Analyte Loss Due to Membrane Filter Adsorption as Determined by High-Performance Liquid Chromatography

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Abstract

The phenomenon of membrane filter adsorption in high-performance liquid chromatography (HPLC) is investigated utilizing 16 brands of filters representing 3 polymeric materials: cellulose acetate (CA), nylon, and polyvinylidene difluoride in a variety of diameters (3, 4, 7, 13, and 25 mm). Sixteen compounds commonly encountered in drug preparations are selected as sample analytes and classified as acidic, basic, and neutral in chemical behavior. Six mobile phase/sample solvent mixtures are included: 3 with methanol–water and 3 with acetonitrile–water as major constituents. When using methanol as the mobile phase organic component, CA, nylon, and polyvinylidene difluoride (PVDF) filters exhibit negligible to moderate adsorption levels with regard to the neutral and basic drug compounds. The acidic drug test compounds are adsorbed by 50% of all 3 filter materials tested in methanol–water. In acetonitrile, neutral compounds are affected by 31.4%, basic compounds are affected by 47.0%, and acidic compounds are affected by 53.6% of the nylon and PVDF filters. CA is incompatible with acetonitrile and is excluded from the study with this solvent.

Introduction

The use of microfiltration in analytical laboratories that employ chromatography is widespread and essential for proper sample preparation, including isolation and concentration. The use of membrane filters also contributes to improved analytical precision and accuracy, as well as protection of the chromatographic system from particulates.

Membrane filters are a type of surface filter that consist of open, colloidal structures prepared from polymeric films (1). Most membrane filter manufacturers provide filters for use with a syringe and needle system that is desirable for filtering small volumes of sample material. Available polymeric membrane filters include cellulose esters (nitrate, acetate) and an assortment of other materials such as regenerated cellulose, nylon (a

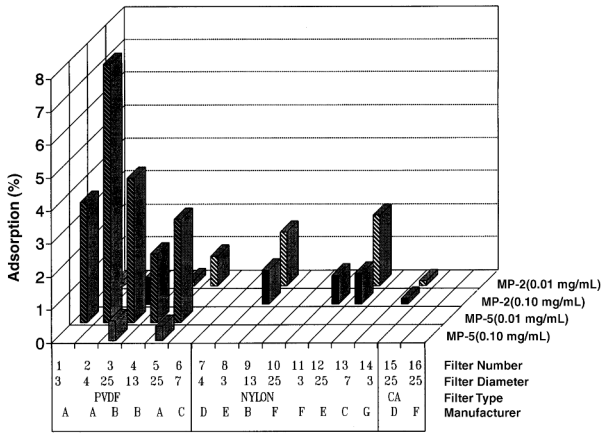

Figure 1. Filter adsorption effect on benzoic acid at 0.01 and 0.10 mg/mL (monitored at 254 nm). Mobile phases MP-2 and MP-5 are methanol–water–acetic acid (60:40:1, v/v/v) and acetonitrile–water–acetic acid (50:50:1, v/v/v), respectively.

Table I. Mobile Phase Compositions

	Component ratio (v/v)				
Mobile phase	Methanol	Acetonitrile	Water	Acetic acid	Potassium dihydrogen phosphate buffer
1	60	–	40	–	–
2	60	–	40	1	–
3*	60	–	–	–	40†
4	–	50	50	–	–
5	–	50	50	1	–
6*	–	50	–	–	50‡

*Final pH adjusted to 5.00 with 20% H_3PO_4.
† 0.0625M KH_2PO_4.
‡ 0.050M KH_2PO_4.

polyamide), PVDF, and polytetrafluoroethylene (PTFE).

Because many of the membrane filter materials are hydrophobic, wetting agents such as glycerol are added to facilitate the passage of aqueous solvents. One membrane filter source (Millipore) chemically modified their fluorocarbon material (PVDF) to attain a hydrophilic character and avoid the use of wetting agents. Another manufacturer (CUNO) has modified their nylon membrane material, yielding a unique positively charged state when wet, resulting in some ion exchange activity toward solute anions.

Membrane filtration using various porous polymeric materials during the final sample clarification step is a common practice in high-performance liquid chromatographic (HPLC) analysis. This separation procedure, which has been the subject of two publications (1,2) is also routinely employed for particle removal from HPLC mobile phase mixtures.

