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Surface energies of hydrophobic interaction chromatography media by inverse liquid chromatography☆

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ABSTRACT

Hydrophobicity of hydrophobic interaction chromatography media is currently ranked according to retention of reference proteins. A new method, suitable for porous media, is presented here to determine the surface energy and its Lifshitz–van-der-Waals, Lewis acid and Lewis base contributions. The theory of van Oss has been adapted for data obtained by inverse liquid chromatography. Furthermore, this method is characterized by the independence of the determination of the phase ratio. The retention of probes with different molecular properties was used to calculate the surface energy and the Lifshitz–van-der-Waals as well as Lewis acid and Lewis base contributions to the surface energy. The media with polymethacrylate backbone had a higher surface energy ($\gamma \approx 200$ mJ/m^2) and Lifshitz–van-der-Waals contribution ($\gamma^{LW} \approx 140$ mJ/m^2) than the agarose-based media ($\gamma \approx 90$–180 mJ/m^2 and $\gamma^{LW} \approx 50$–160 mJ/m^2).

1. Introduction

Surface energy is the general property describing the adsorption strength of surfaces and this concept can be extended to chromatography. For hydrophobic interaction chromatography (HIC) media, this property allows a quantitative description of the surface and provides a ranking according to a physical quantity, i.e., the surface energy expressed in mJ/m^2. Current methods have characterized media on an empirical basis, which has only allowed ranking in a relative manner. The retention of reference proteins in isocratic or gradient elution has been used for this purpose [1,2]. Retention has been also predicted by docking experiments [1,3,4]. Alternatively, quantitative structure property relationship (QSPR) models have provided an indirect insight into hydrophobicity [5,6]. However, none of these methods are able to predict surface energy.

Claessens et al. [7] have classified reversed phase liquid chromatography (RPLC) media with respect to hydrophobicity by various tests as suggested by Engelhardt [8,9], Walters [10], Tanaka [11] and Galushko [12]. In these tests, the hydrophobicitiy was assessed by the hydrophobic selectivity of the chromatographic media for each of two molecular probes. Unfortunately, these assessments proved to be poor descriptors for hydrophobicity. These investigators developed these analyses by using absolute values of retention factors and observed a better correlation by using the logarithm of the retention factor k' as a measure of hydrophobicity rather than from using hydrophobic selectivity. Hydrophobicity is not a definite quantitative physical property because the hydrophobicity is always specific for a certain column and therefore it is dependent on the surface area as well as on the ligand size [13].

Herein, we describe a new method to evaluate hydrophobicity that also uses absolute values of k'. This method compares different surfaces on a rational basis in terms of physical quantities, independent of column dimensions.

Chromatography media for protein separation on the preparative scale are porous beads in the range of 10–300 μm, with pore sizes ranging from 10 nm to 1300 nm. The selection of the best medium with an adequate combination of these dimensions depends on the application for which the media will be used [14]. Chromatography beads are compressible and the extraparticle and therefore also the total porosity depends on the packing density [15]. Generally, natural polymer-based media are more compressible than synthetic polymer-based media.

The determination of surface energies is conventionally done via contact angle measurements [16]. The contact angle measurements of porous media are often erroneous due to soaking effects of the media [17], as well as discrepancies in the determination of the surface angle on non-planar surfaces. For this reason contact angle measurements are rarely applied for this purpose. Reports

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have shown different values of the contact angle with advancing and retracting drops when HIC media were investigated [18]. Thus, a method to determine the surface energy independent of the packing density is needed. Furthermore, the test samples (molecular probes) and the chromatography media must be compatible.

We have developed a method based on inverse liquid chromatography (ILC) in which the retention of the molecular probes serves as a measure for the strength of interaction. The concept of van Oss [19] was adapted for ILC and has been modified to calculate surface energies (see theory Section 3) without requiring the determination of porosity. The chromatographic experiments have shown that the retention time of different molecular probes as defined by the peak maximum is not a suitable measure to describe the different interactions between the molecular probes and the HIC media. As a distinctive criterion, the shape of the peaks was used to gain information about the interaction of each molecular probe with the different column media. By fitting the data with a suitable function, we obtained the parameters required for calculating the surface energies according to the concept of van Oss were obtained.

2. Experimental

Five molecular probes were selected and used to determine the surface energy of three natural polymer-based and two synthetic polymer-based chromatography media.

2.1. Stationary phases

The natural polymer-based media used included Butyl Sepharose 4 Fast Flow (FF), Phenyl Sepharose 6 FF (low sub), and Phenyl Sepharose High Performance (HP) from GE Healthcare (Uppsala, Sweden). We also used the synthetic polymer-based media Toyopearl Butyl-650M, Toyopearl Phenyl-650M and Toyopearl HW-65 from Tosoh Bioscience (Stuttgart, Germany). All media were packed by Atoll (Weingarten, Germany) in MediaScout MiniChrom columns with a column volume of 10 ml.

