



## **CHEMICAL MODIFICATION OF SILICA SURFACE BY BONDING KETOIMINE GROUPS FOR SPE**

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### **ABSTRACT**

The applicability of silica gels, modified with ketoimine groups, in solid-phase extraction was tested. The surface characteristics of the modified silica was determined by elemental analysis, NMR spectra for the solid phases ( $^{29}\text{Si}$  CP MAS NMR), analysis of pore size distribution of the silica support, and nitrogen adsorption-desorption. The newly proposed sorbents with ketoimine groups were used for extraction of endocrine-disruptor compounds, extracted from water and powder milk. The properties of the sorbents were compared with commercial  $\text{C}_{18}$  ones.

Keywords: chemically bonded phases, silica gel, solid-phase extraction (SPE), gas chromatography (GC)

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### **INTRODUCTION**

A vast majority of real samples contain analytes at concentrations that are too low for direct instrumental injection. Preparing a sample by concentrating the species of interest prior to instrumental introduction is often necessary. Currently, solid phase extraction SPE is the most widely used sample preparation method for chromatographic instrumentation [1-4]. SPE becomes a widely used method because it has many advantages, compared with LLE (liquid-liquid extraction). SPE is widely used for the isolation and concentration of various analytes and the clean-up of samples (removal of matrix interferences) in pharmaceutical, clinical, environmental, and food analysis [5-9]. However, the major disadvantage of conventional SPE sorbents, such as RP- $\text{C}_{18}$ , ion exchange and size-exclusion phases, is associated with their inadequate selectivity – matrix interference components

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are co-extracted with the target analytes. Thus, the current research is oriented to developing new, improved sorbents, enabling determination of selected compounds from complex matrices [10,11].

As for the above-mentioned chromatography, extraction, filtration, etc., silica is widely used. Due to the presence of hydroxyl groups in its structure, the silica allows to modify its surface in different ways. More and more new functional groups are being proposed for bonding with silica surface, to improve the physico-chemical properties of silica-based materials, e.g., selectivity. Among other materials, silica modified with ketoimino groups was detected as a particularly useful base for packings for the complexation gas chromatography. Such modified silica has been applied in the separation of such organic compounds as aromatic hydrocarbons, halogenohydrocarbons, and branched aliphatic hydrocarbons [12-14]. Taking into account the chemical properties of silica modified with ketoimino groups, the present authors proposed to apply such packings as sorbents for the SPE technique, to preconcentrate different organic compounds. Specifically, this research work is focused on preconcentration of such harmful compounds as chloroorganic pesticides, alkylphenols, polychlorobiphenyls, tin organic compounds, and Bisphenol A and its derivatives. Most of the above mentioned compounds enumerated have a similar effect on humans as estrogen – a natural hormone, thus possibly disturbing the natural-hormone balance of the human body.

The main goals of our study were the following:

- preparation and determination of properties of new sorbents based on silica modified with ketoimine groups,
- establishing the physico-chemical characteristics of the sorbent obtained,
- practical application of the sorbent in the preconcentration of estrogen-like compounds, such as Bisphenol A and its derivatives,
- establishing the basic conditions for identification and determination of Bisphenol A in SPE-derived extracts by means of gas chromatography with flame-ionization detection (FID).

## EXPERIMENTAL

### Apparatus

Chromatographic separation was performed using the following apparatus:

- Gas chromatography VARIAN CP-3380 equipped with flame ionization detector (FID). A CP-SIL 5 CB (30 m x 0.32 mm; DF = 1.0  $\mu\text{m}$  or 10 m x 0.53 mm; DF = 2.0  $\mu\text{m}$ ) capillary column was used. The analysis was performed at the temperature of 250°C. Helium was the carrier gas. All the work was carried out in a constant flow mode set at 3.5 ml min<sup>-1</sup>.

- Elemental analysis was performed using 2400 CHN Elemental Analyzer (Perkin-Elmer, Norfolk, USA).
- Nitrogen adsorption/desorption isotherms at the temperature of liquid nitrogen were obtained with ASAP 2010 sorptiometer (Micrometrics, Narcross, GA, USA).
- NMR spectra for the solid phases ( $^{29}\text{Si}$  CP MAS NMR) were taken using spectrometer 300 MSL (Bruker, Rhenstteten, Germany).
- A Bakerbond SPE vacuum manifold was used for the elution of SPE columns.