A review of the literature for membrane filters indicates a primary interest focused on extractables (3–7). Minimal information is available in the chemical literature on the topic of membrane filter adsorption. Two published reports describe specific cases of membrane adsorption for prednisone (8) and protein binding (9). A study was also conducted involving the effect of nylon filter adsorption on pharmaceutical preservatives (10). The filters most commonly used in our laboratory include cellulose acetate (CA), nylon-66, and PVDF membranes. We have observed a 5% loss of potassium salicylate

Table II. Analyte Loss via Membrane Filter Adsorption: Methanol Organic Modifier

			Adsorption loss (%)															
			PVDF						Nylon								CA	
Compound	Concentration* (mg/mL)	Mobile phase†	3-mm‡	4-mm‡	25-mm‡	13-mm§	25-mm§	7-mm‖	13-mm§	7-mm‖	4-mm#	3-mm**	25-mm**	3-mm††	25-mm††	3-mm‡‡	25-mm#	25-mm††
Neutral																		
Benzyl alcohol	0.050	1	0.17	0.03	0.36	Z§§	0.63	0.72	Z	Z	Z	Z	I‖‖	0.03	Z	0.37	0.14	Z
Benzyl alcohol	0.300	1	0.04	0.23	0.08	Z	Z	Z	0.26	0.24	Z	Z	Z	Z	Z	0.54	Z	0.09
Guaifenesin	0.030	1	Z	Z	Z	0.04	Z	Z	Z	Z	Z	Z	Z	0.29	0.48	0.65	Z	Z
Guaifenesin	0.300	1	Z	0.40	0.25	Z	0.54	N##	0.33	N	Z	0.30	Z	0.55	0.54	Z	0.06	Z
Hydrocortisone acetate	0.005	1	Z	Z	0.82	Z	0.24	Z	0.01	Z	Z	0.44	0.72	0.42	Z	0.50	0.08	Z
Hydrocortisone acetate	0.050	1	0.39	Z	0.27	Z	0.16	N	Z	N	Z	1.22	Z	Z	0.10	0.52	Z	Z
Methyl paraben	0.005	1	Z	0.21	0.33	0.10	0.30	Z	0.05	Z	Z	Z	0.75	0.17	0.33	0.54	0.38	0.10
Methyl paraben	0.050	1	Z	0.08	0.35	Z	Z	N	Z	N	Z	0.11	0.48	Z	0.22	Z	Z	Z
Acidic																		
Benzoic acid	0.010	2	0.18	Z	0.88	0.18	Z	Z	Z	Z	I	1.62	2.13	Z	Z	0.14	Z	Z
Benzoic acid	0.100	2	Z	Z	Z	Z	0.81	N	Z	N	Z	1.03	0.92	0.86	Z	0.17	Z	Z
Phenol	0.010	2	Z	Z	0.03	0.43	Z	Z	Z	0	Z	Z	Z	Z	I	0.30	Z	Z
Probenecid	0.010	2	1.32	Z	0.69	Z	6.11	Z	0.40	0.37	0.02	Z	5.20	Z	1.37	0.06	Z	Z
Probenecid	0.100	2	Z	N	N	N	4.08	N	N	N	N	N	5.29	N	0.67	N	N	N
Sodium saccharin	0.010	2	4.33	4.16	5.25	I	93.0	Z	3.32	Z	I	8.91	90.9	8.62	78.7	7.18	2.51	Z
Sodium saccharin	0.100	2	Z	Z	0.82	0.71	8.69	N	Z	N	N	0.53	100.0	2.16	10.2	0.96	Z	N
Salicylic acid	0.005	2	Z	Z	0.03	0.52	45.0	Z	0.52	1.12	1.95	0.73	85.8	1.95	20.8	1.69	Z	0.06
Salicylic acid	0.050	2	N	N	N	Z	9.32	N	0.01	0.48	1.16	0.51	60.4	2.63	7.45	0.97	N	N
Sulfadiazine	0.005	2	I	0.32	1.04	Z	0.26	Z	Z	Z	I	I	0.32	Z	0.13	I	I	I
Vanillin	0.005	1	Z	Z	Z	Z	Z	Z	Z	Z	0.20	0.29	0.46	Z	0.33	0.23	0.83	Z
Vanillin	0.050	1	Z	Z	0.08	Z	0.11	N	0.04	N	Z	Z	0.57	Z	0.08	Z	Z	0.45
Basic																		
Albuterol sulfate	0.010	3***	Z	Z	Z	I	Z	Z	Z	Z	Z	Z	I	0.08	I	0.10	Z	I
Pyrilamine maleate	0.005	3	1.61	1.63	2.89	0.40	3.43	Z	Z	Z	Z	0.37	Z	Z	0.75	Z	Z	0.69
Pyrilamine maleate	0.050	3	Z	Z	Z	N	1.03	N	N	N	N	N	N	N	N	N	N	N
Procainamide HCL	0.005	3	Z	Z	0.64	Z	Z	Z	Z	Z	Z	0.20	0.13	Z	I	Z	Z	I
Theophylline	0.005	3	Z	Z	0.40	Z	0.54	N	0.12	Z	Z	Z	Z	Z	I	0.10	0.20	Z

* 20-µL injection volume.
† Mobile phase 1, MeOH–H_2O (60:40, v/v); mobile phase 2, MeOH–H_2O–HOAc (60:40:1, v/v/v); mobile phase 3, MeOH–buffer (60:40, v/v).
‡ Source A.
§ Source B.
‖ Source C.
Source D.
** Source E.
†† Source F.
‡‡ Source G.
§§ Z, no adsorption evident.
‖‖ I, peak interference (extractable).
N, not run due to negligible adsorption observed at the lower concentration.
*** Contains a buffer of 0.0625M KH_2PO_4.

from a multicomponent commercial preparation during the development of an HPLC procedure (11). These results were confirmed by testing analyte standard solutions with a nylon-66 membrane material in a more complete follow-up study (12).

