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Adsorption of plant phenols by polystyrene resins

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Abstract

Adsorption of nine plant phenols by nine polystyrene ion-exchange resins was investigated in an experimental model system. The phenols were adsorbed by Amberlite CG-120 more efficiently than any other acidically charged resins tested in this study. They were also taken up by anion exchangers. Among them Dowex 1-X8 was found to show the strongest effect on the adsorption of the phenolic constituents applied. A comparison of the efficiency of plant phenol adsorption between two different types of the synthetic polymers revealed that the basically charged polystyrenes had more prefarable affinities for phenols than cation exchangers. For example, the ratio of the efficiency between Amberlite CG-120 and Dowex 1-X8 was roughly calculated to be 3: 7 under the present experimental conditions. The adsorption rate of the test phenols was raised mostly by increasing the amount of the resins added, if they were mixed with effective polymers in the incubation model system.

Key words: *plant phenol, polystyrene resin, adsorption*

INTRODUCTION

Most plants, if not all, contain a wide variety of phenolic constituents. Some of these are known to inhibit enzymes during their preparation from plant tissue extracts (Loomis and Battaile 1966). Therefore, the removal of the noxiuos phenols is often desirable for the successful isolation of certain enzymes in a native state. Recently, an insoluble

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vinyl polymer, polyvinyl-N-pyrrolidone (PVP), polystyrene resins containing strongly basic anion-exchange substituents, such as Dowex 1-X8, and an uncharged synthetic polymer, Amberlite XAD-2, have been used to separate phenols from enzyme extracts by mixing them in the homogenization media. Dyer's saffron (*Carthamus tinctorius* L.) can also be easily supposed to contain various phenolic substances ranging from the simple form consisting of low molecular weight (mono-, di-, tri-phenol and so on) to polymeric ones with high molecular size (poly-phenol) which exist in the free state in the cytosol or in a bound form attached to organelles of the living cells. During enzyme preparation, some of the substances, of course, could presumably influence endogenous enzyme activities after disruption of the tissues, cells and/or cellular particles of the saffron plant, because the crude extracts browned gradually, and no active enzyme, other than phenol oxidases could be found in them. A protein extract containing a carthamin-synthesizing enzyme activity is always brown in colour, and moreover, it easily looses the catalytic ability to form carthamin from precarthamin, even though it is stocked in a refrigerator at 2–4°C under reduced pressure. The brown colour can only be partially removed from the extract by dialysis at the cost of a serious decrease in the enzyme activity. The use of a reducing agent, D-araboascorbic acid, was not expected to suppress so efficiently the preparation of the carthamin--synthesizing enzyme which had turned brown. It was necessary, therefore, to examine synthetic polymers to find effective adsorbents of phenols and to use them in the process of enzyme preparation.

As the first step of this study we investigated the adsorption of some plant phenols by polystyrene ion-exchange resins in an experimental model system.

MATERIALS AND METHODS

CHEMICALS

Polystyrene resins, cation exchangers: Amberlite IR-120B, CG-120, IRC-50, Dowex 50W-X8 and anion-exchangers: Amberlite IRA-45, IRA-94, IRA-400, IRA-410, Dowex 1-X8 were supplied by Organo Co., Ltd. (Tokyo, Japan). Pyrocatechol, catechol, caffeic acid, chlorogenic acid and L-3,4-dihydroxyphenylalanine were obtained from Nakarai Chemicals Co., Ltd. (Kyoto, Japan). Safflor yellow A, safflor yelow B, precarthamin and carthamin were prepared from freshly collected saffron florets by the reported methods (Takahashi et al. 1982, 1984a, b, Saito et al. 1983a), some of which were slightly modified to practically convenient ways. The water used throughout this study was carefully deionized and distilled just before starting experiments.

ACTIVATION OF POLYSTYRENE RESINS

Polystyrene resins were dipped in deionized and distilled water, and allowed to stand for a few minutes with stirring, then filtered collecting on a funnel. The washed resins were equilibrated in the desired concentrations (weak ion-exchangers in 0.2 M and strong ion-exchangers in 2.0 M) of acid or base. After long intervals of sufficient equilibration, the resins were transferred to the appropriate volume of deionized and distilled water, and washed exhaustively until free of traced ions. The activated polymers were collected with suction and stored as moist resins before use.