### Chemicals and materials

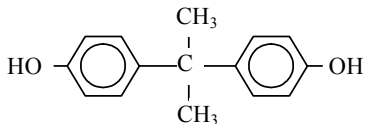
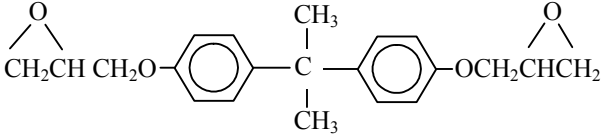
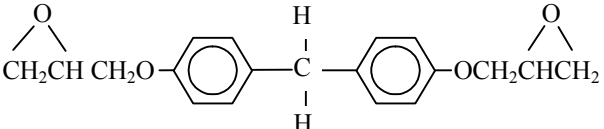
Silica gel (Baker Analyzed<sup>R</sup>) was purchased from J.T. Baker. 3 pentano-2,4-dione derivatives, which were used for modification of the silica surface, were obtained from the Organometallic Chemistry Department of the Adam Mickiewicz University, Poznań, Poland. The solvents to be used for the modification reactions (xylene and hexane, both analytical grade) were purchased either from POCh (Gliwice, Poland), or Fluka (Buchs, Switzerland).

All of the analyzed compounds investigated in the separations were purchased from Sigma–Aldrich, and their purity was equal to or greater than 98%. The structures of some of the test compounds are shown in Table 1.

Water was purified in the Milli-Q apparatus (Millipore S.A. 67120 Molsheim, France).

All standard stock solutions were prepared in methanol and used after proper dilution with the same solvent.

Table 1. Structure of analyzed compounds.

No.	Structure	Compounds analyzed
1.		BPA 2,2-bis- -(4-hydroxyphenyl)- -propane
2.		BADGE 2,2-bis- -(4-hydroxyphenyl)- -propane-bis- -(2,3-epoxypropyl)- -ether
3.		BFDGE bis-(4- -glycidyloxyphenyl)- -methane

### Modification procedure

5 g of dry silica was immersed in a mixture of anhydrous xylene and 3-aminopropyltriethoxysilane. The mixture was boiled for 12 hrs in a vessel equipped with a reflux condenser. The contents were continuously stirred and carefully protected against moisture. Any unreacted silane was extracted with xylene and hexane in a Soxhlet apparatus. Then it was dried under vacuum and finally subjected to the so-called „end capping” reaction with hexamethyldisilazane in order to deactivate free silanol groups remaining at its surface.

The second step was bonding of amino groups using an appropriate derivative of 3-pentano-2,4-dione. As previously, the reaction was performed under continuous stirring in anhydrous xylene and lasted 12 hrs. The system was protected against moisture. The final product was then extracted with xylene and hexane in a Soxhlet apparatus. Finally, silica was dried under vacuum.

The structure of chemically bonded sorbents synthesized is presented in Figure 1.

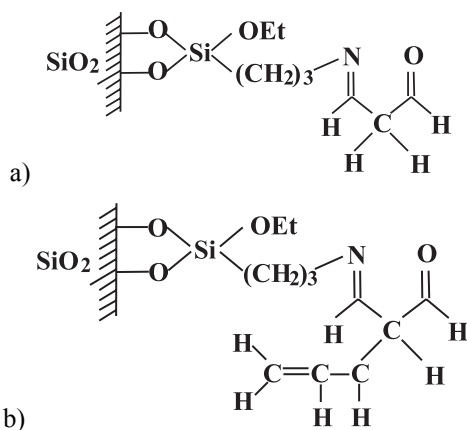


Fig. 1. Structure of chemically bonded sorbents synthesized: (a) packing I, (b) packing II.

