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CHARACTERISTICS OF GLASS BASED PACKINGS AS A SUPPORT IN CHROMATOGRAPHY

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SUMMARY

The preparation and properties of glass beads as a support for gas–liquid chromatography was studied. A comparison between diatomaceous earth type supports and glass beads was made in terms of activity to polar components. The procedure originally suggested by Grob was used to determine specific solute–surface interactions. We unexpectedly found it difficult to deactivate glass beads by conventional means. This is possibly due to the large number of silanol groups remaining on the surface of the support after deactivation. Several methods of deactivation were evaluated.

INTRODUCTION

Glass has been used widely as a support in gas–liquid chromatography (GLC), mainly as a support for open tubular columns (OTC). Numerous publications have dealt with the topic of glass surface modification for gas chromatography and much is known about deactivation and coating of glass[1,2]. One such support for packed column GLC is glass beads. Since much is known about surface modification of glass, glass beads should serve as an excellent support. Glass composition can be well controlled and this material should be superior to diatomaceous type supports since the latter have batch-to-batch variations and contain many metal impurities. Even with these drawbacks, diatomaceous earth type supports remain the overwhelming choice as a support for packed column GLC.

Diatomaceous type supports in packed column GC have a checkered history in terms of deactivation. It has been found to be quite difficult to prepare well deactivated packed columns. Trace analysis of polar components often requires columns with near to perfect deactivation. The inability to prepare sufficiently inert supports is one of the factors that has brought about a decrease in the use of packed columns.

Glass beads have several advantages over other supports. One is that columns packed with glass beads have higher permeabilities than diatomaceous earth type supports[3]. Another is that glass beads of narrow size distribution and spherical shape should also decrease the eddy diffusion term of the van Deemter equation when compared to irregular shaped particles of similar dimensions and thus decrease plate

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height. However, glass beads as a chromatographic support is not without limitation. Early work with glass beads demonstrated poor efficiency due to phase pooling at the contact points of the beads[4,5]. Efforts were made in the early sixties to stabilize stationary-phase coatings by surface roughening techniques but development was discontinued after having achieved only modest success[6,7]. A new concept to overcome the most serious limitations of glass beads, lack of adequate phase loading and film instability, has recently been introduced[8]. From a practical point of view, columns packed with stationary phase stabilized glass beads should occupy a niche between OTC and classical packed columns. This paper deals with the preparation of spherical glass packings and compares their performance to other chromatographic supports, in particular, diatomaceous earth.

EXPERIMENTAL

Materials

Glass beads (Alltech Assoc., Applied Science Labs., Deerfield, IL, U.S.A.; Ferro, Cataphot Division, Jackson, MS, U.S.A.; Phase Separations, Norwalk, CT, U.S.A.), Chromosorb (Johns-Manville, Denver, CO, U.S.A.), and Ultrabond® (Supelco, Bellefonte, PA, U.S.A.) were the supports used in this evaluation. Activity testing of uncoated supports was performed by a coupled column technique, as described previously[9]. The precolumn was a 5 m × 0.53 mm I.D. OV-1 fused-silica OTC (2.65 μm film thickness, Hewlett-Packard, Palo Alto, CA, U.S.A.). Packed columns were prepared from 0.33 m × 1.1 mm I.D. deactivated Pyrex tubing using the procedure suggested by Rijks[10]. The columns were tested (Grob test mix II, Fluka, Ronkoukoma, NY, U.S.A.) following standard procedures[11]. The chromatograph used for the evaluation was a Hewlett-Packard 5890.

Sample capacity was determined by injecting variable amounts of *n*-hexadecane in hexane at a column temperature of 120°C. Overloading was reached when the leading edge of the peak at 10% peak height divided by the tailing edge approached a value of 1.1. *H versus u* curves were obtained on a 1.7 m × 1.1 mm column packed with 3% OV-1 on 80–100 mesh Chromosorb W HP and a 1.0 m × 1.1 mm column packed with 1.5% (three coatings of 0.5%) OV-1 on 80–100 mesh glass beads. The standard used was *n*-hexadecane in hexane with a 1:20 split at a column temperature of 160°C.

Elemental analysis

The glass beads and Chromosorb were digested in a PTFE beaker using 48% HF on low heat overnight. The remaining solution was evaporated to near dryness and diluted with 3 *M* HNO_3 (distilled in-house) and heated to dissolve the residue. The solution was then diluted to 50 ml in a volumetric flask and immediately transferred to a polyethylene bottle. Appropriate standards were prepared and elemental analysis was performed on a Perkin-Elmer 5500 ICP following standard procedures[12].

