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Separation of benzoic acid from volatile fatty acids using Sephadex G-10*

The investigation of the pathway of benzoic acid utilization by methanogenic bacteria led to the problem of separating the benzoic acid substrate from volatile fatty acid (VFA) intermediates. Attempted separations using column, paper or thin-layer chromatography or hydroximate derivatives were unsatisfactory because of identical R_F values, equal elution volumes, or tailing peaks. Gas chromatography also was unsatisfactory.

TABLE I

SEPARATION OF BENZOIC ACID FROM VOLATILE FATTY ACIDS USING SEPHADEX G-10

Sample	2–3 mg with an activity of about 0.1 μC in 0.5 ml
Column	Diameter = 1.27 cm; height = 103.6 cm.
Packing	Sephadex G-10 in phosphate buffer (0.1 M Na_2HPO_4–KH_2PO_4), pH 7.4
Eluent	The same buffer as the packing
Flow	4.5 ml/h as controlled by Cole-Palmer (Model No. 7013F) parastolic pump
Measurement	1 ml of collected sample in 9 ml of phosphors solution (p-dioxane, α-naphthalene, POPOP, PPO), liquid scintillation counting

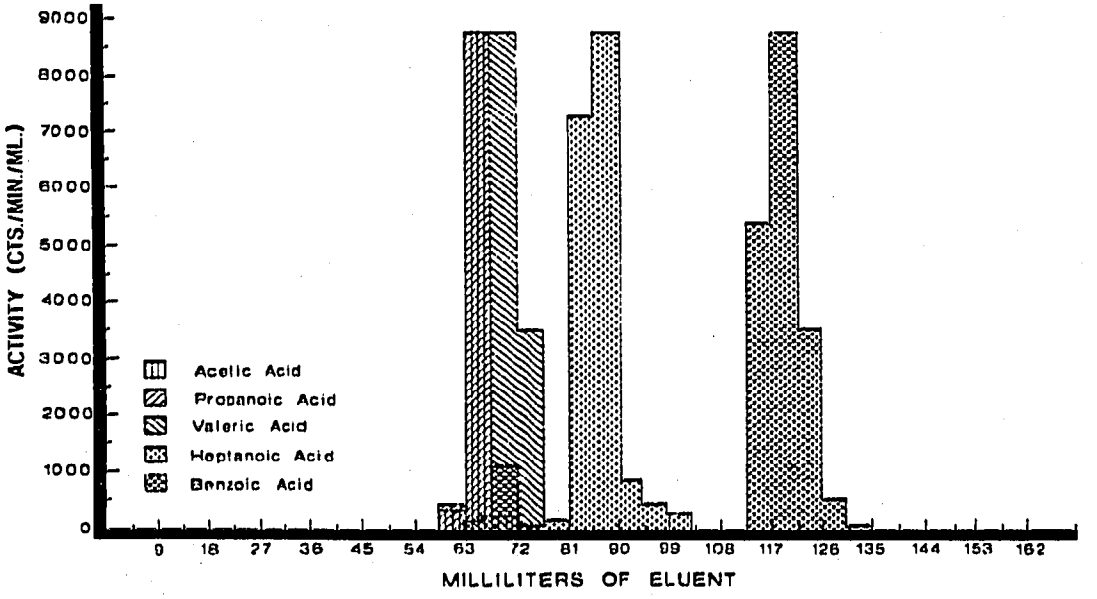

Fig. 1. Separation of benzoate from VFA's.

Benzoic acid, because of its hydrophilic character, steam distills in aqueous solution and sublimes around 100° when dry or crystalline. Methyl derivatives, although satisfactory for benzoic acid, were impractical for fatty acids with a chain

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of fewer than eight carbons. Quantities of VFA's being dealt with were on the order of 1–4 mg (1–4 μmoles).

The ability of highly cross-linked Sephadex G-10 to separate low molecular weight substances suggested its use. Experiments with ^{14}C in various VFA's and benzoic acid were conducted. The conditions are given in Table I. At hourly intervals, using a fraction collector, 4.5 ml samples were obtained. One milliliter of each was used to determine ^{14}C. The ^{14}C tracer was considered to be easier to detect and more sensitive than photometric or chemical techniques, which also may be used. The VFA's isolated from benzoate were further separated and/or identified by gas chromatography.

Results are shown in Fig. 1. Since each experimental run contained slightly different amounts of radioactivity, all peaks were adjusted to a maximum of 8800 c.p.m./ml so that peak shapes and separation could be compared.

Note that lower molecular weight compounds were eluted first. Normally with Sephadex a reverse order is expected. The delayed elution of benzoic acid most likely results from the adsorption phenomena that occur with aromatic compounds in Sephadex columns[1,2]. The reverse separation of the VFA's probably results from some electrostatic phenomena[1]. Sharper isolation of individual VFA's likely could be obtained by collecting smaller volume samples. Multiple overlapping VFA's, once freed of benzoic acid, can be further purified easily.

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1 J.-C. JANSON, *J. Chromatog.*, 28 (1967) 12.
2 R. L. EASTERDAY, *Lab. Management*, 6, No. 8 (1968) 43.

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