

# Adsorption of Organic Compounds by Commercial Filter Papers and Its Implication on Quantitative-Qualitative Chemical Analysis

WIN LOUNG CHIOU and LLOYD D. SMITH

**Abstract** □ Eighteen acidic and neutral organic compounds were studied for adsorption onto Filter I (MF-Millipore filter) with a diameter of 17.5 mm. The adsorption after filtration of 3 ml. of solution ranged from zero to almost 100%. The adsorption to Filter II (Whatman filter), however, was much lower and in some cases negligible. The water-soluble or ionic forms of compounds are less adsorbed. The extent of adsorption for each compound might depend on its concentration, filtration rate, volume of solution filtered, and Filter I size used. The adsorption was found to be reversible and could be represented by the Freundlich adsorption isotherm equation. The water-soluble impurities from both types of filters were also studied. Their absorbances below 230  $m\mu$  were considerably high. The implication of adsorption and impurities from filters on quantitative-qualitative chemical analysis was discussed.

**Keyphrases** □ Filter paper adsorption—organic compounds □ Adsorption—organic compounds on filters □ Impurities—filters □ Solubility, organic compounds—filter adsorption □ Filtration rate effect—filter adsorption

The phenomenon of the possible adsorption of organic or inorganic compounds onto polymeric materials such as nylon (1), polyethylene (2), cellulose dialysis membrane, and regular filter paper has been well recognized in the past. Filter pads,<sup>1</sup> made from cellulose esters or similar polymeric materials, have been widely used in both analytical and biological work (3–14). However, the possible adsorption of chemicals to these filters has been generally underestimated or not noticed by research workers using these products. Some of the reasons for unawareness may stem from a statement by the manufacturer in their brochure (15) that the filters will not generally adsorb components from liquid solutions.

The systematic investigation, reported in this article, of the potential effect of these filters on both quantitative and qualitative chemical analysis was begun after an accidental discovery of the surprisingly high adsorption of a water-soluble organic compound after filtration. It was also stimulated by a report of Saad and Higuchi (5) that a discrepancy of 150 to 200% in the solubility of cholesterol was observed after filtering a saturated aqueous suspension through different pore size of the filters. The authors, however, gave no explanation about the possible cause of this difference. Although there are many types of these filters, only Filter I<sup>2</sup> was selected for thorough studies due to its wide

use. The adsorption to the Filter II<sup>3</sup> was also studied to a lesser extent for the purpose of comparison.

## EXPERIMENTAL

**Materials**—Eighteen acidic or neutral organic compounds were chosen for adsorption studies: *m*-nitrobenzoic acid (Eastman Organic Chemicals); iopanoic acid (Sterling Drug Inc.); hydrocortisone, hydrocortisone acetate, and ethinyl estradiol (The Upjohn Co.); chloramphenicol (Parke, Davis & Co.); benzoic acid (Merck & Co.); phenobarbital (Smith Kline & French Labs.); sodium phenobarbital (Merck & Co.); hexachlorophene (Robinson Laboratory Inc.); salicylic acid (Mallinckrodt Chemical Works); *p*-nitrobenzoic acid (Eastman Organic Chemicals); griseofulvin (McNeil Laboratories, Inc.); warfarin (S. B. Penick & Co.); sodium warfarin (Abbott Laboratories); digitoxin (Eli Lilly & Co.); naphthalene (Matheson Co. Inc.); and spironolactone (Searle & Co.). All the chemicals were reagent grade and were not further purified prior to experiments.

Filter I disks<sup>4</sup> had a prefilter diameter of 17.5 mm. and various pore sizes: 0.025  $\mu$  (VS type), 0.22  $\mu$  (GS type), and 1.2  $\mu$  (RA type). Pyrex microanalysis filter holder (Cat. No. XX10 025 00 from the Millipore Corp.) was used for the support of filtration throughout the experiments. Filter II (W. R. Balston, Ltd., England) employed a round form of disk with the same diameter as Filter I, cut with a pair of scissors and placed into the Millipore filter holder for the filtration study.

