Application of silica extracted from rice husk ash for the encapsulation of AFB1 antibody as a matrix in immunoaffinity columns

Deni Pranowo1, Nuryono1, Ali Agus2, Jumina1, Romsyah Maryam3, FMCS Setyabudi4

1Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia
2Department of Animal Science and Husbandry, Faculty of Animal Husbandry, Universitas Gadjah Mada, Yogyakarta, Indonesia
3Toxicology and Mycology Research Group, Indonesian Research Centre for Veterinary Science
4Department of Food and Agricultural Product Technology, Faculty of Agriculture Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract
The extraction of silica from rice husk ash (RHA) for the encapsulation of aflatoxin B1 antibody (Ab-AFB1) and its application as a matrix in immunoaffinity columns (IACs) were achieved. The RHA extraction was performed using 4 M NaOH, which yielded sodium silicate (Na2SiO3) for the synthesis of silica gel. The obtained silica was used for encapsulating Ab-AFB1 using the sol-gel technique. One milliliter of 1 M Na2SiO3:H2O:H3PO4 (0.43:0.11:0.46) could generate silica gel that was suitable for encapsulating 1.36 mg of Ab-AFB1 at pH 7. After 48 hours of aging, the silica gel modified with Ab-AFB1 (SG-Ab-AFB1) was ground, and packed as the matrix in the IAC for aflatoxins purification. The modified silica gel was characterized using FTIR and SEM. The properties of IAC with SG-Ab-AFB1 were investigated by evaluating AF recovery, binding capacity, and reusability. The recovery of AFB1 was 94.11 ± 4.62%. In addition to AFB1 recovery, the column also retained AFB2, AFG1, and AFG2 with recovery values of 98.22 ± 3.74%, 92.22 ± 7.62%, and 83.00 ± 6.31%, respectively. This column, which contained 0.5 g of SG-Ab-AFB1 had a binding capacity of approximately 50 ng of AFs per column, and could be reused at least 5 times with a recovery of more than 80%.

Introduction
Mycotoxins are toxic secondary metabolites produced by mycotoxigenic fungi. Among 400 types of mycotoxins, aflatoxin B1 (AFB1) is classified in category 1 for carcinogenicity1. Because of the health risks of AFB2, many countries have legislated strict rules to regulate the maximum levels of AFB1 in food and fodder. The European Union has determined the maximum limit of AFB1 to be 5 µg/kg in food, and 20 µg/kg in fodder. In Indonesia, the legal limits of AFB1 are 15 µg/kg for peanut products2, and 100–200 µg/kg for cattle feed concentrates3.

High-Performance Liquid Chromatography-Fluorescence Detection (HPLC-FLD) is a routine method for the analysis of aflatoxins (AFs), which has high sensitivity, selectivity, and accuracy; however, its sample preparation often takes longer, and is more expensive than immunoassay methods, such as ELISA. Prior to analysis by HPLC-FLD, the extraction and purification of AFs are necessary. Immunoaffinity chromatography using immunoaffinity columns (IACs) is a purification technique with high selectivity and recovery of separated molecules, and is based on the interaction between antigens and antibodies4. Although commercial IACs are commonly used, they are relatively expensive and disposable, with limited availability. Recently, many researchers have developed IACs with high recovery and reusability, enabling the analysis of AFs at affordable costs.

For the preparation of IACs based on silica gel, the antibody is often immobilized by encapsulation using the sol-gel technique. This technique imparts more resistant to the matrix used in IACs for organic solvents.
other than those usually used in commercially available IACs, such as sepharose, cellulose, and other organic materials. Silica gels that are synthesized from tetramethyl orthosilicate (TMOS) have been widely used for the purification of bisphenol A (BPA) from fish, deoxynivalenol (DON) from cereals, DON and zearalenone (ZON) from wheat. Bhatia et al. (2000) used sodium silicate solution of silica gels; however, methanol, which might denature the antibody, is also generated during condensation. Bhatia et al. (2000) used sodium silicate (Na$_2$SiO$_3$) for the encapsulation of two enzymes, horse-radish peroxidase and glucose-6-phosphate dehydrogenase, at pH 7 instead of TMOS, and therefore, methanol production and enzyme denaturation were avoided. Moreover, the catalytic activity of the encapsulated enzyme was also maintained at a high level.

