Adsorption (2010) 16: 437–445
DOI 10.1007/s10450-010-9236-z

Influence of temperature and pressure on the preferential adsorption of component of hydroorganic mobile phase in liquid chromatography

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Received: 22 March 2010 / Accepted: 13 May 2010 / Published online: 29 June 2010

Abstract The excess isotherms of methanol and acetonitrile were measured on the series of C18 bonded phases. The measurements were done using the minor disturbance method. The goal of our work was to determine the influence of the temperature on the adsorption of two commonly used solvents. The influence on the mobile phase flow rate on the both organic solvent adsorption was also investigated. The effect of these two parameters was compared on the octadecyl packed columns with different coverage density and on the monolithic Chromolith column. Adsorption of both solvents decreases with the increase of the temperature. The increase of the pressure increases adsorption of methanol but decreases adsorption of acetonitrile.

Keywords Solvent adsorption · Stationary phase · Excess isotherm · Flow rate · Temperature

1 Introduction

Retention in reversed-phase liquid chromatography (RP HPLC) is controlled by the distribution of the solute between the bulk mobile phase and the stationary phase. The interaction between organic bonded ligands and the solute is governed by hydrophobic effect (Gadzała-Kopciuch and Buszewski 2003; Jaroniec 1993).

When the mobile phase is in contact with the hydrophobic surface of the stationary phase, solvent molecules can preferentially adsorb on the surface. The composition of the mobile phase at the surface is different from its bulk composition (Jaroniec 1993; McCormick and Karger 1980; Slais and Kreci 1974). In reversed phase liquid chromatography system, the acting stationary phase is a combination of three components: bonded ligands, adsorbed solvent molecules and residual silanols (Buszewski et al. 1994; Gritti and Guiochon 2005b; Sandi and Szepesy 1999). The solute retention is governed due to adsorption onto the alkylsilica surface, or to partitioning between the layer of bonded alkyl chains and the mobile phase. The combination of these two limitary mechanisms is also possible (Gritti et al. 2007b). The solvent molecules are distributed also between the mobile and the stationary phase and influence chromatographic separations (Berek et al. 1976; Jaroniec 1993, 1996; Kasturi et al. 1994; Piatkowski et al. 2006).

The excess amount of the adsorbed solvent changes with the coverage density (Bocian et al. 2009; Buszewski et al. 2008; Gritti et al. 2007b), with the number of carbon atoms in organic ligands (Bocian et al. 2008a; Kazakevich et al. 2001; Rustamov et al. 2001), with the presence of some polar groups in the ligands structure (Bocian et al. 2008b) and differs between end-capped and non end-capped stationary phases (Bocian et al. 2010; Poplewska and Antos 2005).

As was mentioned by Gritti and Guiochon (2005a), the type of the organic modifier has a big influence on the adsorption and retention of the solute. The retention and the elution in the chromatographic system is a competitive play between solute and organic modifier. In general, the increase of the temperature during chromatographic analysis results

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in decrease of the retention. On the other hand the increase of the pressure causes the increase of the retention, especially for bigger molecules (Guiochon et al. 2006; Neue 1997).

The solvation processes of the stationary phase by mobile phase components (water and organic solvents) depend on the temperature and pressure in the chromatographic system. The equilibrium between the mobile and the stationary phase is changing according to van't Hoff's equation:

$$\left(\frac{\partial \ln K}{\partial p}\right)_T = -\frac{\Delta V^0}{RT} \tag{1}$$

and Laar-Planck equation (Atkins 1997):

$$\left(\frac{\partial \ln K}{\partial T}\right)_p = \frac{\Delta H^0}{RT^2} \tag{2}$$

It is expected that the temperature and pressure influence the distribution of the solvents from the mobile phase to the stationary bonded phases (Berek et al. 1984).

The temperature influence on the solvent adsorption was measured in acetonitrile-water conditions by Kazakevich and McNair (1995) and methanol water condition by Poplewska et al. (2006). As expected, the decrease of the both solvents adsorption was observed with increase of the temperature.