2.2. Mobile phases and molecular probes

We used glycerin (MW_{GLY} = 92.09 g/mol), ethylene glycol (MW_{EG} = 62.07 g/mol), dimethyl sulfoxide (MW_{DMSO} = 78.13 g/mol) and methyl ethyl ketone (MW_{MEK} = 72.11 g/mol), all analytical grade, as the molecular probes. The dilution was 1:2 in HQ-water, except for MEK, where the dilution was 1:6. As mobile phase HQ-water was used. We also performed studies with glucose (0.35M) in a 5% methanol solution.

2.3. Instrumentation and data handles

The columns were connected to a HPLC workstation (Agilent Series 1100 LC, Santa Clara, CA, United States) which was connected to a PC and controlled by Chem Station for LC 3D systems Rev. B. 04.03 (Agilent Technologies, Inc. 1994–2007, 2008). The UV detector response was transferred to the program where the files were exported in CSV format for further handling with Mathematica (Wolfram Research, Inc., Mathematica, Version 8.0, Champaign, IL (2010)).

2.4. Chromatography experiments

The pulse experiments were completed individually for each molecular probe. An aliquot (50 μl of glucose solution; 10 and 20 μl of GLY; 10 and 20 μl of EG; 10 and 40 μl of DMSO and 60 μl of MEK) of the molecular probe was injected into the column. The flow rate for all experiments was 1 ml/min. The continuously detected UV absorption (the glucose solution, GLY and EG were monitored at a λ of 205 nm, MEK was monitored at a λ of 210 nm in each case with a bandwidth of 4 nm; DMSO was monitored at a λ of 250 nm with a bandwidth of 30 nm, the reference wavelength was 400 nm with a bandwidth of 4 nm), increased when the molecular probes reached the detector. The retention behaviors were interpreted as discussed in Section 3.

3. Theory

3.1. The exact solution for the infinite diluted system

We formulated the mass balance for the column, assuming that: the column media is homogeneous, the compressibility of the mobile phase is negligible; the axial dispersion coefficient is constant and independent of the solute concentration; the density and velocity of the mobile phase are constant along the column; the partial molar volumes are the same in both phases; no thermal effects occur; the influence of the heat of adsorption on the band profile is negligible; the experimental parameters fall within the linear region of the isotherm (Henry region); and the mass transfer kinetics are fast (but not infinitely fast) [20].

$$\partial_t c + \frac{1-\epsilon}{\epsilon}\partial_t q_{vol} + u\,\partial_z c - D\,\partial_z^2 c = 0 \tag{1}$$

The solid loading q_{vol} (equilibrium concentration of a component in the stationary phase) and the concentration in the mobile phase c are dependent on time t and on a spatial dimension z in the direction of the column. A single axial dispersion coefficient, D takes into account various contributions to peak broadening. The parameter u is conventionally the interstitial velocity; here it represents the chromatographic velocity and was obtained by using the retention time t_R (peak maximum) of glucose: $u = L/t_R$, with L, the column length. Next we adapted Eq. (1), and we introduced the implicit relation:

$$\lim_{c\to 0}\partial_t q = \underbrace{\partial_c q}_{H}\,\partial_t c \tag{2}$$

Therein, H gives the Henry constant. We introduce a parameter $k' = (1-\varepsilon/\varepsilon)H$ with ε identifying the total porosity. It describes the ratio between the volume of the mobile phase and the column volume.

To give Eq. (1) in a more compact form, we follow [21] and introduce new coordinates: $\xi = z - ut$ and $\tau = t$. Then the mass balance equation can be rewritten:

$$\partial_\tau c = \frac{D}{1+k'}\partial_\xi^2 c \tag{3}$$

A general solution is

$$c = \left(2\sqrt{D\pi\frac{\tau}{1+k'}}\right)^{-1}\int_{-\infty}^{\infty}\phi(\beta')\exp\left(-\frac{(\xi-\beta')^2}{4D(\tau/1+k')}\right)d\beta' \tag{4}$$

where $\phi(\beta)$ represents an initial condition. We insert $\phi(\beta') = \delta(\beta')$ and obtain for the original coordinates:

$$c_{k'} = c_0\frac{1}{2\sqrt{D\pi(t/1+k')}}\exp\left(-\frac{(z-u(t/1+k'))^2}{4D(t/1+k')}\right) \tag{5}$$

Finally, if we assume the response is a superposition of those part of the molecular probes that have interacted, and thus were retarded ($k' > 0$), and the remaining part of these probes that had not interacted with the surface ($k' = 0$), then:

$$c_{k'} = \int_0^\infty (\delta(k'') + \delta(k''-k'))c_{k''}dk'' \tag{6}$$

At which δ represents the Dirac delta function.

