CHROM. 4472

Porous glass as an adsorbent in thin-layer chromatography

A limited study of porous glass (Corning, Code 7935) was undertaken to determine whether initial results warranted further investigation of its potential use as another suitable TLC adsorbent. The intent of this preliminary study of porous glass adsorbent was to ascertain some of its properties, characteristics, and behavior in chromatographing selected pharmaceuticals by the "open column" method.

The literature shows a moderate number of published methods utilizing porous glass for chromatography, mostly gas–liquid and gas–solid procedures. Methods dealing with its use as a TLC adsorbent are very few. Porous glass, fused in the form of plates (Corning, Code 7930), has been used to characterize water-soluble inks[1]. The authors showed that treating the porous glass plates with acidic fluoride solutions or with boiling methanol produced a medium which gave chromatographic separations different from those with untreated porous glass. Powdered glass was used as a TLC adsorbent to separate three dyes. Comparison of results with those obtained by using silica gel and aluminum oxide showed differences in R_F values[2]. A publication in 1964 described the chromatography of three waxes, using porous glass as a TLC adsorbent[3]. The adsorbent was made from porous glass plates (Corning, Code 7930) ground to 200–250 mesh, mixed with plaster of Paris, and applied to plates in the manner described by STAHL[4]. Beeswax, bayberry wax, and spermaceti were compared for TLC development on the ground porous glass adsorbent, Silica Gel G, and aluminum oxide. Results indicated that porous glass produced more spots which were equal to or more distinct than those with Silica Gel G or aluminum oxide. ROUSER *et al.* separated beef brain lipids by two-dimensional TLC with porous glass adsorbent[5]. Three additional methods used porous glass to separate lipids, sugars, and phenols*.

Porous Glass Adsorbent (Corning) is the product of an intermediate phase in the manufacturing process for Vycor® glass. The borosilicate is treated with acid to leach out most of the boron. The process creates a porosity with diameter size of 30–40 Å and a surface area of 200–350 m^2/g. This results in an opalescent product of about 96% silica which is particle sized to about 300 mesh, and which has 24% by volume of void space and a pH of about 4.7 as a 10% aqueous slurry (10% aqueous slurry of Silica Gel G has a pH of about 5.8). Acidic silanol groups produce the surface phenomena; these form hydrogen bonds with electron-donating groups, making it possible to separate acidic, unsaturated, and neutral compounds[6].

Development time with organic solvents in many cases is several times faster than with other conventional adsorbents. Development time for porous glass with water as solvent is about equal to that for Silica Gel G. A 1. in × 3 in. microplate takes about 4 min to develop, using chloroform or ether. The coating can be heated to 450° without change of structure or properties. The product is said to adsorb ambient gases, vapors, and smoke, which may be removed with a 10% spray of hydrogen peroxide followed by reactivation[6]. Binders recommended for plate application are finely divided silica particles (Cabo-sil®, Cabot Corporation, Boston, Mass.), colloidal silica (Ludox SM®, E. I. duPont de Nemours and Company, Inc.), or Boehmite

* Received from Research Department, Corning Glass Works, Corning, N.Y. 14830, U.S.A.

alumina. Calcium sulfate is also used when more water is desired in the adsorbent[6]; however, it is thought to decrease surface area by plugging up some of the pores, resulting in a decrease of load capacity[7].

This study did not include quantitative work, but it has been reported that desired compounds can be determined in the presence of the adsorbent, after zones of interest are scraped off, because settling occurs readily, leaving no suspension in solvent[6]. However, one communication indicated that in eluting compounds from adsorbent, yellow pigments were obtained and caused interference in subsequent measurements such as IR. The analyst believed that the porous glass was catalyzing the polymerization of solvents to produce colored species and that the chromatography by porous glass was not essentially different from that by silica gel[8]. According to another view, the yellow substances were a result of adsorption of organic contaminants from the atmosphere due to the higher adsorptive activity of porous glass adsorbent[7].

Abrasion resistance is claimed to be higher than that of conventional adsorbents[6]. Experience in this laboratory indicated the opposite to be true, although reasonable care will prevent "dust off".

Rapid settling and low load capacities are reported to be disadvantages of porous glass compared to silica gel or aluminum oxide, but purity, uniformity of pore structure, absence of suspension in eluting solvents, its rigid structure, and its low visible background are advantages for TLC work[7].

Experimental

Initial work tentatively supported the previous report that porous glass TLC was not essentially different from that with Silica Gel G in chromatographing pharmaceuticals. Therefore, the experimental design was restricted to comparison with Silica Gel G TLC. Only plates of 1 in. × 3 in. (microslides) were used since they could be made in large numbers and because it was assumed that TLC phenomena would be essentially identical to those obtained with 8 in. × 8 in. plates, except on a smaller scale.