### Solid phase extraction

Five hundred mgs of the modified silica were packed dry into plastic columns with reservoir capacities of 6 mL. Each column contained polyethylene frits to support and cover the filler. The extraction procedure was as follows: prior to initial use, the cartridges were conditioned by passing through: 5 mL of a mixture of methyl chloride and methanol (1:1 v/v), 5 mL of methanol, and 15 mL of deionized water. The columns prepared according to this procedure were injected with a certain amount of the test samples. Once the total amount of the sample was introduced, the sorbent was dried for 10 minutes under vacuum, and the preconcentrated

compounds were washed away with the use of 3 mL of methanol. The extract was dried up and further dissolved in 0.25 mL of methanol. The obtained samples were measured by GC-FID.

## RESULTS AND DISCUSSION

### The properties of modified silica

To investigate the physicochemical properties of the obtained packings, they were subjected to elemental analysis. The contents of carbon, hydrogen and nitrogen were determined and the specific surface area was estimated. The surface concentration of bonded siloxane molecules (denoted by  $\alpha$ ) in  $\mu\text{mol m}^{-2}$  was calculated from the carbon content according to the

$$\text{following equation: } \alpha = \frac{\%C \cdot 10^6}{(100 \cdot n \cdot 12 - \%C \cdot M) \cdot S_{BET}},$$

where:

- $\%C$  – denotes percentage of carbon contribution,
- $n$  – number of carbon atoms in the molecule of bonded silane,
- $M$  – molecular mass of the siloxane,
- $S_{BET}$  – specific surface area [ $\text{m}^2\text{g}^{-1}$ ].

The results are presented in Table 2.

Table 2. Recovery rates for analyzed compounds, for water and powder-milk samples.

Sorbent	Recovery [%]					
	water			powder milk		
	BPA	BADGE	BFDGA	BPA	BADGE	BFDGA
C <sub>18</sub>	92.6	84.8	82.3	91.0	82.7	80.3
Packing I	97.3	88.3	85.1	93.9	84.5	83.2
Packing II	95.5	86.1	83.0	94.0	85.0	82.7

To examine the surface of the obtained sorbents, we used NMR technique for the solid body ( $^{29}\text{Si}$  CP MAS NMR).  $^{29}\text{Si}$  CP MAS NMR spectrum makes it possible to differentiate the silanols in the  $\text{SiO}_2$  structure. Such a spectrum with free different types of silanol [41] is presented in Fig. 2. Geminal silanols (so called twins) are characterized by the  $-90$  ppm signal, free silanols (singles) – by the  $-100$  ppm, and siloxanes – by the  $-110$  ppm. Due to the  $^{29}\text{Si}$  CP MAS NMR technique, it is possible to examine the reactivity of the silanols. As mentioned in the literature, the silanols that are the most reactive at silica modification are the geminal ones.