3.2. Surface energy contributions

van Oss postulated that interactions in condensed media may be divided into apolar interactions (Lifshitz–van-der-Waals, LW) and polar interactions [19]. The polar interactions are defined as comprising all electron-acceptor (Lewis acid) and electron-donor (Lewis base) interactions, denoted by AB. The polar and apolar components of the free energies of interfacial interaction are additive:

$$\Delta G = \Delta G^{LW} + \Delta G^{AB} \tag{7}$$

with

$$\gamma_i = -\frac{1}{2}\Delta G_{ii} \tag{8}$$

The surface energy is partitioned in the same way:

$$\gamma_i = \gamma_i^{LW} + \gamma_i^{AB} \tag{9}$$

The polar contribution to the surface energy is defined as:

$$\gamma_i^{AB} = 2\sqrt{\gamma_i^+ \gamma_i^-} \tag{10}$$

with γ_i^+ representing the electron acceptor (Lewis acid) parameter of phase i, while γ_i^- represents the electron donor (Lewis base) parameter of phase i.

The Dupré equation describes the work between solid (S) and liquid (L) components as:

$$-\Delta G_{SL} = \gamma_{SL} - \gamma_S - \gamma_L \tag{11}$$

By expanding this concept to a description of interactions between two media (1 and 2) which are immersed in a liquid (3) the free energy of interaction is then:

$$\Delta G_{132} = \gamma_{12} - \gamma_{13} - \gamma_{23} \tag{12}$$

where γ_{lm} describes the interfacial tension between two phases l and m. Also the interfacial tension can be divided into polar and apolar contributions:

$$\gamma_{lm}^{LW} = \left(\sqrt{\gamma_l^{LW}} - \sqrt{\gamma_m^{LW}}\right)^2 \tag{13}$$

$$\gamma_{lm}^{AB} = 2\left(\sqrt{\gamma_l^+} - \sqrt{\gamma_m^+}\right)\left(\sqrt{\gamma_l^-} - \sqrt{\gamma_m^-}\right) \tag{14}$$

If both contributions to surface tension are present, polar and apolar, the surface energy Eq. (12) calculates to:

$$\begin{aligned}\Delta G_{ijk} = {} & -2\gamma_j^{LW} + 2\sqrt{\gamma_i^{LW}\gamma_j^{LW}} + 2\sqrt{\gamma_k^{LW}\gamma_j^{LW}} - 2\sqrt{\gamma_i^{LW}\gamma_k^{LW}} \\ & + 2\sqrt{\gamma_j^+}\left(\sqrt{\gamma_i^-} + \sqrt{\gamma_k^-} - \sqrt{\gamma_j^-}\right) + 2\sqrt{\gamma_j^-}\left(\sqrt{\gamma_i^+}\right. \\ & \left. + \sqrt{\gamma_k^+} - \sqrt{\gamma_j^+}\right) - 2\sqrt{\gamma_i^+\gamma_k^-} - 2\sqrt{\gamma_i^-\gamma_k^+} = \{\gamma_{i,j,k}^{\{LW,+,-\}}\}\end{aligned} \tag{15}$$

The difference of the free energy of adsorption is proportional to the logarithm of the equilibrium binding constant K_{eq} [22]:

$$\Delta G_{ijk} = -\frac{k_B T}{A_i}\ln K_{ijk} \tag{16}$$

where k_B is Boltzmann's constant, A_i is the theoretical area an adsorbed molecule of substance i covers on the surface, the values for all molecular probes (listed in Table 1) were calculated by Eq. (17), as described in Ref. [23].

$$A_i = \sqrt[3]{\pi}\left(3\frac{MW_i}{4\rho_i N_A}\right)^{2/3} \tag{17}$$

For weak concentrations, the distribution coefficient of adsorption K_{ijk} equals the Henry coefficient, H_{ijk}, and therefore the Henry coefficient of a substance can be written as a function of the polar and apolar contributions to the surface energies of the substance itself as well as the media which adsorbs the substance and a third substance, the liquid in which the two other substances are immersed:

$$-\frac{k_B T}{A_i}\ln H_{ijk} = \{\gamma_{i,j,k}^{\{LW,+,-\}}\} \tag{18}$$

To calculate the three contributions of the substance k: γ_k^{LW}, γ_k^+ and γ_k^- (the media absorbing a substance i) a set of three equations are necessary (therefore one substance has to be varied), then a linear set of three equations allows the calculation of the three parameters of interest.

Table 1
The theoretical area an adsorbed molecule of substance i covers on the surface; values are given in m^2.

	A_i
GLY	2.962×10^{-19}
DMSO	2.907×10^{-19}
EG	2.474×10^{-19}
MEK	3.394×10^{-19}

3.2.1. Adaption of the van Oss concept

From fitting the experimental data, we obtained the parameter k' which is linked to the Henry coefficient H by the following equation:

$$H_{ijk} = k'_{ijk}\frac{\varepsilon}{1-\varepsilon} \tag{19}$$

Due to the relation described in Eq. (19) the estimation of the Henry coefficient from k'_{ijk} (and vice versa) depends strongly on the flawless and accurate determination of the porosity. To eliminate the influence of the porosity and the accuracy of determination of the porosity, the concept of van Oss was developed further. Another set of equations is set up by a linear combination of four equations of this form:

