

## SPE and GC methods of preconcentration and determination of phenol, o-chlorophenol, and benzene by means of chemically modified silica\*

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In the paper preparation, characteristics and potential applications of two new sorbents with chemically bonded transition metal complexes have been presented. The sorbents have been synthesized by attaching copper (II) chlorides to the silica surface through ketoimine groups. The suggested application area of the proposed sorbents is related with solid-phase extraction (SPE) of aromatic compounds of different structure, e.g., phenol, 2-chlorophenol, and benzene from water matrix. The experiments proved high efficiency of the sorbents, the ranging recovery rates from 88% to 96% and the LOD values from 0.2 for phenol, and 1.0 for o-chlorophenol. Basic characteristics of the sorbents synthesized were determined by the use of elemental analysis and UV-VIS spectroscopy.

### 1. INTRODUCTION

Many organic pollutants, such as phenol, o-chlorophenol, and benzene, present in the environment in trace amounts, due to their chemical stability are not subjected to the bio-degradation. Due to their high toxicity [1] and capability of accumulation, even at low concentration levels, such compounds are of very serious danger for human organisms [2].

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\*This article is dedicated to Professor Roman Lebeda on the occasion of his 65<sup>th</sup> birthday

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Phenol is a pollutant widely occurring in surface water, originating mainly from the sewage of chemical, pharmaceutical, and dye industries. Some processes of water purification, based on chloro-oxidants, lead to formation of some dangerous phenol derivatives [3,4], including chlorophenols showing genotoxic, mutagenic, and carcinogenic properties [5] characterized by high stability [6]. Benzene is a natural fraction of crude oil, and thus that compound may be found in the refinery products [7,8]. Traditionally, benzene was widely used as a solvent in (among others) pharmaceutical industry. However, recently, as this compound is being classified as a 1<sup>st</sup> class carcinogenic factor [9] targeted on bone marrow, its usage is more and more limited.

Among many techniques for the isolation and preconcentration of different organic compounds [10] and metal ions, solid phase extraction SPE is one of the most frequently used [11-14]. The base of the extraction process to the solid phase is related with the retaining of the analyte at the sorbent [15]. This process comprises a division of splitting of the substance molecules between the solid packing and the water. Such division is based on different physico-chemical interactions, including physical sorption, ion exchange, and complexation [16]. Traditional sorption materials are based on silica gel, active carbon, and micropore polymers [15,17]. Recently, stress is put on chemically modified silica, obtained by bonding organic compounds to the active groups at the silica surface. Such modified silica is capable of effectively interacting with the analyte, assuring high recovery rates and high precision as well.

Originally, the SPE application area is restricted to the preconcentration of the analytes showing high hydrophobic properties – the recovery rates for polar compounds are not of sufficient quality [6]. Thus, the work is devoted to the usage of silica sorbents modified by ketoimine groups bonded with copper (II) to the preconcentration of aromatic compounds of different nature, mainly polar phenol, 2-chlorophenol, and non-polar benzene. Further determination of the preconcentrated compounds was performed with the use of a gas chromatograph with the FID detector. The obtained sample recovery rates and detection limits are given in the text, proving high effectiveness of the method. The main goal of the paper is to show a capability of the newly obtained sorbent for an effective concentration of aromatic compounds (both hydrophobic and of polar nature) from water samples.

In our previous work, the silica modified with ketoimine groups bonded with copper (II) was successfully applied as a packing for the complexation gas chromatography [18].

## 2. EXPERIMENTAL

### Apparatus.

- Elemental analysis was performed using a 2400 CHN Elemental Analyzer (Perkin-Elmer, Norfolk, USA).
- VARIAN CARY 100 SCAN UV-Visible Spectrophotometer.
- A gas chromatograph VARIAN CP-3380 equipped with flame ionization detector (FID). A DB-WAX (30 m x 0.25 mm x 0.25  $\mu\text{m}$ ) capillary column was used. The temperature program applied was as follows: the initial temperature 60°C, at 20°C/min to 230°C (15 min) Helium was the carrier gas. All the work was carried out in a constant flow mode set at 1.5 mL·min<sup>-1</sup>.
- A Bakerbond SPE vacuum manifold was used for the elution of SPE columns.
- The water was purified in the Milli-Q apparatus (Millipore S.A. 67120 Molsheim, France).