In addition to the salicylate salts, compounds such as benzoic acid, sodium saccharin, and pyrilamine maleate showed significant adsorption losses on membrane filters during sample clarification in our laboratory.

In order to determine the extent and severity of this adsorption problem, several test compounds were selected in the following categories: aromatic carboxylic acids, amines, a sulfonamide, and some neutral species including steroids. These model compounds were evaluated with respect to their affinity for adsorption on a variety of filter membranes. All filters tested in this study were 0.45-µm porosity and 3–25 mm in diameter. Adsorption losses were determined by comparison of the HPLC responses for each compound with and without membrane filtration.

Treatment of the filter-active sites by prewetting with the appropriate sample solvent (mobile phase) was also investigated. Where significant adsorption occurred at the initial working concentration of 0.005–0.01 mg/mL, further studies were conducted to determine possible adsorption at a greater (10-fold) concentration.

The salicylate adsorption problem previously described was further investigated with respect to functional group position for two related isomers. The overall data from this study resulted in a number of conclusions and recommendations regarding the use of porous membrane filters with drug substance formulations routinely analyzed in pharmaceutical laboratories.

The purpose of this study was to determine the extent of surface adsorption sample loss during membrane filtration based

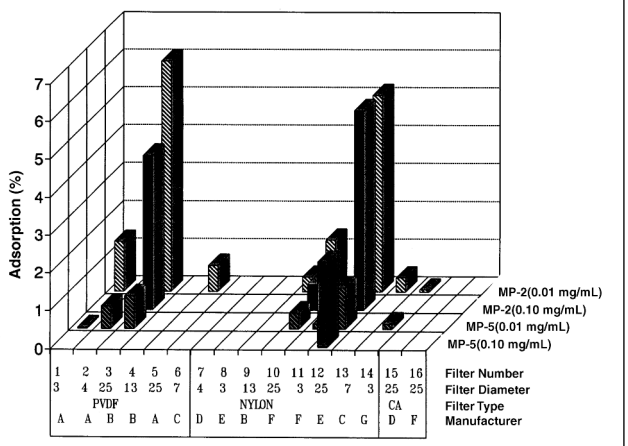

Figure 2. Filter adsorption effect on probenecid, an acidic compound, at 0.01 and 0.10 mg/mL (monitored at 254 nm). Mobile phases MP-2 and MP-5 are methanol–water–acetic acid (60:40:1, v/v/v) and acetonitrile–water–acetic (50:50:1, v/v/v), respectively.

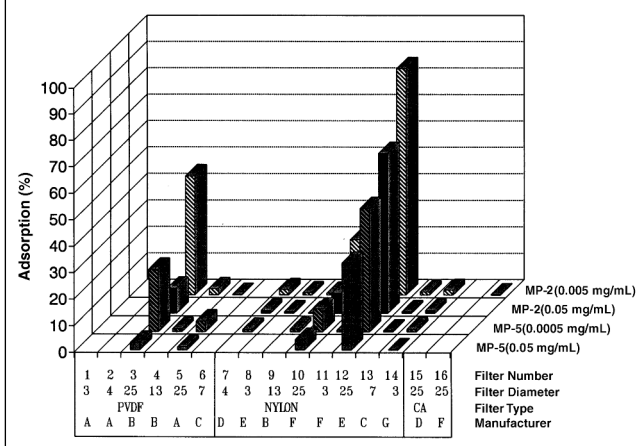

Figure 4. Filter adsorption effect on salicylic acid at 0.005 and 0.05 mg/mL (monitored at 300 nm). Mobile phases MP-2 and MP-5 are methanol–water–acetic acid (60:40:1, v/v/v) and acetonitrile–water–acetic acid (50:50:1, v/v/v), respectively.

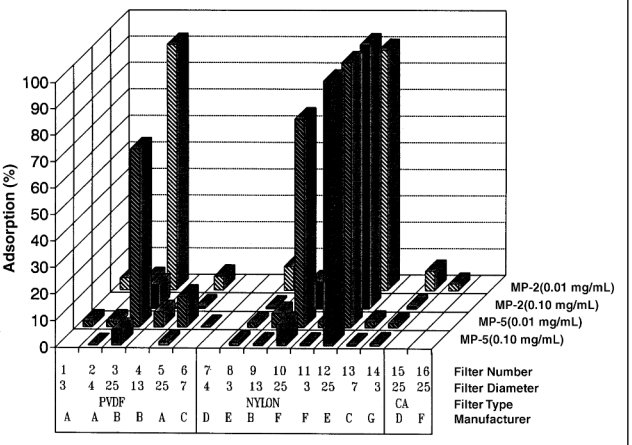

Figure 3. Filter adsorption effect on sodium saccharin, an acidic compound, at 0.01 and 0.10 mg/mL (monitored at 254 nm). Mobile phases MP-2 and MP-5 are methanol–water–acetic acid (60:40:1, v/v/v) and acetonitrile–water–acetic acid (50:50:1, v/v/v), respectively.