The linear combination of two equations (with different molecular probes 1 and 2) is:

$$\begin{aligned}\ln H_{1jk} - \ln H_{2jk} &= \ln\frac{H_{1jk}}{H_{2jk}} = \ln\frac{k'_{1jk}}{k'_{2jk}} + \underbrace{\ln\frac{\varepsilon_1(1-\varepsilon_2)}{\varepsilon_2(1-\varepsilon_1)}}_{\text{for } \varepsilon_1 \approx \varepsilon_2 \to 0} = \ln\frac{k'_{1jk}}{k'_{2jk}} \\ &= \frac{1}{k_B T}(A_1\{\gamma_{1,j,k}^{\{LW,+,-\}}\} - A_2\{\gamma_{2,j,k}^{\{LW,+,-\}}\})\end{aligned} \tag{20}$$

Using k'_{ijk} instead of H_{ijk} has the advantage that there is no need to determine the porosity by an extra measurement. This requires that the ratio of H_{1jk} to H_{2jk} is the same as the ratio of k'_{1jk} to k'_{2jk}, which determines the values of the porosity for different molecular probes to be almost equal, so that the term $\ln(\varepsilon_1(1-\varepsilon_2))/(\varepsilon_2(1-\varepsilon_1))$ can be neglected. From fitting the experimental data, we gain k'_{ijk} directly.

Once the three contributions to the surface energy of a chromatography media are obtained, the Henry coefficient can be calculated by using Eq. (18). As a next step, the porosity can be determined by using the relation between H, k' and ε as described in Eq. (19).

4. Results and discussion

First the molecular probes have been injected to the respective chromatography columns. The retention data of the molecular probes with the media investigated are plotted in Figs. 1a–2c. In

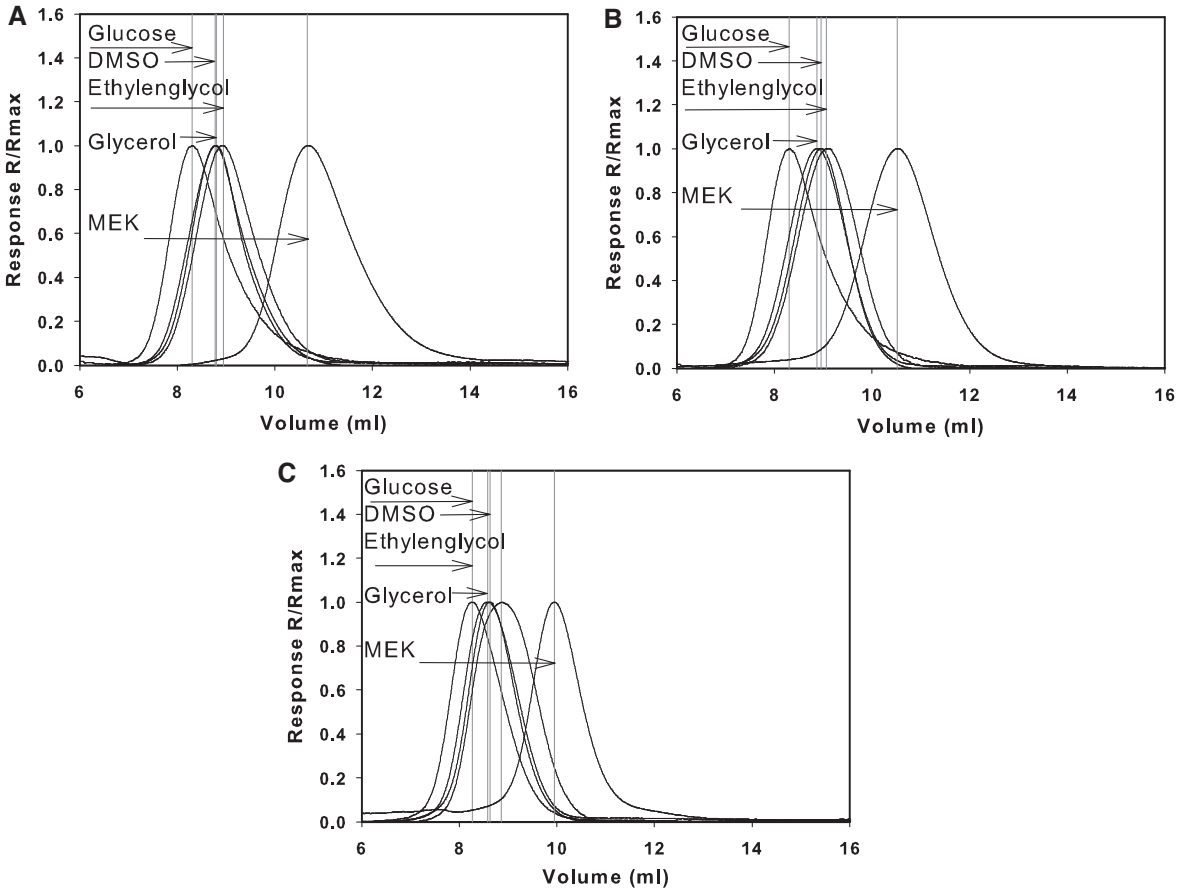

Fig. 1. UV-absorbance over time, normalized to the maximum response. (a) Tosoh Butyl-650M, (b) Tosoh Phenyl 650-M and (c) Tosoh HW 65.