Instead of Na$_2$SiO$_3$, silica could be easily isolated from rice husk ash (RHA), which is economically available as a by-product. In the previous study, RHA, which was combusted at 700–900°C, yielded 16–25% ash that contained a high concentration (87–97%) of silica. Therefore, extraction of silica from RHA using NaOH enabled the generation of Na$_2$SiO$_3$ solution for the synthesis of silica gels.

In the present study, an AF$_B$ antibody (Ab-AF$_B$) was encapsulated in silica gel, which was prepared from the Na$_2$SiO$_3$ that was isolated from RHA. The obtained silica gel that was modified with Ab-AF$_B$ (SG-Ab-AF$_B$) was used in the production of IACs for AF purification.

### Materials and Methods

#### Reagents and chemicals

Polyclonal AF$_B$ antibody (Ab-AF$_B$) at a concentration of 136 mg/mL in PBS was obtained from the Indonesian Research Center for Veterinary Science. The standard solution of AF$_B$ (2.504 µg/mL) and the standard solution of AFS containing AF$_B$ at 1.023 µg/mL, AF$_B$2 at 0.302 µg/mL, AF$_G$ at 0.882 µg/mL and AFG2 at 0.297 µg/mL, were purchased from Sigma-Aldrich (Germany). HCl, NaCl, KCl, NaHPO$_4$, KH$_2$PO$_4$, NaOH, HNO$_3$, and H$_2$OAc were obtained from Merck (Germany). Acetonitrile and methanol of HPLC gradient grade were purchased from JT. Baker (China).

#### Extraction of silica from rice husk ash (RHA)

RHA was collected from brick kilns at Pendowo-harjo, Sewon, Bantul, Yogyakarta. It was cleaned, ground, and sieved using a 200 stainless steel mesh screen (allowing the passage of particles smaller than 0.074 mm). Afterward, silica was extracted from RHA according to the method of Affandi et al. (2009) with modifications. Silica extraction was initiated by boiling and stirring the mixture of RHA (20 g) and 4 M NaOH (160 mL). A black solution was obtained, which was poured into a porcelain crucible, and then, kept for 30 min at 500°C in a muffle furnace (Nabertherm L 3/12, Germany). After the solution was cooled to room temperature, a light gray solid with small cavities was formed, which was then soaked in 200 mL of deionized water overnight. Afterwards, the obtained solution containing 9.14% (w/v) Na$_2$SiO$_3$ was filtered through a Whatman No. 41 filter paper (Whatman Plc, Kent, England), and the filtrate served as sodium silicate (Na$_2$SiO$_3$) solution.

#### Preparation of IAC with SG-Ab-AF$_B$ by the sol-gel technique

The Na$_2$SiO$_3$ solution was diluted with distilled water in various ratios. The pH of 5 mL of its dilution was adjusted to 7 by adding H$_3$PO$_4$. The time required for gel formation using the solution at each dilution was noted. The ratio of the volumes of Na$_2$SiO$_3$, H$_2$O, and H$_3$PO$_4$ in the solution that did not immediately cause gel formation at pH 7 was selected for the preparation of silica gel encapsulated with Ab-AF$_B$ (SG-Ab-AF$_B$).

Approximately 1 mL of the mixture of Na$_2$SiO$_3$, H$_2$O, and H$_3$PO$_4$ at 1 M (with a volume ratio of 0.43; 0.11; 0.46) was mixed with 0.4 mL of phosphate buffer saline (PBS) and 0.1 mL of Ab-AF$_B$ (136 mg/mL Ab-AF$_B$ in PBS) for 5 min. The aqueous gel mixture was aged at 4°C until it reached a constant weight. Because of the drying process and evaporation of water on the orthosilicate molecules during condensation, a xerogel was formed. Accordingly, the encapsulation of Ab-AF$_B$ within the silica gel was achieved simultaneously. Afterward, it was ground, and packed into a 3-mL polypropylene column which was blocked by polytetrafluoroethylene frits at its bottom side. The column matrix was washed with 10 mL of distilled water and 20 mL of PBS, successively. The prepared IAC, which was packed with SG-Ab-AF$_B$, was designated as IAC-SG-Ab-AF$_B$, and stored at 4°C until further analysis.

