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The Significance of the HPLC Time Scale: An Example of Interconvertible Enantiomers**

Albrecht Mannschreck*, Herbert Zinner, and Nikola Pustet

Abstract: Racemization during high-performance liquid chromatography (HPLC) on optically active sorbents generates peak coalescence/decoalescence phenomena. It is shown that they may serve useful in proving the interconvertibility of enantiomers or, in other cases, the chirality of molecules. In addition, racemizations during HPLC at certain temperatures give advice concerning the preparative separation and the subsequent experimental investigation of interconvertible enantiomers. HPLC of (*MP*)-1-dimethylamino-8-dimethylcarbamoylnaphthalene (Fig. 1) on triacetylcellulose at variable temperature and variable flow rate exemplifies these stereochemical applications. The «HPLC time scale» is discussed with reference to the time scale of nuclear magnetic resonance (NMR).

Reversible or irreversible reactions of a substrate molecule during chromatography may cause more than one peak to be detected[1–3]. The special case of interconversion of enantiomers in gas chromatography on optically active sorbents has been described for several racemic mixtures[4]. The coalescence of HPLC peaks of enantiomers has been observed for chiral *N,N*-dimethylthiobenzamides[5,6] and phenanthrene derivatives[5,7].

These findings can be explained by a competition between the processes of chromatographic *separation* of the enantiomers on the optically active sorbent and their subsequent thermal *racemization*. The chromatogram is determined by the interplay of the following parameters: the half-lifetime of the enantiomers and the time intervals they spend on column, i.e. their retention times. This situation is reminiscent of NMR; the similarity between the rate constant of a suitable process and the shift difference between suitable signals results in signal coalescence. Although this NMR time scale has been the basis for many qualitative and quantitative stereochemical investigations, the «HPLC time scale» defined above has not found much stereodynamic applications. The present paper exemplifies such applications by HPLC of a simple organic molecule.

* *Correspondence:* Prof. Dr. A. Mannschreck
Institut für Organische Chemie
Universität Regensburg
Universitätsstrasse 31 – Postfach 397
D-8400 Regensburg
(Bundesrepublik Deutschland)

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1-Dimethylamino-8-dimethylcarbamoylnaphthalene, *m.p.* 95–97 °C, has been prepared from the known[8] corresponding naphthoic acid. No information was available about its chirality. The chromatograms $A(V)$ and $\alpha(V)$ were monitored (Fig. 1) and deconvolved[6] by a personal computer. These deconvolved diagrams show the absorbances and rotation angles of the enantiomers during the elution process. Thus, the relative concentrations of the enantiomers are available as *separate* functions of the retention volume V on a completely experimental basis.

When the temperature is increased (vertical comparison in Fig. 1), the extent of racemization after separation increases. When the flow rate of the eluent is decreased (horizontal comparison in Fig. 1), the on-column times of the enantiomers and, therefore, the extent of racemization after separation increase. In this case, the two capacity factors k' are simply averaged (Fig. 1). When the temperature is increased, however, the k'-values, particularly the one for the second enantiomer, change with temperature; in addition, they are averaged (Fig. 1). The HPLC results for the naphthalene derivative in question are the following: The molecule is chiral and its enantiomers (*M*) and (*P*) (Fig. 1) interconvert at room temperature. Their preparative separation and further investigation will have to be carried out at 0 °C or below.

A half-lifetime of the enantiomers in solution of 9.9 min has been obtained by stopped-flow monitoring[6] of racemization in C_2H_5OH/H_2O, 96:4, at 42.1 °C without preparative enrichment. Thus, the gradual racemization, evident from the chromatograms at variable flow rate and a constant temperature of 42.5 °C in Fig. 1, originates from interconversion of enantiomers with the above half-lifetime. The corresponding barrier $\Delta G_m^{\ddagger} = 96.9 \pm 0.2$ kJ/mol[10] must be due to Aryl–CO rotation. We have determined $\Delta G_m^{\ddagger} = 71.6 \pm 0.9$ kJ/mol (54 °C, $Cl_2CDCDCl_2$) for Aryl–N rotation by coalescence of the two Aryl–N*Me* ^{1}H-NMR signals. Therefore, in the ground state, both the Aryl–NMe_2 and the Aryl–$CONMe_2$ group are twisted out of the ring plane to a considerable extent[11], as shown in Fig. 1.