Table 2
Arithmetical mean of k' values for GLY and DMSO with the standard deviation σ; values have to be multiplied by a factor of 10^{-3}.

	k'_{GLY}	k'_{DMSO}
GE Butyl Seph. 4 FF	35 ± 3	29 ± 9
GE Phenyl Seph. HP	51.6 ± 0.3	16.8 ± 0.4
GE Phenyl Seph. 6 FF (ls)	106 ± 13	56.4 ± 0.7
Tosoh Butyl-650M	64.6 ± 0.2	70.2 ± 0.1
Tosoh Phenyl-650M	51 ± 1	59.48 ± 0.09
Tosoh HW 65	45.7 ± 0.3	49.5 ± 0.6

Table 4
The LW-, Lewis acid (+) and Lewis base (−) contributions to the surface energy γ for the chromatography media investigated; values are given in mJ/m^2.

	γ^{LW}	γ^+	γ^-	γ
GE Butyl Seph. 4 FF	49.4	19.5	28.3	96.4
GE Phenyl Seph. HP	161	1.86	30.9	176
GE Phenyl Seph. 6 FF (ls)	57.9	6.37	34.4	87.5
Tosoh Butyl-650M	144	20.1	53.8	210
Tosoh Phenyl-650M	124	22.5	52.3	193
Tosoh HW 65	156	24.6	42.9	221

Fig. 3, the fit of the retention behavior of DMSO with GE Healthcare Phenyl Sepharose HP media is shown as an example.

The parameter k' (see Tables 2 and 3) is obtained by taking the average value of all measurements of a molecular probe. This was done for the investigated chromatography media. The contributions to the surface energies for the chromatography media were calculated by using the system of equations (see Eq. (20)) and listed in Table 4. γ is calculated by using Eq. (9).

Table 3
Arithmetical mean of k' values for EG and MEK with the standard deviation σ; values have to be multiplied by a factor of 10^{-3}.

	k'_{EG}	k'_{MEK}
GE Butyl Seph. 4 FF	35 ± 2	41 ± 5
GE Phenyl Seph. HP	30.4 ± 0.3	38.9 ± 0.3
GE Phenyl Seph. 6 FF (ls)	58.7 ± 0.9	77.4 ± 0.3
Tosoh Butyl-650M	89 ± 3	312.1 ± 0.3
Tosoh Phenyl-650M	71.6 ± 0.3	235.6 ± 0.1
Tosoh HW 65	80.1 ± 0.6	209 ± 0.4

The synthetic polymer-based media (Tosoh Toyopearl media) exhibited an approximately 2–3 times larger Lifshitz–van-der-Waals contribution to the surface energy than the natural polymer based media (GE Healthcare Sepharose media), except GE Phenyl Sepharose HP which has a Lifshitz–van-der-Waals contribution of the same order of magnitude than the polymer-based media (Table 4). Considering the small size of the molecular probes, one would expect that the molecular probes can enter the space between the ligands attached to the gel surface and come in contact with the backbone media. This would explain the larger surface energy values of the synthetic polymer-based backbone media.

The determination of the surface energy of the ligand free backbone material for the Tosoh materials, Tosoh HW 65 supports this theory. Tosoh HW 65 exhibits a surface energy of about 110% of the surface energy of Tosoh material with ligands, which corresponds with the theoretical concept.

On the other hand, surface energies for polymethacrylate material have been determined and are in the range of 40 mJ/m^2 [24,25]. This value is about five times lower than the values we obtained for the synthetic media (see Table 4, Tosoh materials).

