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Further studies on the adsorption of plant phenols by synthetic polymers

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Abstract

Pyrocatechol, catechol, caffeic acid, chlorogenic acid, safflor yellow A, safflor yellow B, precarthamin and carthamin were effectively adsorbed by insoluble polyvinyl-N-pyrrolidone (PVP) in a neutral buffer solution. These eight phenols also bound with Amberlite XAD resins, however, the rate was found to be far less efficient than that of PVP. The average rate of the phenol binding was calculated as following order (%): PVP (42.7), Amberlite XAD-2 (16.6), Amberlite XAD-4 (10.1), Amberlite XAD-7 (13.0), Amberlite XAD-8 (17.7). No 3,4-dihydroxyphenylalanine was adsorbed by PVP, while the O-dihydroxylic acid could be removed by Amberlite XAD-4, XAD-7 and XAD-8. Data from using different weights of the test polymers showed that the rate of the phenol adsorption rose in proportion to each increasing amount of the adsorbents. PVP also admittedly maintained its predominent capacity for phenol binding over that of each member of the Amberlite resins.

Key words: *plant phenols*, *polyvinyl-N-pyrrolidone*, *Amberlite XAD resin*, *adsorption*

INTRODUCTION

The difficulty in working with plant enzymes arises partially from the fact that the enzyme activities are often seriously affected by endogenous inhibitors during the course of the enzyme preparation. Many phenolic substances are known to act as potential inhibitors of certain enzymes or enzyme systems. To

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reduce the inhibitory action by phenols, phenol-binding compounds have been used in the homogenization media for enzyme extraction (Harel et al. 1964, Jones et al. 1965, Walker and Hulme 1965, Abukharma and Woolhause 1966, Pierpoint 1966, Loomis and Battaile 1966, Anderson and Rowan 1967, Andersen and Sowers 1968, Frirenzuoli et al. 1969). Among these Dowex-1 and insoluble polyvinyl-N-pyrrolidone (PVP) are widely used as polymeric substances for phenol adsorption. A preceding study in this laboratory (Fukushima et al. 1987) has shown that Dowex-1 bound positively with plant phenols including novel types of quinochalcone glycosides such as safflor A, safflor B and precarthamin. However, it had little or no affinity for carthamin which could presumably act as potential inhibitor of an oxidizing enzyme tentatively named "a carthamin-synthesizing enzyme" (Saito et al. 1983a, b, 1985a, b) during the process of the enzyme extraction from the floral tissues of dyer's saffron. Therefore, this work was undertaken to find more effective adsorbents for the pigments as well as other phenols in an incubation model system.

This paper will present the evidence from adsorption tests of plant phenols using ionically charged or uncharged synthetic polymers.

MATERIALS AND METHODS

CHEMICALS

High molecular weight and cross-linked insoluble polyvinyl-N-pyrrolidone (PVP) was purchased from Sigma Co. (St. Louis, Mo., U.S.A.). Polystyrene resins, uncharged Amberlite XAD-2 and XAD-4 or weakly charged Amberlite XAD-7 and XAD-8 were supplied from Organo Co., Ltd. (Tokyo, Japan). Phenols and other chemicals used were from the same source as described previously (Fukushima et al. 1987).

PRETREATMENT OF SYNTHETIC POLYMERS

The powdered PVP was purified by a partially modified method of McFarlane and Vader (1962). PVP (500 g) was mixed with appropriate amount of deionized-distilled water in a beaker. The mixture was stirred for 30 min with a magnetic stirrer and the supernatant discarded by filtration on a Büchner funnel. The washing was repeated two more times. Then the washed PVP was suspended in five times volume per g dry weight of a solution which contained 0.75 g potassium ferricyanide and 40 g sodium carbonate. The suspension was stirred mechanically for 120 h at 22-24°C: the ferricyanide solution was replaced at intervals with a freshly prepared one (10 000 cm^3 in total). When the polymer was no longer discoloured freshly mixed yellow ferricyanide solution, the mixtures were filtered through Toyo Roshi No. 50 papers with suction and the residue was washed several times with deionized-distilled water. Then the polymer was stirred for 1 h prior to filtration with successive volumes of 15% sodium carbonate, 10% hydrochloric acid, deionized-distilled water, acetone and ether. The purified PVP was dried in the air and stocked in a desiccator over silica gel. The dry powder was soaked in water for 24 h, before use, in order to hydrate it and the aqueous paste was applied following incubation process. Polystyrene resins were immersed in 70% methanol for 2-3 h, then they were transferred to fresh methanol and left for several hours. Washing of the polystyrene was carried out in distilled water, methanol, acetone and finally in ether. The washed resins were kept for the experiments.