Etching

Glass beads were washed at room temperature with a 3 *M* NaOH solution that was prepared from a mixture of distilled water and ethanol. Glass beads were etched

with either KHF_2 (Alfa Products, Danvers, MA, U.S.A.), HF, or NH_4HF_2 (BDH Chemicals, U.K.). Solutions in the range 0.1–20% were prepared with distilled water. Etching was performed in a PTFE beaker at temperatures ranging from room temperature to 100°C. The solutions were constantly stirred using a PTFE coated magnetic stirring bar. Etching times ranged from 10 min to 1 h. After etching, the beads were rinsed with distilled water and dried. When etching was performed with KHF_2 the glass beads were washed with a 50% H_2SO_4 solution.

Leaching and dehydration

Glass beads were leached with HCl of varying concentrations ranging from 0.1 to 20%. The temperature range varied from room temperature to 160°C and exposure times varied from a few minutes to overnight. During leaching, the glass beads were placed in 6 in. × 3 in. Pyrex glass vessels and sealed under a vacuum. After leaching, the beads were washed with 50-ml aliquots of 20, 10, 5, 1 and 0.1% HCl. In some situations the glass beads were rinsed with a 0.3% solution of H_3PO_4 after leaching. The beads were then dried and subjected to immediate dehydration under vacuum at 200°C for 2 or 3 h.

Deactivation

Glass beads were deactivated with either hexamethyldisilazane (HMDS, Aldrich, Milwaukee, WS, U.S.A.), octamethylcyclotetrasiloxane (D_4), or polymethylhydrosiloxane (PMHS, Petrarch Systems, Bristol, PA, U.S.A.) following standard procedures[2,13–15]. The glass beads and appropriate amounts of deactivating agent were sealed under vacuum in a Pyrex vessel. A range of temperatures were examined. The H_3PO_4 washed beads were deactivated with HMDS and PMHS. After deactivation, the beads were washed and dried. Glass beads were also deactivated with a non-extractable layer of Carbowax 20M (Supelco, Bellefonte, PA, U.S.A.), following the method suggested by Daniewski and Aue[16].

Cab-O-Sil® HS-5 (Cabot, Tuscola, IL, U.S.A.) was deactivated according to Rutten *et al.*[17,18] and Silanox Grade 101® (Cabot Corporation, Tuscola, IL, U.S.A., now marketed as Tullanox, Tulco, North Billerica, MA, U.S.A.) also gas phase deactivated with Carbowax 20M[19]. In this procedure a glass injector sleeve was packed with 15% Carbowax 20M on 80–100 mesh Chromosorb W AW DMCS and placed in the injector of a GC. The Silanox® was loosely packed in a short length of Pyrex tubing and connected to the injector. The injector and oven were heated at 280°C overnight with helium flowing through the column. After deactivation, the Silanox was washed with methanol.

Coating

Glass beads were coated with either OV-1, OV-101-OH, (Ohio Valley Specialty Chemical, Marietta, OH, U.S.A.), or OV-1-OH (prepared according to Blum[20]) using the layering technique previously described[8]. After coating with OV-101-OH, the glass beads were heated from 100 to 380°C and kept at the final temperature for 12 h. The OV1-OH gum was treated in a similar fashion except that the upper temperature was 330°C.

RESULTS AND DISCUSSION

Chromosorb supports show a large range in batch-to-batch elemental composition. This is particularly prevelent among the two different types of chromosorb, pink and white. These supports contain a significant amount of metal impurities even after extensive acid treatment[21]. Elemental determination of bulk composition of two types of Chromosorb supports is presented in Table I. Fig. 1 presents chromatograms of the test mixture on various Chromosorb supports that were obtained from commercial sources. All supports show activity, except for the Ultrabond variety, which is a Carbowax 20M deactivated material[22]. Large differences in terms of chemical composition and shape have also been found among different manufacturers of glass beads. This is not surprising since some of the materials tested were intended for industrial use. The differences in bulk composition of three different glass beads sources is presented in Table I. Glass beads for chromatography should be made from high quality glass of well defined composition and should contain few irregular shaped beads.

