DOMESTIC GEL SORBENTS FOR THE PURIFICATION OF SUBSTANCES OF PLANT ORIGIN

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Hydrophobic and hydrophilic gels are used as supports for gel chromatography [1]. Of the hydrophilic gels, the best known are the dextran gels – the Sephadexes – and of the hydrophilic gels polystyrene gels and also powdered glass [2]. A special position among gels is occupied by Sephadex ZH-20, showing activity both in aqueous and in organic solvents [2]. In the USSR, methods have been developed for the synthesis of dextran gels [2] of two types – ÉD and DÉD.

The use of gel chromatography for the separation of various substances of plant origin has been described in a number of papers [3-11].

We have studied the swellability and separating capacity of some domestic gel sorbents and have also determined to what extent the hydrophilic gel sorbents correspond in swellability in various solvents and in separating capacity to samples of Sephadexes.

EXPERIMENTAL

Samples of the gels ÉD-1,5 and ÉD-2,0 (particle dimensions 40-120 μ) were first washed with water and then, successively, with 50%, 70%, and 96% ethanols and were dried in a drying chest at 80°C. The degree of swellability was determined by a published method [12]. The following facts can be seen from Table 1: 1) in their absolute capacities for swelling, the gel ÉD-1,5 and Sephadex G-15 are practically identical; and 2) the absolute swellability of the gel ÉD-2.0, in water is considerably greater than that of ÉD-1,5 and is close to that of Sephadex G-25 [1] (no information is given in the literature on the swellability of this Sephadex in other solvents).

In hydrocarbons and chlorinated hydrocarbons the difference in the swellabilities of ÉD-1,5 and ÉD-2,0 is insignificant.

The hydrophilic gels of the SDVp type tested possess the greatest swellabilities in chloroform and benzene. The degree of swelling of the gel SDVp $1 \cdot 10^4$ in ethanols is greater than that of gel SDVp $2 \cdot 10^3$.

TABLE 1. Swellabilities of Hydrophilic and Hydrophobic Gels

Solvent	Hydrophilic gels			Hydrophobic gels	
	Sephadex G-15	ÉD-1,5	ÉD-2,0	SDVp $1 \cdot 10^4$	SDVp $2 \cdot 10^3$
	absolute swellability, ml/g				
Water	3,19	3,38	5,73	—	—
Ethanol	1,82	1,54	1,47	2,24	1,69
n-Butanol	Decreases in volume			2,37	1,81
Acetone	Does not swell			2,85	3,27
Dimethylformamide	3,20	2,98	5,12	3,05	4,65
Benzene	1,38	1,39	1,50	3,36	8,45
Chloroform	1,44	1,66	—	3,36	8,87
Dichloroethane	1,58	1,44	1,30	3,05	6,76
Heptane	Does not swell	—	1,50	3,05	1,70
Toluene		—	1,30	3,30	7,61

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TABLE 2. Results of the Purification of Technical Tannin

Weight of gel	Weight of technical tannin	Amount of purified tannin	Yield‡, %
	g		
6	0,2	0,188	94*
6	0,2	0,190	95†

*The color of the initial tannin was dark brown.
†The purified tannin was slightly grayish.
‡ The yield of purified tannin was calculated as weight/weight.

TABLE 3. Results of the Purification of Technical Hyoscyamine

Gel used	Weight of technical hyoscyamine	Amount of hyoscyamine obtained	Yield, %
	g		
Sephadex SPC-25 (fresh)	0,05	0,047	100*
The same gel after regeneration	0,05	0,046	99†
The same gel after a second regeneration	0,3	0,285	100
Sephadex SPC-25 (fresh)	0,3	0,279	97

*The initial technical hyoscyamine was dark brown and contained 86% of hyoscyamine.
†The purified hyoscyamine was white with a creamy tinge and contained 92% of hyoscyamine.