In preparing plates, 1 part of the absorbent was generally mixed with about 1.1 parts of water when using porous glass containing 12% calcium sulfate binder, or with about 1.3 parts of water when using porous glass containing 3% Boehmite alumina fiber. (In the latter case, the aqueous phase should also contain 8.3 parts of 5% ammonium hydroxide as a deflocculent.) Speed is essential after the slurry has been made to avoid settling problems.

Plates were coated with the Desaga–Brinkmann apparatus to a thickness of about 0.20 mm. (Higher load capacities are obtained with thicker coatings.) The plates were heated at 200° for 30 min to activate the adsorbent and remove all ammonia, then cooled, and stored in a desiccator. Spotting was done with a 10-μl syringe.

Reported solvent systems should be tried first, but it may be necessary to devise one. Suggested load capacities are from 0.1 to 5 μg.

Examples for four pharmaceutical groups follow.

Steroids

0.1 μg each of estrone, estradiol, equilin, testosterone, and progesterone were applied. The solvent system used was benzene–ethyl acetate–water (6 ml : 4 ml : 2 drops).

The spots were visualized by 10% sulfuric acid spray followed by heating at about 300° (hot plate). Spots also show intense fluorescence under UV light. See Fig. 1.

In this example Silica Gel G gave more compact spots, and porous glass with calcium sulfate was second best. Some streaking occurred with both porous glass runs (stretched spots). More polar solvents were tried; they gave tighter spots but with less resolution. Differences of resolution might be due to the degree of adsorbent activation. Both porous glass types showed spots that fluoresced more intensely than on the Silica Gel G plate. A porous glass plate (calcium sulfate) spotted with 10 ng of each of the steroids gave color spots that could still be easily detected.

Barbiturates

1 μg each of amobarbital, mephobarbital, and phenobarbital was applied. The solvent system used was benzene–acetone–methanol–water (8.5 ml:0.75 ml:0.75 ml:1

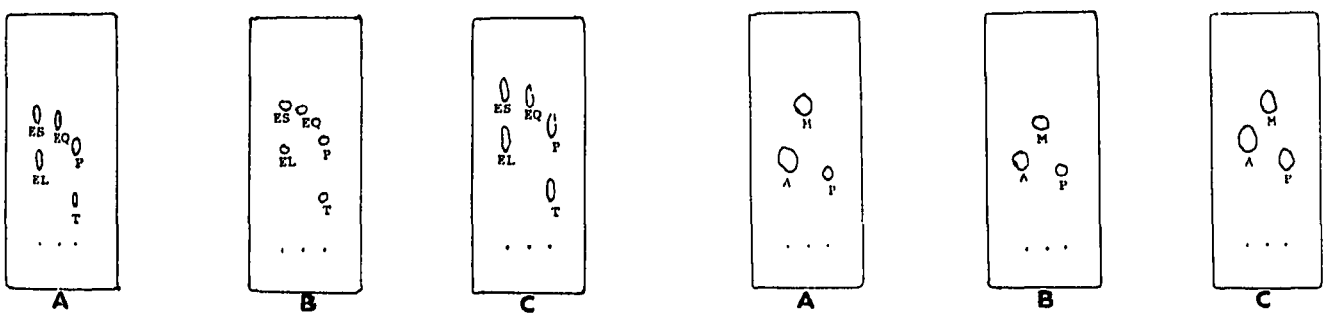

Fig. 1 Fig. 2

Fig. 1. Comparison of TLC of steroids on (A) porous glass with $CaSO_4$, (B) Silica Gel G, and (C) porous glass with Boehmite alumina. ES = Estrone; EL = estradiol; EQ = equilin; T = testosterone; P = progesterone.

Fig. 2. Comparison of TLC of barbiturates on (A) porous glass with $CaSO_4$, (B) Silica Gel G, and (C) porous glass with Boehmite alumina. A = Amobarbital; M = mephobarbital; P = phenobarbital.

drop). The spots were visualized by UV light extinction after a 1 *N* sodium hydroxide spray. See Fig. 2.

Again, the Silica Gel G plate gave tighter spots, but the UV extinction contrast was better with the porous glass plates. Generally there was not much difference between the two adsorbents.

Aspirin, phenacetin, and caffeine

1 μg of each was applied. The solvent system used was water-washed ether–water-washed chloroform–methanol (8 ml:2 ml:2 drops). The spots were visualized by UV extinction for phenacetin and caffeine followed by heating on hot plate (about 300°), after which aspirin fluoresces strongly under UV. See Fig. 3.