Racemizations during HPLC at around room temperature have been detected for other chiral *N,N*-dimethylbenzamides[12,13], *N,N*-dimethylthiobenzamides[5,6,14], helicene hydrocarbons and their derivatives[5,7,12,15], as well as chlorosubstituted triphenylmethyl radicals[16]. As far as the corresponding barriers to interconversion are known, all of them are between $\Delta G_m^{\ddagger} = 88$ and 98 kJ/mol[10,17]. A racemization observable by HPLC may be due to a smaller or larger barrier, if the column is not at room temperature. For barriers of such height, a supplementary technique of investigation is welcome, mainly because the well-known methods using preparative separation and classical equilibration need $\Delta G_m^{\ddagger}$-values of approximately 96 kJ/mol or more at 25 °C[18].

The detection of two HPLC peaks on an optically active sorbent, their coalescence, and their averaging prove the interconvertibility of enantiomers[5,12,15]. On the other hand, the decoalescence of an averaged peak, e.g. upon lowering the temperature, proves chirality[7,12,15]. These conclusions are qualitatively similar to the ones obtained from Dynamic NMR. However, the time scale of NMR is different and generates coalescence of signals between −150 °C and +200 °C, if the barriers are between 20 and 100 kJ/mol[19]. Unlike NMR, the quantitative aspect of HPLC in respect of time scale, i.e. the determination of rate constants from suitable chromatograms, is still at its infancy, although the basic theory has been developed[2–4]. Finally, coalescence/decoalescence phenomena at certain temperatures give information about preparative separability and advice concerning subsequent experimental investigation of interconvertible enantiomers.

The above applications of the «HPLC time scale» to stereochemical problems are possible, if coalescing peaks have been found *by chance*. In the case of suitable molecules, it would seem worthwhile to look for such peaks by changing the temperature and the flow rate *on purpose*.

