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Affinity Techniques

Enzyme Purification: Part B

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[4] Porous Glass for Affinity Chromatography Applications

By H. H. WEETALL and A. M. FILBERT

This report deals with "methods" of attaching ligands to inorganic carriers for affinity chromatography.

An ideal carrier for the affinity chromatography of enzymes should possess a number of desirable characteristics. It should exist as a porous network to allow rapid movement of large macromolecules throughout its entire structure and be uniform in size and shape to exhibit good flow properties. The surface area of the carrier would be necessarily large to allow attainment of a high effective concentration of coupled inhibitor, and the support surface should exhibit little specific attraction for proteins. The material should be mechanically and chemically stable to various conditions of pH, ionic strength, temperature, and the presence of denaturants. The availability of controlled-pore glass provides a material that fulfills many of the above-mentioned "ideal, support criteria," and presents a new and unique carrier for use in affinity chromatography applications.

Preparation of Controlled-Pore Diameter Glasses

Certain sodium borosilicate glass compositions exist (see Fig. 1), which, after heat treatment, can be leached to form a porous glass framework.¹ During heat treatment the base glass separates into two intermingled and continuous glassy phases. One phase, rich in boric oxide, is soluble in acids; the other phase is high in silica and is stable toward acid solutions. The boric acid-rich phase may be leached out of the glass, leaving a porous structure of very high silica content. Porous diameters for these glasses are in the range of 30 to 60 Å, and the pore volume is approximately 28% of the total sample volume.

Larger-pore glasses can be prepared from the same sodium borosilicate glass compositions. After the heat treatment and acid leaching steps, a mild caustic treatment enlarges pore diameters by removing siliceous residue from pore interiors.² By carefully controlling the various physical and chemical treatment parameters, glasses are produced which exhibit extremely narrow pore size distributions, as shown in Fig. 2. The con-

¹ M. E. Nordberg, *J. Amer. Ceram. Soc.* **27**, 299 (1944).

² H. P. Hood and M. E. Nordberg (1940). U.S. Patent No. 2,221,709, "Borosilicate Glass."

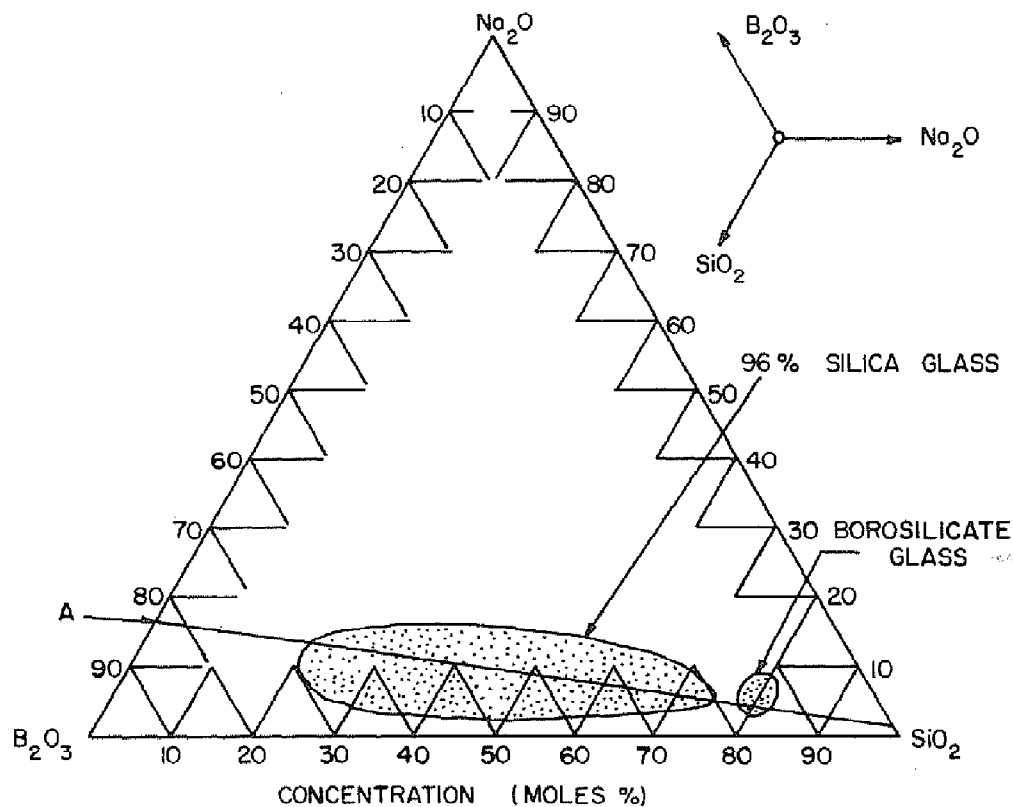


FIG. 1. Phase diagram of sodium borosilicate system.