APPLICATION OF POLYSTYRENE RESINS

Two cm^3 of 50.0 mM citrate-phosphate buffer, pH 7.0 containing weighed amounts (1–50 mg) of resins for testing were poured into a test tube (1.5 × 13 cm) with plant phenol solution (100 nmol cm^{-3} in 50.0 mM citrate-phosphate buffer, pH 7.0). After mixing for 10 s, the mixture was filtered through a paper and the phenol contents in the filtrates were assayed within 50 s with a Shimadzu double-beam spectrophotometer (type 150-02). The rate of the phenol adsorption was determined by subtracting the amount of the phenol remaining in test solutions.

RESULTS

ABSORPTION SPECTRA OF PLANT PHENOLS USED IN THE INVESTIGATION

Prior to the adsorption experiments, nine plant phenols were dissolved in citrate-phosphate buffer, which is routinely used in enzyme extraction (Saito et al. 1983a, b, 1985a, b, 1986, Homma et al. 1985). Each

Table 1

Spectral data of plant phenols

Phenol*	λ max** Band 1, nm	Molecular extinction coefficient, $\varepsilon \times 10^3$
1	276	2.05
2	276	7.20
3	280	2.24
4	286	8.84
5	324	17.19
6	400	2.21
7	411	35.57
8	407	11.69***
9	510	5.56

* See Fig. 1.

** In 50.0 mM citrate-phosphate buffer, pH 7.0.

*** The value was calculated assuming that the molecular weight is 910.

solution was then immediately subjected to spectrophotometric estimation to determine light adsorption maxima of the phenols for testing. The wave-lengths were surveyed from 200 to 500 nm with an absorbance of 0–2.0. Typical results are summarized in Table 1. The λ_{max} (Band 1) and the molar extinction coefficient listed in the table were adopted throughout this investigation for spectrometric assay of the phenol content in each buffer solution.

TIME DEPENDENCE OF THE ADSORPTION OF PLANT PHENOLS BY SYNTHETIC POLYMERS

Just after addition of synthetic resins to the plant phenol containing solutions, spectrophotometric estimation was started and the spectral change followed automatically for 1 min at 24±2°C. Tables 2 and 3 summarize the data of the adsorption velocities obtained from the recording sheets. It is clear that the adsorption rate of plant phenols to synthetic polymers varied according to the compounds examined.

Table 2

Adsorption of plant phenols by cation exchangers

Resin	Phenol. nM carthamin mg resin^{-1} min^{-1}								
	1	2	3	4	5	6	7	8	9
Amberlite IR-120 B	— —	—	—	—	—	4.5	0.4	10.0	—
Amberlite IRC-50	1.7	1.6	7.5	—	7.4	—	—	—	—
Amberlite CG-120	73.9	154.5	73.2	55.5	20.0	—	49.0	40.5	72.5
Dowex 50W-X8	9.6	25.4	—	7.8	—	—	—	1.6	—

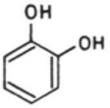
Table 3

Adsorption of plant phenols by anion exchangers

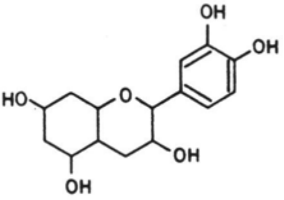
Resin	Phenol, nM carthamin mg resin^{-1} min^{-1}								
	1	2	3	4	5	6	7	8	9
Amberlite IRA-45	4.3	26.2	15.0	37.2	25.9	26.7	6.2	6.6	8.9
Amberlite IRA-94	—	36.3	37.5	70.3	96.6	13.4	0.8	2.9	0.8
Amberlite IRA-400	20.0	40.2	3.5	1.8	67.2	13.4	4.2	26.6	0.8
Amberlite IRA-410	10.4	47.5	56.5	17.7	19.4	4.5	6.6	10.9	18.9
Dowex 1-X8	90.0	263.5	330.0	96.5	205.0	117.8	38.5	199.0	51.0

ADSORPTION OF PLANT PHENOLS TO SYNTHETIC POLYMERS

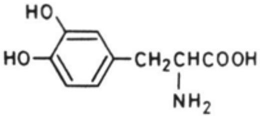
Adsorption of nine phenols was tested at the selected eleven weights of each of the nine polystyrene ion-exchangers. The data are shown



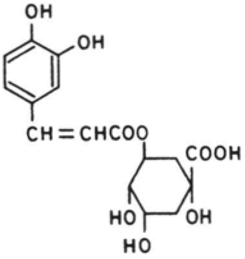
Pyrocatechol (1)



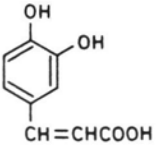
Catechol (2)