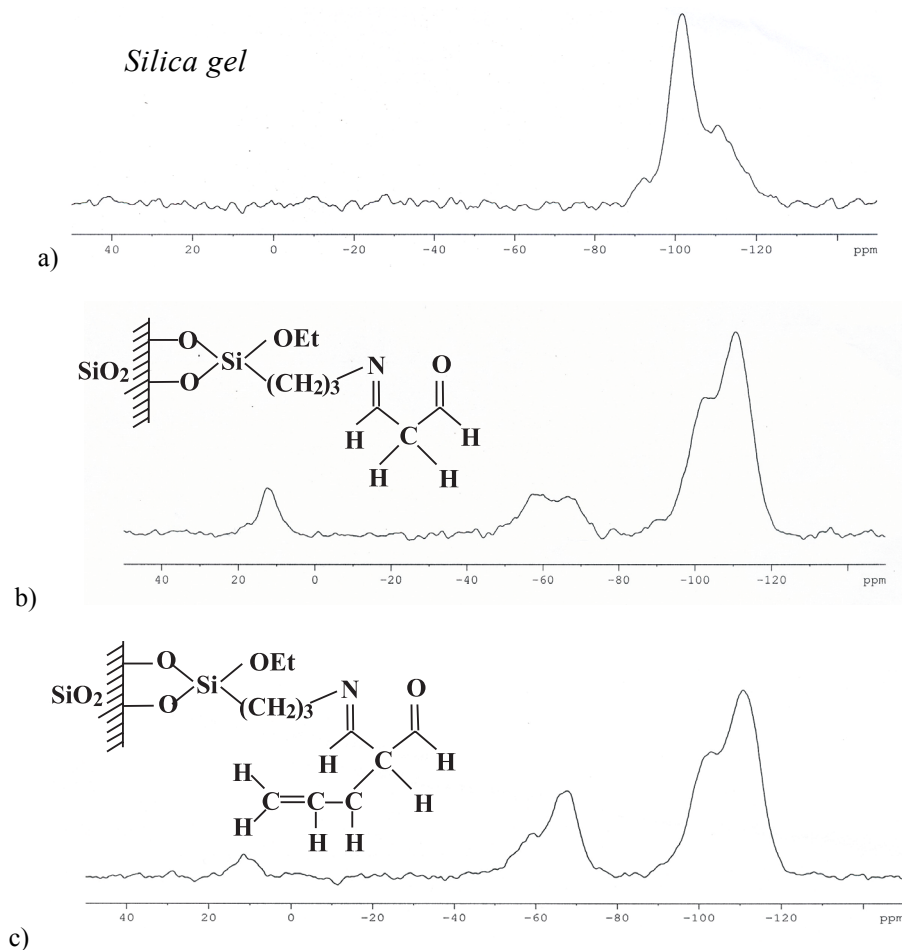


Fig. 2.  $^{29}\text{Si}$  CP MAS NMR spectrum of: (a) unmodified silica, and (b) and (c) sorbent under study.

Spectra  $^{29}\text{Si}$  CP MAS NMR of the unmodified silica are presented in Figure 3a, while the spectra of the sorbent under study are shown in Figures 3b and 3c. An analysis of these spectra confirms that the synthesis reaction took place at the silica surface. During this reaction, the geminal silanols were blocked. The distinct signal at -90 ppm for the spectrum of unmodified silica is definitely weaker for the modified silica. At the same time, the signal at -100 ppm is changed, and this fact proves that the isolated silanols are blocked as well. A clear signal at +12 ppm accompanied by the signals at -46 ppm ( $T_1$ ), -50 ppm ( $T_2$ ), and -64 to -70 ppm ( $T_4 + T_4'$ ) indicates modification of the silica by the trifunctional silane, and the „end capping” process by the use of hexamethyldisilazane.

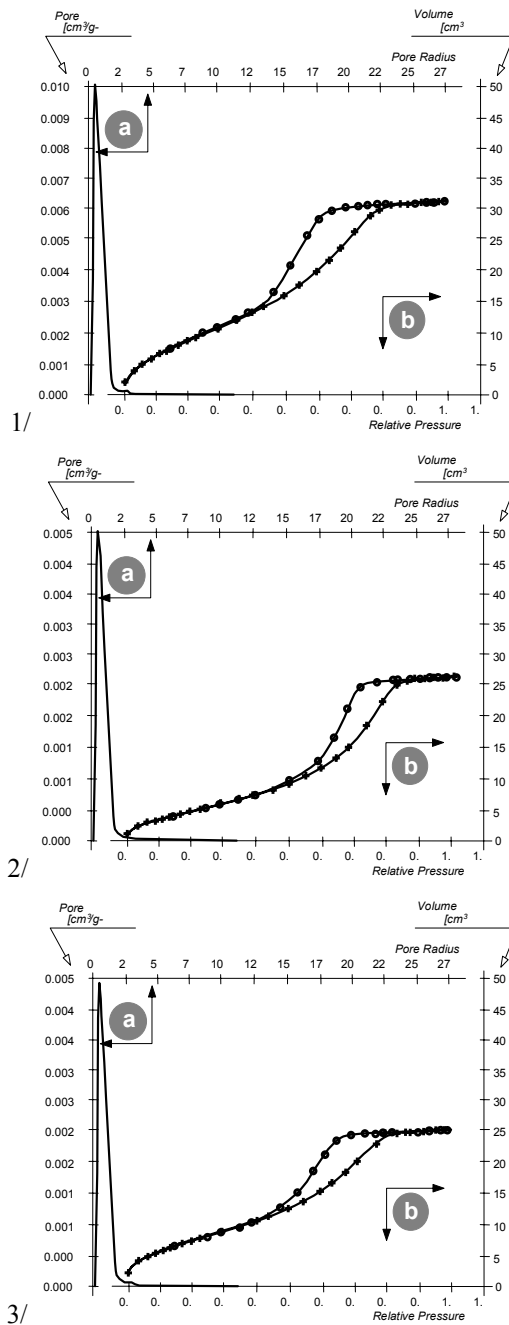


Fig. 3. Pore size distribution of: (a) silica support, and (b): nitrogen adsorption/desorption isotherms, for: 1/ pure silica, 2/ packing I, and 3/ packing II, respectively.

