Macroscopic and Microscopic Behavior of the Eluent in Liquid Chromatography

Ken-ichi FUKUHARA,* Asao NAKAMURA, and Masamichi TSUBOI†

Central Research Laboratories, Ajinomoto Co., Inc. Suzuki-cho, Kawasaki-ku, Kawasaki 210

†*Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113*

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Synopsis. By injecting an isotope pulse into the eluent of liquid chromatography, it has been shown that the eluent flow does not go into the stationary phase, while each of the molecules in the eluent is replaced by an equivalent molecule originally contained in the stationary phase. Meanwhile, an appropriate method for determining the t_0 value is given.

Liquid chromatography is useful, not only for the preparation and/or analysis of chemical substances, but also for deriving some thermodynamic functions of intermolecular interactions. For this purpose, it is convenient to regard a chromatogram as free-energy spectrum and to assign to each molecule in question a proper, standard free energy on the basis of the observed chromatogram.[1,2] It is essential in this procedure to determine the t_0 value, which is the time required for the eluent to go through the column, in other words, t_0 is the retention time of a hypothetical molecule which is kept always in the mobile phase and which never goes into the stationary phase.[2] In the present note, our examination of how to determine t_0 will be briefly discussed.

An example of our present experimental results is shown in Fig. 1. Here is a series of chromatograms in which the eluent is always CH_3CN (50%)+H_2O (50%). In every case, a small amount of a sample solvent was injected at zero time. Whenever its composition was different from that of the eluent (chromatograms b,c,d,e,f, and j), a peak (or a negative peak) appeared 6.7 min after the injection. The peak height depends upon the composition of the sample solvent, but its position (6.7 min) is independent of it. This position is considered to correspond to the total volume of the mobile phase of the particular column now in question. In other words, 6.7 min must be the time required for the sample solvent (having a different index of refraction from that of the eluent) to go through the mobile phase of the column. This is taken as the above-defined t_0 for this particular column system.

Next, in order to confirm the above determination of the t_0 value, the microscopic behavior of the eluent in this system is examined. Whenever an isotopic acetonitrile CD_3CN is involved in a sample solvent, another negative peak appears at a definite position: t_c=7.9 min after the injection, *i.e.*, at a somewhat retarded time (see chromatograms c,g,i, and j). This means that CD_3CN in the present column system, with the CH_3CN (50%)+H_2O (50%) eluent, has a relative retention time (t_c-t_0) of 7.9—6.7=1.2 min. Figure 1 indicates also that, after the injection of isotopic water D_2O, a negative peak appears at t_w=10.5 min (chromatograms e,h,i,j) and that the relative retention time (t_w-t_0) is 10.5—6.7=3.8 min.

In order to determine whether the relative retention times (1.2 min or 3.8 min) come from the microscopic behavior of the individual solvent molecule (acetonitrile or water) in the eluent or from simple isotope effects (CH_3CN–CD_3CN or H_2O–D_2O), radioisotopic water T_2O was examined in this system. When a small amount of T_2O is added to the D_2O injected, a radioactivity peak of T_2O is found to be superimposed on the refractive index peak of D_2O (Fig. 1,f). We could not find any difference among T_2O, D_2O, THO, TDO, and HDO in their retention time t_w, nor could we detect any isotopic effect.

Therefore, it is evident that H_2O has the same retention time t_w as those mentioned above. It is also evident that CH_3CN has the same retention time t_c as that of CD_3CN.