#### Characterization of IAC-SG-Ab-AF$_B$

IAC-SG-Ab-AF$_B$ was characterized using 3 analytical techniques, namely, Fourier Transform Infrared Spectroscopy (FTIR), Atomic Absorption Spectroscopy (AAS), and Scanning Electron Microscopy (SEM). FTIR was performed in order to detect the functional groups of the silica gel and the immobilized antibody. The concentration of silica in the Na$_2$SiO$_3$ solution prepared from RHA was determined using AAS. The microstructures on the surface of the silica gel and the silica gel encapsulated with Ab-AF$_B$ (SG-Ab-AF$_B$) were investigated using SEM.
Analysis of the interaction between the silica gel and AFs

In the loading step, 1 mL of the AF standard solution at 20 ng/mL in methanol and 20 mL of PBS were mixed, and passed through the silica gel column at a rate of 1 drop/sec. Then, the column was washed with 20 mL of PBS, and further eluted with 5 mL of the mixture of MeOH, H₂O, and HOAc (50:49:1, v/v/v). Five milliliters of each solution that passed through the silica gel column during the loading, washing, and elution steps were collected.

Afterward, the solutions were analyzed using HPLC-FLD (Shimadzu, Japan) under optimized conditions. The HPLC-FLD system consisted of a pump (LC10ADVP), a controller (SCL-10A VP), an inline degasser (DGU-14A), and an oven (CTO-10C). The temperature of the column oven was set at 40°C. A guard column (LiChrospher 100 RP-18, 250 × 4 mm, 5 µm, Merck, Germany), and a 100-µL injection loop were used. Detection was carried out at an excitation wavelength of 360 nm and an emission wavelength of 440 nm using a fluorescence detector (RF-10AXL FD) that was coupled to a post-column electrochemical derivatization equipment Coring cell (Coring system Diagnostic GmbH, Germany) in order to enable the detection of AFB1 and AFG1. Data were processed using the LC solution ver. 1.23 SP1 software by Shimadzu. The recovery of AF was calculated based on the linear equation from each calibration curve. Then, the recovery of the AFs was expressed as the percentage of the absolute quantity of AFs from the standard. In addition to the analysis of recovery, the analysis of binding capacity was performed in a manner similar to that of the analysis of recovery; however, various concentrations of the AF standard solution at 10, 20, and 50 ng/mL were used.

Reusability of IAC-SG-Ab-AFB₁

The analysis of reusability was similar to the aforementioned analysis of AF recovery from IAC-SG-Ab-AFB₁, except that the same IAC-SG-Ab-AFB₁ was employed several times. The recovery of AF was carefully noted at every cycle of purification. The steps of one cycle included the following: passage of 20 mL of PBS, loading of 1 mL of the AF standard solution (20 ng/mL in methanol) and 20 mL of PBS into the IAC-SG-Ab-AFB₁, washing with 20 mL of PBS, elution with 5 mL of eluant, and regeneration with 20 mL of PBS.

Results and Discussion

In this study, we found that the moisture content of sodium silicate (Na₂SiO₃) that was obtained from RHA was 80.52%. AAS analysis showed that the concentrations of Si (SiO₂) and Fe (Fe₂O₃) in RHA were 97.14% and 0.022%, respectively. The mass of SiO₂ in 200 mL of Na₂SiO₃ solution was 19.43 g or 9.14% (w/v). In this study, one IAC could be prepared using a mixture of 1 mL of Na₂SiO₃ from RHA, 0.25 mL of demineralized water, and 1.1 mL of 1 M H₃PO₄, at pH 7 with a gelation time of 5 min.

The reaction between SiO₂ and NaOH was suggested to be given by the following equation [14]:

\[
\text{SiO}_2(s) + 2 \text{NaOH(aq)} \rightarrow \text{Na}_2\text{SiO}_3(aq) + \text{H}_2\text{O(l)}
\]

The formation of Na₂SiO₃ can also be given by the following equation [15]:

\[
n \text{SiO}_2 + 2 \text{NaOH} \rightarrow n \text{SiO}_2\text{Na}_2\text{O} \cdot y\text{H}_2\text{O}
\]

where n is the ratio of SiO₂ and Na₂O. In case n = 1, the synthesized molecule is sodium metasilicate (SiO₂Na₂O or Na₂SiO₃).