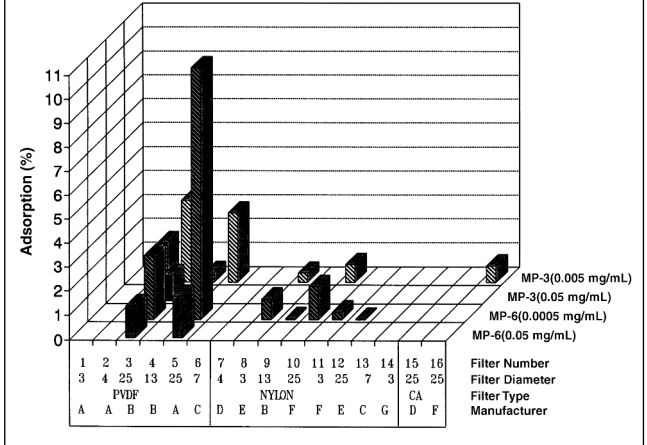

Figure 5. Filter adsorption effect on pyrilamine maleate, a basic compound, at 0.005 and 0.05 mg/mL (monitored at 254 nm). Mobile phases MP-3 and MP-6 are methanol–0.0625M aqueous KH_2PO_4 (60:40, v/v) and acetonitrile–0.050M aqueous KH_2PO_4 (50:50, v/v), respectively.

on the functional groups present in various groups of drug substances and to increase the level of awareness for potential filter adsorption problems during the preparation of pharmaceutical samples for HPLC.

Experimental

Apparatus

The HPLC system consisted of an Altex/Beckman model 100A pump (Beckman Instruments, Fullerton, CA) equipped with a Laboratory Data Control LDC SpectroMonitor III model 1204A variable wavelength detector (Thermo Separations Products, San Jose, CA), a Rheodyne (Cotati, CA) model 7125 sampling valve having a 20-µL fixed loop, and a Hewlett-Packard (Wilmington, DE) model 3380A integrator. The column was a 300- × 3.9-mm-i.d. stainless steel µBondapak C_{18} column of 10-µm particle size (Waters, Milford, MA).

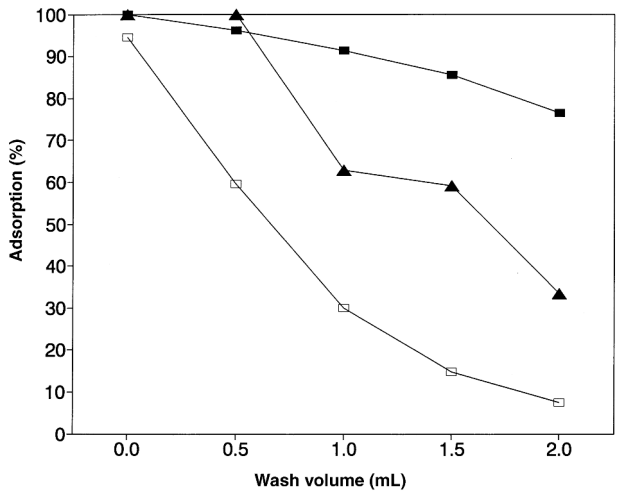

Figure 6. Effect of mobile phase prewash on the filter adsorption of salicylic acid (0.005 mg/mL). The mobile phase was methanol–water–acetic acid (60:40:1,v/v/v). Filter A, 25-mm nylon (□); filter B, 25-mm nylon (■); and filter C, 25-mm PVDF (▲).

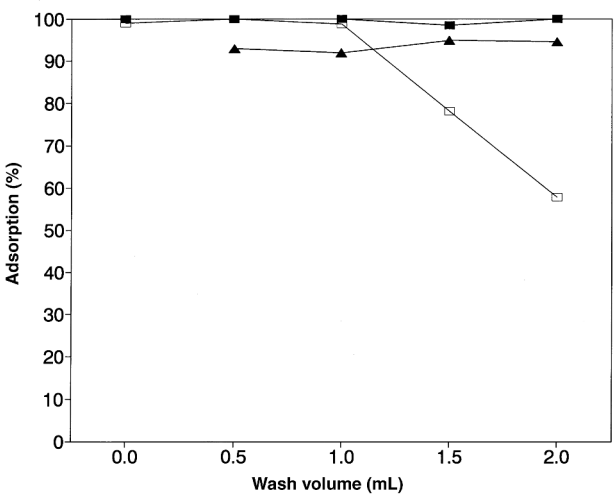

Figure 7. Effect of mobile phase prewash on the filter adsorption of sodium saccharin (0.01 mg/mL). The mobile phase was methanol–water–acetic acid (60:40:1,v/v/v). Filter A, 25-mm nylon (□); filter B, 25-mm nylon (■); and filter C, 25-mm PVDF (▲).