**Preparation of Solutions**—Solutions of relatively water-soluble compounds such as *m*-nitrobenzoic acid, benzoic acid, phenobarbital, sodium phenobarbital, salicylic acid, *p*-nitrobenzoic acid, sodium warfarin, and naphthalene were prepared by dissolving them directly in distilled water or diluted aqueous hydrochloric acid solution. Solutions of relatively water-insoluble compounds such as iopanoic acid, hydrocortisone, chloramphenicol, hydrocortisone acetate, hexachlorophene, griseofulvin, ethinyl estradiol, digitoxin, and spironolactone were prepared by dilution of the concentrated stock solution in 95% ethanol with distilled water. These solutions contained, however, less than 1% ethanol (v/v).

**Adsorption Studies**—Before carrying out adsorption studies, the filter apparatus without the placement of filter disks was thoroughly cleaned with distilled water. Three milliliters of the distilled water or aqueous hydrochloric acid solution was then passed through the apparatus by the suction of a water aspirator. Possible contamination in the filtrate was checked spectrophotometrically by using a Cary 15 spectrophotometer.

The effect of both single and multiple filtrations on adsorption to the filters was studied. In the single-filtration studies, 3 ml. of aqueous solutions of organic substances was filtered through either the Filter I or II disk. The time of the filtration was kept as constant as possible: about 30 sec. for 0.22- $\mu$  Filter I and 1.2- $\mu$  Filter I and Filter II, and about 2.5 min. for the 0.025- $\mu$  Millipore. The absorbance of the solution, usually at the peak absorption wavelength of each

<sup>1</sup> Millipore filter disks, Millipore Corp., Bedford, Mass.

<sup>2</sup> MF Millipore.

<sup>3</sup> Whatman filter paper, No. 4.

<sup>4</sup> All Millipore filter disks used in this investigation were purchased from the the Millipore Corp. in 1969. The lot number for 1.2- $\mu$  pore size is 1145.

**Table I**—Absorbances<sup>a</sup> of the Filtrate after Passing 3 ml. of Distilled Water through Different Filter Paper<sup>b</sup>

Wave-length, m $\mu$	Filter I			Filter II
	0.025- $\mu$	0.220- $\mu$	1.20- $\mu$	
205	0.406	0.133	0.238	0.095
210	0.320	0.110	0.217	0.080
220	0.156	0.073	0.137	0.054
230	0.062	0.040	0.034	0.027
240	0.048	0.018	0.004	0.011
250	0.047	0.017	0.013	0.009
260	0.039	0.013	0.025	0.008
270	0.022	0.013	0.043	0.009
280	0.015	0.010	0.055	0.007
290	0.012	0.005	0.019	0.005
300	0.009	0.003	0.012	0.004
310	0.009	—	—	0.003
320	0.009	—	—	0.002

<sup>a</sup> All values are the average of three runs. <sup>b</sup> Area of the filter paper is 2.5 cm.<sup>2</sup>.

compound, was measured before and after the filtration using a Cary 15 spectrophotometer. Within the concentration range studied, it was found that the absorbances of the solutions of all the compounds followed Beer's law.

In the multiple-filtration studies, four aliquots of 3 ml. each of the aqueous solutions were passed successively through the same 0.22- $\mu$  Filter I pad; the absorbance of each 3-ml. filtrate was then determined. The effect of the rate of filtration on adsorption was also studied by the single-filtration method filtered over 30 and 300 sec.

It was found that both Filters I and II contain a fairly large amount of water-soluble impurities which can be extracted into the filtrate during the filtration process and show optical absorption at various wavelengths. Therefore, control experiments were performed by passing distilled water or appropriate aqueous vehicles through filter disks for all adsorption studies reported in this communication. The extent of adsorption was estimated after making such blank corrections. All experiments were run at least in duplicate. It must be noted the variation of adsorption from different runs was generally very small and insignificant.

**Desorption Studies**—In desorption studies, 3 ml. of aqueous solution was passed through 0.22- $\mu$  Filter I, the absorbance of the filtrate was measured, and the percent adsorbed was determined. The filter pad was then removed, the apparatus was thoroughly cleaned, and a blank check was run to ensure no contamination. The same pad was replaced in the apparatus, and 3 ml. of distilled water or 0.01 N HCl (as in the case of warfarin) passed through the pad. The absorbance of the filtrate was measured and the percent of chemical

washed from the filter pad was determined. Additional two or three similar washings were carried out on the same pad, and the extent of desorption was determined each time.

