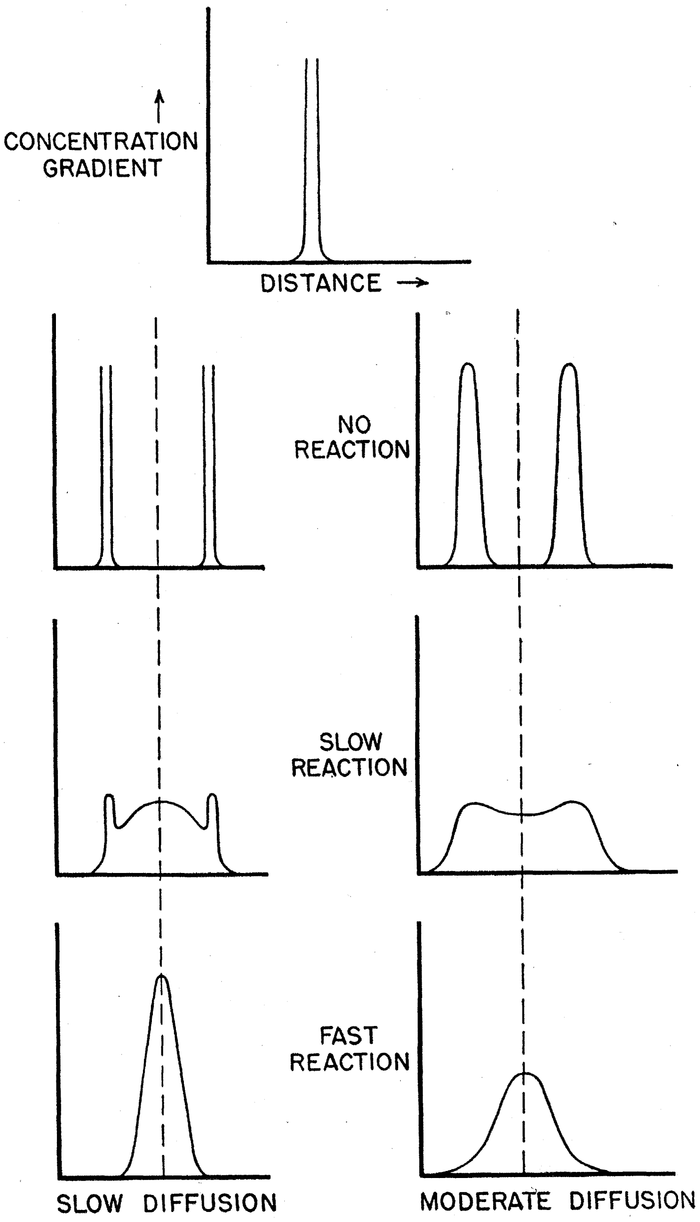

Fig. 2. The anomalous third peak appears (row 3, left) in the schlieren patterns of the distributions of Fig. 1. These patterns also represent directly the corresponding distributions if the material is initially concentrated in a narrow region, as shown at top.

