Cent. Eur. J. Chem. • 7(4) • 2009 • 697–701
DOI: 10.2478/s11532-009-0080-5

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Central European Journal of **Chemistry**

Modified silica surface by phenylboronic acid derivatives as effective sugar sensor

Short Communication

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Received 25 March 2009; Accepted 6 June 2009

Abstract: Silica surface was modified with phenylboronic acid derivatives. The structures of the functionalized supports were characterized by FT-IR spectroscopy, ^{13}C CP/MAS NMR spectrometry, elemental and thermogravimetric analysis. The solid supports were effectively applied as artificial receptors for sugars in aqueous solutions. Alizarin Red S. (ARS) was used to detect colour change upon carbohydrate (sugar) binding. The association constants of the supports studied-ARS complex and the functionalized silica-sugar (glucose) complex were calculated. The parameters obtained were comparable with those for the phenylboronic acids used for the silica gel modification. The competitive binding of the functionalized silica surface with ARS and sugar were studied by UV/VIS measurements.

Keywords: *Silica surface modification • Boronic acid derivatives • Diols • Alizarin Red S.*

1. Introduction

A widely applied method for getting different functional groups immobilized on silica surface is based on modification of the surface with alkoxysilanes. Depending on the functional groups introduced, functionalized silica gel can be applied as phase in chromatography, in extraction of cations, catalytic and ion-exchange reactions or in bioengineering [1-4].

Solid supports having phenyl boronate were found to be useful for separation of multiple hydroxyl group compounds [5-9]. *Cis*-diols form reversible complexes with boronate ions and are found applicable in the construction of sensors for sugars [10]. Formation of boronic ester depends on the binding constants, boronic acid pK_a the pH of the solution, and on relationships among these factors [11]. It is difficult to examine the binding process of boronic acids without the fluorophore that would change fluorescence upon binding to diols. The fluorophore agent can be introduced as a separate reporter and two competing equilibria of boronic acid binding are observed [12]. Alizarin Red S. (ARS) [13] demonstrates the ability of arylboronic acids binding and shows simultaneously a colour change (from deep red to yellow) in aqueous solution (pH 7.4). The addition of sugar generates another equilibrium between boronic acid and the diol compound added. As a result the colour of the solution returns to the initial red. The environment for sensors based on boronic acid is generally phosphate buffer at pH 7.4 that simulates physiological conditions [14].

In our earlier work we reported synthesis of different matrices based on silica gel that were able to complex sodium cations [15]. This paper reports synthesis of two phenylboronic acid derivatives immobilized on silica surface as well as their ability to bind carbohydrate at physiological pH.

2. Experimental

Supports **1-2** were prepared following the procedure outlined in Scheme 1.

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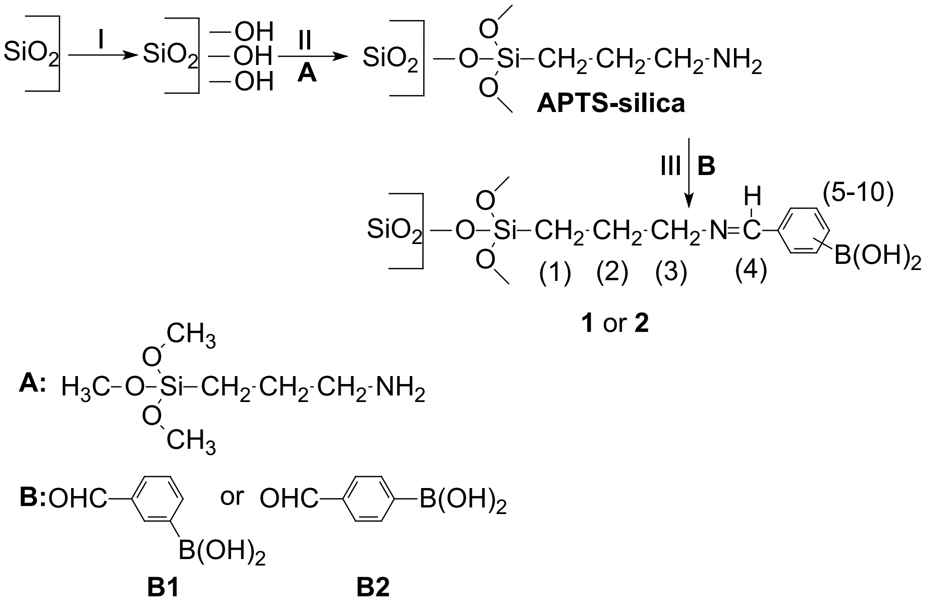

Scheme 1. Synthesis of the supports studied 1-2

Amorphous silica, Syloid® 244 (Grace Davision) was used as the support (according to the provider: composition of about 99.7% SiO_2; particle size 2.5-3.7 microns; pore volume 1.6 mL g^{-1}; specific surface area BET 314 m^2 g^{-1}).

