PRODUCTION OF MODIFIED SILICAS FOR THE ADDITION OF BIOLOGICALLY ACTIVE COMPOUNDS

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Recently, the use of water-soluble carriers for the addition of various biologically active compounds and especially enzymes has received great development [1, 2]. Inorganic matrices, including porous glass, are widely used for the fixation of enzymes [3, 4]. It should be noted that the reaction of modification of inorganic carriers for the addition of enzymes, as well as methods of functional analysis of the sorbents obtained, have been insufficiently studied.

In this work, as the initial inorganic matrix we used macroporous silica silochrome [5], which is characterized by great chemical purity (99.5% SiO₂) and a structure of the pores and value of the surface that can be regulated within broad limits. The capacity of the inorganic carrier is determined by the concentration of OH groups, for the measurement of which the ability of the protonated hydroxyl groups to exchange a H⁺ ion for Ca²⁺ from a solution of Ca(OH)₂ was used [6]. The loss of Ca²⁺ ions is recorded during the titration of samples of the solution (Fig. 1).

The concentration of OH groups on the surface of an industrial sample of Silochrome C-80 is 0.8 × 10⁻³ mole/g, which is two orders of magnitude higher than for Chromosorb P and higher than for porous glasses distributed in the United States. It should be noted that the concentration of OH groups on the surface of Silochrome depends on the conditions of treatment, and in various samples it ranges from 0.2 to 0.9 × 10⁻³ mole/g. To obtain maximum concentration of OH groups, the carrier was rehydroxylated by boiling in water and dried under vacuum at ≤ 200-250 °C.

The modification of Silochrome and production of sorbents with various functional groups were conducted according to the scheme

![Scheme](image)

An important step in the production of fixed biologically active compounds - the modification of the inorganic carrier with the aid of γ-aminopropyltriethoxysilane [7] - was conducted in various organic solvents, as well as in 0.01 N HCl. In all cases we obtained samples of aminoalkylsilochrome (II) with a content of amino group according to the data of elementary analysis and titrimetry within the range of 0.3-0.7 × 10⁻³ mole/g, which is almost an order of magnitude higher than the corresponding indices for porous glasses [8]. For a direct determination of aminoalkyl groups, we used the method used earlier for the determination of protein [9]. The method proved to be comparatively simple and convenient and consists of a determination of the isotope ¹⁴C after acetylation of the aminoalkyl groups of the carrier with ¹⁴C-N-acetoxyxysuccinimide (¹⁴C-NASD). A gas-chromatographic determination of the aminoalkyl groups was also developed. Various methods of determination of the aminoalkyl groups gave close results.

For the addition of biologically active compounds, we obtained silochromes with various functional groups: aminoalkyl (II), aldehyde (III), aminoaryl (IV), and carboxyl (V).

To obtain the carrier (III), Silochrome was treated with 25% glutaraldehyde [8]. In aqueous solutions glutaraldehyde undergoes an aldol condensation [10], which may increase the distance between the matrix and the enzyme, and also convert glutaraldehyde to a polyfunctional reagent. For modification of carriers with glutaraldehyde it is important to observe an optimum ratio between the amount of the aldehyde and that of the aminoxyl groups of the carrier. The concentration of aldehyde groups in the initial glutaraldehyde and on the carrier was determined according to the reaction with hydroxylamine hydrochloride by titration of the HCl liberated [11]. As was found, to obtain a carrier with a large number of aldehyde groups, a very large excess of the aldehyde should be used (Fig. 2).

The carrier with aminoaryl functional groups (IV) was produced by the interaction of aminoalkylsilochrome with p-nitrophenylacetyl chloride, followed by reduction of the nitro group with Na₂S₂O₄ or SnCl₂. The concentration of aminoaryl groups was determined by the spectrophotometric method of analysis of the products of alkaline hydrolysis of (IV) that we developed, based on the production of a diazonium salt from the products, followed by titration of this salt with an alkaline solution of β-naphthol. The sorbent, possessing carboxyl groups (V), was produced by treatment of Silochrome (II) with an aqueous solution of succinimide anhydride. The carboxyl groups were converted to acid chloride groups by treatment with thionyl chloride.

It is known that porous glasses and silochromes have a tendency to dissolve in alkaline media [12]. The Si content in alkaline eluates was determined by the method of [13] for various sorbents (Fig. 3). As was found, both porous glass and Silochrome C-80 dissolve appreciably, losing up to 5% of the weight of the sample during the experiment. Good stability under the conditions of the experiment was exhibited by Chromosorb P, as well as by Silochrome modified with ZrO₂.

**EXPERIMENTAL METHOD**

In the work we used samples of the carrier Silochrome C-80 (Stavropol' Factory of Luminophores and Especially Pure Substances) with the following characteristics: S ≈ 80 m²/g; dpore 350 Å; ΣVpore 1.5 cm³/g; particle size 0.16-0.25 mm.

The radioactivity of samples labeled with ¹⁴C was measured on a gas-discharge BFL-25 counter with a PS-1000 counting setup. Gas-liquid chromatography was carried out on a Varian 1860 chromatograph with an integrator, a Katharometer detector, on a column 200 x 0.6 cm with 3% SE-30 on the carrier Varaport 30 (100-200 mesh), temperature of column 70°, temperature of evaporator 190°, and velocity of the carrier gas helium 40 ml/min.

For the determination of aldehyde groups we used a TTT-Ic Radiometerautotitrator (Denmark). The spectrophotometric determination of aminoaryl groups was performed on a Spekol spectrophotometer (German Democratic Republic).

**Determination of the Hydroxyl Groups of Silochrome.** A weighted sample of Silochrome (50-500 mg) was mixed in 50-100 ml of freshly prepared 0.027 N Ca(OH)₂. Approximately a fivefold excess of Ca²⁺ was used in the reaction. At 5-10 min intervals, samples were collected and titrated with 0.1 N HCl according to phenolphthalein. The kinetic curve of the absorption of Ca²⁺ ions was constructed according to the results of the titration (Fig. 1). The linear portion of the kinetic curve was extrapolated to the y axis and the ion-exchange absorption of Ca²⁺ determined.

**Production of Aminoalkoxysilochrome (II).** To 10 g of Silochrome C-80 in 50 ml of 0.01 N HCl we added 3.2 ml of γ-aminopropyltriethoxysilane and boiled the mixture with mixing for 10 h. The derivative (II) obtained was washed with 500 ml of water, 100 ml of acetone, and then dried for 1 h in a vacuum-drying oven at 80°. For the aminoalkylsilochrome (II) we found: C 2.55, N 0.98%.