Equation for calculating surface coverage from end-capping of chromatographic bonded phases

Junior E. Sandoval

Departamento de Química, Universidad del Valle, AA 25360, Cali, Colombia

Abstract

An equation was derived to calculate the surface density of trimethylsilyl groups (α_{TMS}) on bonded chromatographic stationary phases that have undergone primary as well as secondary ('end-capping') chemical modification. The new equation is an extension of that published by Berendsen-de Galan for calculating primary surface coverage and, likewise, α_{TMS} is calculated in terms of the carbon content (% by weight) of the bonded material before and after end-capping, specific surface area of the starting silica and structural information (molecular weight and number of carbon atoms) of the anchored groups. The new equation is valuable when a thorough characterization of bonded stationary phases is desirable and, if used along with the Berendsen–de Galan equation, it affords total ligand coverage information. Application of the new equation to correct for measurable carbon content of the starting support leads to a more accurate expression for surface coverage from primary as well as secondary bonding. The scope and limitations of the new equation are discussed. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Stationary phases, LC; Surface coverage; End-capping; Trimethylsilyl groups

1. Introduction

In 1978, Berendsen and de Galan [1] developed equations to calculate the surface density of organosilyl groups in bonded silicas used in liquid chromatography. Surface coverage of the chemically bonded phase, α (usually in µmoles of anchored group per m^2 of *native* silica support), is calculated from elemental carbon content (% by weight), specific surface area of the starting silica (most often obtained from BET nitrogen adsorption method measurements) and the structure of the anchored organosilane species (molecular weight and number of carbon atoms). It is now widely accepted that surface coverage rather than percentage of carbon alone is a more suitable criterion to assess the extent of surface modification of a primary bonding procedure [1–3]. This chemical process of surface modification is usually carried out by means of a *substitution* reaction of the type

$$|Si–OH + RMe_2SiX \rightarrow |Si–OSiMe_2R + HX \qquad (1)$$
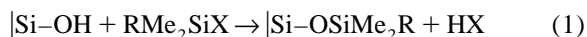
Alternatively, an olefin *addition* reaction can be used as the primary bonding process on a silica surface that has been previously modified with a silicon hydride functionality [4,5]:

$$|Si–H + CH_2{=}CH–R \rightarrow |Si–CH_2CH_2–R \qquad (2)$$
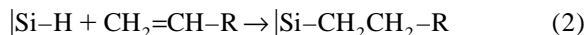
To reduce the effects of silanols left unreacted by the primary bonding process, organosilylated silicas are subsequently end-capped (trimethylsilylated) by

a secondary bonding process with trimethylchlorosilane (TMCS) or with hexamethyl–disilazane (HMDS):

$$|Si–OH + Me_3SiY \rightarrow |Si–OSiMe_3 + HY \quad (3)$$
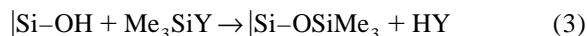
where Y corresponds to –Cl for TMCS or –NHSiMe$_3$ for HMDS.

While the importance of the group density value calculated for the primary bonding step has been extensively emphasized elsewhere [3], the necessity of fully characterizing a bonded phase with respect to the extent of end-capping (α_{TMS}) has received much less attention. The subject of end-capping per se has been and continues to be the core of a number of research reports [6–12], but the lack of appropriate end-capping coverage information has rendered, in most cases, such reports incomplete. The calculation of α_{TMS} is necessary in order to estimate the total effectiveness of the two-step bonding process. The effectiveness factor, η, is defined as the ratio of the number of silanols that actually react, $n_{OH (react)}$, to the total number of them originally present in the underivatized silica surface, $n_{OH (total)}$ [3]. In the case of end-capped silicas, η will be given by

$$\eta = \frac{n_{OH(react)}}{n_{OH(total)}} = \frac{\alpha_1 + \alpha_2}{\alpha_{OH}} \quad (4)$$

where the subscripts 1 and 2 refer to the primary and secondary bonding steps, respectively. In addition to supplementing the characterization of the final bonded phase product, end-capping coverage information may be useful in studies that correlate surface coverage with retention data of selected chromatographic probes [6,7,12].

2. Results and discussion

2.1. Theoretical

See Appendix A for the definition of the different symbols used herein. The basis of the new equation lies on the mass increase resulting from each chemical modification which takes place at the silica surface.

