299. *The Use of Adsorption for Isolation of Aromatic Substances.*

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The effect of competition between adsorbates during the isolation of aromatic substances by adsorption on, and desorption from, suitably deactivated charcoals has been examined. If adequate adsorbent is used, even minor components of complex mixtures can be isolated with good recoveries. The adsorption behaviour of some additional types of compound is reported.

The study of metabolites in body fluids is simplified if a preliminary separation of given types of compound from the complex mixtures normally encountered is possible. Charcoal, suitably pre-treated, can be used [1,2] to isolate aromatic substances even in the presence of large excesses of inorganic or aliphatic substances. The pre-treatment, with long-chain aliphatic substances such as stearic acid or octadecylamine, blocks the most active adsorbing sites on the charcoal surface so that adsorption of aromatic substances on the remaining, less firmly-binding, sites is reversible and the adsorbed substances can be conveniently recovered by elution with aqueous phenol. This method applies to derivatives of benzene, pyrrole, pyridine, indole, purine, and pyrimidine, and depends on the presence of an aromatic ring in the adsorbate but, in general, not on the nature of other functional groups.[2] Bases are recovered in good yield by using a neutral eluant only if the charcoal has been pre-treated with a basic deactivating agent such as octadecylamine.

Body fluids may contain a wide variety of aromatic substances—normal human urine probably contains at least 100 phenols or derivatives of phenols, many in minute quantity. If adsorption is used for isolation, all the components of a mixture should in theory be recoverable provided that adsorption is reversible, and that an adequate amount of adsorbent is used to provide binding sites for all adsorbate molecules. Adsorption can be made very largely reversible by pre-treatment of the charcoals [2] and we now consider the effect of competition between adsorbates, and examine the applicability of the method to some other classes of compound.

Table 1 shows the effect of saligenin (*o*-hydroxybenzyl alcohol) on the adsorption and recovery of phenylalanine. With small amounts of saligenin the recovery of phenylalanine is unaffected, but as the amount of saligenin increases, increasing amounts of phenylalanine remain unadsorbed, with correspondingly poor recoveries. However if the amount of adsorbent is then increased a high recovery of phenylalanine can again be obtained even

[1] Dalgliesh, *J. Clin. Path.*, 1955, **8**, 73.
[2] Asatoor and Dalgliesh, *J.*, 1956, 2291.

in the presence of a three-hundredfold excess of saligenin. Similar results were obtained for the effect of varying amounts of *m*-hydroxybenzoic acid on the recovery of phenylalanine.

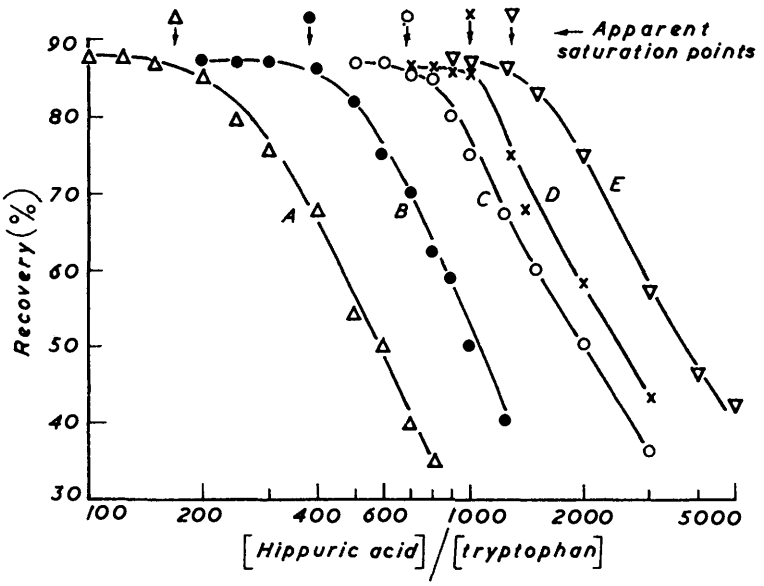
TABLE 1. *Adsorption behaviour of phenylalanine in the presence of saligenin.*

12·5 μmoles of phenylalanine were used in each case. The adsorbent was charcoal deactivated with 8% by weight of stearic acid, and the eluant was 50 ml. of 7% (w/v) of aqueous phenol/g. adsorbent.