**Adsorption Equilibrium Studies**—Two 0.22- $\mu$  Filter I disks were placed in 15-ml. screw-top culture tubes. Five milliliters of various concentrations of griseofulvin or warfarin was added. The tubes were stirred at 100 strokes/min. in a constant-temperature (29  $\pm$  0.5 $^{\circ}$ ) water bath shaker (Eberbach Corp.) for 24 hr. The solutions were drained off the pads and the absorbance at suitable wavelengths was measured. It was found the adsorption equilibrium was reached after 24 hr. of shaking.

## RESULTS AND DISCUSSION

**Impurities from Filters**—As previously noted (5), a significant amount of UV-absorbing impurities could be extracted from Filter I and these interfered with the spectrophotometric assay of the filtrate at 205 m $\mu$  during the study of the water solubility of cholesterol. However, it seems, to date, that no quantitative analysis of the impurities has been reported in the literature. The water-soluble impurities from Filter II also appear to be generally ignored.

Table I shows the absorbances from 205 to 320 m $\mu$  of the filtrates after passing 3 ml. of distilled water through different filters. It is obvious that the absorbance of extracted contaminants increases with the decrease of the wavelength except for the 1.2- $\mu$  Filter I disk which shows a minimum absorbance at 240 m $\mu$ . The absorbance of the impurities below 230 m $\mu$  is so high that it can result in a serious error in the quantitative-qualitative analysis if not taken into consideration. It must be noted the absorbance of filtrates decreased as the volume of the distilled water filtered increased. This is expected because there is only a maximum amount of impurities present. Therefore, the effect of the contaminants on the spectrophotometric measurements will be greater when working with smaller volumes of solutions. It should also be emphasized that the filtering area used in these studies is only about 2.5 cm.<sup>2</sup>. Since much larger filter paper, especially Filter II type, is usually employed for the filtration purpose, the amount of extracted impurities will be considerable. This investigation does not attempt to determine the exact chemical nature of the impurities, although it has been reported in the past that Filter I might contain 2-3% of its dry weight as a detergent (16). It should be noted that the different spectra of the impurities from various pore sizes of Filter I might indicate that they contained different water-extracted contaminants and/or different quantities of such contaminant.

**Adsorption from Single Filtration through Filter I**—Table II lists the wavelength used for each compound, their initial concentrations and absorbances, and the percent adsorbed from the first 3 ml. of the filtering solutions by the 0.22- and 0.025- $\mu$  Filter I pads. It is quite surprising to find all 18 compounds with a wide range of solubility

**Table II**—Adsorption of Various Chemicals by Filter I Pads (17.5-mm. Diameter)

Chemicals	Initial Concn., mcg./ml.	Wave-length, m $\mu$	Initial Absorbance	Chemicals Adsorbed, % <sup>a</sup>	
				0.22- $\mu$	0.025- $\mu$
<i>m</i> -Nitrobenzoic acid (distd. H <sub>2</sub> O)	20	270	0.841	3.2	4.6
Iopanoic acid	10	230	0.675	5.8	0.0 <sup>b</sup>
Hydrocortisone	10	247	0.414	6.1	9.6
Chloramphenicol	20	276	0.649	9.9	14.4
Benzoic acid (0.1 N HCl)	10	230	0.966	14.2	46.6
Benzoic acid (distd. H <sub>2</sub> O)	10	225	0.723	9.1	2.6
Phenobarbital (0.01 N HCl)	20	223	0.392	16.9	23.5
Na-Phenobarbital (distd. H <sub>2</sub> O)	20	240	0.645	6.2	6.5
Hydrocortisone acetate	10	247	0.395	17.0	35.6
Hexachlorophene	20	314	0.328	17.4	64.7
Salicylic acid (0.1 N HCl)	25	303	0.664	21.2	69.5
Salicylic acid (distd. H <sub>2</sub> O)	25	302	0.641	9.2	71.2
<i>p</i> -Nitrobenzoic acid (0.1 N HCl)	10	264	0.612	25.8	46.6
Griseofulvin	10	295	0.717	28.9	89.2
Ethinyl estradiol	10	210	0.353	37.5	100.0 <sup>b</sup>
Warfarin (0.01 N HCl)	10	273	0.329	38.6	94.8
Na-Warfarin (distd. H <sub>2</sub> O)	20	308	0.803	4.7	0.0 <sup>b</sup>
Digitoxin	10	220	0.190	57.9	100.0 <sup>b</sup>
Naphthalene	25	276	0.949	86.0	97.7
Spirolactone	10	242	0.435	99.4	60.2

<sup>a</sup> Values obtained after analysis of a 3-ml. filtrate. <sup>b</sup> Approximate value.