The initial synthesis steps – SiO_2 hydration (I) and silanization procedure (II) have already been described previously [15]. In the final stage (III), Aminopropyl silica (APTS silica) was reacted with 3- or 4-formylphenylboronic acid (Aldrich) in methanol (Aldrich) to give products **1** or **2** respectively.

Elemental analysis found (%): **1** (N, 0.76; C, 6.96), **2** (N, 0.96; C, 9.54).

Elemental analysis of the supports studied was carried out on a Vario ELIII (Elementar, USA) analyzer, while thermogravimetric studies - in a Setaram TGA at the heating rate of 10°C min^{-1} in helium atmosphere. For the infrared measurement silicon wafer penetrable to the infrared beam was used. The IR spectra were recorded on an IFS 66v FT-IR spectrophotometer from Bruker, equipped with a MCT detector (128 scans, resolution 2 cm^{-1}). Nuclear magnetic resonance spectra of the solid samples were obtained on a Bruker DRX 500 MHz spectrometer with 4 mm MAS 1H/BB VTN probehead at room temperature.

Fresh double distilled water was used to prepare aqueous solutions. ARS solution (10^{-4} M) was prepared in phosphate buffer (0.1 M, pH 7.4). The complexes of the supports studied with ARS were prepared by the addition of 45 mg of supports **1-2** (approximately 10^{-3} M assuming the surface modification in 20%). Glucose was used as a model compound of *cis*-diol (10^{-1} M). Each sample was centrifuged and filtered before UV/VIS measurements. Analogous procedures were applied for studies of complexation of 3- and 4-formylphenylboronic acids solutions (10^{-3} M) with ARS and glucose. The association constants (K_1; M^{-1}) of the supports **1**(**2**)-ARS complex in 0.1 M phosphate buffer were determined by Benesi-Hildebrand method [16]. The concentration of the supports studied was used in large excess compared to ARS. It was then gradually increased to give five points for the graph. The dependence of ARS concentration/complex absorbance *vs*. 1/supports **1**(**2**) concentration was linear. The association constants (K_2; M^{-1}) of supports **1**(**2**)-glucose complex in 0.1 M phosphate buffer were determined using fixed concentrations of the supports studied and ARS, and different ranges of glucose concentrations [17]. K_1 divided by the slop of the plot gives the association constant K_2.

The absorbance of solutions was recorded using UV/VIS spectrophotometer from Agilent with a quartz cuvette 1 cm. UV/VIS absorbance spectra were measured at room temperature and 0.1 M phosphate buffer was used as a reference compound. The absorbance of functionalized silica gel treated with ARS and glucose solutions was recorded using UV/Vis/NIR from Varian.

3. Results and Discussion

Supports **1-2** gave similar FTIR spectra differing only in signal intensity (supplemental information). A broad band between 3200 and 3400 cm^{-1} was assigned to the O-H stretching frequency of silianol groups. The broad and intense band at 1100 cm^{-1} was assigned to the siloxane vibration (Si-O-Si). Bands at 808 and 496 cm^{-1} were observed due to Si-O-Si stretching and Si-O-Si bending, respectively. The reaction with a silane coupling reagent, leads to the appearance of bands at 2930 cm^{-1} (region of the C-H stretching vibrations of alkyl groups). Typical N-H stretching band, in 3400-3500 cm^{-1} was not observed due to the participation of the amine group in the Schiff base formation. A band at 1595 cm^{-1} indicated probably on Schiff base formation but it was not confirmed. It could be also associated with the benzene ring. Strong absorption of silica-gel at 1640 cm^{-1} (Si-O stretching vibrations) makes a proper signal assignment in this region impossible. The band of C=N was definitively confirmed by solid state NMR spectrometry.