The chromatographic materials should be free from micropores, i.e., those with diameters below 20Å [15], because mass transfer is slowed down by their presence [16-19]. Figure 3a illustrates that this is the case of the sorbents under study. Moreover, the pore shape of the sorbents should be harmonized. Although this is not easy to verify, analysis of the shape of the hysteresis loop on the adsorption-desorption isotherm can give some information about pore structure.

The hysteresis loop shown in Figure 3b is nearly vertical, with almost parallel branches, therefore, it belongs to type H2 according to the IUPAC classification [15]. This type of hysteresis is usually associated with porous materials consisting of agglomerates or compacts of approximately uniform spheres as fairly regular array. Therefore, they have a relatively narrow pore size distribution.

### Results of SPE

Recoveries of the new sorbents with ketoimine groups in SPE were compared with those of commercially available C<sub>18</sub> phases. The C<sub>18</sub> phase is the most commonly used phase for preconcentration of the organic compounds of our study. For the sake of comparison, we used 2,2-bis-(4-hydroxyphenyl)-propane, 2,2-bis-(4-hydroxyphenyl)-propane-bis-(2,3-epoxypropyl) ether, bis-(4-glycidyloxyphenyl)-methane. Recovery is the most easily comparable parameter of synthesized and commercial sorbents. The recovery tests were performed for the samples of deionized water and powder milk, spiked with known amounts of BPA and other compounds (BADGE, BFDGE, 0.5 µg g<sup>-1</sup>). Recovery results are presented in Table 3.

Table 3. Surface properties of modified silica support.

Sorbent	Elemental analysis (%)			Surface area (m <sup>2</sup> g <sup>-1</sup> )	Surface concentration of silane (µmol m <sup>-2</sup> )	Pore diameter (nm)	Specific pore volume (mL g <sup>-1</sup> )
	C	H	N				
Silica gel	0.05	0.99	–	552	–	6.18	0.88
Packing I	10.70	2.99	2.25	376	4.04	5.29	0.52
Packing II	13.44	3.34	1.96	326	4.37	4.87	0.41



A typical GC-FID chromatogram of the compounds analyzed is presented in Figure 4. In addition, apart from determination of these compounds in standard solutions, we applied our method to the real samples. To this end, we took into consideration samples of water stored in PC-made cans, available from a supermarket, water from canned food, such as popcorn, and powder milk. In Figure 5, we present sample chromatograms of the compounds analyzed (BPA) determined in powdered milk, purchased in Polish supermarkets. Detailed results of our research relating to real water and powder milk samples were presented in [20, 21, 22].

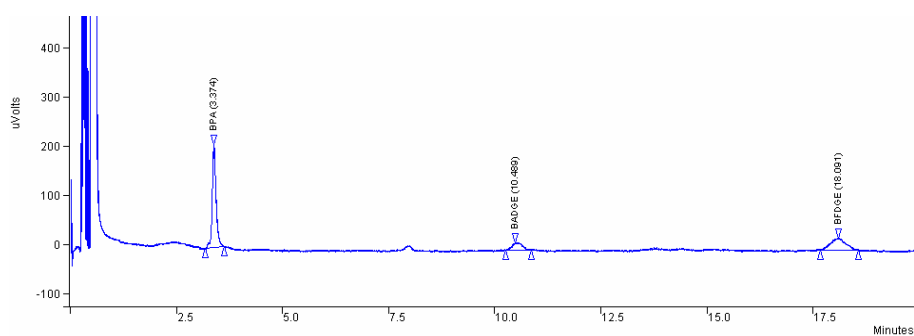


Fig. 4. GC chromatogram of analyzed compounds (BPA, BADGE, BFDGA).