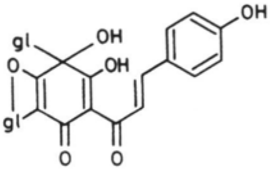
3, 4-Dihydroxyphenylalanine (3)



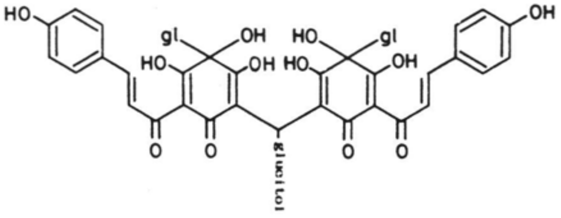
Chlorogenic acid (5)



Caffeic acid (4)

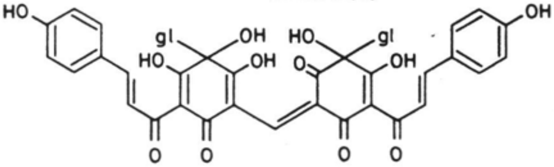


Safflor yellow A (6)



Safflor yellow B (7)

Undetermined



Precarthamin (8)

Carthamin (9)

Fig. 1. Chemical structure of phenols

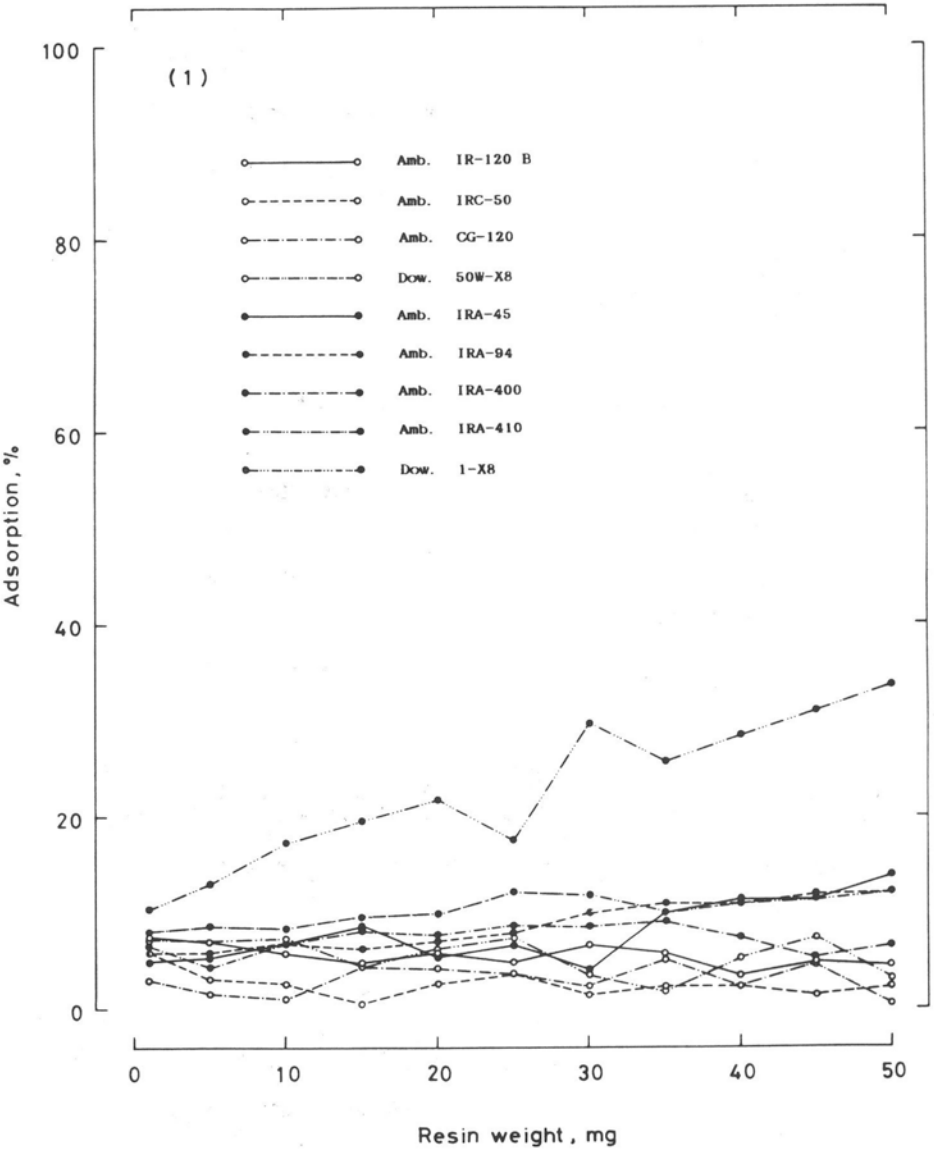


Fig. 2. Adsorption of plant phenols by ionically charged polystyrene resins. Amb. — Amberlite, Dow. — Dowex. (○) — cation-exchange resins. (●) — anion-exchange resins. Numberings of (1), (2), (3)... in each figure denote the phenols used in this study (see Fig. 1). Moisture contents of polystyrene resins were: Amberlite IR-120 B, 5.6%; Amberlite IRC-50, 5.8%; Amberlite CG-120, 6.9%; Dowex 50W-X8, 4.4%; Amberlite IRA-45, 4.5%; Amberlite IRA-94, 6.3%; Amberlite IRA-400, 5.3%; Amberlite IRA-410, 4.3%; Dowex 1-X8, 7.5%. For details of assaying phenol contents see Materials and Methods