Etching and leaching of glass surfaces is relatively well understood[1,2]. Etching has traditionally been carried out on glass to induce surface roughening so that polar stationary phases could be coated efficiently. We adapted this technology to glass beads to increase surface area and allow for higher initial phase loading. The use of high concentrations of HF resulted in the dissolution of the glass. When the concentration was lowered, etching could not be observed. Little or no roughening occurred at intermediate concentrations. In contrast to gaseous HF, which is very effective[23], aqueous HF can obviously not be used to induce roughening. NH_4HF_2 produced no surface roughening at any concentration or temperature but KHF_2 was found to produce coarsely roughened surfaces. It was found that the surface was not etched by removal of glass but was roughened by deposition of a layer of K_2SiF_6[24]. The layer was easily disrupted with strong stirring of the solution. Treatment with dilute H_2SO_4 readily removed the layer. The resulting surface was very active.

Leaching provides a surface composed of a xerogel which constitutes an ideal, metal-free, silanol rich surface that can be adapted to chromatography. Even though the bulk composition of glass beads contains a significant number of metals, leaching should remove these metals from the surface of the glass. Studies have shown that leaching forms a surface depleted of most metals[25,26] and that the bulk metals do not migrate to the surface under ordinary chromatographic conditions[25]. Standard pro-

TABLE I

ELEMENTAL COMPOSITION OF SUPPORTS

Support	*Na*	*K*	*Mg*	*Ca*	*B*	*Al*	*Fe*
Glass beads A	6.5	–[a]	2.9	6.9	–	0.2	0.2
Glass beads B	6.9	–	2.7	7.9	–	0.3	0.2
Glass beads C	3.8	8.0	0.1	0.2	0.3	0.4	–
Chromosorb P NAW	–	–	0.4	–	–	2.2	1.1
Chromosorb W AW	13.5	3.0	0.6	–	–	2.1	0.8

[a] Could not be detected.

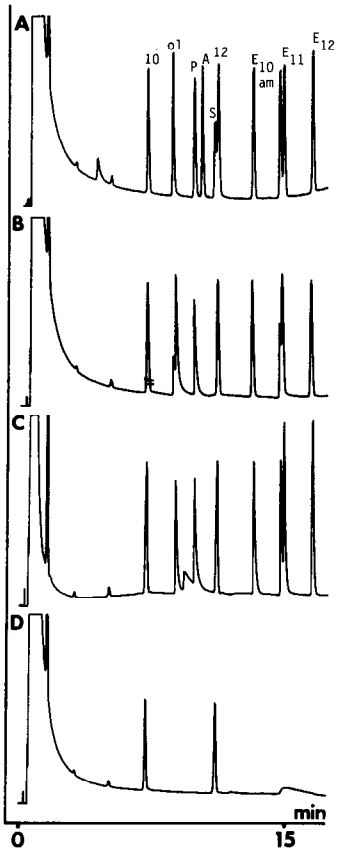

Fig. 1. Chromatograms of test mixture on: (A) Ultrabond; (B) Chromosorb G AW DMCS; (C) Chromosorb W AW DMCS; (D) Chromosorb W AW. Peaks: 10 = decane, ol = 1-octanol, P = 2,6-dimethylphenol, A = 2,6-dimethylaniline, S = 2-ethylhexanoic acid, 12 = dodecane, am = dicyclohexylamine, E_{10}–E_{12} = methyl esters of C_{10}–C_{12} fatty esters.

cedures for leaching capillary columns involve overnight heat treatment at 110–150°C with a 20% metal-free HCl solution[2,25]. The glass beads did not withstand these conditions. The surfaces changed drastically in appearance, showing extensive attack. These conditions are believed to remove most of the metal impurities but leave a very thick hydrated layer that collapses during dehydration. Beads were found to contain small fractures even under less severe conditions. Leaching under very mild conditions, *e.g.* with 5% HCl for 1 h at 110°C, produced smooth surfaces. Excessive leaching, as indicated by surface distortion, was not only related to time and temperature, but also to concentration. Beads treated at temperatures lower than 90°C were insufficiently leached and could not be deactivated.

The deactivation of glass beads with commonly used agents such as HMDS, D_4, or PMHS would appear to be quite routine. The gas phase deactivation with HMDS should yield a surface where most silanol groups have been converted to the corresponding trimethylsilyl moieties. The deactivation of the glass tubing used for

the preparation of test column material was straightforward and yielded perfectly inert surfaces. To our surprise, the use of these agents did not render the surface of the glass beads nearly as inert, in spite of careful optimization of the individual steps. Some residual activity, which most likely is due to remaining silanol groups, always remained. Fig. 2 presents chromatograms of the test mixture with HMDS deactivated glass beads as a function of column length. As the column length was increased overall, activity also increased. The glass beads were also subjected to multiple HMDS treatments. One batch was found to exhibit increased deactivation for the second treatment and an overall decrease in deactivation for the third treatment. D_4, a cyclic octamethyltetrasiloxane, was applied in an inert atmosphere and also in an oxygen atmosphere as suggested by Xu *et al.*[15]. No decrease in activity was observed.