Characterization of IAC-SG-Ab-AFB₁

Identification of the functional groups of silica gel

There appeared to be no substantial difference between the silica gel that was produced using commercial Na₂SiO₃ and that synthesized using the Na₂SiO₃ that was obtained from RHA, as identified by the FTIR spectra in Fig. 1. The FTIR spectra of each sample showed similar absorbance profiles at 3464 cm⁻¹ that were ascribed to Si-OH groups (Si-OH) and hydrogen linkage among the water molecules that were trapped in the silica gel structure. The trapped water molecules also caused a broad absorbance between 3350–3500 cm⁻¹ and at 1620 cm⁻¹. The absorbance at 3749 cm⁻¹ was attributed to the stretching vibrations of the Si-OH groups. It was also reported that the absorbance at 933 cm⁻¹ indicated symmetric stretching vibrations of the Si-OH groups [16]. The absorbance that was attributed to the stretching vibrations of the Si-O-Si groups was observed at 1080 cm⁻¹, and also indicated the folding and rocking vibrations of the Si-O-Si groups at 786 cm⁻¹ and 462 cm⁻¹, respectively.

The FTIR spectrum of SG-Ab-AFB₁ had no incidence protein groups since there are no the amide groups since there are no the amide groups at 786 cm⁻¹.
groups N-H stretching vibration at 3100 cm\(^{-1}\) and 3300 cm\(^{-1}\), the amide groups C=O stretching vibration at 1600–1690 cm\(^{-1}\), the C-N stretching vibration and N-H bend vibration at 1480–1575 cm\(^{-1}\). The above findings were observed because of a small quantity of protein (1 mg antibody) that was encapsulated in 500 mg of silica gel. Even though the presence of the Ab-AFB\(_1\) molecules in the silica gel was not proven in the FTIR analysis, it could be confirmed by the interaction with the antigen molecules (AFs), as described in a subsequent section.

**Microstructure characterization**

The differences in size and shape between the silica gel synthesized from RHA and the silica gel modified with Ab-AFB\(_1\) are shown in the scanning electron micrographs (SEM) in Fig. 2. These differences could be observed because of the differences in the rate of solvent evaporation. If the evaporation rate were high, the surface of the silica gel would be rough\(^{17}\). In this study, the evaporation time of the silica gel synthesized from RHA was set as 48 h, and hence, the surface of silica gel was smooth. Meanwhile, both the gels had pores, whose sizes were in the range of 0.2–5.0 \(\mu\)m.

The size of the particles and pores determined the quality of the column regarding the encapsulation of Ab-AFB\(_1\). The bigger the pore size, the easier the release of the Ab-AFB\(_1\) molecules from the silica gel. This could happen because the interaction between the Ab-AFB\(_1\) molecules and the silica gel was based on physical interaction rather than chemical bonding. If the pore size were too small, the interaction of the analyte molecules with the binding sites on the Ab-AFB\(_1\) molecules would be difficult.

**Interaction between the silica gel matrix and the AF molecules**

The interaction between the AF molecules and the silica gel synthesized from RHA is illustrated in Fig. 3. A portion of the AF molecules was retained in the column matrix, whereas almost all of them were eluted with the mixture of MeOH, \(\text{H}_2\text{O}\), and HOAc (50:49:1, v/v/v) at pH 3. The possible links between the AF molecules and the polymeric structure of silica are hydrogen bonds, since AF molecules have oxygen atoms that can interact with the silanol groups of the silica gel polymer. According to Baxter et al. (1997), because of their acidity, silanol groups have a tendency to form hydrogen bonds not only with each other, but also with other specific residues in various organic molecules. In addition, these silanol groups have the capability to form hydrogen bonds in simple dimeric configurations or polymeric configuration to strengthen the 3D structure of silica gel\(^{18}\). Therefore, the silica gel molecules and the AFB\(_1\) molecules could interact and be linked via hydrogen bonds.

The quantity of retained AFs in the silica gel synthesized from RHA was 5.79% as shown in Figure 3 at solution code E1. Based on the result, the interaction between the column matrix and the AF molecules was

---

1. Baxter et al. 1997.
2. See Fig. 3 for a detailed illustration.
The number of fractions obtained at the end of the loading (L), washing (W), and elution (E) steps were 4 (L1, L2, L3, and L4), 4 (W1, W2, W3, and W4), and 1 (E1), respectively.

