

Epoxy- and diol-modified silica: optimization of surface bonding reaction

B. Porsch

Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague 6 (Czech Republic)

(First received May 4th, 1993; revised manuscript received June 22nd, 1993)

ABSTRACT

The 3-glycidyoxypropyltrimethoxysilane–silica bonding reaction was investigated. The carbon and bonded epoxide content after the bonding reaction and the vicinal diol and total hydroxy group content after the subsequent opening reaction were determined. The highest yield of bonded epoxide groups was obtained in water at pH 8.5 and ambient temperature. The corresponding diol phase behaves well in the size-exclusion chromatography of poly(ethylene glycol)s in water. Neither the silane bonding reaction nor the subsequent epoxide opening reaction give rise to diol structures only; formation of glycol ethers cannot be avoided. The bonding reaction in toluene gives mostly surface-bonded glycol–ether structures.

INTRODUCTION

High-performance size-exclusion chromatography (SEC) has become a well accepted technique for the characterization of water-soluble polymers [1]. The column packings used may be organic or silica-based. Although the wider pH stability [2] favours organic packings, silica-based materials are frequently preferred because of their higher efficiency and more robust nature. Among them diol-bonded silica [3,4] is very popular. Its hydrophilic moiety is believed to be a vicinal diol obtained by simultaneous opening of the oxirane ring in the bonding reaction [3] with 3-glycidyoxypropyltrimethoxysilane (GPTMS) in water at pH 3.5 and 90°C. The oxirane intermediate was shown to be useful for coupling a variety of ligands used as ion exchangers [5] and affinity packings [6]. To prevent the oxirane ring from opening, the silane bonding reaction is usually carried out at pH 5–6 [5,7,8].

In fact, GPTMS is an exception among other silanes used to prepare bonded phases in the sense that the oxirane ring is highly reactive [9].

The formation of the desired diol grouping might be accompanied by some by-products and their formation could be highly pH and temperature dependent; all of them may be expected to be less hydrophilic. Thus, methanol formed by hydrolysis of GPTMS may participate in oxirane opening and form the corresponding glycol ethers, the diol grouping formed may react with another oxirane ring and also polyaddition may take place. Moreover, both bonded and unbonded GPTMS may participate in these reactions. At the same time, the starting silane is hydrolysed and forms the corresponding trisilanol during the initial part of the bonding reaction in aqueous solution; silanol polymerization giving cyclic oligosiloxanes [10] could then compete with the surface bonding reaction and these cyclic siloxane structures may be also bonded to the silica surface.

The complexity of the bonding reaction may be responsible for the differences in ionic and hydrophobic interactions among diol phases of a different origin [11]. Hydrophobic interactions arise from hydrocarbon spacer groups and the number of hydroxy groups should play a decisive

role [12]. Thus, diol-bonded silica adsorbs [13] oligo(ethylene glycol)s and cannot be used for SEC of these polymers if water is used as a mobile phase.

There is a lack of knowledge [8] concerning a detailed description of the chemical species which exist on diol-modified silica. Therefore, the bonding reaction was studied in detail; bonded oxirane, vicinal diol, total hydroxy and CH contents were determined as a function of temperature, pH and time. The aim was to find conditions giving the maximum surface coverage of oxirane groups on silica (epoxy activation) and, after opening oxirane rings, to obtain a diol phase with a high coverage of hydroxy groups.

EXPERIMENTAL

Materials

The silica used (Separon SGX, mean particle diameter $d_p = 10 \mu\text{m}$, specific surface area $480 \text{ m}^2/\text{g}$) was obtained from Laboratory Instrument Works, Prague (present manufacturer Tessek, Prague, Czech Republic). The 3-glycidyloxypropyltrimethoxysilane and poly(ethylene glycol)s ($M_r = 200, 2000$ and $20\,000$) were purchased from Fluka (Buchs, Switzerland).

Analytical-reagent grade reagents used in functional group analysis and organic solvents were supplied by Lachema (Brno, Czech Republic). Doubly distilled water was used throughout.

Silica modification

Silica was activated by boiling (30 min) in dilute hydrochloric acid (1:10, v/v), washed with water until neutral, boiled in water (30 min), washed and dried at 150°C .

Aqueous solutions (5%) of GPTMS were prepared by adding the silane dropwise to water, keeping the pH of stirred solutions at given value with KOH (10^{-3} – 10^{-1} M) or HCl (10^{-3} M) solutions. A PHM-64 pH meter (Radiometer, Copenhagen, Denmark) was used. Silicas were added in the ratio of 1.0 g of silica to 1.5 g of GPTMS and the reactions were performed with periodic swirling in sealed flasks or under reduced pressure (8–9.5 kPa) at 24 – 26°C . Reactions at higher temperatures were performed in a

drying oven. The bonded-phase supports were filtered and washed with water, dioxane and diethyl ether and dried at laboratory temperature and then at 85°C for 3 h. Bonding reactions in toluene were performed under reflux or at laboratory temperature in sealed flasks; again, 5% solutions of the silane reagent and the ratio of 1 g of silica to 1.5 g of GPTMS were used and toluene replaced water in the washing procedure. Oxirane ring opening was achieved during 3 h at 85°C (drying oven, periodic swirling) in $0.1 \text{ M H}_2\text{SO}_4$; washing and drying were identical to those above after the bonding reaction in water.