**Chemicals and materials.** Silica gel (Baker AnalyzedR) was purchased from J.T. Baker. 3-pentano-2,4-dione derivatives, which were used for the modification of the silica surface were obtained from the Metaloorganics Department of Adam Mickiewicz University, Poznań, Poland. The solvents used for the modification reactions (xylene and hexane - analytical grade) were purchased either from POCh (Gliwice, Poland), or Fluka (Buchs, Switzerland). Phenol, o-chlorophenol, benzene were obtained from Fluka (Buchs, Switzerland). All standard stock solutions were prepared in methanol and used after proper dilution with the same solvent.

**Procedure of modification.** 5 g of dry silica was immersed in a mixture of anhydrous xylene and 3-aminopropyltriethoxysilane. The mixture was boiled for 12 h in a vessel equipped with a reflux condenser. The contents were continuously stirred and carefully protected against the moisture. Unreacted silane was extracted with xylene and hexane in a Soxhlet apparatus. After that the whole system 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 the silica 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 h. The system was protected against the moisture. The final product was extracted subsequently with xylene and hexane in a Soxhlet apparatus. Finally, silica was dried under vacuum.

During the last stage copper (II) was bound to the silica surface. Dry and partially modified silica obtained in the previous stage was immersed in an anhydrous tetrahydrofurane solution of copper (II) chloride, and left there for 7 days at room temperature. The system was protected against moisture. Finally, the silica was filtered off, the excess of  $\text{CuCl}_2$  was extracted with tetrahydrofurane in a Soxhlet apparatus, and the silica was dried. A similar process was applied in one of our previous experiments [19] devoted to determination of Bisphenol A in some mixtures.

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

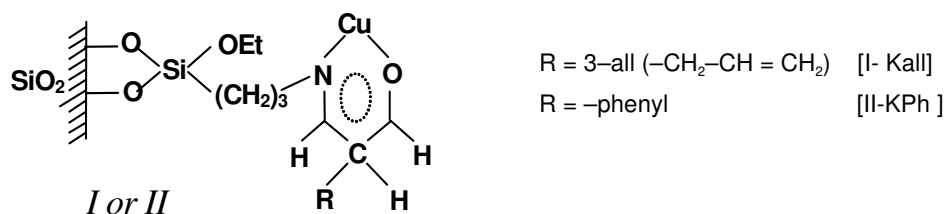


Fig. 1. Structure of synthesized chemically bonded sorbents: [I – Kall], [II – KPh]

**Sample preparation / Solid phase extraction.** Enrichment preconcentration of the analyte from the water samples was performed on little columns with a sorbent. The columns were conditioned before introducing the samples by passing in turn: 5 mL of acetonitrile and 15 mL of deionized water for phenol and o-chlorophenol, and 6 mL of 2-propanol and 6 mL of deionized water for benzene.

Some amount of the analyzed water spiked with some amount of the compounds under study injected into the columns. Once the total amount of a sample was put, the sorbent was dried for 10 minutes under vacuum, and the preconcentrated compounds were washed away by the use of 5 ml of acetonitrile for phenol and o-chlorophenol, and 5 mL of dichloromethane for benzene, respectively. The extract was dried up and further dissolved in 1 mL of the solvent previously used for the elution. The obtained samples were further analyzed by GC/FID.

### 3. RESULTS AND DISCUSSION

**The properties of the modified silica.** To investigate physicochemical properties of the obtained packings, they were subjected to the elemental analysis. The results are presented in Table 1. Based on these results one may draw conclusion that a chemical reaction at the silica surface resulted in bonding copper to the ketoimino groups.

Tab. 1. Elemental analysis of the sorbents under study.

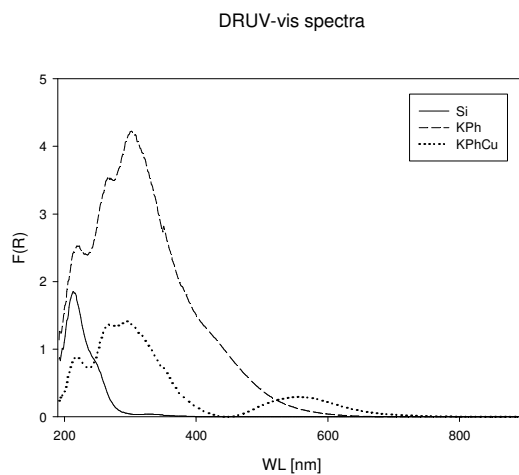
Sorbent	Elemental analysis, %			
	C	H	N	Cu
Silica gel	0.05	0.99	-	-
[I - Kall]	8.04	2.34	1.89	1.7
[II - KPh]	6.61	2.34	1.83	3.7

In order to confirm the formation of bonding between a metal and the silane-bonded ketoimine group, UV spectra were recorded. Similar research was performed for the silica serving as supports in the SPE technique. For all the spectra taken for different ketoimines complexed with copper, one may observe the rising part of the curve at 600÷700 nm (Figure 2a). The solid curve indicates out the unmodified silica, the dashed curve – the silica modified with the phenyl-ketoimine groups, while the dotted curve – with phenyl-ketoimine bonded with the copper complex.