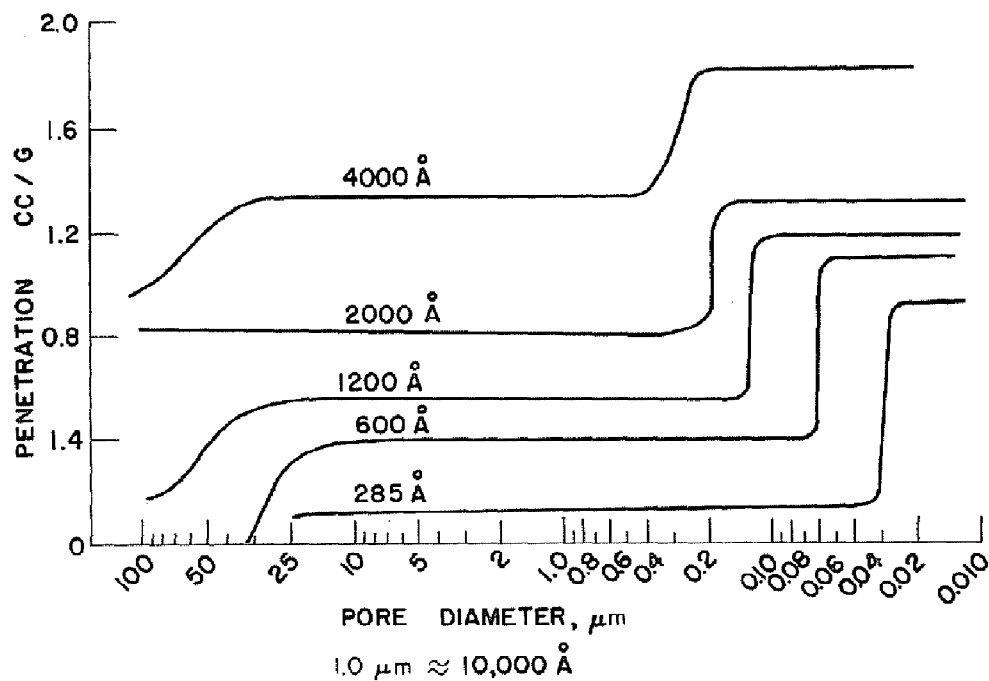


FIG. 2. Pore size distribution of controlled-pore diameter glass supports. All values were determined by the mercury intrusion method.

trolled-pore glass, thus produced, is a 96% silica glass, with 3–5% B_2O_3 content, and trace quantities of several metal oxides.

Surface Area of Controlled-Pore Glass

The surface area of controlled-pore glasses is a function of pore diameter and pore volume. Surface area increases with increased pore volume and decreases with increasing pore diameter, as is shown by the data in the table.

Surface Properties

The surface properties of controlled-pore glass are greatly affected by the thermal history of the glass, heat treatment playing a significant role in determining the ratio of adjacent to single hydroxyl groups which exist on the glass surface. Infrared spectra of the glass change as a function of temperature. On a wet silica surface three bands can be identified as 3747, 3660, 3450 cm^{-1} , and 1650 cm^{-1} . The bands at 3450 and 1650 cm^{-1} are typical of the vibrations associated with molecular water. When temperature is raised to about 150°, these two bands disappear, but the bands at 3747 and 3660 cm^{-1} become more evident. As the temperature is raised further, the band at 3660 cm^{-1} slowly disappears. At 500° it is observed only as a tail on the low frequency side of the 3747 cm^{-1} band. At 800°, only the band at 3747 cm^{-1} remains. These bands are attributed to the vibrations of, respectively, surface hydroxyl groups spaced sufficiently far apart that they do not interact with each other, surface hydroxyl groups so close together they are hydrogen-bonded to each other, and molecular water physically adsorbed on the surface of the silica (Fig. 3).