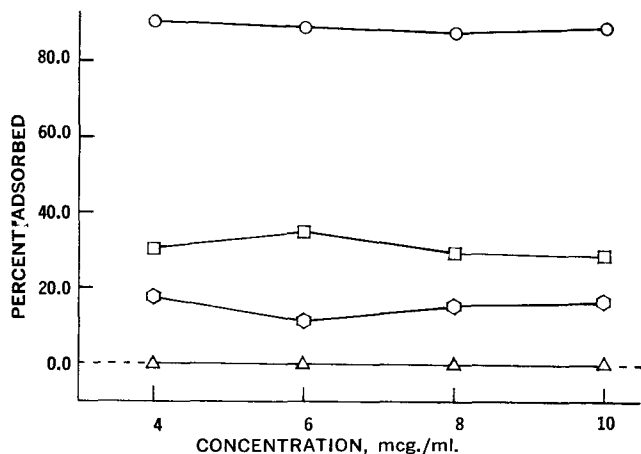


Figure 1—Percent of griseofulvin adsorbed after passing 3 ml. of solutions through different filter paper. Key:  $\circ$ , 0.025- $\mu$  Filter I;  $\square$ , 0.22- $\mu$  Filter I;  $\diamond$ , 1.20- $\mu$  Filter I; and  $\triangle$ , Filter II.

and chemical structure were adsorbed by the 0.22- $\mu$  Filter I, ranging from 3.2 to 99.4%. This is contrary to the statement given by the manufacturer. A range of zero to almost 100% adsorption was found for the 0.025- $\mu$  Filter I.

From Table II several interesting points seem worth discussing.

1. Adsorption by Filter I probably exists for every organic compound. Hence, one should check this possibility whenever they are intended to be used for quantitative separation. A warning of the possible adsorption should be stated in the brochure by the manufacturer.

2. It is recommended that the adsorption problem should be checked when using other types of Millipore filters (Duralon and Mitex). They were not studied in this investigation.

3. As will be further discussed later, the extent of adsorption seems to relate to the pore size of filters: the smaller the pore size, the more the adsorption. This might be due to the higher specific surface area available for adsorption in the smaller pore size filters. This is demonstrated in the table, where a majority of compounds show higher adsorption by the 0.025- $\mu$  Filter I. It is, however, possible that the presence of different amounts or nature of the water-extractable impurities may also affect the degree of adsorption. The effect of the removal of these impurities on adsorption seems worth further study.

4. The water solubility of compounds also appears to relate to the adsorption: the lower the solubility, the stronger the adsorption. This is illustrated by the generally lower adsorption of the more soluble compounds listed at the top of the table (iopanoic acid is an exception). One interesting example is hydrocortisone and hydro-

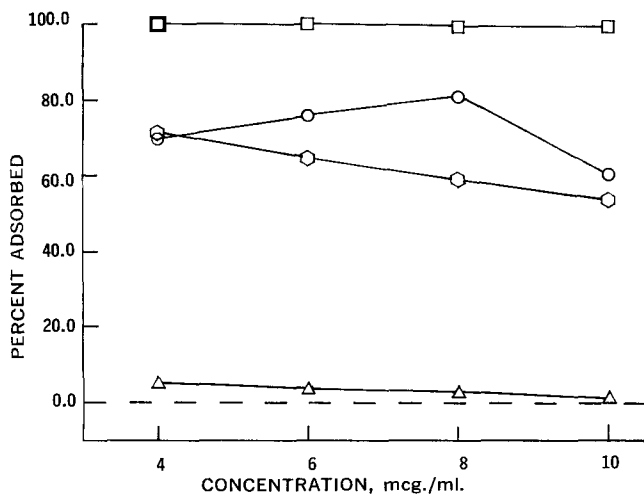


Figure 2—Percent of spironolactone adsorbed after passing 3 ml. of solutions through different filter paper. Key:  $\square$ , 0.22- $\mu$  Filter I;  $\circ$ , 0.025- $\mu$  Filter I;  $\diamond$ , 1.20- $\mu$  Filter I; and  $\triangle$ , Filter II.