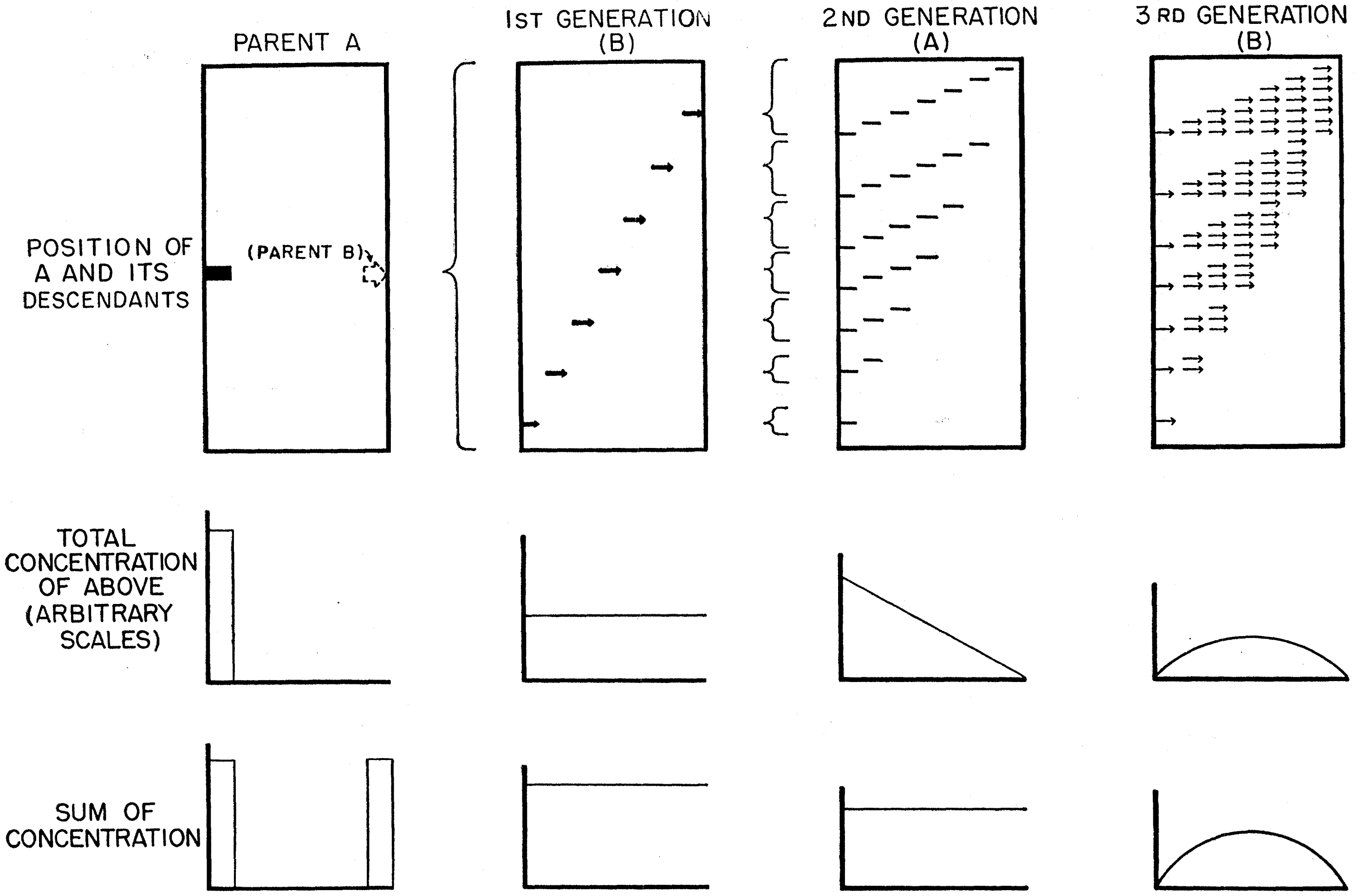

Fig. 3. The position of the groups of particles after successive reactions in an isomerizing and migrating system (top panels). It is only after the third reaction that a contribution to the central peak is made (middle and bottom rows).

verse the argument and from a discussion of concentrations draw a conclusion about schlieren patterns. Thus, if we can show that a narrow initial region gives a central concentration peak, this will account for the central peak of a schlieren pattern from a layered system (as in Fig. 1).

Successive generations. It will simplify matters if we assume that species *A* has zero mobility while *B* has a positive one, both species are present in equal amounts, and that diffusion is completely negligible. At the beginning, let all the particles be concentrated in a very narrow region. Once migration has started (that is, once the potential has been applied), the particles in the *B* form are always moving to the right while those in the *A* form are stationary at whatever point they were last formed by the reaction.

In order to follow the motion of the particles we may divide them into successive generations according to the number of times they have reacted. Thus, the ones which are still in their original *A* or *B* state may be called the parent generation. Those that have undergone one reaction and are now in the other state (for example, *B*'s formed from the original *A*'s) will be the first generation, those that have reacted twice and are therefore back to the original state will be the second generation, and so on.

Let us now look at our system a short time after migration has started and see whether and why a third peak has already formed. For convenience let us divide the time from the starting moment into still shorter intervals, say seven in number, each sufficiently short so that the fraction α of particles which have reacted during each is quite small. Figure 3 shows schematically the state of our system at the end of the seventh interval. In its upper part each panel is devoted to a single generation. Solid lines or arrows indicate the position of particles having a common history of successive reactions starting from the original *A*. We shall refer to these as "groups." Descendants of the original *B*'s are not shown, but their behavior is exactly symmetrical.

Arrows indicate groups which are moving at the instant considered, that is, are in the *B* state, the first and third generation. The lines represent stationary groups, that is, *A*'s of the parent and second generation.

Let us now consider in detail the position of each group of each generation and the contribution it makes to the total observed concentration.

In accordance with our premises, those particles which remain in the *A* state and thus form the parent group did not move and are shown by a short line at the origin in the left panel of Fig. 3. The corresponding parent group of *B* has moved to the right and is indicated by the outlined arrow.

