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Separation of vitamins K_2 on capillary columns of methylated Sephadex

In the course of studies of the potential uses of methylated Sephadex for the separation of lipid soluble compounds[1] it was considered of interest to study the separations that could be obtained with a series of isoprenologue compounds. A series of vitamins K_2 were chosen since the effluent could be easily monitored by measurement of the U.V. absorption.

Experimental

Vitamins $K_{2(10)}$–$K_{2(40)}$ were generously supplied by Drs. O. WISS AND U. GLOOR, Hoffmann-la Roche, Basel, Switzerland.

Sephadex G-25 fine and superfine (kindly supplied by Dr. B. GELOTTE, Pharmacia, Uppsala, Sweden) were methylated as described previously[1]. Columns having a diameter of 2 cm were prepared with about 25 g of methylated Sephadex G-25, fine[1]. The samples (0.2–1 mg) were applied to the columns in 0.5–1 ml solvent. Capillary columns were prepared in teflon tubing (outer and inner diameters 2.3 and 1.5 mm, respectively) in the following way:

A small piece of glass wool and a 2 cm piece of stainless steel capillary tube (O.D. $^1/_{16}$ in., I.D. 0.25 mm, cut to a tip in the distal end) were inserted into the distal end of a teflon tubing about 2 m in length. The tubing was filled with the solvent to be used for the chromatography. The proximal end was connected with a stainless steel tubing (O.D. $^1/_{16}$ in., I.D. 0.6 mm, length 5 cm) silver soldered to a stainless steel cylindrical reservoir (O.D. 30 mm, length 100 mm) which contained a slurry of methylated Sephadex G-25, superfine, in the same solvent. The upper end of the

cylindrical reservoir was connected to a nitrogen tank and a pressure of about 1–2 kp/cm^2 was applied. The slurry passed slowly through the capillary into the teflon tubing. Clogging was prevented by vibrating the reservoir with a Vibro-graver (Burgess Vibrocrafters Inc., Grayslake, Ill.). When the teflon tubing was completely filled with the gel the pressure was released and the tubing was disconnected from the reservoir. An injection port (see Fig. 1) was attached to the proximal end of the teflon column and connected to another cylindrical reservoir (38 × 300 mm) which contained the solvent to be used. Pressure, 1–3 kp/cm^2 (depending on the solvent used and the length of the column), was applied to the system.

The sample was dissolved in a suitable solvent and about 5–50 μg in about 5 μl was injected into the column (usually about 10 mm below the gel surface at the top of the column). The flow rate was kept at 0.5–0.6 ml/h.

Vitamins K_2 appearing in the effluent were determined by measurement of the absorption at 270 nm. With the capillary columns, U.V. measurements did not permit the collection of fractions small enough to make use of the full separating efficiency of the columns. At a later stage of the work Dr. E. HAAHTI kindly lent us one of his platinum chain–flame ionization detectors[2]. This permitted a continuous monitoring of the effluent from the capillary columns.

Results

Several different solvent mixtures were tried. Satisfactory results were obtained with chloroform–methanol–heptane, 1:1:2. The separation of vitamins $K_{2(40)}$, $K_{2(20)}$ and $K_{2(10)}$ on a 25 g column is shown in Fig. 2. Since only small amounts of vitamins K_2 were available to us the capacity of the columns could not be tested.

The separation of vitamins $K_{2(40)}$–$K_{2(10)}$ on a capillary column is shown in Fig. 3. It is possible that a higher column efficiency would be obtained with methyl-

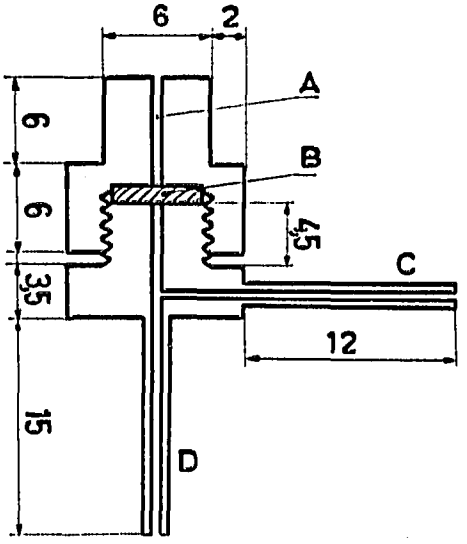

Fig. 1. Schematic drawing of stainless steel injection port used for capillary columns. All dimensions are in mm. (A) 0.6 mm hole guiding the 50-mm needle of a Hamilton syringe during sample injection; (B) silicone rubber membrane; (C and D) capillary tubing, $^1/_{16}$ in. O.D. 0.6 mm I.D., connected to the teflon column (D) and to the solvent reservoir (C).