In reversed phase chromatography, the influence of pressure on the chromatographic behavior is related to the hydrophobic interactions involved in the retention mechanism. The importance of the changes in the retention factor and the saturation capacity with a change in the average column pressure will vary with the hydrophobicity of the molecule (Liu et al. 2003). Thus, the differences between methanol and acetonitrile adsorption with variation of pressure are expected.

2 Theory

The thermodynamic void volume of the column (V_M) is obtained by integrating the plot of the retention volume (V_R) of the perturbation peaks (from 0 to 100% of the organic modifier) (Kazakevich and McNair 1995):

$$V_M = \frac{1}{C_{\max}} \int_{C=0}^{C=C_{\max}} V_R(C)dC \tag{3}$$

where $C_{\max}$ is a maximum concentration of the organic modifier in the mobile phase.

When V_M is known, the excess amount of the adsorbed organic modifier per unit amount of stationary phase (Γ) can be calculated (Kazakevich et al. 2001):

$$\Gamma(C) = \frac{1}{S} \int_0^C (V_R(C) - V_M)dC \tag{4}$$

where S is a total surface area of the stationary phase [m^2].

The maximum concentration of the adsorbed solvent (C_{ads}) in/on stationary phase can be found by extrapolating the slope of the excess isotherm in a linear region to the y-axis or it can be calculated as the intercept parameter of the straight line fitted to the linear region of the excess isotherm (Gritti and Guiochon 2005a).

In this work, we used the minor disturbance method to measure the excess adsorption isotherms of methanol and acetonitrile from water onto in-house made non-endcapped C18 bonded silica packing materials. The goal of our work was to determine the influence of the temperature on the methanol and acetonitrile adsorption on the octadecyl stationary bonded phase. The influence on the mobile phase flow rate, proportional to the back pressure, on the both organic solvent adsorption was also investigated. The effect of these two parameters was compared on the octadecyl packed columns with different coverage density and on the monolithic Chromolith octadecyl column.

3 Experimental

3.1 Instruments

The home-made phases under study were packed into 125 × 4.6 mm stainless steel columns. All columns were packed using a DT 122 packing pump (Haskel, Burbank, CA, USA) under the pressure of 50 MPa. Technical details of the procedure are described in earlier papers (Buszewski et al. 1997, 1991).

An HP Model 1050 (Agilent Technologies, Waldbronn, Germany) liquid chromatograph equipped with four-channel gradient pump, an autosampler with a 100-µL loop, diode array detector and computer data acquisition station.

Temperature was controlled using liquid thermostat Julabo F12 (Julabo Labortechnik GmbH, Seelbach, Germany).

3.2 Materials

The solid support of in-house made phases was Kromasil® 100 (Akzo Nobel, Bohus, Sweden). Five chromatographic columns with different surface coverage densities were studied. All the columns were prepared from the same batch of silica particles. The silica was chemically modified with octadecyldimethylchlorosilane (Wacker GmbH, Munich, Germany). Morpholine was purchased from Sigma-Aldrich Chemie (Steinheim, Germany). The characteristics of these stationary phases are listed in Table 1.

Table 1 Coverage density of stationary bonded phasesc

Phase code	Carbon load (P_c) [%]	Hydrogen load (P_H) [%]	Coverage density (α_{RP}) [μmol/m^2]
# 1	2.42	0.93	0.33
# 2	7.55	1.73	1.11
# 3	10.88	2.24	1.68
# 4	17.27	3.14	2.95
# 5	18.70	3.33	3.27

One commercially available chromatographic column Chromolith Performance RP-18e 100 × 4.6 mm (Merck KGaA, Darmstadt, Germany) was used in the study.

All solvents were HPLC "*isocratic grade*", purchased from Scharlau Chemie S.A. (Barcelona, Spain). Water was purified using Milli-Q system (Millipore, El Paso, TX, USA). All eluents were degassed in ultrasonic bath under vacuum.

Geometrical parameters of the packed columns and bare silica gel (pore volume, external porosity and surface area) were discussed in details in previous paper (Bocian et al. 2009).