Filters

The membrane filter sources were Gelman Sciences (Ann Arbor, MI), Schleicher and Schuell (Keene, NH), Millipore and Xydex (Bedford, MA), Micron Separations (Westborough, MA), Vangard International (Neptune, NJ), and CUNO (Meriden, CT).

Chromatographic conditions

The column flow rate was 1 mL/min. Detection was performed at 254 nm for all test compounds except albuterol sulfate at 276 nm, ethinyl estradiol at 281 nm, and salicylic acid at 300 nm. The absorbance range was set at 0.05 AUFS. All experiments were carried out at ambient temperature with an integrator chart speed of 0.5 cm/min.

Reference material and reagents

Benzyl alcohol, benzoic acid, phenol, sodium saccharin, phosphoric acid, and glacial acetic acid were obtained from Mallinckrodt (Paris, KY). Guaifenesin, hydrocortisone acetate, methylparaben, pyrilamine maleate, and ethinyl estradiol were purchased from K & K Laboratories (Plainview, NY). The compounds probenecid and procainamide hydrochloride were received from the United States Pharmacopeial Convention, Inc. (Rockville, MD); vanillin was from Fisher Scientific (Fairlawn, NJ); salicylic acid was from Sigma Chemical Co. (St. Louis, MO); sulfadiazine was from Lederle Laboratories (Pearl River, NY); albuterol sulfate was from Schering-Plough (Kenilworth, NJ); theophylline was from Knoll Pharmaceuticals (Whippany, NJ); methanol, acetonitrile, and potassium dihydrogen phosphate (KH_2PO_4) were received from EM Science (Cherry Hill, NJ).

All reagents were HPLC or AR grade. Distilled, deionized water was passed through 0.2-µm Versapor membrane filters (Gelman Sciences).

Six mobile phase/sample solvents were prepared and used as appropriate for the compound type under investigation (acidic, neutral, or basic). All six solutions were filtered through a 47-mm-diameter, 0.45-µm-porosity nylon membrane (CUNO) prior to use. The mobile phase compositions used in the study are presented in Table I.

Standard preparations

The reference standard solutions for all compounds included in this study ranged in concentration from 0.005 to 0.05 mg/mL depending on their respective absorptivities (molar extinction coefficient) at the wavelength of interest. For those compounds that exhibited significant membrane filter adsorption, additional standard solutions were prepared at 10–20 times the initial concentration (0.05–0.10 mg/mL). This protocol was designed to determine whether a greater concentration of analyte would be less affected due to an increased saturation of the active adsorption sites present on the membrane filter.

Procedure

Each standard was accurately weighed and placed in a 100-mL volumetric flask. The appropriate mobile phase solution was added, sonicated for 2 min, and then shaken mechanically for 30 min to achieve complete dissolution. Twenty microliter volumes of each standard solution were injected into the chromatograph using the following sequence: unfiltered, filtered, filtered, unfiltered. The average response of the unfiltered versus filtered injections was used to calculate filtration (adsorption) loss in percent. Two-milliliter-capacity syringes having a Luer glass tip were supplied by Popper and Sons (Hyde Park, NY) and used throughout the study. The injection syringe was filled to its 2-mL capacity with sample solution, and the attached membrane filter "prewashed" with the first 1.5 mL of the syringe contents. The remaining 0.5 mL of the syringe contents was used to fill the 20-µL capacity injection loop. The flow rate through the filters was approximately 9–12 mL/min depending on solvent composition and filter diameter.

Table III. Analyte Loss via Membrane Filter Adsorption: Acetonitrile Organic Modifier