From the definition of p_i

$$p_1 = \frac{m_1}{W_1} \times 100 \quad (5)$$

$$m_1 = W_1 \frac{p_1}{100}$$

Likewise

$$p_2 = \frac{m_1 + m_2}{W_2} \times 100 = \frac{W_1 \frac{p_1}{100} + m_2}{W_2} \times 100$$

Solving for m_2

$$m_2 = \frac{W_2 p_2 - W_1 p_1}{100} \quad (6)$$

2.1.1. The original Berendsen–de Galan equation (α_1)

Basis: W_1 (g) of bp_1.

The number of μmoles of G_1 per m^2 of native silica is given by

$$\alpha_1 = \frac{10^6 m_1}{C n_1 A_0} = \frac{10^6 W_1 p_1}{100 C n_1 A_0}$$

but $S_0 = A_0 / W_0$, thus

$$\alpha_1 = \frac{10^6 p_1}{100 C n_1 S_0} \left(\frac{W_1}{W_0} \right) \quad (7)$$

The term W_1 / W_0 corrects for the mass increase of the silica upon derivatization. Such a mass increase relates W_1 and W_0 as follows:

$$W_1 = W_0 + m_1 \frac{M_1}{n_1 C}$$

Combining with Eq. (5) and solving for W_1 / W_0

$$\frac{W_1}{W_0} = \frac{1}{1 - \frac{p_1 M_1}{100 C n_1}} \quad (8)$$

Substituting in Eq. (7) and rearranging, one obtains Berendsen–de Galan's equation:

$$\alpha_1 = \frac{10^6 p_1}{S_0 (100 C n_1 - p_1 M_1)} \quad (9)$$

2.1.2. The equation for end-capping coverage (α_2)

The derivation of an expression for α_2 follows a similar path to that of α_1. To simplify many inter-

mediate expressions (vide infra), the temporary variable ϵ_i is introduced:

$$\varepsilon_i = \frac{M_i}{100Cn_i} \qquad (10)$$

As a result, a somewhat simpler expression can be obtained for W_1/W_0 (Eq. (8))

$$\frac{W_1}{W_0} = \frac{1}{1 - p_1\varepsilon_1} \qquad (8')$$

Going back to α_2, the number of μmoles of G_2 per m^2 of native silica is given by

$$\alpha_2 = \frac{10^6 m_2}{Cn_2 A_0} = \frac{10^6 m_2}{Cn_2 S_0 W_0}$$

Replacing m_2 with Eq. (6), yields:

$$\alpha_2 = \frac{10^6}{100Cn_2 S_0}\left(\frac{W_2}{W_0}p_2 - \frac{W_1}{W_0}p_1\right) \qquad (11)$$

W_2 is the result of two mass increases

$$W_2 = W_0 + m_1\frac{M_1}{n_1 C} + m_2\frac{M_2}{n_2 C}$$

Combining with Eqs. (5) and (6)

$$W_2 = W_0 + \frac{W_1 p_1}{100}\frac{M_1}{n_1 C} + \frac{(W_2 p_2 - W_1 p_1)}{100}\frac{M_2}{n_2 C}$$

Inserting Eq. (10)

$$W_2 = W_0 + W_1 p_1 \varepsilon_1 + (W_2 p_2 - W_1 p_1)\varepsilon_2$$

Rearranging

$$\frac{W_2}{W_0}(1 - p_2\varepsilon_2) = 1 + \frac{W_1}{W_0}p_1(\varepsilon_1 - \varepsilon_2)$$

Combining with Eq. (8′) and solving for W_2/W_0

$$\frac{W_2}{W_0} = \frac{1 - p_1\varepsilon_2}{(1 - p_1\varepsilon_1)(1 - p_2\varepsilon_2)} \qquad (12)$$

Inserting Eqs. (8′) and (12) into Eq. (11) and rearranging

$$\alpha_2 = \frac{10^4(p_2 - p_1)}{Cn_2 S_0 (1 - p_1\varepsilon_1)(1 - p_2\varepsilon_2)} \qquad (13)$$

Finally, combining with Eq. (10) and rearranging

$$\alpha_2 = \frac{10^8 Cn_1(p_2 - p_1)}{S_0(100Cn_1 - p_1 M_1)(100Cn_2 - p_2 M_2)} \qquad (14)$$

