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NOTE

The Separation of Water-Soluble Organic Diastereomers by Chromatography on Sephadex

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Abstract

We have investigated the influence of the degree of cross-linking of Sephadex gels and the polarity of the eluent on the separation of *cis*- and *trans*-cinnamic acids and concluded that the physical basis for the separations is hydrophobic interactions between the solute and the cross-linkages in the gels.

The separation of chemically and physically similar nonvolatile organic compounds such as cis and trans diastereomers of alkenes is often a tedious process. In previous studies we had observed that a mixture of *cis*- and *trans*-cinnamic acids could be separated by chromatography on Sephadex G-25, one of several types of cross-linked dextrans sold by Pharmacia Fine Chemicals and used primarily for gel-filtration separations based on differences in molecular size. While chromatography on Sephadex has been used to separate small organic molecules (*1*), it is ordinarily employed to fractionate polymeric materials such as proteins. As expected, a survey of the literature uncovered only a few examples of the separation of diastereomers in aqueous solution. Kundu and Maenza (*2*) noted that mixtures of maleic (cis) and fumaric (trans), of isocrotonic (cis) and crotonic (trans), and of coumaric (cis) and coumarinic (trans) acids could be

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separated by chromatography on Sephadex G-10, eluting with 0.5% ammonium bicarbonate. However, mesaconic (cis) and citraconic (trans) acids could not be fractionated by this method. Since the trans diastereomer eluted first in cases where separation was obtained, these authors concluded that the basis for the separation was a gel-filtration phenomenon in which the larger effective sizes of the trans isomers resulted in their smaller elution volumes. Subsequently, Andersson reported the separation of cis and trans diastereomers of halogenated crotonic (*3*) and acrylic (*4*) acids by the same procedure.

Our observations that *cis*-cinnamic acid had a smaller elution volume than its trans isomer under all conditions employed (see Tables 1 and 2) appeared inconsistent with the results and explanation of Kundu and Maenza. A more appropriate rationale seemed to be in terms of adsorption of the hydrocarbon portion of these molecules to the cross-linkages in Sephadex via hydrophobic interactions (*5*). In order to more fully investigate the physical nature of this type of chromatography and to attempt to derive conditions for maximum separating efficiency, we have studied the effect of the degree of cross-linking, type of gel, and eluting solvent polarity on the separation of *cis*- and *trans*-cinnamic acids (*6*). Our results from this study, reported below, support the conclusion that

TABLE 1

Effect of Gel Type on the Separation of *cis*- and *trans*-Cinnamic Acids[a]

Gel	V_t (ml)	V_0 (ml)	V_e (ml)		K_{av}		Flow rate (ml/hr)
			cis	trans	cis	trans	
G-10	93	35	84	196	0.85	2.78	24
G-15	93	34	86	167	0.88	2.26	16
G-25	93	35	84	111	0.85	1.31	28
G-75	93	29	84[b]	98[b]	0.85	1.08	10
LH-20	93	29	85	163	0.88	2.09	4
P-2	89	32	46	58	0.25	0.46	15

[a] Each column (1.1 × 80 cm i.d. glass) was poured and eluted with 0.05 *M* potassium phosphate (pH 8.0) containing 0.1 *M* potassium chloride. One milliliter samples of a stock solution containing 0.5 mg/ml of a mixture of *cis*- and *trans*-cinnamic acids in the same buffer were applied. The eluent was monitored with an ISCO UV Monitor at 254 nm, and each diastereomer was collected and identified by its UV spectrum. V_0 was the elution volume of Blue Dextran 2000 from Pharmacia.

[b] A broad, unresolved peak with the leading and trailing edges containing pure *cis*- and *trans*-cinnamic acids, respectively.