Table 1 Recovery of AFs from IAC-SG-Ab-AFB1

| Batch | Column No | Recovery (%) of AFs | Repeatability | RSD (%) |
|-------|-----------|---------------------|---------------|---------|
|       |           | B1 | B2 | G1 | G2 |  |    |    |
| 1     | 1         | 90 | 93 | 89 | 78 |  |  |  |
| 2     | 1         | 81 | 100| 84 | 76 |  |  |  |
| 3     | 1         | 99 | 107| 92 | 84 |  |  |  |
|       | Repeatability | 90.00 | 100.00 | 88.33 | 79.33 |  |    |    |
| 1     | 2         | 88 | 86 | 80 | 80 |  |  |  |
| 2     | 2         | 84 | 89 | 84 | 71 |  |  |  |
| 3     | 2         | 109| 107| 100| 91 |  |  |  |
|       | Repeatability | 93.67 | 94.00 | 88.00 | 80.67 |  |    |    |
| 1     | 3         | 98 | 97 | 104| 85 |  |  |  |
| 2     | 3         | 98 | 102| 100| 91 |  |  |  |
| 3     | 3         | 100| 103| 97 | 91 |  |  |  |
|       | Repeatability | 98.67 | 100.67 | 100.33 | 89.00 |  |    |    |
| 1     | Reproducibility | 94.11 | 98.22 | 92.22 | 83.00 |  |    |    |
|       | RSD (%)   | 4.62 | 3.74 | 7.62 | 6.31 |  |    |    |

Recovery of AFs from IAC-SG-Ab-AFB1
The mixture of Na2SiO3, H2O, and H3PO4 at a concentration of 1 M and Ab-AFB1 could be used for producing three IAC-SG-Ab-AFB1 columns for every batch. Then, each column in each batch was analyzed to observe the recovery values of repeatability. Meanwhile the values of recovery from all the columns of the 3 batches were also used to calculate reproducibility. Table 1 shows the recovery of AFs from the IAC-SG-Ab-AFB1 with values more than 80% and RSD values less than 10% for all the three batches, which indicates that an acceptable level of purification was achieved with each column in every batch. Gonzales et al. (2010) suggested the use of the Horwitz table to determine the criteria for the acceptability of recovery.[19]. Based on those criteria, acceptable recovery was determined to be 60–115% at an analyte concentration of 10 ng/g, and thus, IAC-SG-Ab-AFB1 met the requirements for purification of AFs from the actual samples.

Binding capacity of IAC-SG-Ab-AFB1

Binding capacity is described as the maximum quantity of analyte that is bound to the antibody molecules in the column. For instance, the binding capacities of several commercial columns were established as approximately 1–300 ng/column. The evaluation of the binding capacity of IAC-SG-Ab-AFB1 at different concentrations of AFs is shown in Fig. 4. The values of AF recovery from IAC-SG-Ab-AFB1 at 20, 40, and 50 ng of AFs exceeded 98, 97, and 93%, respectively. Therefore, the binding capacity of the IAC-SG-Ab-AFB1 produced in this research study was estimated to be approximately 50 ng of total AFs per column.

Reusability of IAC-SG-Ab-AFB1
As shown in Fig. 5, IAC-SG-Ab-AFB1 that was produced in this research study could be reused at least 5 times with recovery values up to 80%; however, loss in recovery was observed thereafter. Compared with previously described IACs for the analysis of different mycotoxins, the reusability of our IAC-SG-Ab-AFB1 appeared to be lower. Reiter et al. (2011) created an IAC for OTA analysis with a reusability of 7 times[20]. Brenn-Struckhofova et al. (2007) successfully produced an IAC that could be reused 20 times for the analysis of DON[21]. The major factors that influenced the loss of column reusability in this study could be the acidity of the eluent (pH 3), methanol concentration (50%), and the possible leaching of antibody from the column matrix after denaturation at the time of elution.
Conclusion

In the present study, AFB$_1$ antibody (Ab-AFB$_1$) was successfully encapsulated in the silica gel that was synthesized from rice husk ash, and it enabled the production of IAC-SG-Ab-AFB$_1$ with the recoveries of AFB$_1$, AFB$_2$, AFG$_1$, and AFG$_2$ at 94.11 ± 4.62%, 98.22 ± 3.74%, 92.22 ± 7.62, and 83.00 ± 6.31%, respectively. Moreover, the same IAC-SG-Ab-AFB$_1$ could be reused at least 5 times with a recovery of more than 80%.

Acknowledgements

This research study was funded by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia (Ristekdikti – Indonesia) through the Institution Collaboration Grant Scheme (LPPM-UGM/1402/LIT/2013).