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[9] Column, CONBRIO-TAC, 250 × 5 mm, d_p = 15–25 µm, from Perstorp Biochem, Lund, Sweden. Void volume[5] 3.16 mL at 20°C.
[10] The subscript «m» refers to the mobile phase in the absence of a sorbent, i.e. these barriers were determined under non-chromatographic conditions. In principle, kinetic parameters in *solution* and during *sorption* in the stationary phase may differ[1–4].
[11] The doubly twisted ground state and the unknown excited state(s) are probably responsible for the unusual luminescence, e.g. in C_2H_5OH: λ = 545 nm (Intens. ≡1), Stokes shift ≈ 230 nm, at +20°C; λ = 485 nm (Intens. = 24), at −196°C (H. Kunkely, H. Zinner, A. Mannschreck, unpublished result). In this respect, a joint study has been initiated with E.M. Kosower and D. Huppert, Tel-Aviv; cf. E.M. Kosower, D. Huppert, *Annu. Rev. Phys. Chem. 37* (1986) 127.
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[17] A very similar range of barriers is predicted from the crude assumption that HPLC peak coalescence is observed, if the half-lifetime of enantiomers is of the order of magnitude of their retention times.
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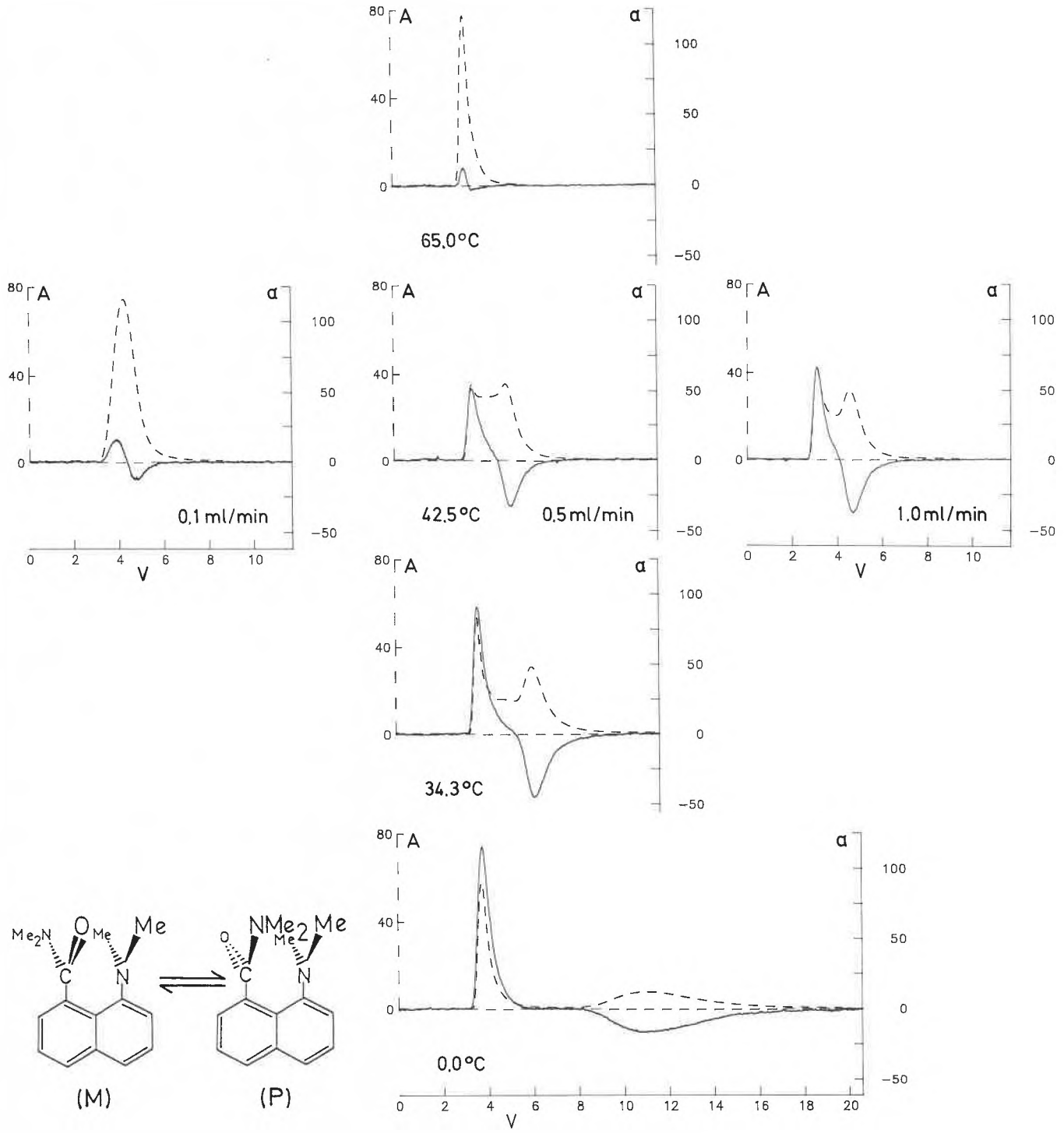

Fig. 1. HPLC of 0.13 mg of (MP)-1-dimethylamino-8-dimethylcarbamoylnaphthalene in C_2H_5OH/H_2O, 96:4, on non-dilute triacetylcellulose I[5,9]. A: absorbance at λ = 325 nm in arbitrary units; α: rotation angle at λ = 365 nm in mdeg; V: retention volume (V = 0 upon injection) in mL. Horizontal comparisons refer to variable flow rate of the eluent at 42.5°C; vertical comparisons refer to variable temperature at 0.5 mL/min. The capacity factors at 0.0°C are 0.28 and 2.6; the averaged capacity factor at 65.0°C amounts to 0.09. At 42.5°C, the capacity factors at 1.0 mL/min are approximately 0.1 and 0.4; the averaged capacity factor at 0.1 mL/min amounts to 0.3.