Deactivation with PMHS was also somewhat disappointing. An attempt was made to wash the beads with a 0.3% H_3PO_4 solution after leaching[2]. The beads proved to be extremely sticky after washing with H_3PO_4. After washing with H_3PO_4 and dehydrating, the beads were deactivated with either HMDS of PMHS. Deactivation of these beads with HMDS failed, but deactivation with PMHS provided almost perfect deactivation. This is believed to be due to H_3PO_4 serving as an acid catalyst. Fig. 3 presents a chromatogram of the test mixture with PMHS deactivated

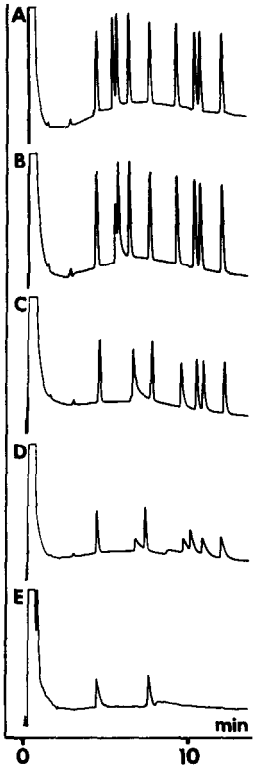

Fig. 2. Chromatograms of test mixture on HMDS deactivated glass beads of various lengths: (A) 4 cm; (B) 8 cm; (C) 14 cm; (D) 28 cm; (E) 50 cm. Preparation conditions: 80–100 mesh glass beads cleaned with NaOH–ethanol and washed with 50-ml aliquots of 20, 10, 5, 1 and 0.1% HCl; dehydrated at 200°C for 2 h.

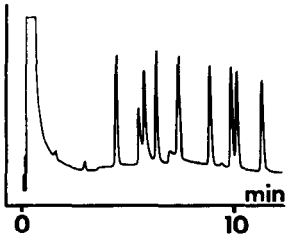

Fig. 3. Chromatogram of test mixture on glass beads washed with 0.3% H_3PO_4 and deactivated with PMHS.

glass beads. After PMHS deactivation the beads were still extremely tacky. H_3PO_4 has been found to be undesirable because it attacks the glass surface[25].

Another deactivation procedure which consists of deactivation with Carbowax 20M proved to be very effective in reducing the surface activity. These beads exhibited excellent deactivation as shown in Fig. 4A. Unfortunately, the stability of the Carbowax 20M layer is questionable. It is known that Carbowax 20M deactivated supports are not stable above 300°C and are very susceptible to degradation by traces of oxygen[16]. We decided to test the Carbowax 20M deactivation for different time periods and temperatures. Stability testing revealed that deactivation deteriorated slightly after heating for 2 h at 280°C. Raising the temperature to 300°C for short periods of time did not cause noticeable deterioration. Prolonged heating at 320°C caused serious disruption of the protective layer as evidenced by severe tailing of all components, as shown in Fig. 5. It is possible to use Carbowax 20M deactivated beads at temperatures up to 300°C for only short periods of time.

The coating procedure previously advocated[8] was found to be very effective in providing an even layer of phase. Silanox proved to be an excellent means for removing the stickiness of individual particles and producing free-flowing glass beads. Attempts were also made to directly deactivate the surface of the glass with OV-101-OH. It was observed that a new substance had actually been formed, which appears to be a

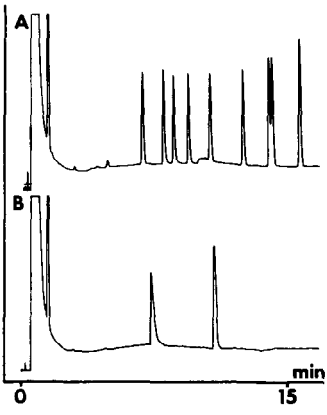

Fig. 4. Chromatogram of test mixture on: (A) Carbowax 20M deactivated glass beads; (B) after dusting with 4.3 mg of Silanox.