In chloroform, dichloroethane, benzene, and toluene, the swelling of gel SDVp $1 \cdot 10^4$ is little more than half that of gel SDVp $2 \cdot 10^3$. The samples of gels were kindly given to us by S. B. Makarova and E. V. Egorov.

We investigated the separating capacity of the gels ÉD-1,5 and Sephadex G-15 comparatively for the case of the purification of technical tannin isolated from sumach leaves. Separation was performed in glass columns with a diameter of 1.5 cm and a height of 30 cm containing 6 g of gel swollen for 24 h in distilled water, the height of the layer being 17 cm. On the top of the layer of gel was placed a solution of 0.2 g of technical tannin in 1 ml of ethanol. Elution was performed with distilled water and the issuing filtrate was collected in 10-ml fractions in a measuring cylinder. The rate of flow was 170 liters/$h \cdot m^2$. The amounts of tannin in the individual fractions were determined by means of the reaction with a 3% solution of ferric chloride, with which tannin gives a blue coloration. The eluate containing the tannin was colorless, all the colored impurities remaining in the top layer of the gel.

The tannin was extracted from the combined aqeuous filtrates with n-butanol (in the presence of NaCl) until the reaction for tannin was negative. The aqueous butanolic solution was evaporated under vacuum to a small residue, which was then placed in a vacuum desiccator where, on standing, crystals of purified tannin deposited (Table 2).

It can be seen from Table 2 that gel ÉD-1,5 can purify tannin from accompanying substances with a yield of 94.5%. The same results were obtained in experiments on the purification of tannin on Sephadex G-15.

We also decided to determine the possibility of purifying combined tropane alkaloids (technical hyoscyamine isolated from scopolia roots by the adsorption method) [13] on the cation-exchange resin Sephadex SPC-25.

The Sephadex SPC-25 (4 g) was first swollen in distilled water for 24 h and was then placed in a column with a diameter of 1.5 cm. The height of the layer was 16 cm. On the layer so obtained was deposited a solution of 0.05 or 0.3 g of hyoscyamine in 1 ml of ethanol. The alkaloids were eluted from the layer of gel with a 0.1 N solution of sulfuric acid. The eluate containing the alkaloids was colorless, the colored impurities remaining in the top layer of the gel. The presence of alkaloids in the filtrate was checked by the qualitative reaction with Dragendorff's reagent. The sensitivity of the reagent for hyoscyamine is 3-5 μg.

The combined sulfuric acid filtrates were made alkaline, and the alkaloids were extracted from them with chloroform. The chloroform was distilled off on the water bath under vacuum to a small residue, from which the purified combined alkaloids were precipitated by the addition of petroleum ether.

The colored impurities were subsequently eluted from the gel with distilled water made alkaline to pH 9 and 50%, 70%, and 96% ethanol until the filtrate and the layer of gel were colorless and, finally, with distilled water. The gel washed in this way was used for subsequent experiments (Table 3).

As can be seen from Table 3, in the purification of technical hyoscyamine a product of good quality was obtained in practically quantitative yield. Furthermore, the possibility has been shown of the satisfactory regeneration of the gel. All this shows the efficiency of the use of cation-exchange gel sorbents for purifying alkaloids from extractive substances and resins.

The quantitative determination of the hyoscyamine in these cases was performed by the method described previously [14]. The results obtained show the desirability of organizing the production of hydrophilic cation-exchange gels.

SUMMARY

1. The degrees of swellability of the gels ÉD-1,5, ÉD-2,0, SDVp $1 \cdot 10^4$ and SDVp $2 \cdot 10^3$ and also of Sephadex G-15, in water and nine different organic solvents have been determined.

2. The possibility has been shown of purifying technical tannin on Sephadex G-15 and the domestic gel ÉD-1,5 and of purifying technical hyoscyamine on the cation-exchange Sephadex SPC-25.

3. On the basis of the results on swellability and on the purification of technical tannin, gel ÉD-1,5 has been shown to be equivalent to Sephadex G-15.

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