Saligenin (μmoles)	Molar ratio of saligenin to phenylalanine	Adsorbent (g.)	Recovery of phenylalanine (%) Filtrate	Eluate	Saligenin (μmoles)	Molar ratio of saligenin to phenylalanine	Adsorbent (g.)	Recovery of phenylalanine (%) Filtrate	Eluate
20	1·6	1	3	94	4000	320	1	52	44
40	3·2	1	3	92	8000	640	1	73	26
80	6·4	1	3	92	8000	640	2	55	45
160	12·8	1	5	87	8000	640	4	21	73
400	32	1	13	81	4000	320	2	21	79
800	64	1	18	71	4000	320	3	10	84
1600	128	1	34	60	4000	320	3·5	5	89
3200	256	1	44	50	4000	320	4	5	89

Fig. 1 shows the effect of excess of hippuric acid on the recovery of tryptophan. For a given amount of adsorbent, recoveries of tryptophan are little affected by excess of hippuric acid until a point is reached, which we have called the "apparent saturation point", at

FIG. 1. *Recoveries (%) of 1 μmole of tryptophan in presence of excess of hippuric acid, varying amounts of charcoal deactivated with 8% by weight of stearic acid being used as adsorbent.*



Adsorbent: *A*, 125 mg.; *B*, 250 mg.; *C*, 500 mg.; *D*, 750 mg.; *E*, 1000 mg.

which presumably insufficient suitable adsorption sites remain available for binding all the tryptophan. Again, by increasing the amount of adsorbent good recoveries of tryptophan can be restored. The "apparent saturation points" in Fig. 1 are plotted against amount of adsorbent in Fig. 2. If adsorption were completely reversible this should give the straight (broken) line; the slight deviation which occurs shows a small degree of irreversible adsorption. However, from the practical aspect of recovering substances from solution, a very large excess of hippuric acid makes no appreciable difference to the recoverability of tryptophan from solution, provided adequate adsorbent is used. A similar set of curves was obtained for the recovery of anthranilic acid in the presence of excess of hippuric acid.

The adsorption behaviour of various other substances is shown in Table 2. Pterins are well recovered; phenobarbitone (5-ethyl-5-phenylbarbituric acid), which contains a true aromatic ring, is also well recovered, but barbituric acid and quinalbarbitone [5-allyl-5-(1-methylbutyl)barbituric acid] are only partially recovered.

TABLE 2. *Adsorption behaviour of miscellaneous substances on charcoal deactivated with stearic acid.*

Substance	Molar concentration of adsorbate	Stearic acid used for deactivation (%)	Recovery Filtrate	Recovery Eluate
5-Hydroxyindolylacetic acid	0·0005	4	0	75
Xanthopterin	0·0005	4	0	86
*iso*Xanthopterin	0·00005	4	0	100
2 : 4 : 6-Trihydroxypteridine	0·005	4	0	97
	0·0005	4	0	100
Barbituric acid	0·005	8	3	41
	0·0005	8	2	44
Phenobarbitone	0·005	8	3	88
	0·0005	8	0	100
Quinalbarbitone	0·005	8	0	50
	0·0005	8	0	66

Under the standard conditions [2] histidine was largely unadsorbed. We therefore determined the adsorption behaviour of histidine, histamine, and urocanic acid at various pH values. Results obtained with stearic acid-deactivated charcoal are shown in Table 3. Results with octadecylamine-deactivated charcoal were similar. Maximal adsorption of