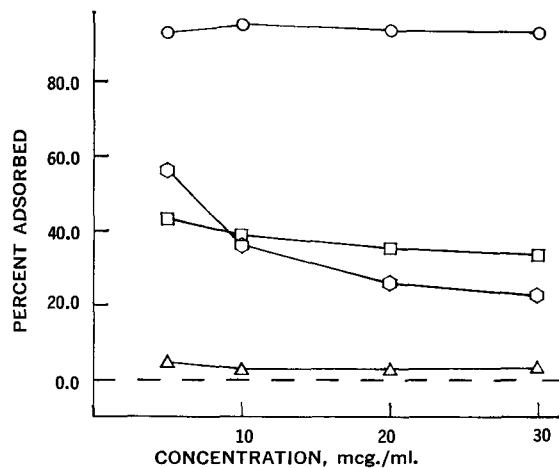


Figure 3—Percent of warfarin adsorbed after passing 3 ml. of solutions through different filter paper. Key:  $\circ$ , 0.025- $\mu$  Filter I;  $\square$ , 0.22- $\mu$  Filter I;  $\diamond$ , 1.20- $\mu$  Filter I; and  $\triangle$ , Filter II.

cortisone acetate. Both have similar chemical structures. However, hydrocortisone acetate, with much less water solubility, shows greater adsorption.

5. The ionic forms are generally much less adsorbed than their nonionic forms. This is clearly shown by warfarin, phenobarbital, benzoic acid, and salicylic acid. It indicates that the ionic bonding between organic adsorbates and filter adsorbents is not significant in contributing to adsorption. The adsorption between them may mainly be due to hydrophobic and hydrogen bonds.

6. The importance of the van der Waals force in causing adsorption is demonstrated by the high adsorption of naphthalene, a fully conjugated flat hydrocarbon.

7. It should be pointed out that the concentrations of several compounds listed in Table II such as hydrocortisone acetate (17), ethinyl estradiol (17), digitoxin (18), griseofulvin (6), and spironolactone (the authors' preliminary study) are approximately equal to their water solubility. A serious error in the solubility value may be made if one uses these filters and ignores their adsorption problem. It is also possible the discrepancy of the reported solubilities of many compounds in the literature (5, 17) might be attributed to the impurity, contamination and adsorption by the filters. Cholesterol might represent a good example. It is quite startling to find from the literature that this biologically important steroid has an extremely wide range of reported water-solubility values: 0.025 mcg./ml. at 30° from Saad and Higuchi (5); about 2.0 mcg./ml. from the Merck Index (18) and Gemant (19); 52 mcg./ml. from Lange and Amund-

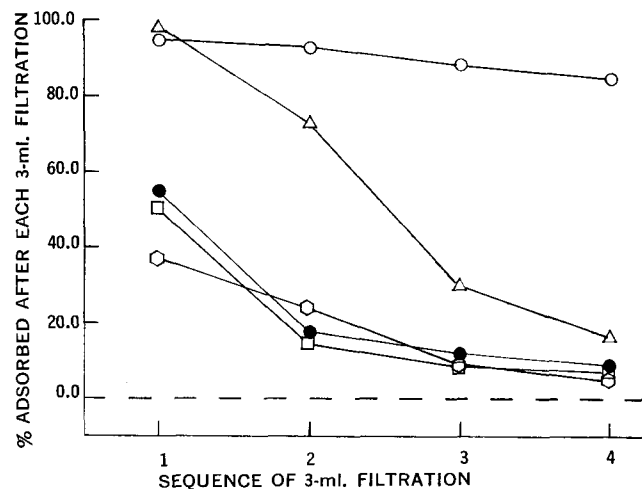


Figure 4—Adsorption of four chemicals by 0.22- $\mu$  Filter I pads after repetitive filtration of 3 ml. aqueous solution. Key:  $\circ$ , naphthalene (initial concentration, 7.6 mcg./ml.);  $\triangle$ , spironolactone (10 mcg./ml.);  $\bullet$ , warfarin (0.01 N HCl, 10 mcg./ml.);  $\square$ , griseofulvin (5 mcg./ml.); and  $\diamond$ , ethinyl estradiol (10 mcg./ml.).