2.2. Scope and limitations

Although relatively more complex due to the additional bonding, the new equation (Eq. (14)) resembles that derived by Berendsen–de Galan (Eq. (9)). In the special case where $p_1 = 0$, the expression for α_2 converges into that for α_1, as expected. For Eq. (14) to have a practical meaning, p_2 must be statistically greater than p_1, which is often the case. It should be recalled that the effectiveness of end-capping can also be assessed by chromatographic means. This approach can be much more sensitive than measuring the increase of carbon loading after end-capping, and is particularly useful when the later is negligible [13]. Similarly with the Berendsen–de Galan equation, when applying Eq. (14), it is assumed that the bonded phase has been thoroughly washed with appropriate solvents after each modification step to remove any physically adsorbed material (e.g., organosilane dimers) that may form. More importantly, application of Eq. (14) also assumes that alkyldimethylsilyl groups (Eq. (1)) are not exchanged by TMS groups during the end-capping reaction (Eq. (3)). This assumption is of paramount importance because such a substitution might eventually result in a decrease in carbon content, that is, $p_2 < p_1$ [8,12]. For instance, end-capping of octadecyl–silicas by a high temperature silylation procedure often results in a significant decrease in carbon content, suggesting extensive reactivity of the octadecylsiloxane group towards substitution under such conditions [12]. Unfortunately, the extent at which this substitution occurs is not readily known, precluding calculation of α_{TMS} and invalidating any $\alpha_{octadecyl}$ value.

The use of the new equation is illustrated in the following cases taken from the literature. A typical application of the end-capping coverage equation can be found in a relatively recent work by Nagae et al., where the results from a new method that produced a more densely end-capped C_{18}-bonded stationary phase are presented [10]. Notice (see Table 1) that the authors reported coverage information for end-capping but failed to discuss the equations they used to accomplish such results. Nevertheless, their fig-

Table 1
Surface density of alkyldimethylsilyl and trimethylsilyl groups obtained by two end-capping procedures[a,b]

R	M_1	n_1	p_1	M_2	n_2	p_2	This work, Ref. [10]		This work, Ref. [10]		α_{total}	η^d (%)
							$\alpha_1^{\ c}$	α_1	α_2	α_2		
C_1-A	72.2	3	3.96	–	–	–	3.98	4.17	–	–	3.98	50
C_1-B	72.2	3	4.83	–	–	–	4.95	4.96	–	–	4.95	62
C_{18}-A	310.6	20	8.40	72.2	3	10.04	1.31	1.31	2.13	2.13	3.44	43
C_{18}-B	310.6	20	8.40	72.2	3	10.43	1.31	1.31	2.66	2.67	3.97	50

[a] Data taken from Ref. [10].
[b] A refers to 'conventional' end-capping, while B refers to a newly developed procedure.
[c] Specific surface area (S_0) of the starting silica was 300 m^2/g.
[d] Assuming α_{OH} to be 8 μmol/m^2 (see Eq. (4)).

ures agree very well with those from the new equation reported here. Notice also that the reported end-capping procedure affords a significantly higher total effectiveness of surface modification. There are numerous examples in the literature where similar applications of the new equation are possible.

Perhaps a more enlightening application of the new equation can be found in a report by Chen and McCown [14]. These authors conducted a quite cumbersome gravimetric procedure to evaluate coverage from both primary bonding as well as end-capping. It should be pointed out that the mass increases of the silica upon chemical modifications were actually measured in their procedure, while the coverage equations (Eqs. (9) and (14)) simply correct mathematically for such mass increases and, hence, require less data. Notice (see Table 2) that the results obtained from the coverage equations agree very well with those obtained from the time-consuming, labor-intensive procedure of Chen and McCown's.

There is another potential application of the new equation that relates to the determination of ligand density from primary bonding. While one expects a native silica support to be 'fully hydroxylated' (free from any chemically anchored organic moiety), one often finds a measurable carbon load in this presumably underivatized material (that is, $p_0 > 0$, typically 0.1–0.3% w/w, but sometimes well above 1%). It may be necessary to correct for this 'background' carbon in the starting silica material. In a simplistic approach of subtracting the 'background' carbon percentage of the starting silica from that of the primarily bonded support and then proceeding to using the Berendsen–de Galan equation significant error is introduced into the resulting ligand density figure. The use of a simple subtraction to correct for background carbon leads to the following modification of the Berendsen–de Galan equation to express α_1, now represented by α_1' to denote its shortcoming:

$$\alpha_1' = \frac{10^6(p_1 - p_0)}{S_0[100Cn_1 - (p_1 - p_0)M_1]} \qquad (15)$$