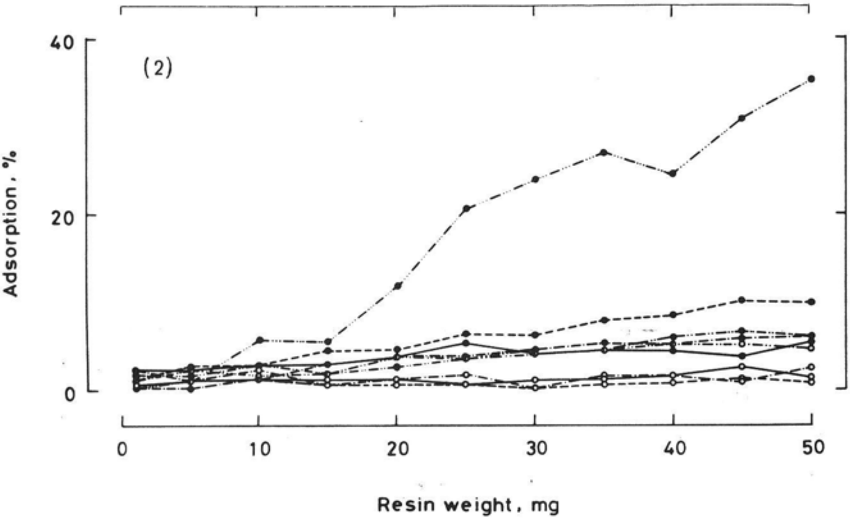


Fig. 3. See description of Fig. 2

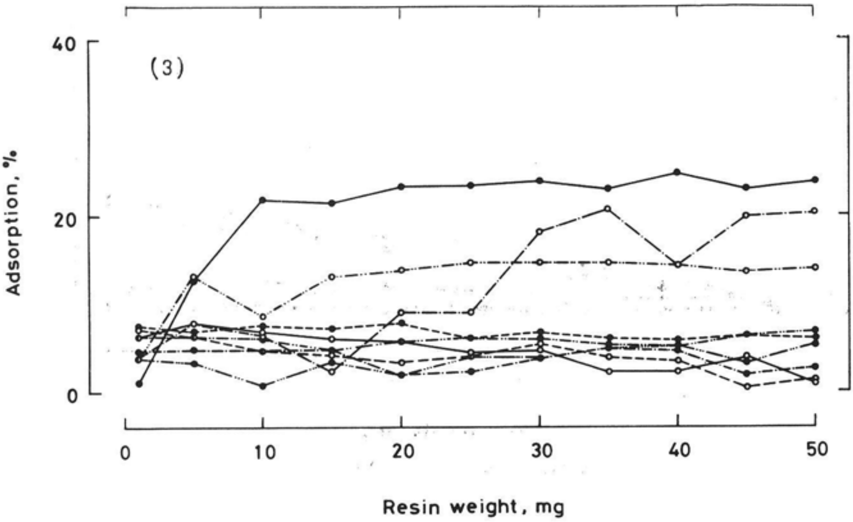


Fig. 4. See description of Fig. 2

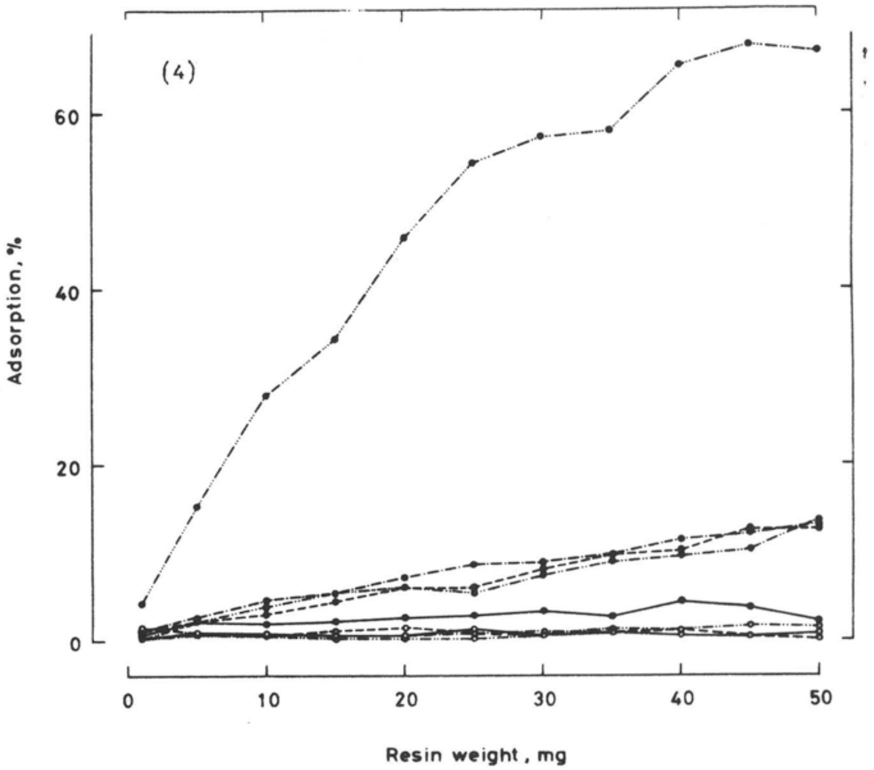


Fig. 5. See description of Fig. 2

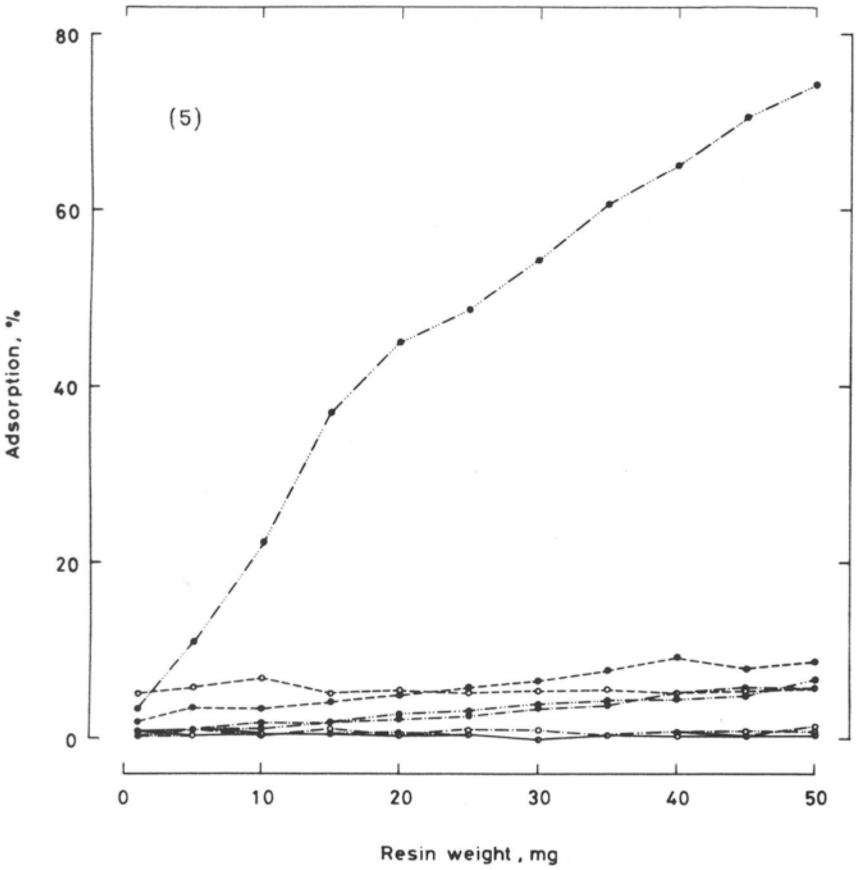