Analytical procedures

There is a broad range of analytical techniques available for the determination of oxirane, vicinal diol and hydroxy functions [14]. In the case of surface-bonded groups, a decrease in reactivity [15] had to be expected and, therefore, the most efficient procedures were selected after testing several possibilities found in the literature [14]. The main criterion was the reproducibility of the end-point determination as a function of the speed of the titration (epoxy groups) and of the duration of the oxidation (acetylation) in the case of back-titrations.

Direct titration of epoxy groups of the sample suspended in acetic acid with standard (0.1 M) perchloric acid in acetic acid in the presence of an excess of tetraethylammonium bromide (25% solution in acetic acid) to the crystal violet end-point, described by Jay [16], was evaluated as the best alternative.

The periodate oxidation method [14] was found to be the best for determination of vicinal diol groups: the bonded silica sample after reaction (30 min) with periodic acid (0.1 M) solution was partially neutralized with saturated sodium hydrogencarbonate solution, then sodium arsenite solution (0.06 M) was added and the excess of arsenite was back-titrated with iodine (0.0125 M) to the starch end-point. Blank titration was performed in the same way.

Perchloric acid-catalysed acetylation [17] was used to determine the total content of hydroxy groups: a silica sample was treated (60 min) with acetic anhydride (0.25 M) in ethyl acetate containing 0.006 M HClO_4 , then pyridine–water

(9:1) was added, the sample was filtered and washed with methanol and the filtrate was titrated with sodium hydroxide (0.15 M) in methanol to the cresol red-thymol blue mixed indicator end-point. Again, the same blank titration was performed. Using unmodified silica no silanol interference was observed.

The precision of the epoxide and vicinal diol determinations was estimated as $0.02 \mu\text{mol}/\text{m}^2$; for total hydroxy group determination *ca.* $0.06 \mu\text{mol}/\text{m}^2$ was obtained.

Chromatography

The SEC equipment consisted of a VCR 40 HPLC pump (CSAS Development Works, Prague, Czech Republic), a Rheodyne (Cotati, CA, USA) Model 7125 injection valve with a 100- μl loop, an R401 differential refractometer (Waters, Milford, MA, USA) and a Servogor 2S recorder (Goerz Electro, Vienna, Austria). Stainless-steel columns (250 \times 8 mm I.D., supplied by Tessek) were packed by a slurry technique at 30 MPa with methanol-dioxane (1:1, v/v) as the slurry liquid.

RESULTS AND DISCUSSION

To avoid opening of oxirane ring during the bonding reaction, pH 4.9 was selected and the influence of reaction temperature and time was

investigated (Table I). The theoretical content of bonded epoxide ligand in $\mu\text{mol}/\text{m}^2$ was calculated from carbon analysis. From the calculated loss of epoxide due to the bonding reaction, it is immediately seen that heating decreases the content of epoxides considerably. A similar decrease is seen if a longer reaction time at ambient temperature is chosen. Both the carbon and measured epoxide content are low in all cases. Primary aminopropyl (C_3 ligand) phases bonded to the same silica matrix [18] exhibit typical values of 5-6% C and 2.4-2.7 $\mu\text{mol}/\text{m}^2$ NH_2 groups; in the present instance *ca.* 10-12% C should be expected for the C_6 ligand. The conversion parameters, $X = \text{vic.-OH}/\text{found epoxide content}$ and $Y = \text{vic.-OH}/\text{epoxide content calculated from \%C}$, allow a comparison of samples with various carbon contents and indicate a complex behaviour. Assuming no loss of epoxide during the bonding reaction and oxirane ring opening exclusively to diol, we obtain $X = Y = 2$. If the oxirane ring is opened during the bonding reaction only to diol, $X > 2$ and $Y = 2$ should be obtained. The values of $Y < 2$ may be interpreted as a result of side-reactions, formation of ether bonds during bonding and/or a ring-opening reaction. Then, if epoxide loss is low (or none) in the bonding reaction and ether formation during sulphuric acid treatment proceeds, $X < 2$ may be found. Experimental X

TABLE I
REACTION OF GPTMS WITH SILICA IN WATER AT pH 4.9

$X = \text{vic.-OH}$ ($\mu\text{mol}/\text{m}^2$): epoxide ($\mu\text{mol}/\text{m}^2$) content from titration; $Y = \text{vic.-OH}$ ($\mu\text{mol}/\text{m}^2$): epoxide ($\mu\text{mol}/\text{m}^2$) ligand calculated from carbon content; reaction described under Experimental.