On the other hand, the true expression for α_1 entails

Table 2
Surface density of alkyldimethylsilyl and trimethylsilyl groups for different alkyl (R) chain lengths[a]

R	M_1	n_1	p_1	p_2	Ref. [14]			This work		
					$\alpha_1^{\ b}$	α_2	α_{total}	α_1	$\alpha_2^{\ c}$	α_{total}
C_{18}	310.6	20	10.720	11.047	3.259	0.769	4.028	3.238	0.846	4.084
C_8	170.4	10	6.430	6.602	3.700	0.326	4.026	3.682	0.378	4.060
C_3	100.2	5	3.540	3.584	3.919	0.088	4.007	3.915	0.087	4.003
C_1	72.2	3	2.202	2.202	4.000	0.000	4.000	3.996	0.000	3.996

[a] Data taken from Ref. [14].
[b] Specific surface area (S_0) of the native silica was 160 m^2/g.
[c] In all cases $M_2 = 72.2$ and $n_2 = 3$.

the use of Eq. (9) in a more suitable form (again, see Appendix A for definition of subscripts):

$$\alpha_1 = \frac{10^8 C n_0 (p_1 - p_0)}{S_0 (100 C n_0 - p_0 M_0)(100 C n_1 - p_1 M_1)} \quad (16)$$

Notice that this equation also assumes a detailed knowledge of the structure of the species already present on the starting silica. Although not generally available, this information can be inferred from the manufacturing processes of the silica and/or from spectroscopic analysis. One can anticipate the starting species to be an alkoxy group, probably a methoxy or an ethoxy, remaining from the hydrolytic manufacturing process of colloidal silica that employs a tetraalkoxysilane as a starting material [15]. Alternatively, such a species may originate from a final washing step of the native silica with a volatile alcohol according to the reaction

$$|Si–OH + R–OH \rightarrow |Si–OR + H_2O \quad (17)$$

To assess the error incurred when using Eq. (15) rather than Eq. (16) to calculate the primary surface coverage, it is useful to consider a relative error expression shown below:

$$\%error = \frac{\alpha_1' - \alpha_1}{\alpha_1} \times 100$$

$$= \frac{(100 C n_0 - p_0 M_0)(100 C n_1 - p_1 M_1)}{C n_0 [100 C n_1 - (p_1 - p_0) M_1]} - 100 \quad (18)$$

This relative error value does not depend on the specific surface area of the silica, but does so on the amount and structure of both species G_0 and G_1. Fig. 1 illustrates the case for a C_{18}-bonded phase end-capped with TMS moieties as a function of background carbon and total carbon percentages. The use of Eq. (15) always introduces a negative bias in the calculated coverage. The magnitude of such bias increases almost linearly with increasing p_0, but is affected to a much lesser extent by p_1. Moreover, for given p_1 and p_0 values, the bias is greater in the case of shorter alkyl chain lengths (not shown), as expected from the smaller carbon contribution of such groups to the total carbon load of the bonded phase.

An important corollary of using Eq. (16) is that a more accurate expression for α_2 should also take into account the presence of background carbon in the starting silica. Following a derivation sequence very similar to that shown above, one arrives at the following equation for end-capping coverage:

$$\alpha_2 =$$

$$\frac{10^8 C n_0 (p_2 - p_1)(100 C n_1 - p_0 M_1)}{S_0 (100 C n_0 - p_0 M_0)(100 C n_1 - p_1 M_1)(100 C n_2 - p_2 M_2)} \quad (19)$$

Similarly to Eq. (9), it is assumed that the alkoxy groups (Eq. (17)) are not exchanged by alkyldimethylsilyl or TMS groups during either of the bonding steps (Eqs. (1) or (3)). The validity of this assumption should be confirmed experimentally since, due to the relatively labile nature of alkoxy species on silica, the exchange of this species during the primary bonding reaction is more likely than that of alkyldimethylsilyl groups by TMS groups during the end-capping reaction. Spectroscopic monitoring (e.g., by solid-state ^{13}C NMR) of a 'native' silica containing a measurable alkoxy surface coverage that is subjected to organosilylation (Eqs. (1) or (3)) should be sufficient to clarify this issue.

As expected, when $p_0 = 0$, this expression for α_2 converges into Eq. (14).