Fig. 6. See description of Fig. 2

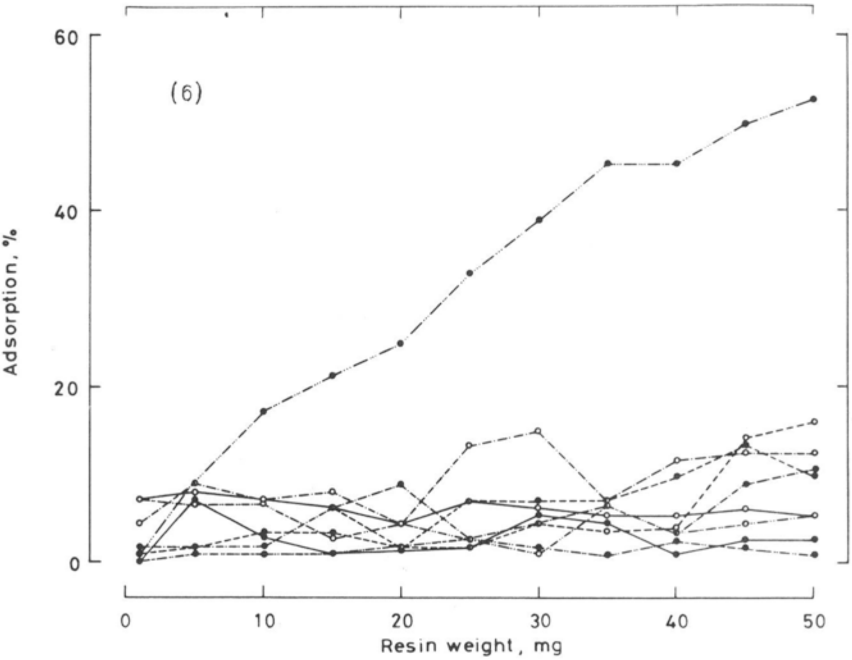


Fig. 7. See description of Fig. 2

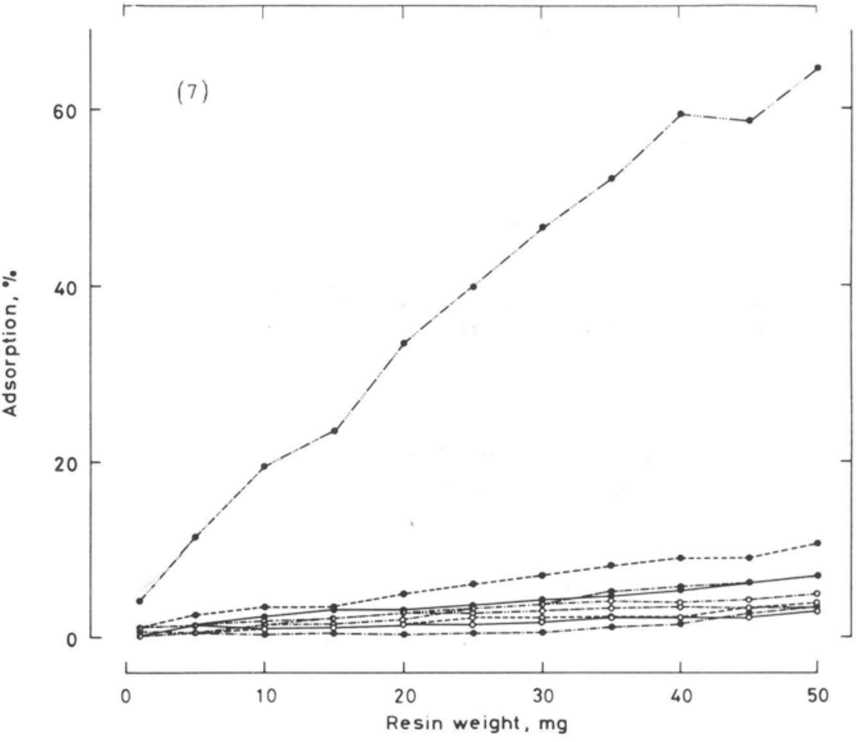


Fig. 8. See description of Fig. 2

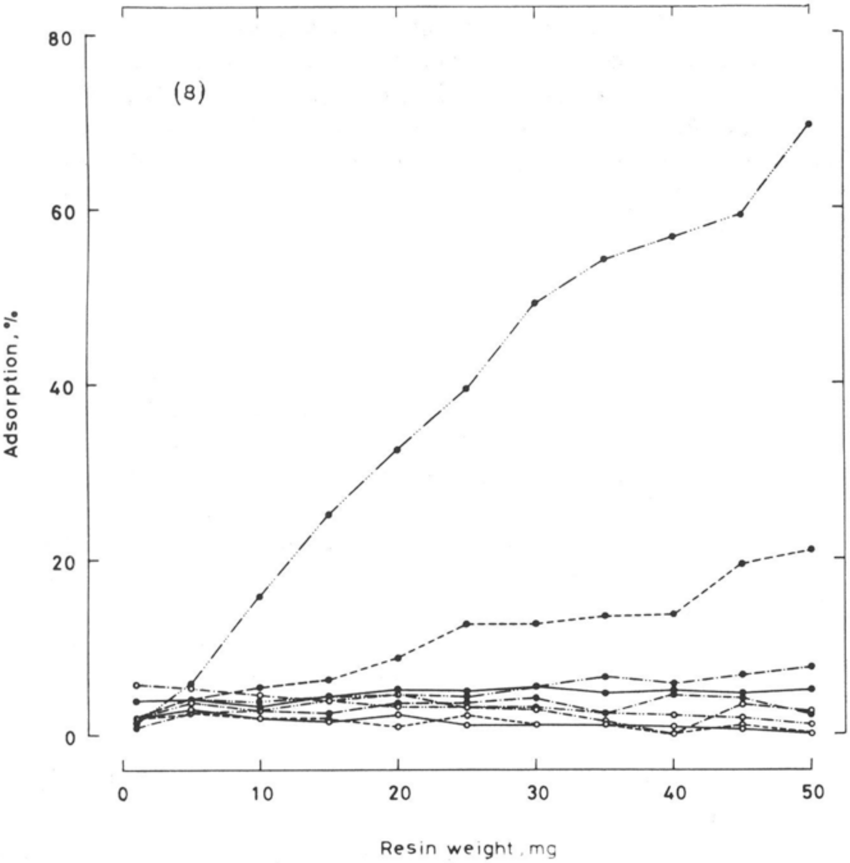