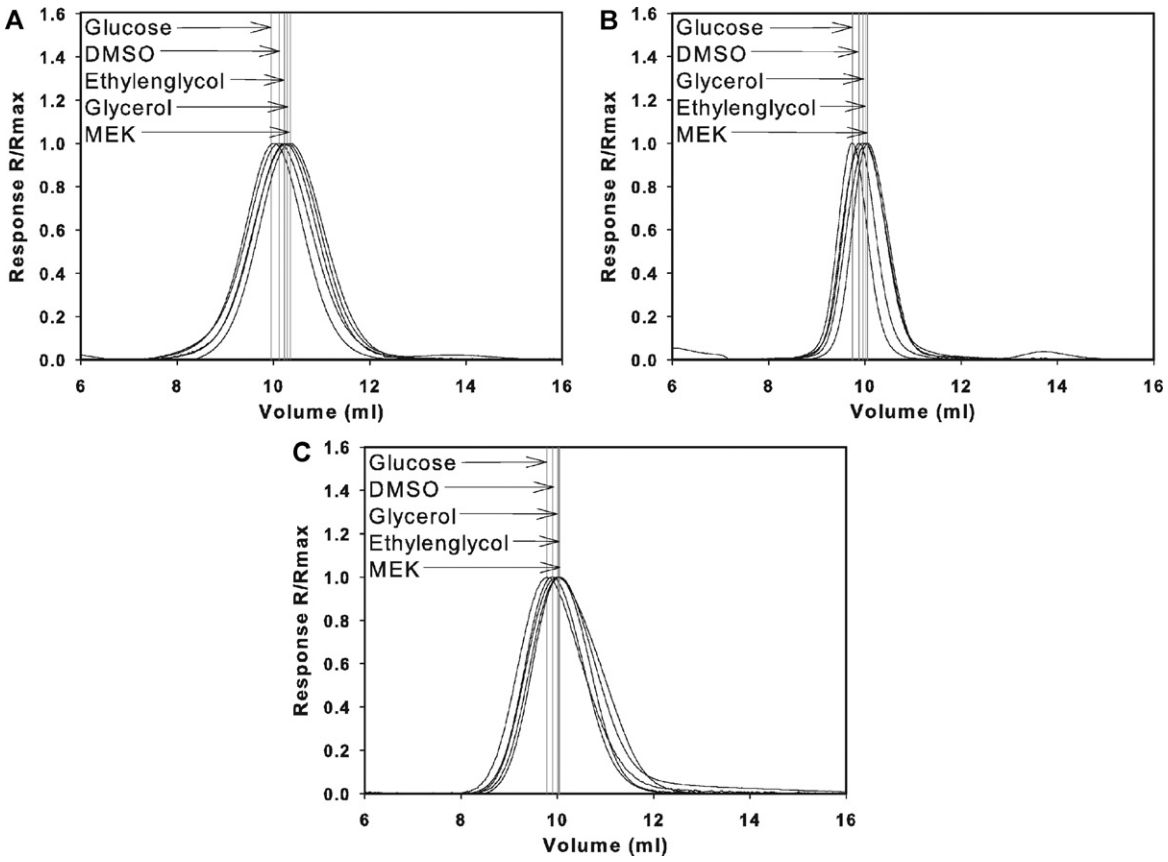

Fig. 2. UV-absorbance over time, normalized to the maximum response. (a) GE Butyl Seph. 4 FF, (b) GE Phenyl Seph. HP and (c) GE Phenyl Seph. 6 FF (low sub).

The most common method for determination of surface energies of planar surfaces is contact angle measurement. Gindl et al. [30] showed that the outcome is dependent on the selected algorithm. The data must be interpreted with care and an absolute value of surface energy is definitely not obtained by either contact angle or inverse chromatography.

Ujimoto and Kurihara [26] used 1-alkanols for determination of hydrophobicity of chromatography material without ligands. They found a lower hydrophobicity for agarose-based media than polymethacrylate-based ones. Furthermore, our method can be used to determine batch to batch variations of chromatography media. Riske et al. [27] suggested that the retention of lysozyme depends on the source of material and packing quality of the column as well as the extra column volume and injection profile. Our method is independent of the packing density and thus our method is, in principle, easily transferable to other labs.

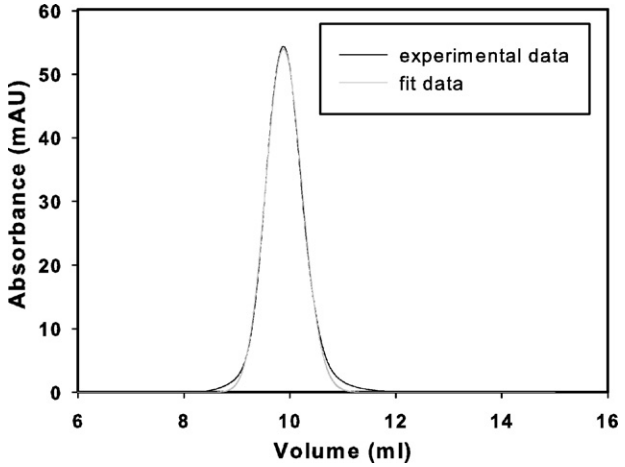

Fig. 3. Comparison of experimental data with data of the fit for the combination DMSO with GE Phenyl Sepharose HP media.

Jennissen [28,29] has proposed a critical hydrophobicity for purification of proteins. This approach is limited to actual HIC media and is confined to a certain protein purification problem. Knowledge of the surface energy makes our theory generally applicable.

The results of calculating the Henry coefficients based on Eq. (18) by using the values of Table 4 are listed in Table 5.

Table 5
The Henry coefficients of the molecular probes used (GLY, DMSO, EG, MEK) for the chromatography media investigated.

	H_{GLY}	H_{DMSO}	H_{EG}	H_{MEK}
GE Butyl Seph. 4 FF	0.96	0.80	0.94	1.1
GE Phenyl Seph. HP	1.3	0.41	0.74	0.95
GE Phenyl Seph. 6 FF (ls)	2.2	1.2	1.2	1.6
Tosoh Butyl-650M	0.99	1.1	1.4	4.8
Tosoh Phenyl-650M	0.99	1.2	1.4	4.6
Tosoh HW 65	0.53	0.58	0.93	2.4

Table 6
The total porosity calculated for all resins.