INCUBATION OF SYNTHETIC POLYMERS IN PHENOL SOLUTIONS

Weighed polymers (1-50 mg) were separately dipped in an aliquot of 50 mM citrate-phosphate buffer, pH 7.0, for a few minutes at 24°C. Two cm^3 of phenol solution (0.1 μmol cm^{-3} in 50 mM citrate-phosphate buffer, pH 7.0, each) was added and stirred with the solution containing a test polymer for 10 s, then the mixture was filtered through a glass fibre filter paper (Toyo Roshi Co., Ltd., 55 mm × 5.5 cm), and immediately the filtrate was subjected to spectrophotometric estimation for the phenol content.

RESULTS

The affinity of plant phenols for synthetic polymers was compared by using PVP and Amberlite XAD resins in an experimental model system. Differences in the mechanism by which the test phenols are fixed were suggested by the specific affinities with the polymers examined. The time course of the phenol adsorption by PVP and Amberlite resins is summarized in Table 1. The data prove clearly that PVP adsorbs phenols about four times better compared with the average rates of XAD resins, though it has no affinity for 3,4-dihydroxyphenylalanine which can be removed by XAD-4, XAD-7, and XAD-8. Among polystyrene resins, XAD-8 is the most effective, followed by XAD-2 and XAD-7 follows this, while XAD-4 is the poorest adsorbent. A comparison of the affinity of each XAD for test phenols reveals some potential characteristics presumably derived from the chemical and/or physical properties. For example, XAD-7 bound 3,4-dihydroxyphenylalanine most efficiently, though it showed the poorest affinity for catechol. On the other hand, XAD-2 bound caffeic acid (about 21%) while it exhibited poor affinity for 3,4-dihydroxyphenylalanine under the present experimental conditions.

Table 1

Adsorption of plant phenols by synthetic polymers

Polymer	Phenol, μM mg^{-1} polymer min^{-1}								
	1	2	3	4	5	6	7	8	9
PVP	0.57	0.42	0	0.86	0.42	0.80	1.34	1.72	1.71
Amberlite XAD-2	0.52	0.16	0.01	0.37	0.27	0.06	0.42	0.47	0.79
Amberlite XAD-4	0.35	0	0.11	0.26	0.24	0.01	0.19	0.40	0.13
Amberlite XAD-7	0.09	0.01	0.34	0.14	0.28	0.18	0.22	0.36	0.03
Amberlite XAD-8	0.22	0.29	0.23	0.12	0.40	0.14	0.20	0.40	0.53

1 – Pyrocatechol, 2 – catechol, 3 – 3,4-dihydroxyphenylalanine, 4 – caffeic acid, 5 – chlorogenic acid, 6 – safflor yellow A, 7 – safflor yellow B, 8 – precarthamin, 9 – carthamin.

A study on varied amounts of five different polymers in adsorption of phenols is illustrated in Figures 1-9. In this experiment PVP also maintained predominance over Amberlite resins, showing that the rate of the phenol adsorption rose progressively on increasing the amount of the vinyl polymer. On the contrary, only a slight increase was observed in pyrocatechol, catechol, caffeic acid, chlorogenic acid, safflor yellow B and precarthamin, even though XAD weights were increased (see Figs. 1-9).