Fig. 9. See description of Fig. 2

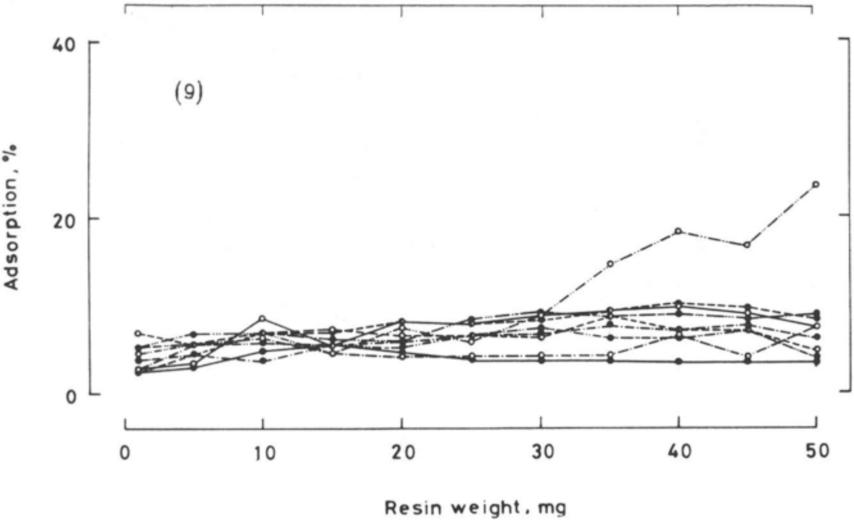


Fig. 10. See description of Fig. 2

in Figs. 1–10. Differences in the phenol adsorption exhibited by the polymers are obvious from the characteristic patterns illustrated in each figure. On the whole, basically charged resins are effective, though the efficiencies varied according to the exchangers used. Acidic were resins mostly less effective on the phenol adsorption, except for Amberlite CG-120, whose value was relatively high.