Finally, it should be mentioned that, as with the Berendsen–de Galan equation [1,2], application of the equation introduced here is limited to monomeric phases prepared from monofunctional reagents such as those described by Eqs. (1)–(3). Although the need for end-capping phases prepared from di- or trifunctional alkylsilanes may be greater due to the formation of extra silanols, the use of a coverage equation (Eqs. (9), (14), (16) or (19)) is hindered by the unknown contributions of the various organosilane species to the molecular weight figure, M_1. Thus, in the reaction of silica with di- and trifunctional alkylsilanes, one or two linkages with the silica surface are formed per silane molecule, leading to a stoichiometric factor f that, on average, lies between 1 and 2 [3]. In contrast, $f = 1$ for monofunctional silanes, since a single linkage results for each silane molecule that reacts with the silica surface. Recall that in this case the atomic weight of hydrogen must be subtracted from the molecular weight of the anchored group G_1 to account for one hydrogen lost during the process (see Appendix A).
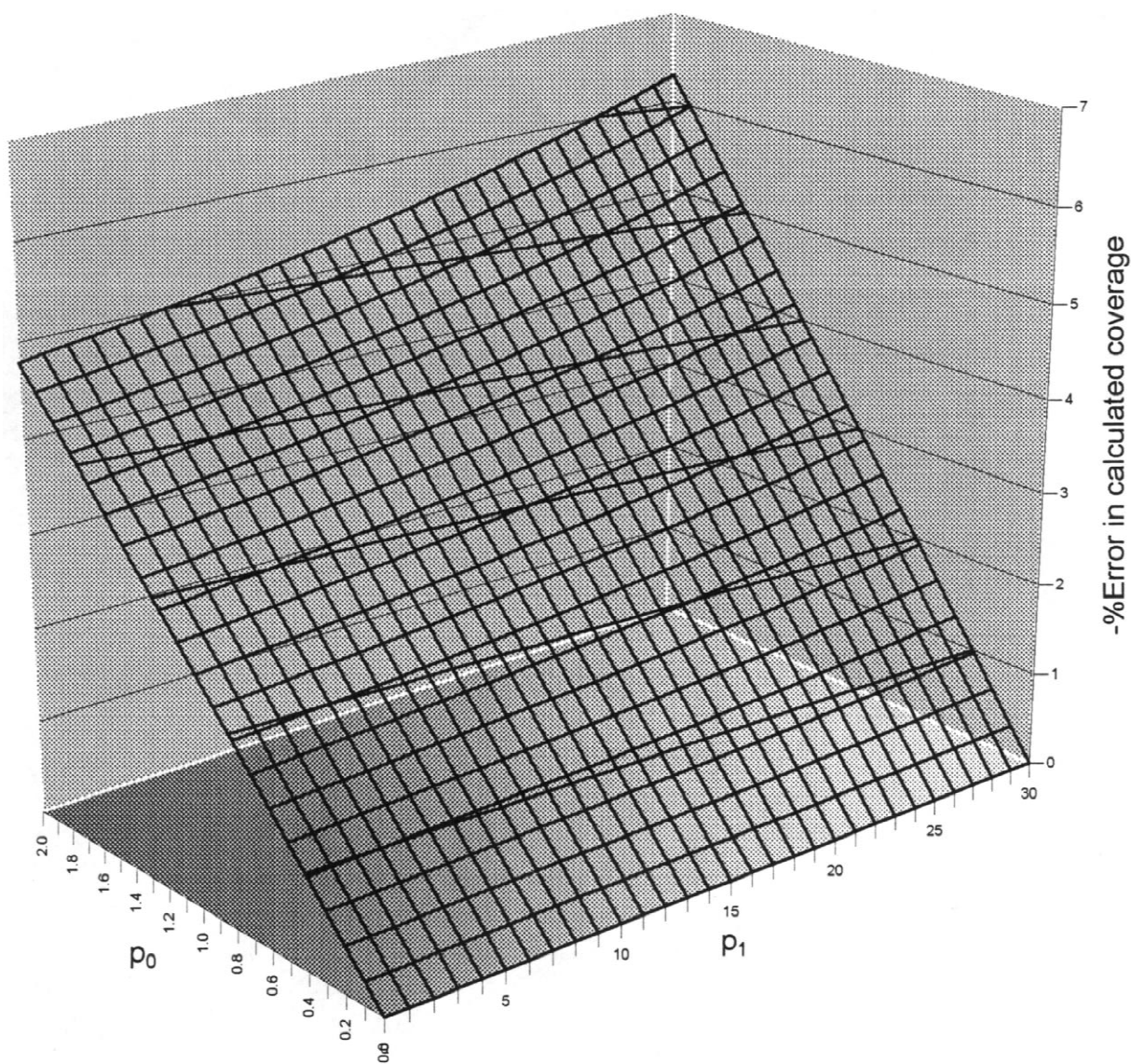
Fig. 1. The magnitude of the relative error (incurred when the net carbon load in an octadecyldimethylsilyl-modified silica is taken as the difference between the carbon percentages after and before chemical modification) as a function of carbon contents of the starting silica and total carbon contents of the end-capped phase. It is assumed that the carbon load prior to bonding is due to methoxy moieties (that is, $M_0 = 14.03$ and $n_0 = 1$). See text for details.