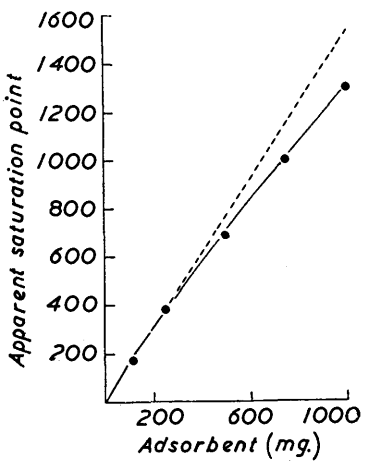


FIG. 2. *"Apparent saturation points" from Fig. 1 plotted against amount of adsorbent.*

histidine occurs at pH *ca.* 7, whilst histamine and urocanic acid are maximally adsorbed at alkaline and acid pH, respectively. Presumably adsorption occurs primarily through the uncharged molecules, as suggested by Phelps and Peters.[3] Recoveries of glyoxalines on elution with phenol are not satisfactory. However, histamine can be eluted almost quantitatively with phenol–hydrochloric acid.

We examined various substances as potential deactivating agents for charcoal, but none proved equal to stearic acid or octadecylamine. Those examined were surface-active agents, such as cetyltrimethylammonium bromide and sodium *p*-*n*-dodecyl- and -*n*-octyl-benzenesulphonates, and aromatic substances such as anthracene, 1 : 5-dihydroxynaphthalene, 1 : 5-dihydroxyanthraquinone, and naphthalene-1 : 4 : 5 : 8-tetracarboxylic acid. In addition various substances were examined as potential eluants: these included aqueous solutions of benzyl alcohol, aniline, and pyridine and aqueous ethanolic solutions of

[3] Phelps and Peters, *Proc. Roy. Soc.*, 1929, *A*, **124**, 554.

6-methylheptyl alcohol, 1-methylheptyl alcohol, and *n*-hexyl alcohol; none was as effective as aqueous phenol.

TABLE 3. *Adsorption behaviour of glyoxalines at various* pH *values, charcoal deactivated with* 8% *by weight of stearic acid being used as adsorbent.*

Figures under filtrate and eluate represent percentage recoveries in appropriate fractions. 1 g. of adsorbent and 25 ml. of 0·005M-solutions of adsorbate were used. Elution was by 50 ml. of 7% (w/v) aqueous phenol.

pH for adsorption	Recovery of histidine		Recovery of histamine		Recovery of urocanic acid	
	Filtrate	Eluate	Filtrate	Eluate	Filtrate	Eluate
2·2	100	1	96	0	13	28
3·0	89	6	96	0	17	31
4·0	85	7	92	0	17	34
5·0	52	21	72	0	21	65
6·0	33	25	53	0	27	73
7·0	22	31	32	0	30	50
8·0	44	31	4	0	64	21
9·0	55	33	4	0	86	13
10·0	74	33	4	0	103	0

Experimental.—The preparation of adsorbents and the standard procedure for adsorption and elution have been described.[2] In experiments with glyoxalines McIlvaine buffer (Na_2HPO_4–citric acid) was used for pH 2·2—8, and Clark and Lubs buffer (H_3BO_3–KCl–NaOH) for pH 9 and 10. In the competition experiments the amount of the minor adsorbate was dissolved in sufficient aqueous solution (adjusted to pH 4 with acetic acid) to contain the appropriate amount of major adsorbate.

Methods of estimation. 5-Hydroxyindolylacetic acid was estimated with 1-nitroso-2-naphthol.[4] Pterins were estimated by ultraviolet absorption in 0·1N-sodium hydroxide at 255 mμ (xanthopterin), 340 mμ (*iso*xanthopterin), or 275 mμ (2 : 4 : 6-trihydroxypteridine). Barbiturates were estimated by ultraviolet absorption in pH 10 borate buffer at 257 mμ (barbituric acid) or 240 mμ (phenobarbitone and quinalbarbitone).[5] Other substances were estimated as previously described.[2]

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[*Received, November 29th,* 1957.]

[4] Udenfriend, Weissbach, and Clark, *J. Biol. Chem.*, 1955, **215**, 337.
[5] Goldbaum, *Analyt. Chem.*, 1952, **24**, 1604.