DISCUSSION

Adsorption of plant phenols by polystyrene ion-exchange resins has been investigated to find effective and/or to detect a possible mechanism of the interaction between phenols and polymers in an experimental model system. The data presented above provide that the anion exchangers tested in the range of 1–50 mg mostly acted as positive phenol removing effectors, while the cationic resins showed lower affinities for plant phenols, except for Amberlite CG-120. The poorest affinity of the cation exchangers for phenols may be safely explained by the recognition that the hydroxyls on the aromatic ring(s) of phenols are positively charged in aqueous solutions, due to which the polymers containing acidic cation-exchange substituents are not allowed to form a stable binding with phenols. Lam and Shaw (1970) suggested that the superiority of Dowex 1-X8 was due to its ability to bind ionically with negatively charged polyphenols. However, the ionic form of the synthetic polymers cannot be the sole explanation of the efficiency of the exchanging resins, because conspicuous discrepancies have been found in the adsorption rate of phenols among the polymers. This suggests that adsorption of phenols by the resins is predominantly due to the chemical structure, molecular size, particle form and/or ionic strength in solutions. Differences in phenol adsorption support this through the following fact that the resins show different affinities for 3,4-dihydroxyphenylalanine and carthamin in a neutral buffer solution (Figs. 1–10). Based on the results it can be said that the above listed effective resins should be considered for use in combination with other polymeric adsorbents to achieve optimal extraction of plant phenols from crude tissue extracts.

Further studies on the examination of other phenol adsorbents are now under way in our laboratory.

REFERENCES

Homma T., Sawaguchi M., Kawai H., Takanashi Y., Saito K., 1985. Preparative studies on the isolation of an enzyme associated with carthamin synthesis in *Carthamus tinctorius* L. Acta Soc. Bot. Pol. 54: 403–416.

Lam T. H., Shaw M., 1970. Removal of phenolics from plant extracts by grinding with anion exchange resin. Biochem. Biophys. Res. Commun. 39: 965–968.

Loomis W. D., Battaile J., 1966. Plant phenolic compounds and the isolation of plant enzymes. Phytochemistry 5: 423–438.

Saito K., Takahashi Y., Wada M., 1983a. Distribution and variation of the catalytic activity of the enzyme responsible for carthamin synthesis in safflower seedlings. Z. Naturforsch. 38c: 724–729.

Saito K., Takahashi Y., Wada M., 1983b. Enzymic synthesis of carthamin in safflower. Biochim. Biophys. Acta 756: 217–222.

Saito K., Takahashi Y., Wada M., 1985a. Formation of carthamin by a partially purified enzyme from safflower seedlings. Experientia 41: 59–61.

Saito K., Takahashi Y., Wada M., 1985b. Preparation of an enzyme associated with carthamin formation in *Carthamus tinctorius* L. Z. Naturforsch. 40c: 819–826.

Saito K., Fukushima A., Takahashi M., Takahashi Y., 1986. Comparative studies on the activities of a carthamin-synthesizing enzyme, monophenol monooxygenase and peroxidase in vegetative tissues of safflower. Biochem. Physiol. Pflanzen 181: 633–643.

Takahashi Y., Miyasaka H., Tasaka S., Miura I., Urano S., Ikura M., Hikichi K., Matsumoto T., Wada M., 1982. Constitution of two coloring matters in the flower petals of *Carthamus tinctorius* L. Tetrahedron Lett. 23: 5163–5166.

Takahashi Y., Saito K., Yanagiya M., Ikura M., Hikichi K., Matsumoto T., Wada M., 1984a. Chemical constitution of safflor yellow B, a quinochacone C-glycoside from flower petals of *Carthamus tinctorius* L. Tetrahedron Lett. 25: 2471–2474.

Takahashi Y., Wada M., Saito K., 1984b. Purification and some characteristics of precarthamin, a precursor of carthamin, isolated from the florets of *Carthamus tinctorius* L. Acta Soc. Bot. Pol. 53: 187–198.

Adsorpcja fenoli roślinnych na żywicach polistyrenowych

Streszczenie

W doświadczalnym układzie modelowym, badano adsorpcję dziewięciu fenoli roślinnych na dziewięciu polistyrenowych, jono-wymiennych żywicach. Amberlite CG-120 adsorbował fenole najskuteczniej spośród innych, użytych w tych badaniach kationitów. Fenole były również adsorbowane przez wymieniacze anionowe. Spośród nich Dowex-1 okazał się najsilniejszym odsorbentem badanych związków fenolowych. Porównanie skuteczności adsorpcji fenoli roślinnych przez dwa różne typy syntetycznych polimerów wykazało, że polistyreny z ładunkiem zasadowym miały lepsze powinowactwo do fenoli niż wymieniacze kationowe. Na przykład, stosunek wydajności Amberlitu CG-120 i Dowex 1-X8 wyniósł ok. 3:7, w stosowanych w pracy warunkach doświadczalnych. Wartość adsorpcji testowanych fenoli była znacznie zwiększona przez dodanie wzrastających ilości żywic, gdy je mieszano z efektywnymi polimerami w modelowym układzie doświadczalnym.