**Table III—Effect of Filtration Rate on Adsorption by Filter I Pad, 0.22- $\mu$  Pore Size**

Chemicals	% Adsorbed	
	30 sec. <sup>a</sup>	300 sec. <sup>a</sup>
Salicylic acid (0.1 N HCl; 25 mcg./ml.)	21.2	27.3
<i>p</i> -Nitrobenzoic acid (0.1 N HCl; 10 mcg./ml.)	25.8	46.6
Griseofulvin (distd. H <sub>2</sub> O; 10 mcg./ml.)	28.9	29.1
Warfarin (0.01 N HCl; 30 mcg./ml.)	33.7	36.6

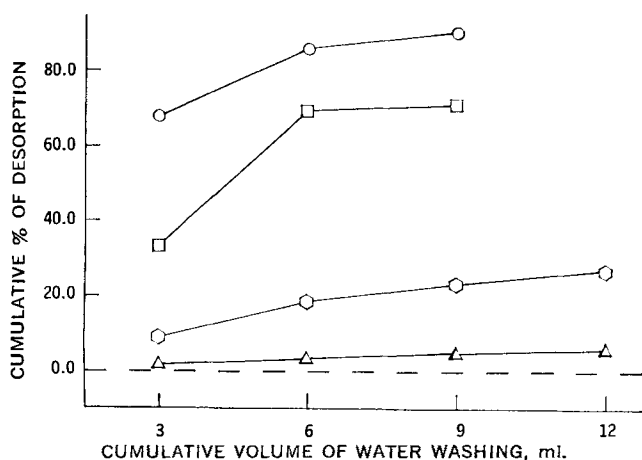
<sup>a</sup> Filtration time of 3 ml. aqueous solution.

son (20); and 2600 mcg./ml. from the "Handbook of Chemistry and Physics" (21) which cites the value from Dehn (22). Although the solubility of a compound may vary with its particle size, crystalline form, and electric charge of particles (23), it is believed these factors could not explain a 10,000-fold difference.

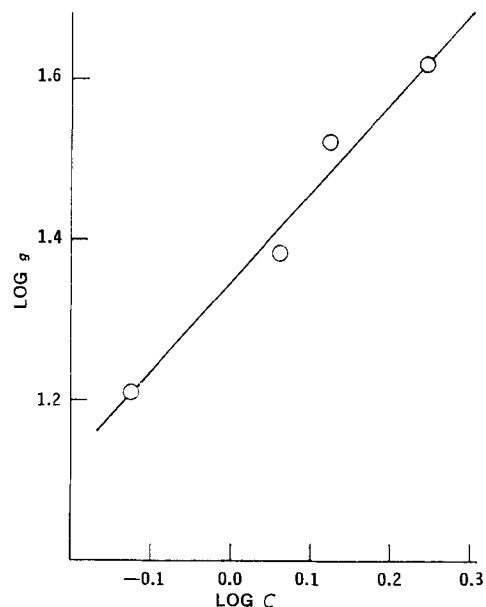
**Adsorption by Filters I and II versus Concentrations**—The percent of adsorption at different concentrations of three compounds, griseofulvin, spironolactone, and warfarin, by Filter II and three different pore sizes of Filter I is shown in Figs. 1-3. The percent adsorbed was found to vary with the type of filters, Filter I versus Filter II, the pore size of Filter I, and finally the concentration of each compound. The decreasing degree of adsorption by the four different filters is generally in the following order: 0.025- $\mu$  Filter I > 0.22- $\mu$  Filter I > 1.2- $\mu$  Filter I > Filter II.

**Adsorption from Multiple Filtration through Filter I**—The percent of adsorption from the multiple filtration of five compounds, *i.e.*, spironolactone, griseofulvin, ethinyl estradiol, warfarin, and naphthalene, through the 0.22- $\mu$  Filter I is shown in Fig. 4. The extent of adsorption of all compounds studied decreased as the volume of filtering solutions increased. This is not unexpected because the adsorption site is usually saturable. The adsorption capacity of Filter I toward the naphthalene molecule is, however, surprisingly high, as evidenced by the least steepness of the slope in Fig. 4, and 85% of naphthalene was still adsorbed in the fourth filtration through the same filter.