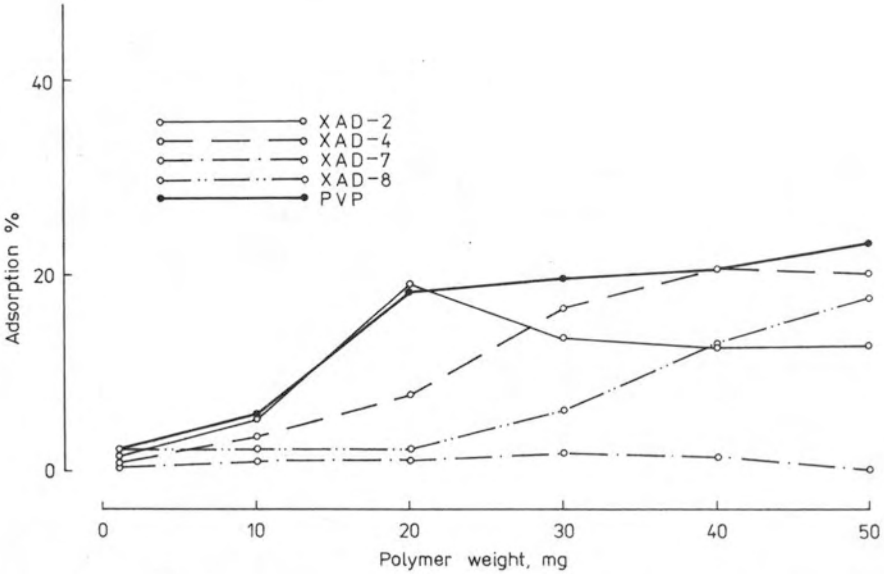

Fig. 1 Adsorption of pyrocatechol by synthetic polymers. Moisture contents of polymers were: Amberlite XAD-2, 4.4%; Amberlite XAD-4, 5.1%; Amberlite XAD-7, 8.2%; Amberlite XAD-8, 7.6%; PVP, 9.8%

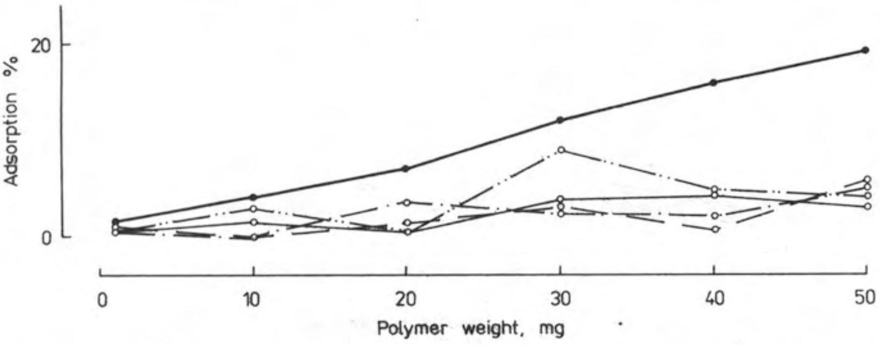

Fig. 2. Adsorption of catechol by synthetic polymers. Legend as on Fig. 1

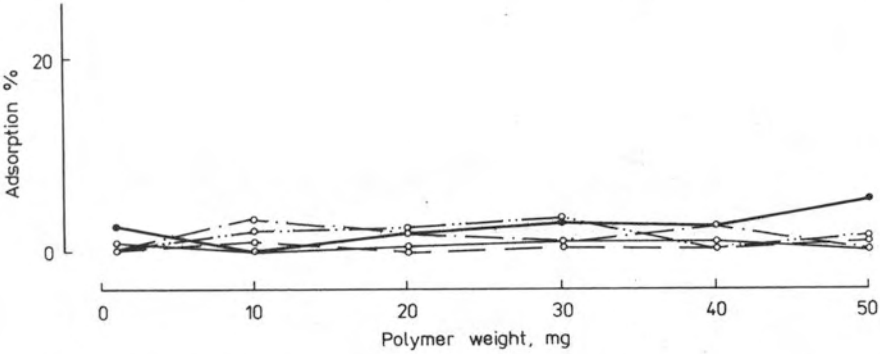

Fig. 3. Adsorption of 3,4-dihydroxyphenylalanine by synthetic polymers. Legend as on Fig. 1