The mass of the packing material present in each of the five columns studied in this work may be calculated using the method presented by Gritti et al. (2007b), Giaquinto et al. (2008). The total surface areas of silica gel in the column may be calculated using known carbon load and specific surface area of the bare silica gel. The amount of the silica gel in all columns is almost constant with the differences less than 7%. We can assume that all columns have the same silica surface area which is consistent with data presented in Gritti et al. (2007b). Calculations of the silica surface in the column and other geometrical parameters of these phases were presented in previous paper (Bocian et al. 2009).

3.3 Methods

For excess isotherm measurements each column was equilibrated with mobile phase of decreasing concentration of organic solvent in water (100, 98, 94, 90, 80, 70, 60, 50, 40, 30, 20, 10, 8, 4, 0%) by pumping at least 30 mL of solvent mixture. Perturbation of the base line was done by 5-μL injection of the mixture with higher concentration of organic solvent than the plateau concentration. The signal was detected with a UV detector at 195 and 200 nm. Injections were repeated three times in each measurement.

Measurements were carried out with flow rate 0.2, 0.5, 1, 1.5 and 2 ml/min at the temperature 295 K. For the temperature dependences the measurements were carried out in 278, 288, 295, 308 and 323 K (5, 15, 22, 35 and 50 °C) with the flow rate 1 ml/min.

4 Results and discussion

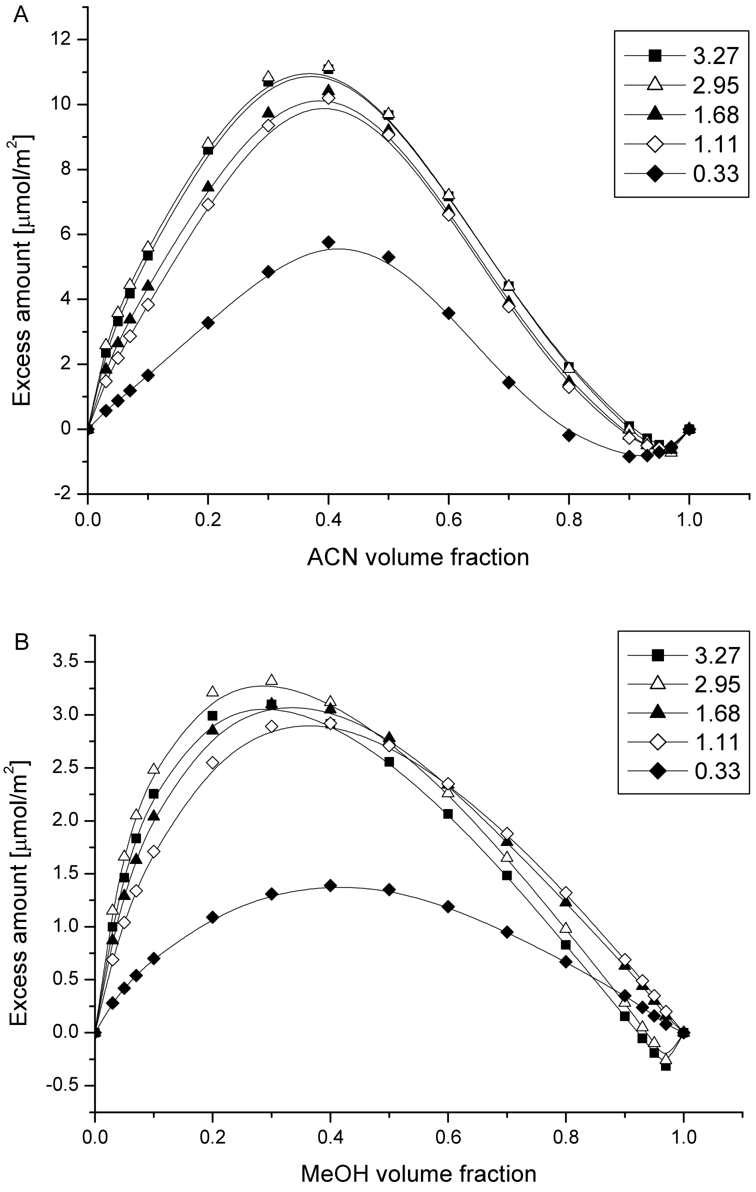
Measurement of the excess adsorption isotherm of methanol and acetonitrile from the binary mobile phases were done. As the adsorbents a series of octadecyl bonded phases with different coverage density were chosen. The excess isotherms of methanol and acetonitrile are presented in Fig. 1. All the curves behave in a similar manner. However, excess isotherms of methanol measured on low coverage density column do not exhibit a negative excess which is observed for adsorption on the high coverage phase (Fig. 1B). This indicates that the preferential type of interaction is hydrophobic (Kazakevich and McNair 1995). In the case of methanol, preferential adsorption of water on the residual silanols is observed only when the coverage density of the bonded ligands high. However, significant amount of methanol may be adsorbed near the silica surface through the hydrogen bonds with residual accessible silanols.