The spectra taken for the complexes of copper and the substrates for the synthesis of allyl-ketoimine as well as for the final complex with copper in a solution are presented in Figure 2b. Similar to the figure above, the solid curve points out to the unmodified silica, the dashed curve – the silica modified with the allyl-ketoimine groups, while the dotted curve – with phenyl-ketoimine bonded with the copper complex.

**Calibration graph. Recovery.** Dependencies between the peak area and the compound concentration were determined as a result of chromatographic analysis of water samples with added phenol, o-chlorophenol, and benzene, respectively. Based on these dependencies, the calibration graphs were prepared. To this end, some standard solutions were used with the concentration level of the compounds under study ranging from 2.5 to 50  $\mu\text{g}\cdot\text{mL}^{-1}$ . The final peak area was taken as an average of three experiments in turn. The calibration plot was described with the general equation:  $y = ax + b$ , where  $y$  is the peak area, and  $x$  – the amount of determined compound in  $\mu\text{g}\cdot\text{mL}^{-1}$ . For the observed range of concentration values, linearity of the calibration curves for the calibration coefficients greater than 0.9998 is observed.

a)



b)

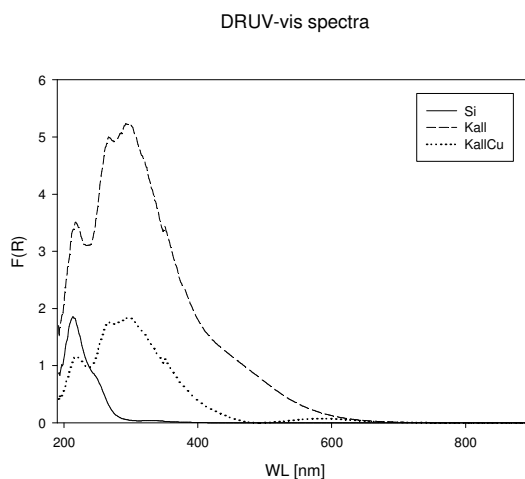


Fig. 2. DRUV-vis spectra of (a) sorbent with the chemically bonded 3-phenyl-pentane-2,4-dione, and (b) the sorbent with chemically bonded 3-allyl-pentane-2,4-dione.

The detection limits were defined as the amount of the compounds producing a peak three times higher than the noise level recorded for a matrix without the component determined. The obtained parameters of the calibration curves for the analyzed compounds are reported in Table 2.

Tab. 2. Parameters of calibration curves, recovery rates, and detection limits for the analyzed compounds.

Determined values	a	b	r <sup>2</sup>	LOD µg·L <sup>-1</sup>
Phenol	22.69	40.64	0.9998	0.2
o-chlorophenol	18.62	-19.55	0.9999	1
Benzene	171.86	-421.15	0.9998	0.5

Recovery tests were performed for the deionized water, with significant, known amount of phenol, o-chlorophenol, and benzene added (0.5 and 5 µg / 500 mL). These tests were performed using the above described method. The obtained results are given in Table 3. The obtained calibration curves are characterized by very good linearity. The method is characterized by good recovery rates as well as low detection limits.

Tab. 3. Results of recovery tests for the 0.5 µg and 5 µg of phenol, o-chlorophenol, and benzene solution in 500 mL of water (n=5).