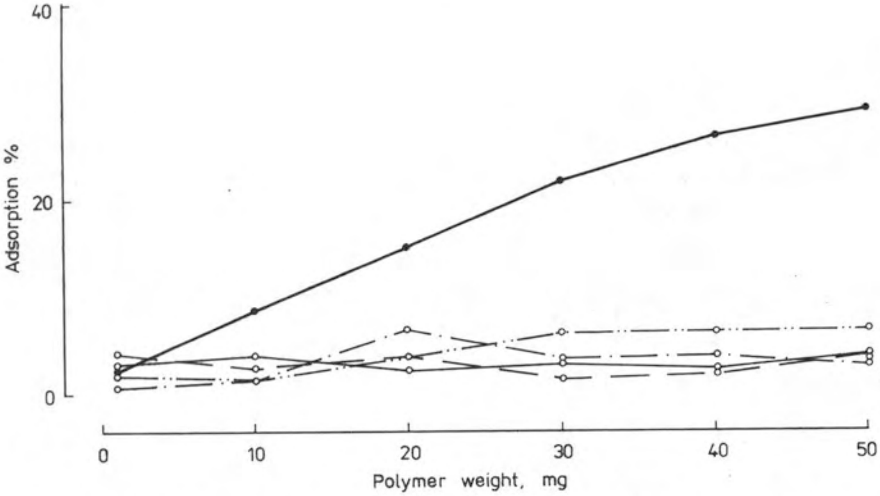

Fig. 4. Adsorption of caffeic acid by synthetic polymers. Legend as on Fig. 1

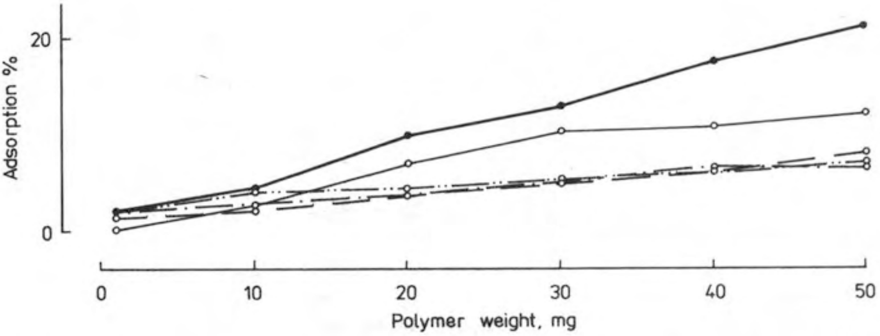

Fig. 5. Adsorption of chlorogenic acid by synthetic polymers. Legend as on Fig. 1

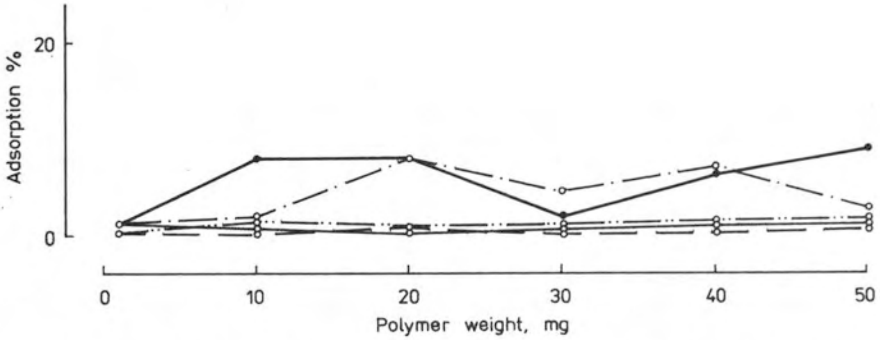

Fig. 6. Adsorption of safflor yellow A by synthetic polymers. Legend as on Fig. 1

DISCUSSION

This study was carried out to find synthetic polymers with strong affinity for plant phenols, with which we attempted to remove phenolic inhibitors which reduce enzyme reactivity. The findings from the present studies clearly indicate that there are some comparable characteristics in the pattern of phenol adsorption by the test polymers with different chemical compositions. On addition of PVP to a neutral solution, plant phenols were removed progressively as the polymer weights were increased (Figs. 1-9). The differences in the phenol adsorption by PVP must be reflected by a certain chemical interaction between polymer and phenol. In general, polymers containing proton-accepting groups in their molecules are supposed to bind easily with phenols through hydrogen binding (Loomis and Battaile 1966). Therefore, the test phenols could combine with PVP, which retains strong proton-drawing groups, presumably via binding with the carbonyl oxygens of the pyrrolidone polymer, though the reason why PVP showed no affinity for 3,4-dihydroxy-