The excess amount of adsorbed methanol and acetonitrile increases with the surface coverage increase until the effect of the decreasing surface area becomes significant (Gritti et al. 2007b). When the modeled surface area of the stationary phase is used in the calculations (Bocian et al. 2009), the excess amount of adsorbed organic solvents increases in whole range of coverage density. It suggests that organic modifier molecules can not penetrate between bonded ligands if their coverage density is high.

All the excess isotherms of acetonitrile exhibit negative part at large acetonitrile concentration. It corresponds to adsorption of water. The amount of excessively adsorbed water on the residual silanols increases with the decreasing amount of water molecules in the mobile phase. The negative part of the excess isotherms decrease with the increase of the coverage density (Fig. 1A).

The maximum excess adsorption of water decreases when the surface coverage density increases. This result is consistent with the reduced number of residual silanols in the structure of bonded phase and with a reduced access of the mobile phase components to the silica surface when the thickness of the bonded layer increases.

Fig. 1 Comparison of acetonitrile (**A**) and methanol (**B**) excess isotherms



4.1 Comparison between packed and monolith bed

In the Fig. 2 the excess isotherms of methanol and acetonitrile on the Chromolith phase are presented. All isotherms behave in similar manner with small negative excess which correspond to adsorption of water molecules on the residual silanols. Acetonitrile is much stronger adsorbed on all tested stationary phases than methanol. On the Chromolith column, the adsorption of methanol is relatively higher than on the packed beds. Chromolith column and packed homemade column have very similar surface chemistry so the origin of interaction between solvent molecule and adsorption centers in the stationary phase should be the same. The main difference between this two types of columns is the pore distribution. The monolith stationary phase has much higher permeability in the comparison with packed bed (Guiochon 2007). It influences the mass transfer in the monolithic bed. The differences of the flow rate influence on the adsorption between packed and monolith bed is expected.

4.2 Influence of the temperature on the solvent adsorption

There have been many studies on the temperature dependence of the retention factor under linear conditions. However, only few studies were done on the influence of the temperature on the saturation capacity or on the other numerical coefficients of an isotherm (Guiochon et al. 2006).

The temperature dependences were investigated on two packed columns with radically different coverage densities (0.33 and 3.27 µmol/m^2) and on a monolithic column. As