The second panel shows the position of the first generation, that is, of particles which were originally *A* but reacted to become *B* in one of the small time intervals and remained in that state. Being *B*, these groups (*7*) are moving to the right and are represented by arrows. The group formed in the first interval migrated all this time and is therefore farthest from the origin, as indicated at the top of the second panel. The group formed last is still at the origin, as shown at the bottom of this panel, while groups formed in intermediate times have reached intermediate positions. Hence this generation produces a uniform concentration of particles between the positions of the two parent groups, as shown schematically in the figure below the panel.

While the first generation was migrating in this way, some of it reacted during each interval to form the second generation in the immobile *A* form which was thus "shed" along the way. The position of the groups thus formed in each time interval by the corresponding groups of the first generation is shown in the third panel. It may be seen that farthest from the origin only one group of the first generation could contribute in only one period, so that only one second generation group is in this extreme position. Near the origin, however, there was always a first generation group during each interval, so that seven second generation groups have been shed there. Thus the concentration contribution of this second generation decreases linearly from left to right, as shown below in the figure. However, the symmetrical contribution from the parent *B* group exactly compensates, so that the total contribution of the second generation is still uniform, as shown at the bottom of the figure.

The third generation, whose positions are indicated in the right panel, is again formed by moving *B*'s. Each group indicated by a thin arrow has been formed by one of the groups of the second generation in one time interval. Those near the origin had to be formed by the second generation stranded there (and only during the last interval—otherwise they would have moved away). Those farthest from the origin could not have arrived there unless they were in the moving *B* form throughout the time considered. They must therefore descend from the initially formed group of the first generation (and be formed by the second generation in the first interval of its existence). Hence groups of the third generation located at both extremes have a relatively restricted ancestry and their number is correspondingly small.

On the other hand, the third generation groups located in the middle can be derived from any of the second generation groups on the left of center (in the appropriate period), so that their number is relatively large.

Thus the contribution of this third generation to the total concentration is largest in the middle, and this effect is reinforced by the symmetrical behavior of the groups derived from parent *B*, as indicated in the lower part of the figure. This is therefore the origin of the third peak.

Since we have chosen a very short total time, it is clear that the amount of material in each successive generation decreases rapidly. Hence the peak due to the third generation will be a small one and will be superimposed on a larger (but still small) uniform background formed by the preceding generations. By continuing the above analysis one can readily establish that each generation beyond the third also contributes to the peak, but these contributions are clearly negligible.

Conclusion

This graphical reasoning thus shows that from the very beginning of electrophoresis of an isomerizing system a third peak forms between the two peaks of unreacted isomers. This peak is, however, very small at this stage and is therefore normally obscured by diffusion. With time it develops and finally becomes predominant. Whether the three peaks are resolved depends on how rapid diffusion is compared to electrophoretic migration. If diffusion permits good resolution of the two initial peaks, the third one becomes visible between them and gradually absorbs them. If diffusion is too rapid to permit resolution, the shape of the single visible peak changes from a regular gaussian form due to ordinary diffusion alone through a complicated distortion to another regular gaussian due to combined electrodiffusion and diffusion (*2; 8*).

References and Notes

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7. Essentially we compare the number of groups for each position within each generation. If the sizes of these groups were different, our conclusion would be weakened. In fact, however, all the groups within each generation are *exactly* equal. This equality is due to the fact that within each time interval a fraction α of particles then within a group reacts and forms a new group. Hence, if the original number of particles is N, in the first approximation there are αN particles in each group of the first generation, $\alpha^2 N$ in each group of the second generation, and so on. In addition, however, the size of any group decreases with time because of the reaction by a factor $(1 - \alpha)$ after the first interval, $(1 - \alpha)^2$ after the second one, and so forth. This attrition is, however, proportionately the same whether it is exerted on the preceding generations or on the one considered, and at the end of n intervals it always amounts to a factor of $(1 - \alpha)^n$. For example, the first generation group formed in the second period from the parent *A* will be $\alpha N(1 - \alpha)^2$. The second generation group formed from this three intervals later will be $\alpha[\alpha N(1 - \alpha)^2](1 - \alpha)^3$, and at the end of the seventh period its size will be $\{\alpha[\alpha N(1 - \alpha)^2](1 - \alpha)^3\}(1 - \alpha)^2 = \alpha^2 N(1 - \alpha)^7$. Thus the size of a group depends only on the total time and the generation, and not on the timetable of its formation. In contrast, the position of a group depends, as we shall see, greatly on this timetable.
8. This work was supported by the National Science Foundation.