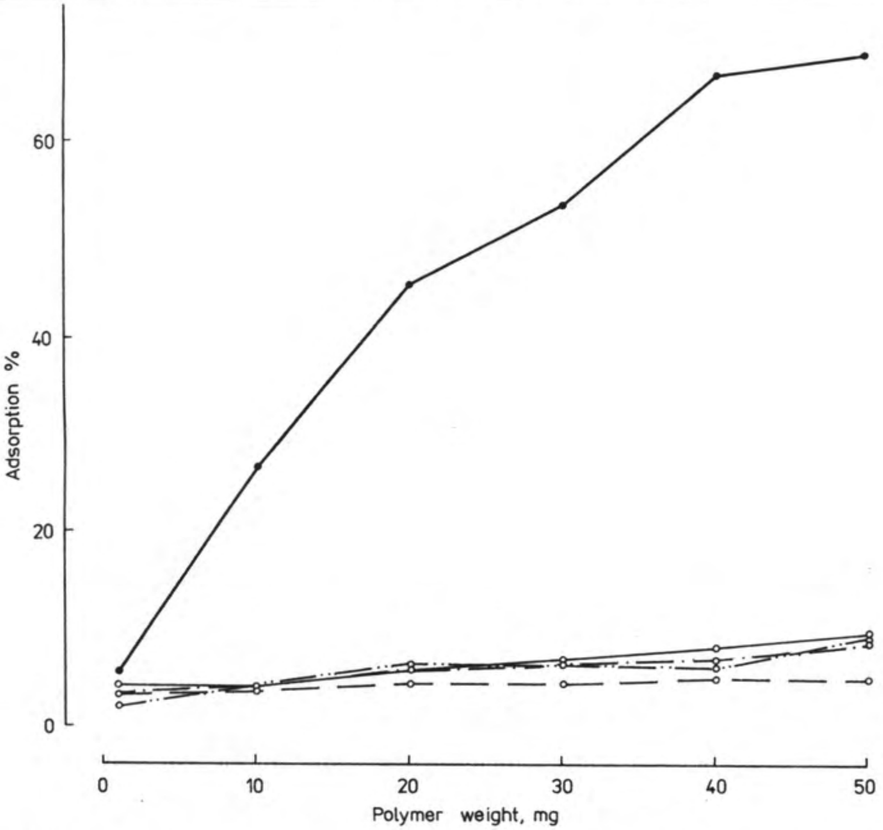

Fig. 7. Adsorption of safflor yellow B by synthetic polymers. Legend as on Fig. 1