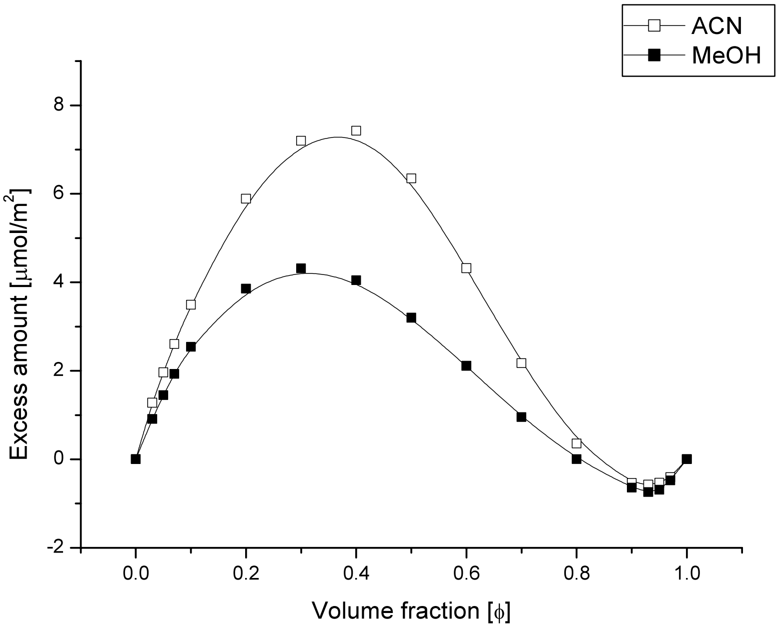


Fig. 2 Excess isotherm of methanol and acetonitrile on Chromolith phase

seen in Fig. 3 adsorption of both solvents decreases with the temperature increase in nonlinear manner. Similar results were observed previously by Kazakevich and McNair (1995) and Poplewska et al. (2006). However, in each paper only one commercial column were studied.

In the case of methanol (Fig. 3A), the highest decrease was observed on high coverage density phase (3.27 µmol/m^2) in the range 278–288 K. This decrease was about 30% of the initial value. For low coverage density phase, the decrease of the adsorbed methanol with increase of the temperature was much smaller. The relative decrease of methanol adsorption in the whole rage of tested temperatures (278–323 K) was 55, 40 and 25% for low coverage phase (0.33 µmol/m^2), high coverage phase (3.27 µmol/m^2) and Chromolith phase respectively.

It is known that the adsorption of methanol is much weaker than adsorption of acetonitrile (Bocian et al. 2009; Gritti and Guiochon 2005a; Gritti et al. 2007b). In the case of acetonitrile adsorption, the dominant interaction of molecules with the stationary bonded phase surface is the hydrophobic effect. Another situation is with methanol. Methanol molecule can interact with bonded ligands due to hydrophobic interaction but polar interaction with residual silanols are very common (Bocian et al. 2010).

As it can be expected, the temperature influences acetonitrile adsorption much stronger (Fig. 3B). The relative decrease of acetonitrile adsorption in the whole range of tested temperatures 278–323 K was 70, 45 and 50% for low coverage phase (0.33 µmol/m^2), high coverage phase (3.27 µmol/m^2) and Chromolith phase, respectively. As seen, the stronger adsorption of solvent causes the stronger influence of the temperature on this process.