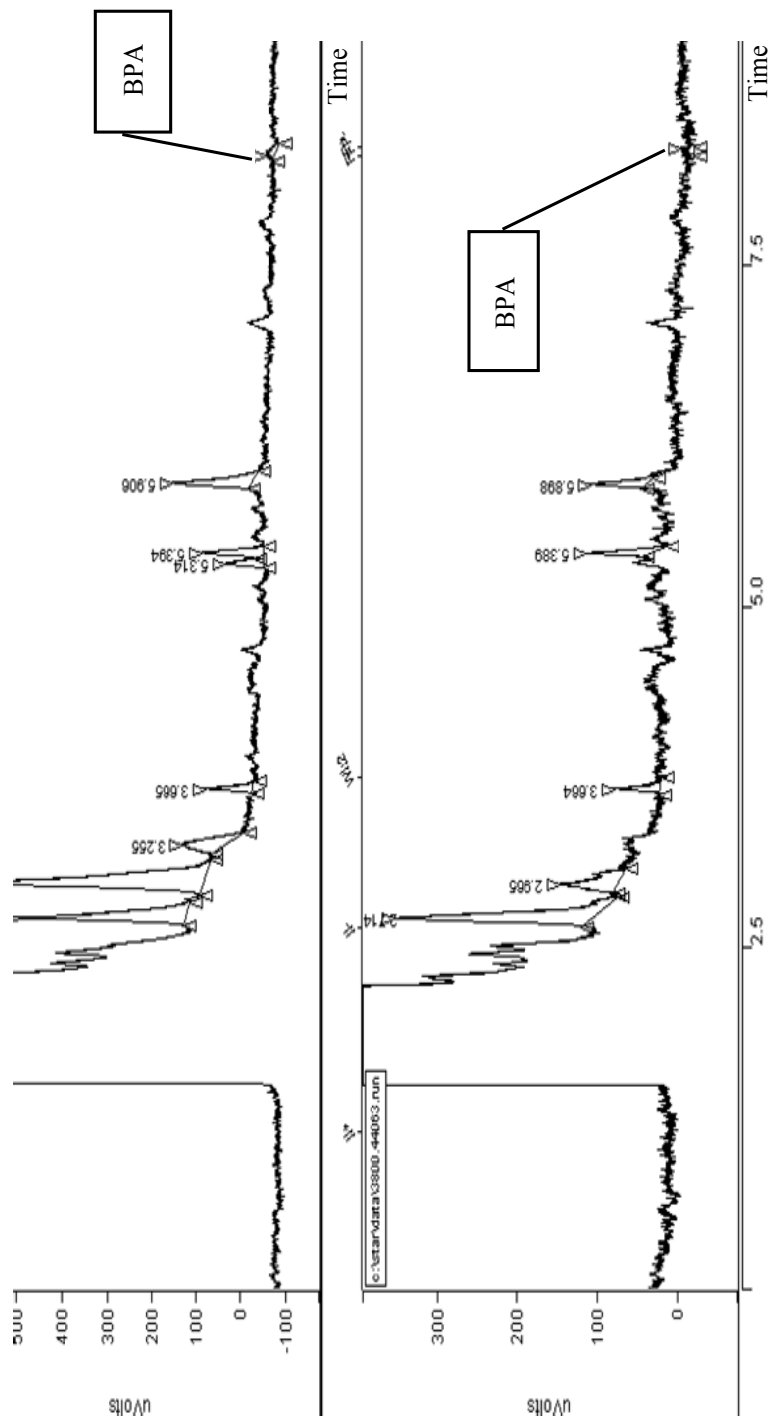


Fig. 5. Typical GC/FID chromatograms of powdered milk samples: (a) not spiked, (b) spiked with BPA.

## CONCLUSION

In this work two new sorbents are proposed for the SPE method with chemically bonded ketoimine groups. The way of preparation and the physico-chemical properties of these sorbents are presented. Some results are given for the application of these sorbents in the preconcentration and further determination of bisphenol A (BPA) and other compounds (BADGE, BFDGE) using solid-phase extraction (SPE) and gas chromatography. Based on the obtained results, we proved that the new sorbents with ketoimine groups may be used for the preconcentration of the above compounds, and the recovery rates of these new sorbents are higher than the rates obtained for the commercial C<sub>18</sub> phase.

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