In the case of polymeric phases, f multiplied by the atomic weight of hydrogen is the correct figure to be subtracted. Although not customarily available, this stoichiometric factor can be readily estimated from relative signal strengths in solid-state ^{29}Si NMR spectra [16].

3. Conclusions

A new equation that extends the usefulness of the Berendsen–de Galan coverage equation has been developed. Such an equation permits calculation of the TMS-coverage originating from end-capping of a

bonded phase, and hence, provides information on total effectiveness of the overall chemical surface modification procedure. Further application of the new equation to correct for measurable carbon content of the starting silica leads to an accurate expression for ligand density resulting from primary as well as end-capping surface modifications.

Appendix A. List of symbols used in this work

Subscripts:

0	Refers to the *native* (*unmodified*) silica
1	Refers to the *primary* bonding step
2	Refers to the *secondary* bonding (end-capping) step

Symbols:

bp_i	Bonded phase i
α_i	Surface coverage of anchored group G_i (μmoles of G_i per m^2 of native silica)
p_i	Percentage of carbon (wt./wt.)
n_i	Number of carbon atoms per anchored group G_i
C	Atomic weight of carbon
M_i	Molecular weight[1] of anchored group G_i
W_i	Mass (g) of stationary phase
S_0	Specific surface area (m^2/g) of the native silica
A_0	Surface area (m^2) of W_0 (g) of native silica
m_i	Mass (g) of carbon in W_i (g) of bp_i

References

[1] G.E. Beredensen, L. de Galan, J. Liq. Chromatogr. 1 (1978) 561.
[2] G.E. Berendsen, K.A. Pikaart, L. De Galan, J. Liq. Chromatogr. 3 (1980) 1437.
[3] K.K. Unger, Porous Silica – Its Properties and Use as Support in Column Liquid Chromatography, Elsevier, Amsterdam, New York, 1979.
[4] J.E. Sandoval, J.J. Pesek, Anal. Chem. 63 (1991) 2634.
[5] M.C. Montes, C. van Amen, J.J. Pesek, J.E. Sandoval, J. Chromatogr. A 688 (1994) 31.
[6] C. Dewaele, P. Mussche, M. Verzele, J. High Res. Chromatogr. Chromatogr. Comm. 5 (1982) 616.
[7] S.A. Tomellini, S. Hsu, S.D. Fazio, R.A. Hartwick, J. High Res. Chromatogr. Chromatogr. Comm. 8 (1985) 337.
[8] B. Buszewski, Chromatographia 28 (1989) 574.
[9] Z. Matus, R. Ohmacht, Chromatographia 30 (1990) 318.
[10] N. Nagae, Y. Hatano, D. Ishii, Chromatography 14 (1993) 45R.
[11] N. Nagae, D. Ishii, Am. Lab. 27 (1995) 20.
[12] Y. Sudo, J. Chromatogr. A 737 (1996) 139.
[13] N. Tanaka, H. Godell, B.L. Karger, J. Chromatogr. 158 (1978) 233.
[14] W. Cheng, M. McCown, J. Chromatogr. 318 (1985) 173.
[15] G. Cox, J. Chromatogr. A 656 (1993) 353.
[16] E. Bayer, K. Albert, J. Reiners, M. Nieder, D. Müller, J. Chromatogr. 264 (1983) 197.

---

[1]In the case of the most common *substitution* reaction (see Eqs. (1) and (3)), the atomic weight of hydrogen must be subtracted to account for *one* hydrogen lost during the process.