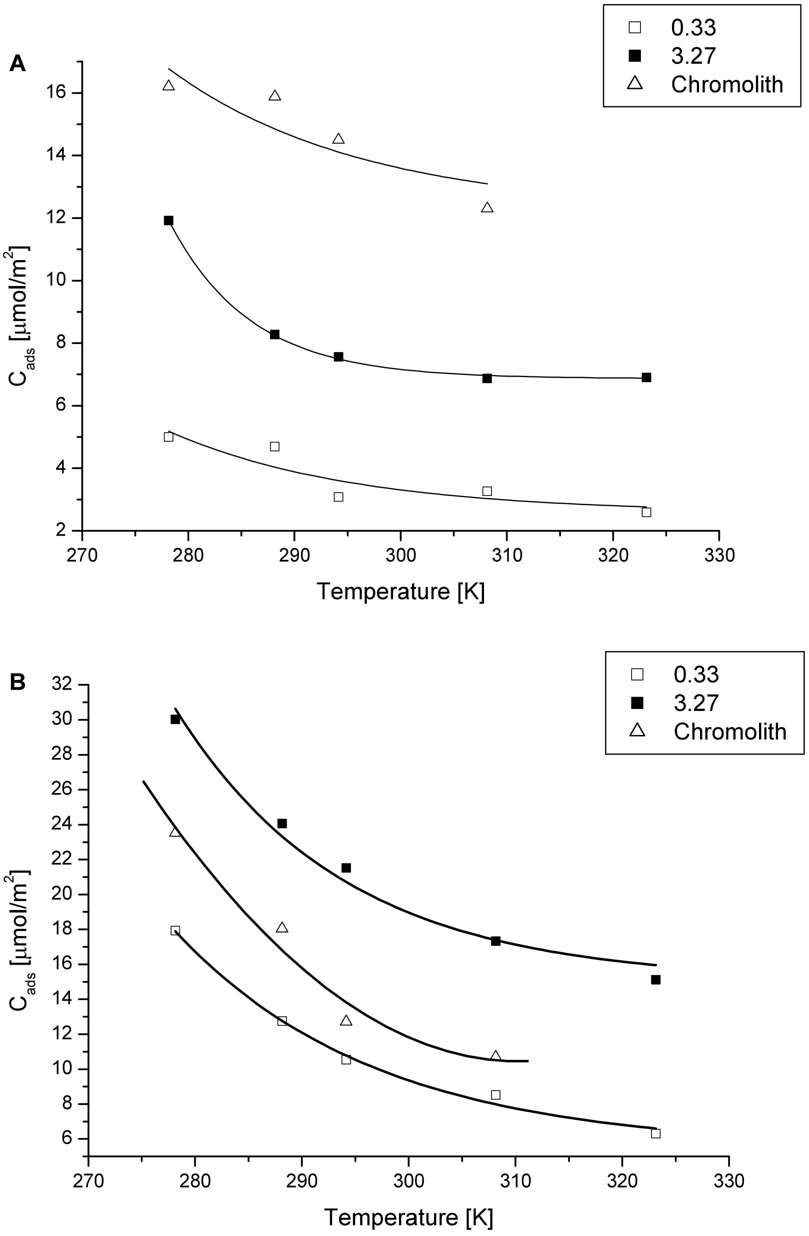
The decrease of the solvent adsorbed amount with the temperature can be caused by two effects. First, if the adsorption process is exothermal, the solvent adsorption decreases with increasing temperature according to van't Hoff equation (Guiochon et al. 2006; Neue 1997). Second, the increase of the temperature affects geometric parameters of the stationary phase. As was reported, the dead volume of the column decreases with the temperature increase (Ahmad and Guiochon 2006; Gritti et al. 2007a; Kazakevich and McNair 1995). It is caused by conformational changes of the bonded ligands. As a result the surface area of the interface stationary phase—mobile phase accessible for solvent molecule also decreases. This can reduce the amount of adsorbed solvent.

4.3 Influence of the flow rate on the solvent adsorption

Changing of the mobile phase flow rate through the column results a proportional variation of the back pressure along the column. These changes seem to be linear, at least in the range of pressures that are accessible in preparative liquid chromatography (usually less than 200 bar) (Guiochon et al. 2006). In excess adsorption measurement, the composition of the mobile phase is changing from pure organic solvent to pure water. It causes the back pressure changes parabolic during the analysis and it is difficult to use back pressure as a parameter in this investigation. However, pressure is proportional to the flow rate all time during the analysis on given column. In this case the maximum of adsorbed solvent is plotted over the flow rate.

It is important to notice that large molecules tend to have a larger difference in their molar volumes than smaller

Fig. 3 Influence of the temperature on the maximum concentration of the adsorbed solvent (C_{ads}): methanol (**A**) and acetonitrile (**B**)



ones if they are adsorbed on the surface from the solution. The effect of pressure on the adsorption is usually rather small, often almost negligible for compounds having a small molecular mass (McGuffin and Chen 1997). Changes of the pressure has important effect on the adsorption of peptides and other macromolecules (Liu et al. 2003; Zhou et al. 2003). As seen in Fig. 4, the pressure influence is also observed in the adsorption of organic solvent molecules from the binary hydro-organic mobile phase despite of their low molecular volume.

In the case of acetonitrile (Fig. 4A), the slight decrease of the adsorption (about 10%) with pressure increase in tested range is observed for packed columns. Opposite tendency is observed on the Chromolith phase which exhibit high increase especially for higher flow rate. This effect is probably caused by difference in the mass transfer between packed and monolithic bed. Faster mass transfer in the monolithic column causes increase of the adsorption with back pressure increase. In the packed column mass transfer of the solvent to the surface in pores is lower so the increase of the flow rate may hamper the diffusion to the stationary phase—mobile phase interface. Obviously it depends on the type of the molecules. On the packed columns, changes in the organic solvents adsorption with pressure do not depend on the surface coverage density with bonded organic ligands.