It can now be stated that the molecules constituting the solvent are retained to a proper extent in the stationary phase, while the solvent sample as a mac-

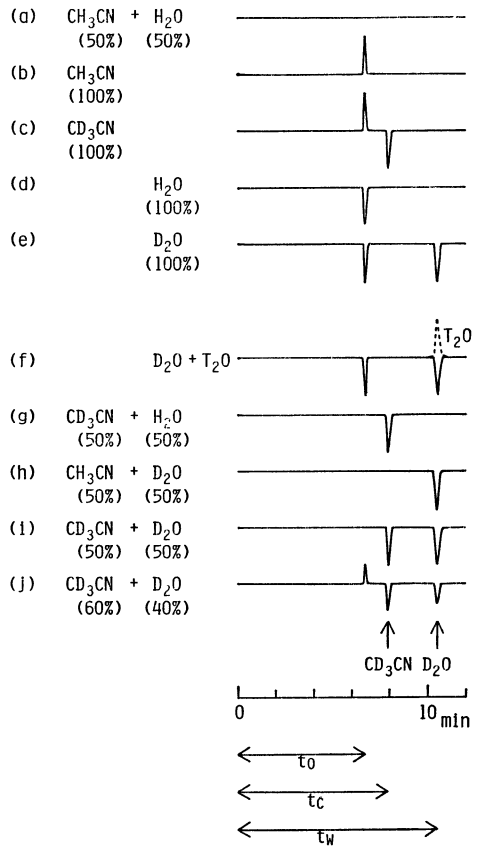

Fig. 1. A series of chromatograms for the determination of t_0, t_c, and t_w values.
Column, Hitachi #3013-N (8ϕ×500 mm); eluent, CH_3CN: H_2O: 1 M phosphate buffer (pH 7)=50 : 49 : 1 (v/v); flow rate, 2 ml/min; temperature, 50 °C; detection, refractive index.

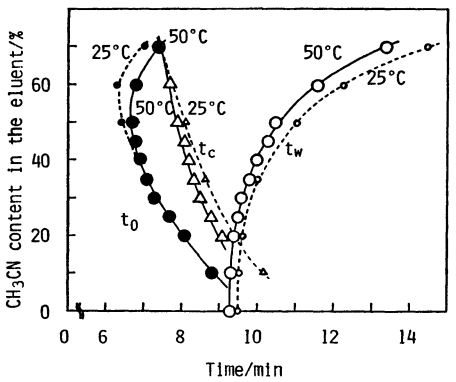

Fig. 2. The values of t_0, t_c, and t_w in a chromatograph system (Hitachi #3013-N with CH_3CN+H_2O as eluent), as functions of the CH_3CN content in the eluent.
Here t_0 is the time required for the solvent to go through the mobile phase (—●—, at 50 °C; --●--, at 25 °C); t_c is the time required for the CD_3CN molecule to come out from the column after its injection (—△—, at 50 °C; --△--, at 25 °C); and t_w is the time required for the D_2O (or HOD or T_2O) molecule to come out from the column after its injection (—○—, at 50 °C; --○--, at 25 °C).

roscopic mass goes through the mobile phase without any retention. In other words, the sample solvent does go down through the mobile phase without changing its composition, but a certain amount of microscopic exchange of the solvent molecules is considered to take place between the mobile phase and the stationary phase. Without an isotopic injection, we cannot detect such a retention of the solvent or eluent molecule (Fig. 1,a), but it is evident that a similar microscopic molecular exchange takes place in every eluent used.

As for how to determine t_0, we have now found an answer: we should inject a solvent pulse with a slightly different composition from that of the eluent. Quite often one does this without intention, for the solvent of a sample solution is actually often slightly different in its composition from that of the eluent, and this causes a small peak in chromatogram which corresponds to t_0. One might think that an isotopic solvent should directly indicate t_0, but actually this does not work, as has been shown here.

We have made similar series of experiments, with different eluents, to see how the t_0, t_c, and t_w values depend upon the eluent composition. These results are given in Fig. 2. As may be seen in the figure, the effective volume of the mobile phase gives a minimum at about 50% of CH_3CN. The retention time (t_w) of D_2O increases, while that (t_c) of CD_3CN decreases, with the increase in the CH_3CN content in the eluent. There is a small temperature effect for each of the t_0, t_c, and t_w values.

References

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2) M. Tsuboi, *Gendai Kagaku (Chemistry Today)*, April **1981**, 46—53.