It was uncertain whether the better resolution for aspirin using porous glass with $CaSO_4$ was due to differences in activation, binders, or adsorbent characteristics. The test was tried again on all three types of adsorbent microslides after they had been sprayed with a 10% ammonium hydroxide solution and activated at 100° and at 200°, respectively, for 30 min each. The results showed no essential difference from the illustrated chromatograms. Untreated Silica Gel G (no ammonia or heat activation)

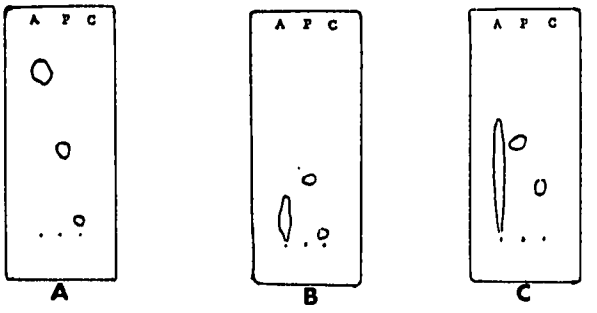

Fig. 3

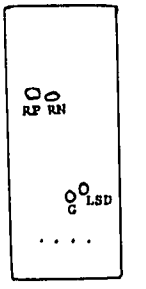

Fig. 4

Fig. 3. Comparison of TLC of aspirin (A), phenacetin (P), and caffeine (C) on (A) porous glass with $CaSO_4$, (B) Silica Gel G, and (C) porous glass with Boehmite alumina.

Fig. 4. Comparison of TLC of the alkaloids reserpine (RP), rescinnamine (RN), ergotamine (G), and LSD on Silica Gel G and on two types of porous glass. Spots did not move with either type of porous glass adsorbent. Only Silica Gel G microplate is illustrated.

again did not resolve aspirin. Contrast in detection by UV extinction was better with both types of porous glass plates than with Silica Gel G. As a note of interest, in one chromatogram where the spotting sequence was A to P to C, aspirin was detected in trace amounts in the phenacetin and caffeine columns even though the spotting syringe was rinsed twice before each compound was spotted. It is estimated that aspirin was detected at a contamination level of less than 0.01 μg.

Alkaloids

0.1 μg each of reserpine, rescinnamine, ergotamine, and LSD was applied. The solvent system used was water-washed chloroform–acetone (2:8). The spots were visualized by UV fluorescence. See Fig. 4.

No movement occurred with either type of porous glass, even when pure acetone was used, reflecting the higher acidity of the porous glass compared to Silica Gel G. Differences in resolution between porous glass and Silica Gel G are indicated for weakly alkaline substances.

Discussion

Porous glass adsorbent showed a TLC behavior essentially similar to that of Silica Gel G for five steroids, three barbiturates, phenacetin, and caffeine. Steroid spots were somewhat elongated when porous glass was used. Differences noted with aspirin appeared to be due to a combination of binder and adsorbent characteristics. Differences for the four alkaloids tested were due to the more acidic nature of porous glass. In all cases, speed of development was two or three times faster with porous glass when organic solvents were used.

Unfavorable aspects of porous glass are its rapid settling, low load capacity, and the reported formation of yellow substances which interfere when the adsorbent is eluted for subsequent quantitative determination (IR). Low load capacity may be due to the relatively small pore size (30–40 Å diameter) which does not contribute much usable surface area to the adsorbent for those organic molecules which are about 10 Å or more in diameter. This large molecular size and surface tension leave little room for those molecules to move in and out of the pore openings. Silica Gel G

is superior to porous glass in chromatographing spots compactly. The two adsorbents are not equivalent.

Porous glass adsorbent may be useful for TLC when a more acidic adsorbent is preferable, when better detection contrast is required, when speed of development is a consideration, or, in certain instances, when resolution with Silica Gel G is unsatisfactory. Further investigation is warranted.

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Enhanced photoemulsion sensitivity at low temperatures used in radiochromatography

Recently a method of detecting tritium and radiocarbon in thin-layer radiochromatography has been developed. By adding scintillators to the thin-layer media, the small β particle energies are converted into light (β-radioluminescence)[1,2]. The detection sensitivity is greatly increased by lowering the temperature when detecting the light by photographic methods[3,4] but not by photomultiplier detection[2,5]. Upon lowering the temperature from 20° to −78° in the applied scintillators, anthracene and 2,5-diphenyloxazole, an increase in detection sensitivity less than 5% is found by photomultiplier detection, while for photographic detection a factor of *ca.* 25 is quoted for the sensitivity increase.

Thus we conclude that the film material, which in fact has been cooled down together with the radiochromatograms, is responsible for the main temperature variation in the overall detection sensitivity. In the film emulsions, back reactions might be prominent, either reducing the extent of latent image formation or producing