The adsorption of methanol increases with the increase of the pressure (see Fig. 4B). In tested range, the increase of the methanol adsorption about 20–30% is observed. Much

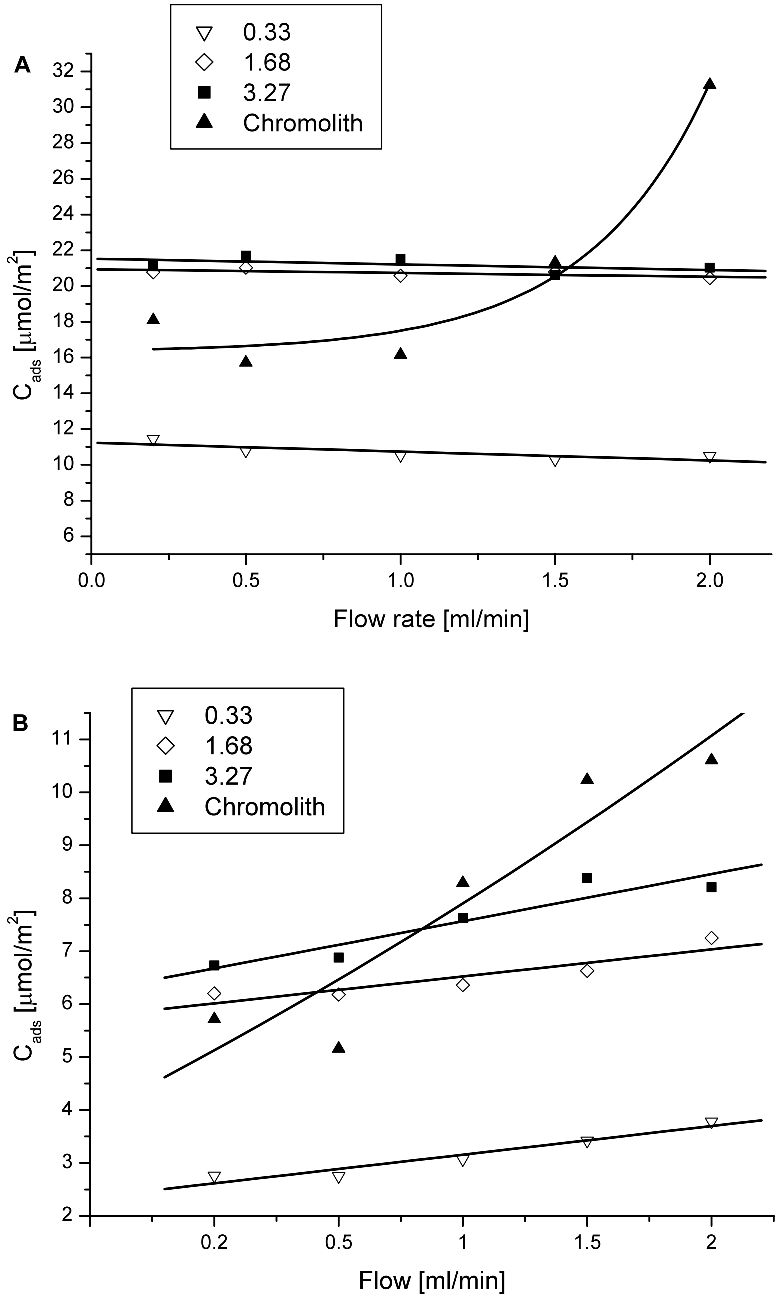


Fig. 4 Influence of the flow rate on the maximum concentration of the adsorbed solvent (C_{ads}): acetonitrile (**A**) and methanol (**B**)