The solid state, ^{13}C CP-MAS NMR spectra of supports **1**-**2** demonstrate the presence of three peaks at 10.09 (9.76), 21.81 (21.77) and 41.85 (42.31) ppm assigned to the carbon atoms in a propyl group numbered as 1, 2 and 3, and corresponding to support **1** (**2**) respectively. The signals of the aromatic carbon atoms are found in the range of 127.87-134.68 (127.06-134.13) ppm (carbons 5-10). The formation of imine group on the surface is confirmed by the peak at 165.44 (163.25) ppm (C=N group), numbered as 4 (supplemental information).

The results of elemental analysis of the supports studied allow the calculation of the amount of ligands attached to the surface of silica gel [18]. The percentage of nitrogen in the functionalized silica-gel **1** gave a value of 0.543 mmol g^{-1}, while in support **2** - 0.686 mmol g^{-1}. According to the results obtained, the degree of functionalization of the silica-gel is high. It suggests that the synthesis procedure applied for the support studied is very effective. Thermogravimetric curves show loss of mass by supports **1**-**2** as an effect of continuous heating in a helium atmosphere. First mass loss occurs in the region from 60 to 140°C (mass loss of about 1.5%) and is caused by abstraction of the physically adsorbed solvent applied in the synthesis. Further mass loss is observed until 630°C (mass loss of about 5%) due to elimination of part of the ligand (the decomposition of functional groups) and some silanol groups (see supplemental information).

The properties of phenylboronic acids (substrates) and supports **1**-**2** were investigated by UV/VIS studies carried out in phosphate buffer (pH 7.4). Fig. 1 presents the absorption spectra of ARS with and without 4-formylphenylboronic acid and glucose. The addition of acid causes a wavelength shift of maximum absorbance from 521 to 461 nm indicating complex formation between boronic acid and ARS. However, this complex is unstable in the presence of *cis*-diol (glucose). Competitive complex is obtained and the absorption of unbounded ARS is observed. This behaviour is in agreement with the previously described mechanism of boronic acid competitive binding [12]. An analogous procedure was applied for the modified silica gel. Schiff base systems are sensitive to the aqueous solution but the buffer environment ensures their stability. Initially, Alizarine Red S. solution was added to the modified silica surface, which resulted in colour change of the

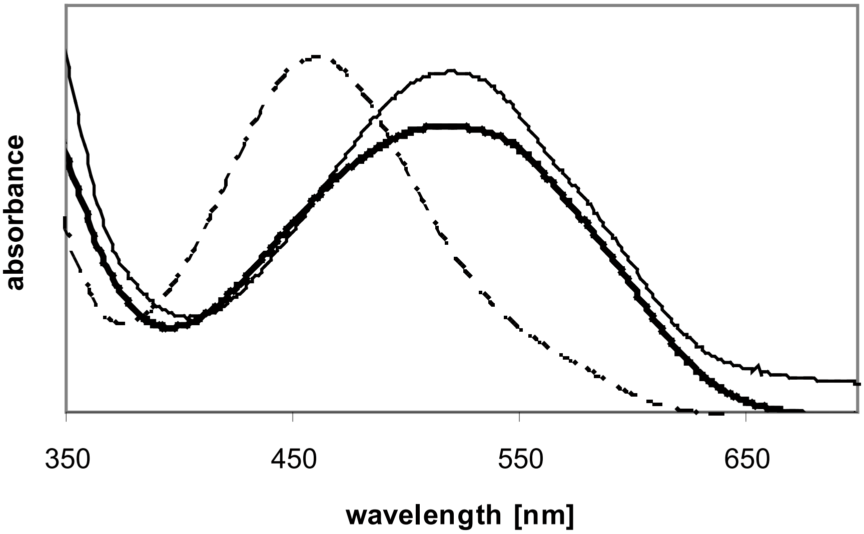

Figure 1. Absorbance of ARS in phosphate buffer (—); ARS with 4-formylphenylboronic acid (- - -); ARS with 4-formylphenylboronic acid and glucose (▬)