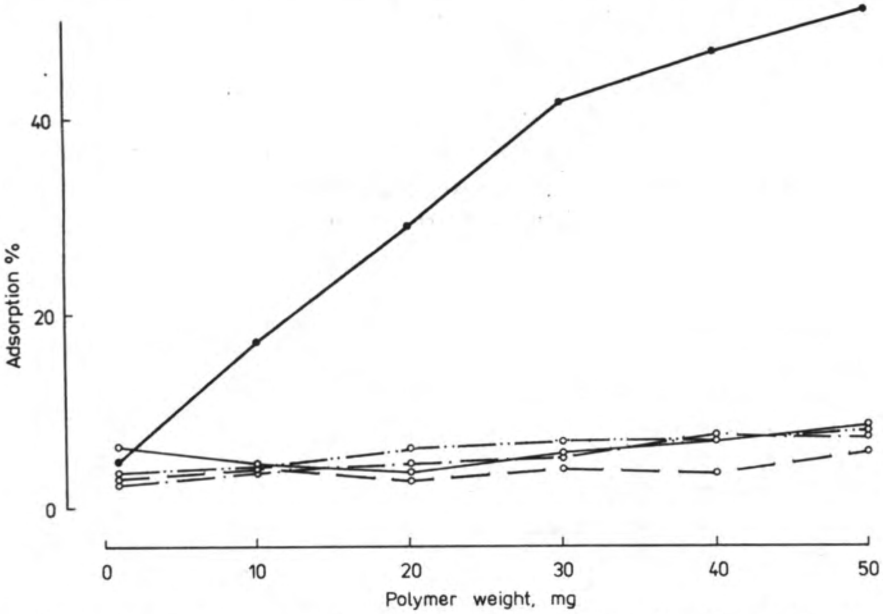

Fig. 8. Adsorption of precarthamin by synthetic polymers. Legend as on Fig. 1

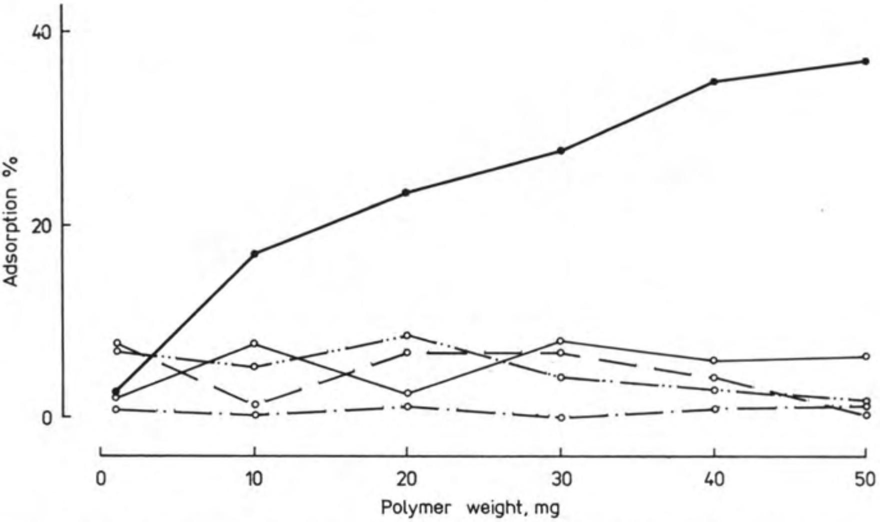

Fig. 9. Adsorption of carthamin by synthetic polymers. Legend as on Fig. 1

phenylanine is unknown. Amberlite XAD-2 and XAD-4 have been used by several workers as useful polyphenol adsorbents in the enzyme extraction processes (Loomis 1974, Gray 1978). In the present experiments we tested Amberlite XAD resins, -2, -4, -7 and -8, to examine their binding capacities with each of nine different phenols and noticed that further complexities might be involved in the phenol binding mechanism of these XAD polymers. XAD-8, XAD-7 and XAD-4 bound positively with 3,4-dihydroxyphenylalanine which is well-known to be a strong phenolic inhibitor for many endogenous plant enzymes. XAD-4 and XAD-7 adsorbed little or no pyrocatechol, which could be bound by XAD-8 and XAD-2. This clearly suggests that XAD resins have a relatively narrow specificity for adsorption of phenolic substances. The limited and lower affinity of the micro-porous polystyrene beads for phenols makes them unsuitable as phenol adsorbents. Based on the data, Amberlite XAD resins seem not to be as effective as generally declared. The above results contrast with those of Croteau et al. (1973), Loomis (1974) and Gray (1978) who demonstrated that Amberlite XAD-2 and XAD-4 were effective adsorbents either of peppermint monoterpens or potato, walnut and spinach polyphenols.

In this study we have provided new evidence that PVP adsorbed no 3,4-dihydroxyphenylalanine, Amberlite XAD resins were inferior to PVP in the phenol adsorptive capacity. These findings will surely give useful suggestions to the method of enzyme extraction from plant materials.

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Dalsze badania nad adsorpcją fenoli roślinnych przez syntetyczne polimery

Streszczenie

Pirokatechol, katechol, kwas kofeinowy, kwas chlorogenowy, safflor żółty A, safflor żółty B, prekartamina i kartamina były adsorbowane przez nierozpuszczalną poliwinyl-N-pirrolidynę (PVP) w neutralnym roztworze buforowym. Również żywice Amberlit XAD wiązały tych osiem

fenoli, ale znacznie mniej wydajnie niż PVP. Pod względem średniego poziomu wiązania fenoli można polimery uszeregować następująco (w %): PVP – 42,7; Amberlit XAD-8 – 17,7; Amberlit XAD-2 – 16,6; Amberlit XAD-7 – 13,0 i Amberlit XAD-4 – 10,1. 3,4-Dwuhydroksyfenyloalanina nie była adsorbowana przez PVP, natomiast aminokwas O-dwuhydroksylowy był adsorbowany przez Amberlity XAD-4, XAD-7 i XAD-8. Poziom adsorpcji fenoli rósł proporcjonalnie do wzrostu ilości adsorbenta. PVP utrzymał również swoją wiodącą pozycję jeśli chodzi o pojemność w wiązaniu fenoli w porównaniu z każdą z żywic Amberlit.