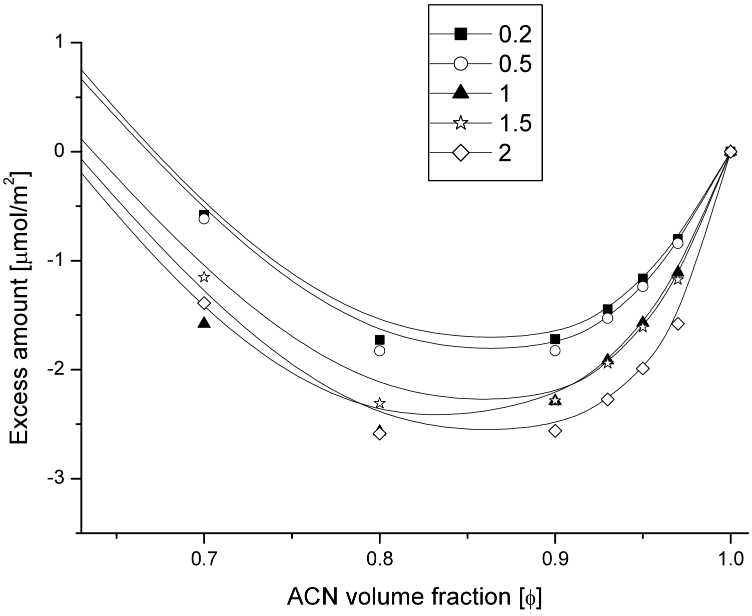
higher influence of the pressure is observed on the Chromolith phase. The increase in the tested flow rate exceeds 50%.

The decrease of the acetonitrile adsorption on the packed columns may be connected with preferential adsorption of water on the residual silanols. As seen in Fig. 5, the adsorption of water increases with an increase of the pressure (flow rate). In the binary system, when increase of the water adsorption with pressure is higher than increase of acetonitrile adsorption, water molecules may displace acetonitrile molecules from the surface of the stationary phase. The displacement may be illustrated by following equation (Gritti and Guiochon 2007):

$$[organic]^{adsorbed} + [\mathrm{H_2O}]^{mobile} \Leftrightarrow [organic]^{mobile} + [\mathrm{H_2O}]^{adsorbed} \quad (5)$$

In methanol-water system, there is a competitive adsorption of water and methanol molecules on the residual silanols. Adsorption of water from methanol-water mobile phase is much smaller than in the case of acetonitrile. It seems that possibility of hydrogen bond creation between methanol and silanols causes the preferential adsorption of methanol molecules on the silica surface. Therefore the in-

Fig. 5 Negative part of the acetonitrile excess isotherm obtained in the measurement with different flow rate of the mobile phase



crease of methanol adsorption with pressure increase is observed.

5 Conclusions

The temperature and the pressure are important parameters which influence chromatographic separation. These parameters influence retention of the solutes and solvation processes of the stationary phase by solvent molecules from mobile phase.

The adsorption of methanol and acetonitrile decreases with the increase of the temperature. These changes are better visible for stronger solvent—acetonitrile, which has relatively higher adsorption. The highest decrease of adsorption was observed on the stationary phase with low coverage density.

The pressure influence on the solvent adsorption is not ambiguous. The adsorption of acetonitrile decreases with increase of the pressure whereas adsorption of methanol increases. It can be connected with competitive adsorption of water.

The big differences are observed when packed and monolithic column are compared. First, the adsorption of methanol on Chromolith column is relatively higher in the comparison with packed bed. The increase of the both solvent adsorption on the monolith column with pressure is also much higher than in the case of packed column.

Acknowledgements This work was sponsored by Ministry of Science and Higher Education (KBN, Warsaw, Poland, Grant No. N N204 136637 for 2009–2011 period). Financial support from European Social Found, Polish National Budget, Kujawsko-pomorskie Voivodship Budget (within Sectoral Operational Programme Human Resources)—"Krok w przyszlosc II" and "Stypendia dla doktorantów 2008/2009-ZPORR" is gratefully acknowledged. The authors thank Akzo Nobel (Bohus, Sweden) for kind donation of the Kromasil 100 silica gel used in this study.

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