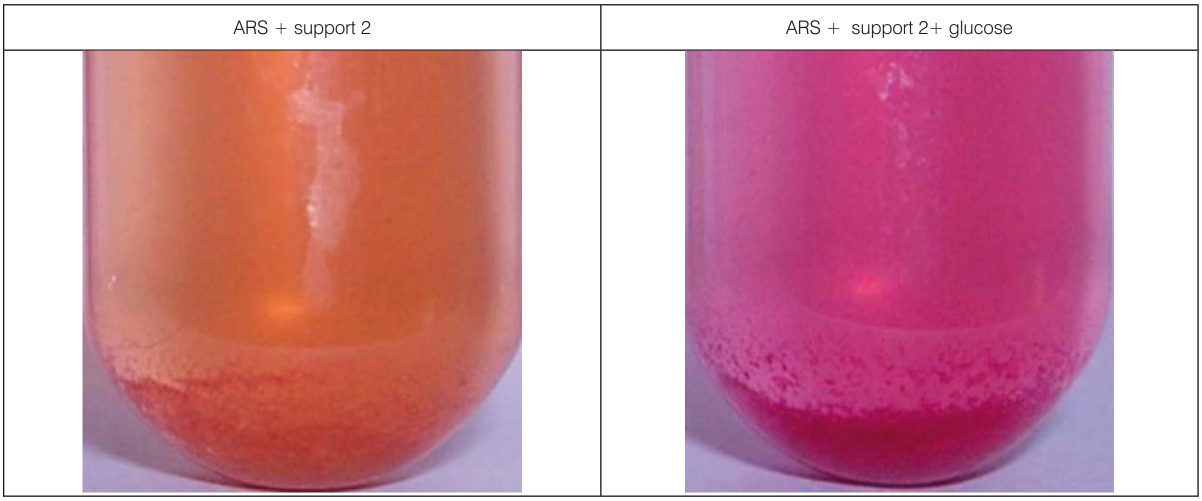

Figure 2. The influence of ARS and glucose solution on the color change of the support 2 from yellow to red

functionalized silica gel (from white to yellow). However, addition of the model compound (glucose) brought back the initial colour immediately upon mixing. The color change of the support **2** studied is presented in Fig. 2. The addition of the supports studied caused a significant decrease in the absorbance of ARS solution at 521 nm. After the addition of the glucose solution, the signal intensity at maximum wavelength increases again. On the other hand, the supports studied that are treated with ARS solution exhibit absorption band at 460 nm, which confirms complex formation between the functionalized silica gel and ARS. Further addition of glucose solution leads to disappearance of the absorption band at 460 nm.

The absorbances of the supports studied at different concentrations were used to calculate the association constant of support **1**(**2**)-ARS complex by Benesi-Hildebrandt equation. The parameters obtained were as follows: K_1=1.5×10^2; for support **1** and K_1=1.1×10^2 for support **2**. The K_1 values are in agreement with literature data. The compounds that contain 1,2-dihydroxyphenyl unit are characterized by high affinities to phenyl boronic acids [11,17]. K_1 were further used to calculate the association constants of support **1**(**2**)-glucose complex (K_2). In general, glucose has lower values of K_2 constants if compared with fructose and catechol. Indeed, the values obtained were 3.1 (3.5) for the support **1**(**2**)-glucose complexes. It indicates that glucose is characterized by weak affinity to phenylboronic acid derivatives (the supports studied). Nevertheless, the supports studied demonstrate similar behaviour of *cis*-diol sensing as their solution counterpart. The modified solid supports could be applied as a separation tool for *cis*-diols.

The two supports studied have very similar features. They differ only in the position of boronic acid substituents. The analogous synthesis procedure applied for both systems results in similar amount of the ligand attached to the silica surface. The association constants of the supports discussed-ARS (glucose) complex also demonstrate insignificant differences. According to the results obtained, the supports studied could be applied alternatively as separation tools because their effectiveness in *cis*-diol sensing is the same. The position of boronic acid substituent in the phenyl ring does not affect the complexing ability of *cis*-diols by the supports **1**-**2**.

In conclusion, we found that two silica surfaces modified with boronic acid derivatives are effective complexing agents of *cis*-diols. The boronate ester formation was monitored in the presence of the optical reporter-Alizarine Red S. The method described here could be used for fast and easy tests for presence of sugar in samples. The functionalized solid surface, sensitive to the presence of *cis*-diols, allows for monitoring of the process quantitatively and qualitatively. It could be applied to any analysis concerning sugars as a preliminary test.

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