**Effect of Filtration Rate on Adsorption**—From the practical point of view, it is also interesting and important to know whether the filtration rate will affect the adsorption, since different rates may be employed at different occasions by the same or different workers. The results obtained from four compounds after filtering through 0.22- $\mu$  Filter I are shown in Table III. A 10-fold difference in the filtration rate did indeed result in a change in the degree of adsorption. The slower the filtration rate, the more was



**Figure 5—Cumulative desorption of four chemicals from 0.22- $\mu$  Filter I pads after previous filtration of 3 ml. aqueous solution. Key: ○, spironolactone (initial filtered concentration, 10 mcg./ml.); △, naphthalene (7.6 mcg./ml.); □, salicylic acid (H<sub>2</sub>O; 25 mcg./ml.); and ◇, warfarin (0.01 N HCl; 20 mcg./ml.).**



**Figure 6—Freundlich adsorption isotherm of griseofulvin (0.22- $\mu$  Filter I).**

the adsorption. Some compounds like *p*-nitrobenzoic acid and salicylic acid are sensitive to the rate while some are not.

**Desorption Studies**—Desorption experiments on four compounds, *i.e.*, spironolactone, naphthalene, salicylic acid, and warfarin, were run to study further the binding nature of adsorption on Filter I. The results are shown in Fig. 5. The fact that the adsorbed compounds could be washed from the filter indicates a reversible characteristic of the adsorption. The strong van der Waals bonding between the naphthalene and Filter I is once again demonstrated by the least desorption of naphthalene. Only 6% of the adsorbed naphthalene was washed away by 12 ml. of distilled water.

**Adsorption Equilibrium**—Table IV represents equilibrium adsorption data for griseofulvin and warfarin. The percent adsorbed was found to be almost independent of initial concentration. The plots of Freundlich adsorption isotherms are shown in Figs. 6 and 7 in which *q* represents micrograms of compounds adsorbed by the two filter disks used for each study, and *c*, in micrograms per milliliter, is the concentration of the solutions at equilibrium.

## SUMMARY

1. Eighteen acidic and neutral organic compounds with a wide range of water solubility and chemical structure were studied for their adsorption to Filters I and II. The adsorption on the 0.22- and 0.025- $\mu$  Filter I varied from zero to almost 100% during the single-filtration studies. The adsorption on Filter II was low or negligible.

2. Water-soluble compounds generally showed a lower tendency for adsorption.

**Table IV—Data Showing Equilibrium Adsorption of Griseofulvin and Warfarin by 0.22- $\mu$  Pore Size Filter I**

Chemicals	Initial Concn., mcg./ml.	% Adsorbed
Griseofulvin	4.0	81.2
	6.0	80.7
	8.0	83.5
	10.0	82.5
Warfarin	5.0	99.4
	10.0	98.8
	20.0	98.4
	30.0	98.4

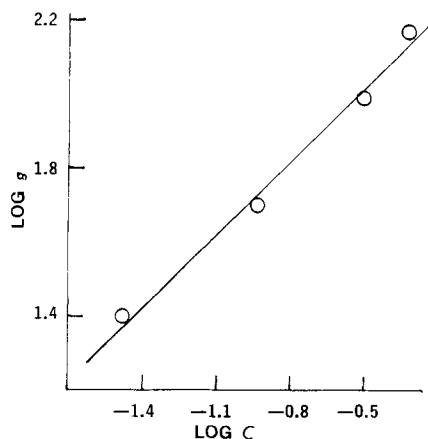


Figure 7—Freundlich adsorption isotherm of warfarin (0.22- $\mu$  Filter I).

3. Ionic salts were less adsorbed than their neutral forms.
4. The adsorption of a compound might vary with its concentration, volume of the solution filtered, filtration rate, and the pore size.
5. The adsorption was found to be reversible. The adsorption at equilibrium could be represented by Freundlich adsorption isotherm plots.
6. Water-soluble impurities from Filters I and II were studied spectrophotometrically. Their absorbances below 230  $m\mu$  were found to be considerably high.
7. The possible effects of adsorption and impurities from filters on the quantitative-qualitative chemical analysis were discussed.

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#### ACKNOWLEDGMENTS AND ADDRESSES

Received August 22, 1969, from the College of Pharmacy, Washington State University, Pullman, WA 99163

Accepted for publication December 11, 1969.

This investigation was supported in part by NIH Grant FR056866