			Adsorption loss (%)													
			PVDF						Nylon							
Compound	Concentration* (mg/mL)	Mobile phase†	3-mm‡	4-mm‡	25-mm‡	13-mm§	25-mm§	7-mm‖	13-mm§	7-mm‖	4-mm#	3-mm**	25-mm**	3-mm††	25-mm††	3-mm‡‡
Neutral																
Ethinyl estradiol	0.020	4	Z§§	Z	Z	Z	0.55	Z	0.27	Z	Z	Z	0.27	Z	Z	0.54
Benzyl alcohol	0.050	4	Z	Z	0.04	Z	Z	Z	0.38	Z	Z	Z	Z	Z	0.14	0.08
Guaifenesin	0.030	4	Z	0.01	0.29	Z	Z	Z	0.67	Z	Z	0.94	0.55	1.22	0.37	Z
Hydrocortisone acetate	0.005	4	Z	Z	Z	Z	Z	Z	0.85	Z	Z	0.11	0.75	Z	0.58	0.40
Methyl paraben	0.005	4	Z	0.19	Z	Z	Z	Z	0.34	Z	Z	Z	Z	Z	Z	Z
Acidic																
Benzoic acid	0.010	5	3.64	7.82	3.12	2.08	4.36	Z	I‖‖	Z	I	I	I	I	I	Z
Benzoic acid	0.100	5	Z	Z	0.43	Z	0.60	N##	N	N	N	N	N	N	N	N
Phenol	0.010	5	I	Z	0.32	Z	1.60	Z	0.04	Z	I	2.06	Z	Z	0.57	Z
Phenol	0.100	5	N	N	N	N	1.53	N	N	N	N	0.59	N	N	N	N
Probenecid	0.010	5	0.03	0.57	Z	Z	0.85	Z	Z	Z	Z	Z	1.07	0.13	0.41	0.12
Probenecid	0.100	5	N	N	N	N	N	N	N	N	N	N	2.25	N	N	N
Sodium saccharin	0.010	5	2.09	2.11	11.4	5.69	67.1	0.05	5.25	2.06	Z	1.59	100.0	2.17	78.6	1.53
Sodium saccharin	0.100	5	Z	0.18	1.39	Z	5.94	N	0.71	0.37	N	1.00	100.0	0.50	8.07	0.87
Salicylic acid	0.005	5	Z	Z	3.93	0.98	23.5	Z	1.04	0.40	0.83	Z	46.6	0.62	8.55	1.06
Salicylic acid	0.050	5	N	N	1.00	Z	2.81	N	Z	N	N	N	32.7	N	3.96	0.20
Sulfadiazine	0.005	5	Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	0.23	0.05	Z	Z
Vanillin	0.005	4	I	Z	Z	Z	0.17	Z	Z	Z	4.39	Z	0.01	0.23	0.11	Z
Basic																
Albuterol sulfate	0.010	6***	Z	I	0.61	I	0.66	Z	I	Z	Z	Z	0.81	Z	I	Z
Pyrilamine maleate	0.005	6	Z	Z	10.5	Z	2.64	Z	0.05	Z	Z	0. 83	0.02	0.30	1.36	Z
Pyrilamine maleate	0.050	6	N	N	1.69	N	1.30	N	N	N	N	N	N	N	Z	N
Procainamide HCL	0.005	6	1.01	Z	1.35	Z	0.49	Z	1.98	Z	1.80	4.38	3.85	1.44	Z	3.12
Procainamide HCL	0.050	6	Z	N	0.05	N	N	N	Z	N	Z	0.11	Z	Z	N	0.01
Theophylline	0.005	6	Z	0.28	0.17	Z	Z	Z	0.97	0.34	Z	1.45	Z	0.73	0.77	0.35
Theophylline	0.050	6	N	N	N	N	N	N	N	N	N	Z	N	N	N	N

* 20-µL injection volume.
† Mobile phase 4, CH_3CN–H_2O (50:50, v/v); mobile phase 5, CH_3CN–H_2O–HOAc (50:50:1, v/v/v); mobile phase 6, CH3CN–buffer (50:50, v/v).
‡ Source A: Millipore.
§ Source B: Gelman Sciences.
‖ Source C: Xydex.
Source D: Vanquard.
** Source E: CUNO.
†† Source F: Schleicher and Schuell.
‡‡ Source G: Micron Separations.
§§ Z, no adsorption evident.
‖‖ I, peak interference (extractable).
N, not run due to negligible adsorption observed at the lower concentration.
*** Buffer, 0.050M KH_2PO_4.

Results and Discussion

Tables II and III display all filter data in a quantitative format. The letter "I" represents peak interferences present due to extractables, the letter "N" represents "not run due to negligible adsorption at the lower analyte concentration", and the letter "Z" signifies "no adsorption evident".

Tables II and III also show the type of mobile phase organic modifier used (methanol or acetonitrile). These solvents were selected based on their popular use in HPLC. In addition, the tables display analyte identity and concentration, mobile phase composition, filter diameter, and filter type.

The data in Tables II and III display analyte loss due to adsorption ranging from 0.01 to 100.0%. A summary of the results observed in Tables II and III support the following conclusions: 38% of all test filters examined show adsorptive effects for 4 basic compounds, 51.5% show adsorptive effects for the acidic compounds, and 41.1% show adsorptive effects for the neutral analytes. Due to the incompatibility of CA filters and acetonitrile in the mobile phase, this combination was excluded from the study.

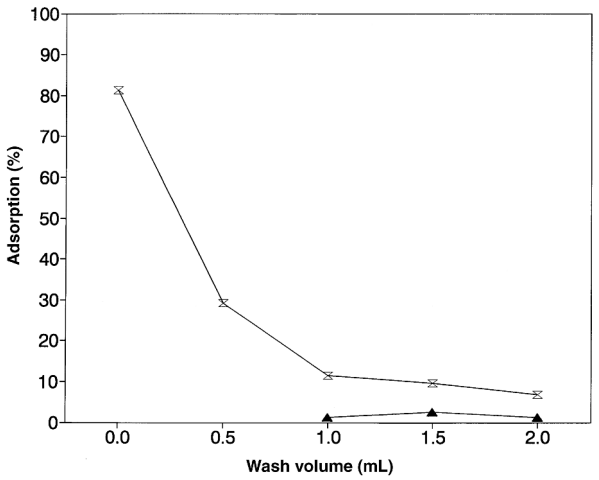

Figure 8. Mobile phase prewash effect on filter adsorption for procainamide hyrochloride (0.005 mg/mL) using filter C and for pyrilamine maleate (0.005 mg/mL) using filter D. The mobile phase was acetonitrile–0.050M aqueous KH_2PO_4 (50:50, v/v). Filter C, 25-mm PVDF (▲); filter D, 25-mm PVDF (⧖).