	ε
GE Butyl Seph. 4 FF	0.96
GE Phenyl Seph. HP	0.96
GE Phenyl Seph. 6 FF (ls)	0.95
Tosoh Butyl-650M	0.94
Tosoh Phenyl-650M	0.95
Tosoh HW 65	0.92

Table 7
Arithmetical mean of k' values for GLY and DMSO with the standard deviation σ, determined by using the Retention volume via momentum, and via peak maximum; values have to be multiplied by 10^{-3}.

	k'_{GLY}		k'_{DMSO}	
	Moment.	Peak max.	Moment.	Peak max.
GE Butyl Seph. 4 FF	26	26 ± 3	17	17 ± 3
GE Phenyl Seph. HP	21	24.7 ± 0.8	9.9	15.6 ± 0.4
GE Phenyl Seph. 6 FF (ls)	74	26 ± 6	38	10.8 ± 0.7
Tosoh Butyl-650M	18	60 ± 1	37	57.8 ± 0.7
Tosoh Phenyl-650M	3.9	39 ± 1	4	47.7 ± 0.3
Tosoh HW 65	60	38 ± 1	28	44 ± 0.6

The results of calculating the porosity based on Eq. (19) by using the values of Tables 4 and 5 are listed in Table 6.

The synthetic polymer based media exhibit marginal lower porosities, compared to Sepharose based media.

5. Determination of k' via Retention volume, surface energies, Henry constants and porosities with this method

The conversion from a volumetric interpretation to the surface is through the relation ship shown in Eq. (18). k' and the Henry constant are dimensionless numbers in our case.

The term A (see Eq. (17)) is the link between volume and surface because it describes the theoretical area/footprint of an adsorbed molecule and thus the retention data can be converted into a surface energy. The outcome is based on the assumption of the area which interacts with the surface. We used always the same algorithm for the calculations of the A value. Slightly different values are found in literature. This maybe a source of error, but this is not only valid for our method, it has also an impact on contact angle measurement and is often neglected.

It is not clear if the molecular probe partitions between the liquid phase and the layer of ligands and to which extent the backbone is involved in the retention of the probe. Only a molecular model would help, but then we cannot expect an average value of the surface energy anymore.

We have used three different methods to evaluate the retention data one by deconvoluting the peak using Eq. (6) the others by simply measuring the first moment respectively the peak maximum. All methods give similar results. So this indicates that the algorithm itself is stable. As control experiments we have calculated the porosities from surface energies via Henry constant and compared to experimental data. We found good agreement. The molecular probes were selected by the criterion of solubility in water, this makes them suitable for investigation of HIC media.

Tables 7–11

6. Conclusion

With the described procedure, the Lifshitz–van-der-Waals and the Lewis acid and Lewis base contributions to the surface energy according to van Oss' theory can be obtained for porous beads. Therefore the hydrophobicity, expressed in terms of the Lifshitz–van-der-Waals contributions to the surface energy can be quantified. The Lifshitz–van-der-Waals contribution to the surface energy of synthetic polymer-based media (Tosoh Toyopearl) is, as obtained by the method described, 2–3 times larger than the same contribution of natural polymer based media (GE Healthcare Sepharose media), except GE Healthcare Phenyl Sepharose HP media, which Lifshitz–van-der-Waals contribution is in the same range as the synthetic polymer-based media. Beside the convenience of having a fast and independent procedure for the determination of the phase ratio, this method has the advantage of being suitable for porous media.

Table 8
Arithmetical mean of k' values for EG and MEK with the standard deviation σ, determined by using the Retention volume via momentum and via peak maximum; values have to be multiplied by 10^{-3}.

	k'_{EG}		k'_{MEK}	
	Moment.	Peak max.	Moment.	Peak max.
GE Butyl Seph. 4 FF	12	25 ± 2	49	33 ± 4
GE Phenyl Seph. HP	21	30 ± 1	31	33.9 ± 0.4
GE Phenyl Seph. 6 FF (ls)	53	24.1 ± 0.4	96	26.9 ± 0.8
Tosoh Butyl-650M	210	78 ± 5	280	288 ± 1
Tosoh Phenyl-650M	16	61 ± 2	230	230.4 ± 0.8
Tosoh HW 65	50	76 ± 5	270	203.5 ± 0.8

Table 9
The LW-, Lewis acid (+) and Lewis base (−) contributions to the surface energy γ for the chromatography media investigated, determined by using the Retention volume via momentum (left column) and via peak maximum (right column); values are given in mJ/m^2.

	γ^{LW}		γ^+		γ^-		γ	
GE Butyl Seph. 4 FF	81.1	93.7	3.59	13.5	72.3	30.4	113	135
GE Phenyl Seph. HP	188	123	7.42	15.6	31.5	24.7	219	162
GE Phenyl Seph. 6 FF (ls)	131	169	5.82	6.96	39.3	25	161	195
Tosoh Butyl-650M	308	168	88	16.2	14.3	56	379	228
Tosoh Phenyl-650M	32.9	148	98.1	23.3	103	55.2	234	220
Tosoh HW 65	338	168	1.75	27.8	69.3	42.5	360	237

Table 10
The total porosity calculated for all resins, initially k' was determined by pulse shape method (identical Table 6), via momentum and via peak maximum.

