Note

Marine polymers

Part I. A new procedure for the fractionation of agar

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It has been demonstrated[1,2,3] that agarose, the major component in commercial agar, is a more desirable matrix for gel-electrophoresis, -diffusion or -chromatography than the unfractionated product. The purified material also has better gel characteristics[4,5]. The separation of this low sulfate-containing component from the contaminating and more highly sulfated agaropectin has been accomplished previously by acetylation[1,6], precipitation[7-10], and adsorption[11]. Generally, these methods are elaborate, provide poor yields of agarose, or require the use of expensive chemicals. A facile, potentially low-cost procedure for the high-yield fractionation of agar is now reported.

Brauns[12] has recently shown that chitin can be used to adsorb the lignosulfonates present in the spent liquors from the sulfite pulping of wood. The adsorption is presumably a consequence of the formation of ionic bonds between the sulfonate groups in the lignin and amino moieties in the adsorbent. Since the impurities associated with the agarose are highly sulfated, it could be anticipated that application of this interpolymeric adsorption technique to commercial agar would lead to their preferential retention as an insoluble, polymeric salt. This in fact proved to be the case and it is shown that commercial agar can be fractionated by using both chitin and its *N*-deacetylated derivative, chitosan. Two experimental systems have been investigated. The first was a homogeneous procedure in which agar, dissolved in formamide, was admixed with an acidic aqueous solution of chitosan, whereupon the co-precipitated polymeric sulfate separated from the agarose-rich, supernatant liquor.

The second system involved a heterogeneous phase reaction in which solid chitin or chitosan was stirred with a solution of agar in formamide and the undissolved solid separated from the supernatant liquid.

The criterion used to determine the extent of reaction for both systems was the sulfur content of the polymer recovered from the supernatant liquid. Nitrogen analyses were carried out to determine the extent of contamination of the product by the solvent or reactants. Negligible nitrogen was found in all cases. The yields and sulfur contents of the starting material and products are collected in Table I.

The results shown in Table I clearly demonstrate that both chitin and chitosan remove a substantial proportion of the sulfated polymer (agaropectin) from agar. The

TABLE I

YIELD AND SULFUR CONTENT OF AGAROSE FRACTIONS FROM AGAR

Fractionated product	*Fractionation procedure*	*Adsorbent*	*Polymer yield, %*	*Sulfur content, %*
Agar	—	—	100	0.92
Agarose	Homogeneous	Chitosan	60	0.15
Agarose	Heterogeneous	Chitin	72	0.45
Agarose	Heterogeneous	Chitosan	68	0.38
Agarose[7]	Homogeneous	R^4N^+	47	0.14

homogeneous system is predictably more efficient than the heterogeneous process. For the latter, the particle surface area and the time of contact are obviously critical. At a later stage it is hoped to determine the effect of these variables. The use of a wholly aqueous procedure is currently being investigated, as is the applicability of the procedure to the purification of other anionic polymers. Preliminary results indicate that alginic acid, the sulfated water-soluble extracts of *Ulva lactuca*, and also cellulose sulfate can all be precipitated with chitosan.

EXPERIMENTAL

Materials. — Commercial chitin was obtained from the Eastman Kodak Company, Rochester, N. Y., and chitosan was obtained by alkaline *N*-deacetylation of chitin by the method of Meyer and Wehrli[13]. Commercial agar was obtained from Difco Laboratories, Detroit, Michigan, U. S. A.

Procedures. — The homogeneous purification reaction was carried out by using a 2% solution of agar in formamide mixed 2:1 (*v/v*) with a solution of chitosan (0.5%, *w/v*) in dilute acetic acid (3%, *v/v*). The solution was maintained at an apparent pH of 4.5. After stirring for 1 h the precipitate was removed by centrifugation and the supernatant was diluted with water (4 vols), whilst the pH was maintained at the same level. The insoluble agarose gel precipitated. After collection over a glass filter, it was sequentially washed with ethanol and ether, and then air-dried.

The heterogeneous phase reaction was also carried out on a 2% solution of agar in formamide. The solution was stirred for 4 h with an equal weight of chitosan or chitin. After centrifugation, the clear solution was treated with ethanol (3 vols), whereupon the agarose precipitated, and was collected as before.

The nitrogen content of the polymers was determined by a modified Kjeldahl method[14], and the sulfur content was measured by neutron activation analysis with the nuclear reactor facilities of the University of Washington. The polymer samples (0.5–0.6 g) were irradiated for 5 min in a neutron flux of approximately 1.8×10^{20} neutrons.cm^{-2}.sec^{-1}. After irradiation and appropriate cooling, the samples were counted by integrating the 3.10 MeV photopeak of ^{37}S by using a 3 in. × 3 in. NaI(Tl) crystal and multichannel analyzer.

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