The rehydration of a silica surface depends on its previous thermal

CONTROLLED-PORE GLASS PHYSICAL PROPERTIES

Pore diameter (Å)	Pore volume SA (M^2/g) at PV = 0.70	cc/g 1.00
75	249	356
125	149	214
175	107	153
240	78	111
370	50	72
700	27	38
1250	15	21
2000	9	13

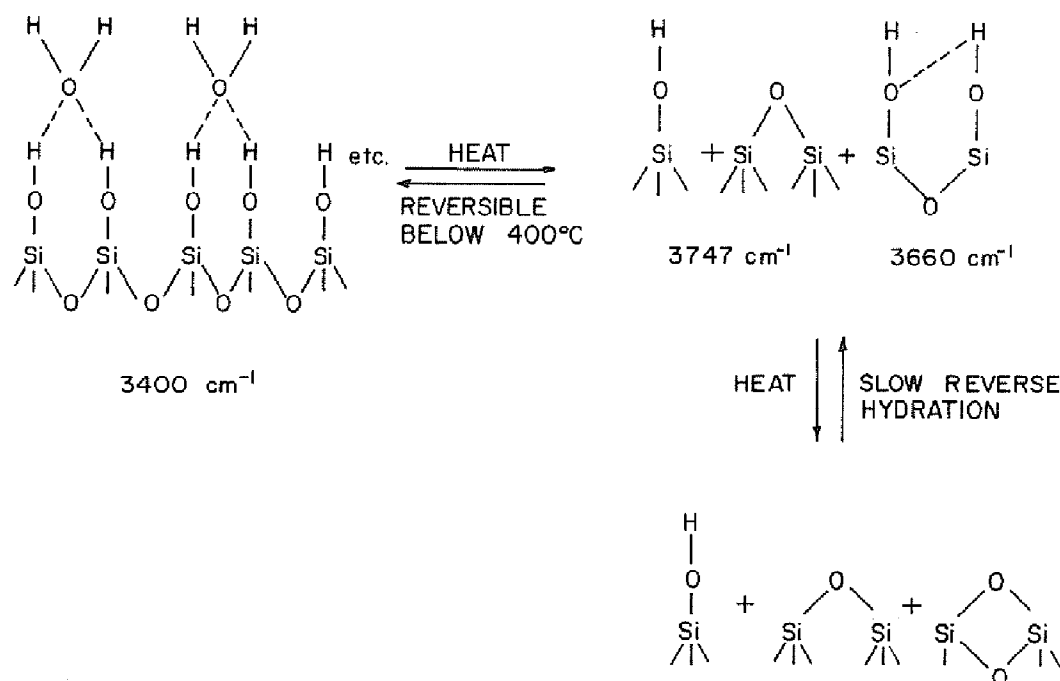


FIG. 3. Dehydration of a silica surface.

history. Up to about 400° , the dehydroxylation of the surface is reversible, but above this temperature the removal of the adjacent hydroxyl groups from the surface causes the silica to become hydrophobic. Indeed, after extensive heating at 850° , a controlled-pore glass surface will not readily adsorb water unless the hydroxylated surface is reconstituted by a drastic treatment such as boiling in concentrated nitric acid. In this case, the water adsorption is associated with the hydrogen-bonded hydroxyl groups and occurs on these groups in preference to the freely vibrating group. In fact, removal of the freely vibrating group from the surface by silanization techniques has little effect on the water adsorption that occurs at low partial pressures.

In addition to the surface silanols, the presence of boron in the glass results in the formation of surface Lewis acid sites as shown in Fig. 4. Although B_2O_3 analysis for the bulk glass is only in the order of 3% with a B:Si ratio of 1:20, infrared studies indicate a much higher surface concentration of boron. Thus, the heat treatment and leaching procedures not only increase surface area of the glass, but also change its surface composition. One now finds a surface boron concentration of 2.4×10^{20} boron atoms per gram of glass and 8 surface silanol groups per square millimicron. The resulting porous glass, with its surface hydroxyls and Lewis acid sites, exhibits a slight negative charge in aqueous media, and this in large part explains the strong adsorption of basic materials.

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