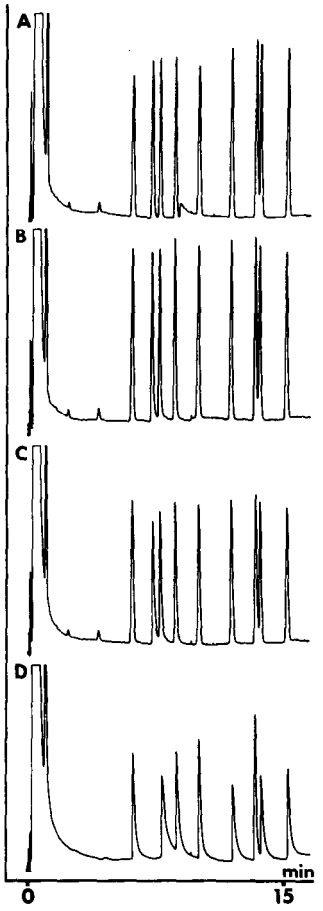

Fig. 5. Chromatogram of test mixture on Carbowax 20M deactivated glass beads after heating for 2 h at: (A) 220°C; (B) 280°C; (C) 300°C; (D) 320°C.

cyclosiloxane[27]. When the gumified OV-101-OH phase was used, the glass beads were not deactivated as well and no cyclosiloxanes were observed.

Silanox, which is produced by gas phase deactivation of Cab-O-Sil HS-5 with HMDS, was found to be very active. This activity was demonstrated in an experiment where Carbowax 20M deactivated beads were dusted with only 4.3 mg of the Silanox. Fig. 4B shows a chromatogram. The observed activity is surprising in view of the extensive literature that appeared in the early seventies for Silanox-modified glass capillary columns[28–32]. These columns were mainly used for the analysis of steroid mixtures and critical activity testing was obviously not performed. Attempts to deactivate fully hydroxylated Cab-O-Sil with HMDS failed. The Cab-O-Sil clumped together during the drying–dehydration process. It was not possible to control the particle size. An attempt was made to use a gas phase Carbowax 20M procedure to deactivate Silanox. The Silanox still exhibited some activity after this treatment and the activity reappeared after washing with methanol.

TABLE II

SAMPLE CAPACITY OF 80–100 MESH GLASS BEADS WITH 1–5 COATINGS OF OV–1

No. of coatings	*Sample capacity (ng)*
1	130
2	300
3	400
4	600
5	900

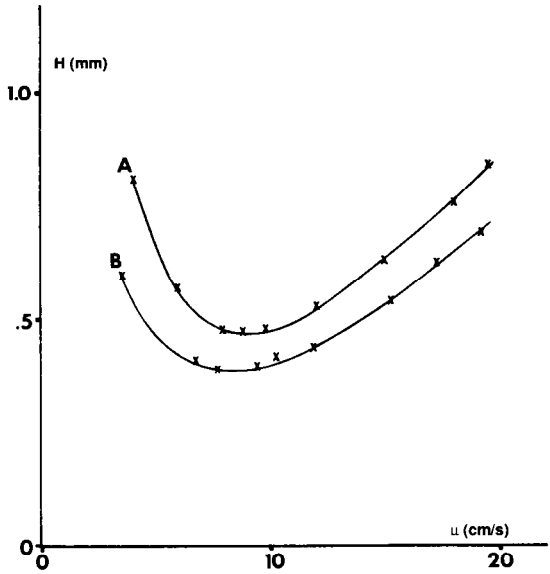

Fig. 6. *H vs. u* curve for: (a) 3% OV-1 on 80–100 Chromosorb W HP; (B) 1.5% (three Coatings) OV-1 on 80–100 mesh glass beads.

The major advantage of packed columns over OTC is their large sampling capacity. Table II presents the sample capacities for glass beads at different phase loadings. As expected, sample capacity increases significantly as the phase loading is increased. In terms of efficiency, glass beads should be superior to diatomaceous earth type supports of comparable size. *H versus u* values of glass beads were found to be smaller than those of Chromosorb, as seen in Fig. 6.

CONCLUSION

The deactivation of capillary tubing produced a well deactivated surface, but the deactivation of glass beads has unexpectedly proved to be very difficult. Common deactivation agents cannot render a surface perfectly inert[33]. The introduction of new deactivation techniques that could produce more inert surfaces should make this support more popular, especially for preparative column GLC.

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