	ε		
	Pulse shape meth.	Moment.	Peak max.
GE Butyl Seph. 4 FF	0.96	0.97	0.97
GE Phenyl Seph. HP	0.96	0.98	0.95
GE Phenyl Seph. 6 FF (ls)	0.95	0.93	0.96
Tosoh Butyl-650M	0.94	0.88	0.95
Tosoh Phenyl-650M	0.95	0.94	0.96
Tosoh HW 65	0.92	0.96	0.92

Table 11
The total porosity calculated for all resins, determined by the retention volumes of glucose in methanol ($\varepsilon = (V_R - V_0)/V$); where V_R is the retention volume and V_0 the bypass volume, obtained with via momentum and via peak maximum.

	ε	
	Moment.	Peak max.
GE Butyl Seph. 4 FF	0.99	0.99
GE Phenyl Seph. HP	0.97	0.96
GE Phenyl Seph. 6 FF (ls)	0.95	0.97
Tosoh Butyl-650M	0.86	0.82
Tosoh Phenyl-650M	0.88	0.85
Tosoh HW 65	0.84	0.82

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References

[1] A. Mahn, M.E. Lienqueo, J.C. Salgado, J. Chromatogr. A 1216 (2009) 1838.
[2] C. Machold, K. Deinhofer, R. Hahn, A. Jungbauer, J. Chromatogr. A 972 (2002) 3.

[3] V.P. Shanbhag, Estimation of surface hydrophobicity of proteins by partitioning, in: G.J. Harry Walter (Ed.), Aqueous Two-Phase Systems, Methods in Enzymology, vol. 228, Academic Press, 1994, p. 254 (Chapter 23).
[4] A. Mahn, G. Zapata-Torres, J. Asenjo, J. Chromatogr. A 1066 (2005) 81.
[5] J. Chen, T. Yang, S.M. Cramer, J. Chromatogr. A 1177 (2008) 207, 5th Hydrophobic Interaction Chromatography/Reversed-Phase Liquid Chromatography Bioseparation Conference.
[6] A. Ladiwala, F. Xia, Q. Luo, C.M. Breneman, S.M. Cramer, Biotechnol. Bioeng. 93 (2006) 836.
[7] H. Claessens, M. Van Straten, C. Cramers, B. Jezierska, M. Buszewski, J. Chromatogr. A 826 (1998) 135.
[8] H. Engelhardt, M. Jungheim, Chromatographia 29 (1990) 59.
[9] H. Engelhardt, M. Arangio, T. Lobert, LC–GC 15 (1997) 856.
[10] M.J. Walters, J. Assoc. Off. Anal. Chem. 70 (1987) 465.
[11] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721.
[12] S.V. Galushko, Chromatographia 36 (1993) 39.
[13] Á Sándi, Á Bede, L. Szepesy, G. Rippel, Chromatographia 45 (1997) 206.
[14] A. Jungbauer, J. Chromatogr. A 1065 (2005) 3, 1st Monolith Summer School.
[15] G. Carta, A. Jungbauer, Protein Chromatography: Process Development and Scale-Up, Wiley-VCH, 2010.
[16] A.W. Adamson, Physical Chemistry of Surfaces, Wiley, 1990.
[17] V.M. Starov, S.A. Zhdanov, S.R. Kosvintsev, V.D. Sobolev, M.G. Velarde, Adv. Colloid Interface Sci. 104 (2003) 123.
[18] J.D. Andrade (Ed.), Surface and interfacial aspects of biomedical polymers, Plenum Press, 1985.
[19] C.J. van Oss, Interfacial Forces in Aqueous Media, Taylor & Francis Group, LLC, 2006.
[20] G. Guiochon, S. Shirazi, A. Katti, Fundamentals of Nonlinear and Preparative Chromatography, 1994.
[21] S.J. Farlow, Partial Differential Equations for Scientists and Engineers, Dover Pubns, 1993.
[22] R. Bonomo, L. Minim, J. Coimbra, R. Fontan, L.M. da Silva, V. Minim, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 844 (2006) 6.
[23] M. Millitzer, Zur Ad- und Desorption von Proteinen an hydrophoben Adsorbentien. Ph.D. Thesis. Universität Erlangen-Nürnberg, Erlangen, 2007.
[24] M. Wulf, P. Uhlmann, S. Michel, K. Grundke, Prog. Org. Coat. 38 (2000) 59.
[25] C.J. van Oss, R.J. Good, M.K. Chaudhury, Langmuir 4 (1988) 884.
[26] K. Ujimoto, H. Kurihara, J. Chromatogr. 301 (1984) 57.
[27] F.J. Riske, M.A. Smith, C. Zhang, K.H. White, Biotechnol. Appl. Biochem. 54 (2009) 157.
[28] H. Jennissen, A. Demiroglou, J. Chromatogr. A 1109 (2006) 197.
[29] H. Jennissen, Int. J. Bio-Chromatogr. 5 (2000) 131.
[30] M. Gindl, G. Sinn, W. Gindl, A. Reiterer, S. Tschegg, J. Colloids Surf. A 181 (2001) 279.