THIO DERIVATIVES OF MACROPOROUS SILICAS.

3. SYNTHESIS OF MACROPOROUS SILICA CHEMISORBENTS WITH GRAFTED POLYMER COATINGS

V. I. Lozinskii and S. V. Rogozhin

We have previously reported the preparation of 2-pyridyl disulfide and o-nitrophenyl disulfide derivatives of macroporous silicas (silochromes) [1, 2], which are used in the chemispecific chromatography [3] of thiol-containing biopolymers. Unfortunately, at basic pH values, the stability of the silica matrix to the hydrolytic action of the aqueous medium is low. In addition, marked nonspecific adsorption on silochromes of substances such as peptides and proteins is observed [4]. Improvements in matrix stability in aqueous media are achieved by modification of the surface using zirconium or aluminum salts [4, 5] and a reduction in the amount of nonspecific adsorption with a concurrent increase in the support's capacity for functional groups is achieved by the addition of a suitable polymer coating to the inorganic surface [6-8].

In the present work, such improved matrices composed of macroporous silica with a surface modified using Al₂O₃ [9] and with grafted polymer coatings were employed for the preparation of chemisorbents with o-nitrophenyl disulfide (S-NPS) groups. The use of a similar type of composite material for the preparation of supports for biospecific chromatography was described recently [10].

Silochrome with 1130 Å pore diameter containing 3% Al₂O₃ was the starting inorganic adsorbent. Then, a polyacrylic acid or polymethyl acrylate polymer coating was grafted onto the inorganic surface by our previous method [7]. After thorough washing to remove soluble homopolymers by extraction for several hours using boiling solvent in a Soxhlet extractor and subsequent drying, we obtained products (I) and (VI). The support with the grafted polyacrylic acid surface (I) contained 6.2% C (1.7 mmole CO₂H groups/g), while the support with polymethyl acrylate coating (VI) contained 8.7% C (1.8 mmole CO₂CH₃ groups/g).

Further modification of the functional groups of the grafted polymers was achieved using the previously proposed S-NPS derivatives of 2-mercaptoprothamine (V) or thioglycolic acid (X) [11]. To add these compounds to the supports, we used activated N-hydroxysuccinimide esters [12]. An S-NPS support (III) was obtained in two steps from (I) with ~140 μmoles S-NPS groups/g, i.e., the modification affected about 8% of the CO₂H groups present under the experimental conditions.

\[
\begin{align*}
&\text{CH}_2\text{COOH} \rightarrow \text{CH}--\text{COOH} \rightarrow \text{CH}--\text{COOSu} \rightarrow \text{CH}--\text{CONHCH}_2\text{CH}_2\text{S}--\text{NPS} \rightarrow \text{CH}--\text{CONHCH}_2\text{CH}_2\text{S}--\text{NPS} \rightarrow \\
&\text{Su--succinimide} \rightarrow \\
&\text{I} \rightarrow \text{II} \rightarrow \text{III}
\end{align*}
\]


0568-5230/81/3008-1547$7.50 © 1982 Plenum Publishing Corporation 1547
A possible reason for such a low yield may lie in the poor wettability or swelling of the polyacrylic acid polymer coating in the solvents employed either in the transesterification step (pyridine) or the aminolysis step (abs. CH₂Cl₂). Such a low yield was not found in the preparation of silochrome chemisorbents with analogous active groups but without a polymer coating using the same solvents for the same synthetic steps [2].

The effect of the correctness of solvent selection in carrying out the heterophase reactions with combined matrices was clearly seen in working out the conditions for preparing chemisorbent (VIII), which was synthesized using support (VI) with grafted polymethyl acrylate according to the scheme

```
CH--COOCH₃
CH₃
(VI)
```

```
H₂NCH₂CH₂NH₂ (IX)
CH--CONHCH₂CH₂NH₂
CH₂
(VII)
```

```
1. NPS--SCH₂COOH(X)+HOSu+DCC
2. CH₂COOSu (XI)
CH--COOCH₂CH₂NH₂
CH₂
(VIII)
```

```
DCC — N,N'-dicyclohexylcarbodiimide  HOSu — N-hydroxysuccinimide
```

Treatment with anhydrous ethylenediamine (IX) was used to introduce amino groups to the insoluble phase. The aminolysis was carried out by heating adsorbent (VI) at reflux in a dioxane solution with a 100-fold excess of (IX). Carrying out this reaction without dioxane, which serves as the solvent for polymethyl acrylate, led to a reduction in the amount of amino groups in (VIII) by a factor of 2-2.5. The adsorbent obtained after the introduction of S-NPS groups to the NH₂-support (VII) by the action of the N-hydroxysuccinimide ester of acetic acid (XI) in order to block the unreacted amino groups. Preliminary experiments showed that acetylation using 1:1 Ac₂O–NEt₃ led to a loss of about 50% of the S-NPS group capacity of the support, while the use of (XI) did not cause a noticeable drop in the amount of these groups. It is interesting that the acetylation virtually does not occur in dioxane (the amount of titrable amino groups did not change), while this acylating agent in CH₂Cl₂ blocked about 3/4 of the NH₂ groups present. This is apparently another case of discrepancy in the interaction of these two solvents relative to the chemically modified polymer coating.

Determination of the functional group content on the supports after each step of the chemical transformations permitted us to estimate the reaction yields (Table 1). This table shows that the addition of (X) to (VII) proceeds in good yield (51%), but some of the NH₂ groups (~40 μmole/g) become unavailable for titration. Further acetylation also incompletely blocks the amine functional groups of the chemisorbent (VIII).

The IR spectra of derivatives (VI), (VII), and (VIII) (Fig. 1) show qualitative differences in the course of modification of the inorganic support. The ester carbonyl stretching band (1730 cm⁻¹) in spectrum 2 of the sorbent with grafted polymethyl acrylate (VI) is clearly seen. After treatment with ethylenediamine, the intensity of this band decreases considerably, though it is not completely absent from spectrum 3, indicating incomplete conversion of all the available CO₂CH₃ groups. Bands appear at 1650 cm⁻¹ (amide I) and 1570 cm⁻¹ (amide II) and also a broad band arises in the vicinity of 3400 cm⁻¹ (vN–H). A small new peak at 1520 cm⁻¹ (vC–NO₂) may be seen after the introduction of S-NPS to the support while the intensity of the 3400 cm⁻¹ band decreases.

The S-NPS group content of (VIII) may be readily altered by varying the reagent excesses and contact time. Thus, for example, an increase in the time of treatment of (VII) with the N-hydroxysuccinimide ester of (X) from 6 h to 3 days at 4°C led to an increase in the content of the desired groups in the insoluble phase from 150 to 245 μmole/g.