TABLE 2

Effect of Solvent on the Separation of *cis*- and *trans*-Cinnamic Acids on Sephadex G-10[a]

Elution solvent	V_t (ml)	V_0 (ml)	V_e (ml) cis	V_e (ml) trans	K_{av} cis	K_{av} trans	Flow rate (ml/hr)
Deionized water	93	34	56[b]	56[b]	0.37	0.37	25
0.5% ammonium bicarbonate	93	34	73	157	0.66	2.08	26
0.1 *M* potassium chloride	93	34	69	166	0.59	2.22	28
1.0 *M* potassium chloride	93	33	87	219	0.90	3.10	30
0.05 *M* potassium phosphate, pH 8	93	33	89[c]	262[c]	0.93	3.82	30
0.5 *M* potassium phosphate, pH 8	93	38	172[d]	—[d]	2.44	—	36
0.05 *M* trisodium citrate	93	33	109	292	1.26	4.32	28

[a] The same procedure and column were used as in Table 1. The column was equilibrated with 500 ml of the elution solvent before use.

[b] The peaks were not resolved, but the leading and trailing edges were enriched in *cis*- and *trans*-cinnamic acids, respectively.

[c] The peaks were much broader than in the first chromatography shown in Table 1.

[d] The peak for *cis*-cinnamic acid was so broad as to be almost undetectable while that for trans was not detected.

hydrophobic interactions between the cinnamic acids and Sephadex are responsible for the separations obtained.

Table 1 contains data which demonstrate the effect of cross-linking on the separation of the two diastereomeric acids. Clearly, the K_{av} [defined (*7*) as $V_e - V_0/V_t - V_0$ where V_e, V_0, and V_t are the elution volume of solute, void volume, and total column volume, respectively, and where K_{av} is proportional to the degree of interaction between the solute and gel (*8*)] for the trans diastereomer increases markedly as the gel is changed from G-75 to G-10 (from least to most cross-linked), strongly suggesting that the degree of cross-linking is influential. Interestingly, LH-20, which is G-25 that has been exhaustively hydroxypropylated, gives improved separation when compared to the more highly cross-linked Sephadex G-15, a further indication that the interactions responsible for separation are hydrophobic. For comparison, chromatographic separation of the cis and trans isomers of cinnamic acid was made using Bio-Gel P-2, a hydrophilic polyacrylamide gel sold by Bio-Rad having nearly the same degree of cross-linking as G-25. Although separation was achieved, both diastereomers possessed a much lower adsorption coefficient as reflected in their low K_{av} values.

If our postulate that adsorption to the gel is due to hydrophobic interactions is correct, then it is expected that increasing the "polarity" of the solvent by increasing its ionic strength would increase K_{av} for both stereoisomers. In Table 2, are data recorded for separations using water with varying concentrations of salts as the eluent and Sephadex G-10 as adsorbent. Virtually no separation in deionized water, the eluent of lowest ionic strength, was obtained. Increases in potassium chloride or potassium phosphate concentrations in the eluent resulted in substantial increases in the adsorption of both diastereomers and enhancements in separations. Importantly, 0.05 *M* potassium phosphate is more effective than 1.0 *M* potassium chloride in increasing the adsorption of *trans*-cinnamic acid (V_e of 262 ml compared to 219 ml); adsorption of the cis isomer is nearly the same in both solvents. This suggested that the best type of salt for increasing the interaction between gel and trans isomer and maximizing separation is one that is highly charged and thus capable of interacting with a large number of water molecules. Accordingly, 0.05 *M* trisodium citrate gave a larger K_{av} value than 0.05 *M* potassium phosphate.

The data presented are consistent with separation of the cinnamic acids on the basis of hydrophobic interactions due to the cross-linkages of Sephadex G. We have shown that adsorption to the gel is a function of the degree of cross-linking and the ionic strength of the solvent. The results of this study suggest that adsorption chromatography on Sephadex G could be very useful as a method for separation of water-soluble diastereomers on an analytical or preparative basis using materials that are relatively inexpensive and reuseable.

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