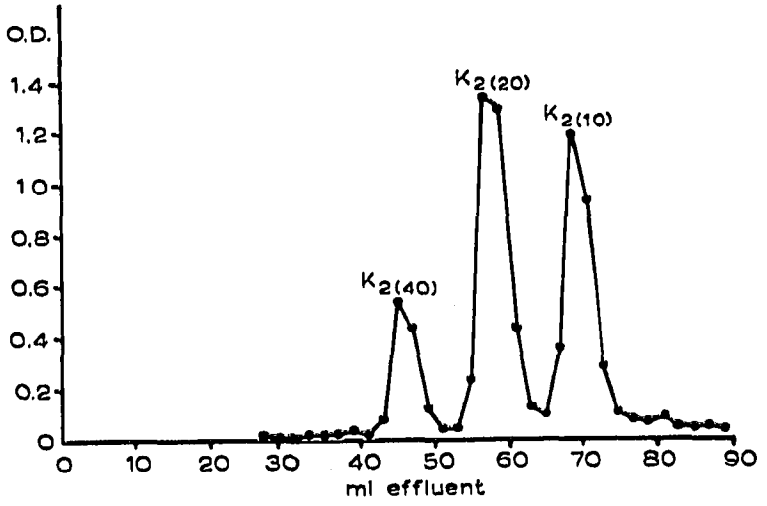

Fig. 2. Separation of 0.2–0.3 mg each of vitamins $K_{2(40)}$, $K_{2(20)}$ and $K_{2(10)}$ on a 30 × 2 cm column of methylated Sephadex G-25 in chloroform–methanol–heptane (1:1:2). Flow rate: 0.7 ml/min.

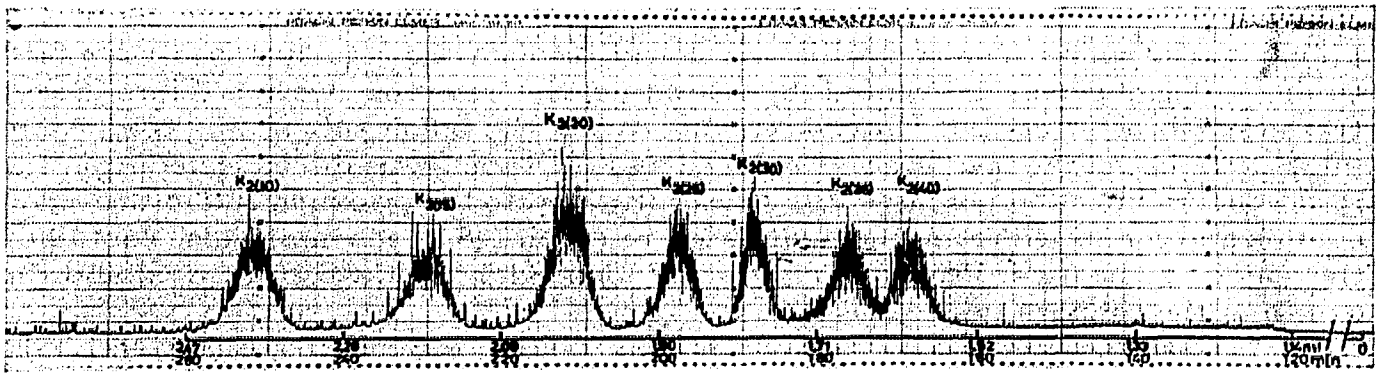

Fig. 3. Separation of about 20 μg each of vitamins $K_{2(40)}$–$K_{2(10)}$ on a 1750 × 1.5 mm column of methylated Sephadex G-25, superfine, in chloroform–methanol–heptane (1:1:2). The effluent was monitored with a flame ionization detector[2].

ated Sephadex having a more uniform particle size than the superfine grade used in this column.

The mechanisms responsible for the separations have not been elucidated. The compounds are eluted in an order of decreasing molecular weight and in an order of increasing polarity. From this and previous studies[1] it appears reasonable to assume that the separations are effected through a combination of gel filtration and partition chromatography between a stationary gel–solvent phase and a less polar mobile phase.

Several methods for the separation of vitamins K_2 have been published previously (for a review see ref. 3). The present technique using methylated Sephadex could be used as a complement to these methods. It is non-destructive, and the separations can be carried out rapidly on a micro- or macroscale. The columns can be used repeatedly over long periods of time and in this respect chromatography on methylated Sephadex can be compared with gas–liquid chromatography which is less suitable for the separation of vitamins K_2.

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