Sorbent	Recovery rate ± SD, %		
	Phenol	o-chlorophenol	Benzene
[I - Kall] (cf. Fig. 1)	94.6 ± 1.0* 95.1 ± 1.3**	82.0 ± 1.5**	93.2 ± 1.2**
[II - KPh]	94.3 ± 1.6* 94.4 ± 1.3**	95.6 ± 1.4**	96.6 ± 0.9**

\* sample 0.5 µg / 500 mL, \*\* sample 5 µg / 500 mL

#### 4. CONCLUSIONS

In this work some sorbents were proposed for the SPE method with chemically bonded ketoimine groups and copper (II). A way of preparation and physico-chemical properties of these sorbents are presented. There are given some results of application of these sorbents for preconcentration and further determination of phenol, o-chlorophenol and benzene by means of the solid-phase extraction (SPE) and GC-FID methods. Based on the obtained results, we proved that new sorbents with the ketoimine groups may be used for efficient preconcentration of the trace amounts of these compounds, with the determined recovery rates at the level of 88÷96%.

One may observe a significant decrease of the process efficiency for higher analyte concentration. This fact is related to the breakthrough.

The experiments taken proved that the silica modified with the ketoimine groups and copper may be used for preconcentration of phenol, o-chlorophenol, and benzene solutions of different concentrations and extracted from high-volume matrices. Taking into consideration the fact that in the real environmental samples the concentration of organic compounds ranges from  $\text{ng}\cdot\text{L}^{-1}$  to  $\mu\text{g}\cdot\text{L}^{-1}$ , and the sample amount usually does not exceed 500 to 1000 mL, probability of a breakthrough is small for the components under study, thus the newly proposed sorbents may be successfully applied as effective SPE sorbents.

## 5. REFERENCES

- [1] A. Li, Q. Zhang, J. Chen, Z. Fei, C. Long, W. Li, *React. Funct. Polym.*, 49, 225 (2001).
- [2] D. G. Kim, M. W. Jung, I. R. Paeng, J. S. Jae-Seong Rhee, K. Paeng, *J. Microchemical Journal*, 63, 134 (1999).
- [3] M. Cernakova, A. Zemanovicova, *Folia Microbiol*, 43, 411 (1998).
- [4] H. Liu, P. K. Dasgupta, *Anal. Chem.*, 68, 1817 (1996).
- [5] J. Michałowicz, W. Duda, J. Stufka-Olczyk, *Chemosphere*, 66, 657 (2007).
- [6] T. Saitoh, S. Matsuhima, M. Hiraide, *J. Chromatogr. A*, 1040, 185 (2004).
- [7] R. Schnatter, *J. Toxicol. Environ. Health. Part A*, 61, 433 (2000).
- [8] J. Roma-Torres, J. P. Teixeira, S. Silva, B. Laffon, L. M. Cunha, J. Méndez, O. Mayan, *Mutation Research*, 604, 19 (2006).
- [9] T. Eining, W. Dehnen, *J. Chromatogr. A*, 697, 371 (1995).
- [10] T. Saitoh, T. Kondo, M. Hiraide, *J. Chromatogr. A*, 1164, 40 (2007).
- [11] D. F. Rendle, *Talanta*, 51, 1235 (2000).
- [12] J. Stevens, M. Crawford, G. Robinson, L. Roenneburg, *J. Chromatogr. A*, 1142, 81 (2007).
- [13] U. J. Nilsson, *J. Chromatogr. A*, 885, 305 (2000).
- [14] J. S. Fritz, J. Masso, *J. Chromatogr. A*, 909, 79 (2001).
- [15] Y. Picó, M. Fernández, M. Ruiz J., G. Font, *J. Biochem. Biophys. Methods*, 70, 117 (2007).
- [16] V. I. Beketov, V. Z. Parchinskii, N. B. Zorov, *J. Chromatogr. A*, 731, 65 (1996).
- [17] N. Fontanals, R. Marcé, M. F. Borrul, *J. Chromatogr. A*, 1152, 14 (2007).
- [18] W. Wasiak, I. Rykowska, *Chromatographia*, 48, 284 (1998).
- [19] W. Wasiak, I. Rykowska, *Anal. Chim. Acta*, 378, 101 (1999).
- [20] I. Rykowska, *Chemia Analityczna* 51, 399 (2006).

## CURRICULA VITAE



**Wiesław Wasiak** was born in Margonin (Poland) in 1947. He works for Adam Mickiewicz University in Poznań, Poland. He received MSc in chemistry in 1970, PhD in 1976, DSc in 1989, and title of Professor – in 1999. He was the Vice-Dean of the Faculty of Chemistry of Adam Mickiewicz University from 1990 to 1996, and the Member of the Senate from 1999 to 2000. He has been the Head of the Department of Analytical Chemistry since 2001. He has been a member of the Committee for Analytical Chemistry PAS (since 1999), and the Head of Commission



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