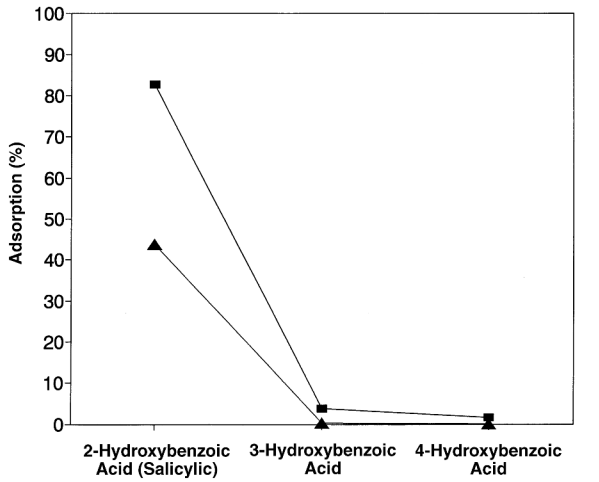

Figure 9. Effect of salicylic acid isomer functional group placement on filter adsorption (1.5 mL prewash, 0.005 mg/mL each compound). The mobile phase was acetonitrile–0.050M aqueous KH_2PO_4 (50:50,v/v). Filter B, 25-mm nylon (*n*); filter C, 25-mm PVDF (▲).

Figures 1–5 illustrate the most significant adsorption effects encountered in this study. The results indicate a greater degree of filter adsorption for acidic compounds and lower levels of adsorption for the basic compounds. Neutral compounds exhibited the lowest adsorption levels for the three different filter materials tested.

A review of the study data with regard to precision provided a mean coefficient of variation (CV) of 0.41% (n = 26) for a combination of two filter types (nylon and PVDF) and five analytes (benzyl alcohol, guaifenesin, hydrocortisone acetate, methyl paraben, and vanillin).

Fifty percent of the PVDF filter test material exhibited adsorption losses for benzoic acid ranging from 0.18 to 7.82%, whereas 30.4% of the nylon filter material displayed adsorption losses of 0.14–2.13%, as shown in Figure 1.

Figure 2 indicates the adsorption of probenecid (an acidic compound) ranging 0.03–6.11% by 50.0% of the PVDF test filters and 0.02–5.29% by 68.4% of the nylon test filters. This graph clearly shows that 25-mm-diameter filters yield the largest adsorption values due to their significantly greater surface area.

Figure 3 details the loss of sodium saccharin (an acidic compound) by 72.7% of the PVDF filters over a range of 0.05–93.0%, whereas 86.2% of the nylon filters exhibited adsorption effects in the 0.37–100.0% range, with the 25-mm-diameter filters displaying the highest adsorption levels. The bar graph in Figure 3 includes a sodium saccharin (0.01 mg/mL) adsorption loss of 78.7% with filter number 10 (nylon, 25-mm diameter) and mobile phase number 2, which is obscured by four larger adsorption losses observed for nylon filter number 12 (25-mm diameter). This was due to limited rotation of the 3-D bar graph presentation. One of the two CA filters gave a single adsorption value of 2.51%.

Filter adsorption effects on salicylic acid are shown in Figure 4, where 52.9% of the PVDF test material displays an adsorption range of 0.03–45.0%, and 92.8% of the nylon filters tested exhibit salicylic acid losses of 0.01–85.8%. The CA filters yielded negligible adsorption effects.

Figure 5 depicts filter adsorption of the basic compound pyrilamine maleate, where 55.6% of the PVDF filters tested indicate adsorption in the 0.40–10.5% region, and 41.2% of the nylon filters show analyte loss in the 0.02–1.36% range. The CA filters exhibited one adsorption value of 0.69%.

A study was conducted to investigate adsorption effects on a variety of 25-mm filters with respect to four compounds before and after filter prewash treatment (Figures 6–8). The filter prewash was the sample solution.

Included in the study were two acidic compounds (salicylic acid and sodium saccharin) and two basic compounds (pyrilamine maleate and procainamide hydrochloride). Figures 7 and 8 lack data points that are below the abscissa and represent interference peak material leached from a PVDF filter in the

presence of sodium saccharin and procainamide hydrochloride, respectively. These absent data points coincide with minimal filter prewash treatment (0–0.5 mL mobile phase).