References

1) International Agency for Research on Cancer (IARC): Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins, in “IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, vol. 56” WHO Press, Lyon, France (1993)
2) BPOM: Penetapan Batas Maksimum Cemaran Mikroba dan Kimia dalam Makanan, No HK.00.06.1.52.4011, (2009), Badan Pengawas Obat dan Makanan, Jakarta
3) BSN: Pakan konsentrat-bagian 1: sapi perah. SNI no. 3148-1:2009, (2009), Badan Standarisasi Nasional, Jakarta
4) Reiter, E.V., Vouk, F., Böhm, J., Razzazi-Fazeli, E.: Aflatoxins in rice – a limited survey of products marketed in Austria. Food Control, 21, 988-991 (2010)
5) Podlipna, D., Cichna-Markl, M.: Determination of bisphenol A in canned fish by sol–gel immunoaffinity chromatography, HPLC and fluorescence detection. Eur Food Res Technol, 224, 629-634 (2007)
6) Brenn-Struckhofova, Z., Cichna-Markl, M.: Determination of bisphenol A in wine by sol–gel immunoaffinity chromatography, HPLC and fluorescence detection. Food Addit Contam, 23, 1227-1235 (2006)
7) Li, Y., Wang, Z., De Saeger, S., Shi, W., Li, C., Zhang, S., Cao, X., Shen, J.: Determination of deoxynivalenol in cereals by immunoaffinity clean-up and ultra-high performance liquid chromatography tandem mass spectrometry. Methods, Immunoaffinity Methods and Related Methods, 56, 192-197 (2012)
8) Brenn-Struckhofova, Z., Füreder, C., Cichna-Markl, M., Razzazi-Fazeli, E.: Co-isolation of deoxynivalenol and zearalenone with sol–gel immunoaffinity columns for their determination in wheat and wheat products. J Chromatogr A, 1216, 5828-5837 (2009)
9) Bhatia, R.B., Brinker, C.J., Gupta, A.K., Singh, A.K.: Aqueous sol–gel process for protein encapsulation. Chem Mater, 12, 2434-2441 (2000)
10) Enymia, S., Sulistiharini, N.: Pembuatan silika gel kering dari sekam padi untuk bahan pengisi karet ban. Jurnal Keramik dan Gelas Indonesia, 7, 1-8 (1998)
11) Souza, M.F. de, Magalhães, W.L.E., Persegil, M.C.: Silica derived from burned rice hulls. J Mat Research, 5, 467-474 (2002)
12) Kalapathy, U., Proctor, A., Shultz, J.: An improved method for production of silica from rice hull ash. J Bioresource Technology, 85, 285-289 (2002)
13) Affandi, S., Setyawan, H., Winardi, S., Purwanto, A., Balgis, R.: A facile method for production of high-purity silica xerogels from bagasse ash. Adv Powder Technol, 20, 468-472 (2009)
14) Ghosh, R., Bhattacherjee, S.: A Review study on precipitated silica and activated carbon from rice husk. J Chem Eng Process Technol, 4 (2013)
15) Besbes, M., Fakhfakh, N., Benzina, M.: Characterization of silica gel prepared by using sol–gel process. Phys Procedia, Proceedings of the JMSM 2008 Conference 2, 1087-1095 (2009)
16) Aguilar, H., Serra, J., González, P., León, B.: Structural study of sol–gel silicate glasses by IR and raman spectroscopies. J Non-Cryst Solids, 355, 475-480 (2009)
17) Pecoraro, É., Davolos, M.R., Jafelicci, M.: Silica morphology characterized by SEM - the effects of the solvent treatment and the drying process. J Braz Chem Soc, 6, 337-341 (1995)
18) Baxter, L., Cother, L.D., Dupuy, C., Lickiss, P.D., White A.J.P., Williams, D.J.: Hydrogen bonding to silanols, electronic...
19) González, A.G., Herrador, M.Á., Asuero, A.G.: Intra-laboratory assessment of method accuracy (trueness and precision) by using validation standards. J Talanta, 82, 1995-1998 (2010)

20) Reiter, E.V., Cichna-Markl, M., Tansakul, N., Shim, W.-B., Chung, D.-H., Zentek, J., Razzazi-Fazeli, E.: Sol–gel immunoaffinity chromatography for the clean up of ochratoxin A contaminated grains. J Chromatogr A, Advanced Food Analysis, 1218, 7627-7633 (2011)

21) Brenn-Struckhofova, Z., Cichna-Markl, M., Böhm, C., Razzazi-Fazeli, E.: Selective sample cleanup by reusable sol–gel immunoaffinity columns for determination of deoxynivalenol in food and feed samples. Anal Chem, 79, 710-717 (2007)