The data displayed in Figures 6–8 indicate significant adsorption of the two acidic compounds and one basic compound (pyrilamine maleate) while in contact with nylon and PVDF filters. The second basic compound (procainamide hydrochloride) exhibited low-level adsorption on the PVDF filter. Where similar filter types are present in Figures 6 and 7, the nylon or PVDF products from two separate manufacturers are represented.

An additional investigation of the isomers of salicylic acid (2- ,3-, and 4- hydroxybenzoic acid) illustrates the effect of functional group position versus filter adsorption. The concentration of each compound was 0.005 mg/mL. All filters were prewashed with 1.5 mL of sample solution prior to injection. Figure 9 indicates significant adsorption of 2-hydroxybenzoic acid (salicylic acid) and negligible adsorption of 3- and 4-hydroxybenzoic acids on the nylon and PVDF filters tested. Salicylic acid is a hydrogen donor that may contribute to hydrogen bonding with the amide functional groups in nylon-66 membrane filter material. Hydrogen bonding can contribute to the adsorption losses observed with this filter product versus a variety of acidic compounds. In contrast, the hydrophilic PVDF membrane filter matrix may have fewer active sites available for interaction with acidic compounds such as salicylic acid or sodium saccharin. Losses observed using PVDF may be related to electrostatic attraction generated between the analyte and filter material (1).

The potential membrane filter adsorption problem was initially observed in some commercial pharmaceutical formulations containing potassium salicylate (3) and was confirmed by testing standard solutions containing the same analyte. Adsorption of the salicylate by the nylon membrane filter was observed with and without the presence of a sample matrix and was found to be significant in both cases (a 5% loss in the sample).

Membrane filter adsorption effects can be reduced by saturating the filter with a few milliliters of sample solution during the injection step. Filter extractables are removed during this process, and the adsorptive sites are gradually occupied by the sample matrix ingredients. When the available active sites are occupied, additional filtration will no longer reduce adsorption effects (1). The compound of interest may be tested by injecting a standard solution or the sample solution into the liquid chromatograph with and without filtration in order to observe any response differences. If significant losses are observed, consideration should be given to the use of the same type of membrane filter material from another manufacturer or the choice of a different membrane filter material.

Conclusion

Analyte loss is commonly encountered via membrane filter adsorption of acidic, basic, and neutral compounds of pharmaceutical interest. Acidic compounds exhibit the greatest adsorption losses, basic compounds exhibit an intermediate amount, and neutral compounds exhibit the least losses.

The filter materials included in this study (CA, nylon-66, and PVDF) were those commonly used in our laboratory for pharmaceutical applications. Potential filter adsorption may also apply to other filter materials currently in use, such as PTFE, polysulfone, and polypropylene.

The primary objective of this project was to determine the extent of membrane filter adsorption with regard to some common analytes encountered in the analysis of pharmaceuticals by HPLC. This study was also conducted in order to raise awareness of this potential problem with respect to the use of HPLC as a determinative step and, finally, demonstrate how to minimize adsorption effects if encountered.

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References

1. T.D. Brock. *Membrane Filtration: A Users Guide and Reference Manual*. Science Tech, Madison, WI, 1983, pp 20, 80, 254, and 272.
2. M. Cheryan. *Ultrafiltration Handbook*. Technomic Publishing Co., Lancaster, PA, 1986, pp 27, 35, 39, and 43.
3. J.M. Johnson and C.H. Harris. Selecting the most effective filtration media for HPLC analysis of saccharides. *J. Chromatogr. Sci.* **25:** 267–69 (1987).
4. A. Riva. La filtrazione in HPCL. *Inostri Servizi Speciali (ICP)* **14:** 41–46 (1986).
5. J.C. Merrill. Avoiding problems in HPLC through filtration. *Am. Lab.* **19:** 74–891 (1987).
6. T.H. Meltzer. *Filtration in the Pharmaceutical Industry*. Marcel Dekker, New York, NY, 1987, pp 174–81.
7. A. Otsuki and K. Fuwa. Identification of an organic compound leached from a membrane filter. *Talanta* **24:** 584–86 (1977).
8. J.W. Munson. Analytical techniques in dissolution testing and bioavailability studies. *J. Pharm. Biomed. Anal.* **4:** 717–724 (1986).
9. M.L. Mayer. Selecting filters for chromatographic applications. *LC–GC* **14:** 902–905 (1996).
10. D.E. Guilfoyle, R. Roos, and S.L. Carito. An evaluation of preservative adsorption onto nylon membrane filters. *Lab. Info. Bull. No. 3400*. Vol 5. U.S. Food and Drug Administration, Rockville, MD, 1–18 (1989).
11. M. Carlson and R.D. Thompson. Adsorption losses of some pharmaceutical compounds during membrane filtration with nylon-66. *Lab. Info. Bull. No. 2887*. Vol. 1. U.S. Food and Drug Administration, Rockville, MD, 1–6 (1985).
12. M. Carlson and R.D. Thompson. Determination of p-aminobenzoic and salicylic acid salts in pharmaceuticals by high-performance liquid chromatography. *J. Liq. Chromatogr.* **10:** 997–1009 (1987).

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