Sample No.	Temperature (°C)	Duration (h)	Elemental analysis		Titration		Epoxide loss (%)	X	Y
			Carbon (%)	Ligand ($\mu\text{mol}/\text{m}^2$)	Epoxide ($\mu\text{mol}/\text{m}^2$)	vic.-OH ($\mu\text{mol}/\text{m}^2$)			
1	90	0.5	3.45	1.00	0.12	0.98	86.4	7.17	0.98
2	45	0.5	2.45	0.71	0.19	0.64	72.3	3.26	0.91
3	26 ^a	2.0	1.30	0.38	0.27	0.44	46.7	1.62	1.17
4	26 ^a	7.5	2.40	0.69	0.50	0.85	28.5	1.74	1.24
5	26 ^a	54	5.98	1.73	0.79	2.00	54.6	2.55	1.16
6	Ambient	168	7.06	2.04	0.50	2.58	75.3	5.12	1.26

^a Under vacuum.

and *Y* values (Table I) confirm a considerable formation of ether bonds in both reaction steps; *Y* values indicate that the ether formation increases with increasing temperature. A disappointing result was obtained: neither higher temperature nor time can be used to increase the content of unopened epoxide.

Therefore, the next step was to look in detail into the pH dependence of the bonding reaction of GPTMS at ambient temperature. In this respect, some controversy is found in the literature. Regnier and Noel [3], using pH 3.5, pointed out that basic bonding conditions and extended reaction times increased the amount of bonded phase, but no decrease in protein adsorption was observed. Another report [19] shows that reaction at pH 6 gives a material with inferior chromatographic characteristics and recommends the use of lower pH. Stout and DeStefano [20] used pH 8.8 and 90°C and obtained packings exhibiting good performance. According to Plueddemann [10], using epoxy functional silanes in aqueous solutions, the pH of the solution must be maintained above 4 to prevent hydrolysis of the oxirane ring to a glyceryl ether. It is worth mentioning that the mechanism of oxirane ring opening is different [9] in acidic and base media. Alkylloxiranes form corresponding ether and primary hydroxy groups capable of further addition of another oxirane

ring under acid catalysis; basic catalysis gives mainly secondary hydroxy groups of low reactivity towards another epoxide group. Hence the basic catalysis might decrease the formation of higher adducts. When methanol adds to the oxirane ring, the same effect should operate in the first step owing to the formation of a secondary hydroxy group. Hydrolysis of trialkoxysilanes to silanetriols is rapid [10]; a threefold molar excess of methanol to epoxide may be expected to be formed in the initial period of the bonding reaction.

The results of the GPTMS bonding in water at ambient temperature and different pH are summarized in Table II. It is seen that the lowest epoxide loss is achieved at pH 8.5. The decrease in *Y* at low pH indicates more side-products in agreement with the previous scheme. The small increase in *X* in this case of high epoxide loss also confirms considerable ether formation during the opening reaction, in agreement with Plueddemann [10].

Consequently, the reaction at pH 8.5 giving the lowest loss of epoxide and highest conversion parameter *Y* was studied further. The effects of reduced pressure (expected to decrease the methanol content in the reaction mixture) and reaction time and reproducibility of bonding were investigated. To obtain a deeper insight into the bonding reaction, the determination of

TABLE II
pH DEPENDENCE OF GPTMS-SILICA BONDING REACTION IN WATER

X and *Y* as in Table I. All experiments under vacuum; 24-26°C

pH	Duration (h)	Elemental analysis		Titration		Epoxide loss (%)	<i>X</i>	<i>Y</i>
		Carbon (%)	Ligand ($\mu\text{mol}/\text{m}^2$)	Epoxide ($\mu\text{mol}/\text{m}^2$)	<i>vic.</i> -OH ($\mu\text{mol}/\text{m}^2$)			
3.1	2	2.18	0.52	0.27	0.48	58.3	1.81	0.75
4.9	2	1.30	0.38	0.27	0.44	30.0	1.64	1.15
5.5	2	1.39	0.40	0.23	0.40	45.6	1.79	0.97
7.0	2	2.79	0.81	1.00	1.48	16.0	1.49	1.25
7.0	5	4.09	1.19	1.58	2.25	18.8	1.42	1.15
8.5	2	6.78	1.96	1.71	—	13.4	—	—
8.5	1	7.19	2.08	1.90	—	9.0	—	—
8.5	1	7.11	2.06	1.79	2.90	12.5	1.60	1.40
9.0	2	7.59	2.19	1.81	—	17.0	—	—

total hydroxy group content was added and a conversion parameter $Z = \text{total OH } (\mu\text{mol/m}^2) / \text{epoxide } (\mu\text{mol/m}^2)$ calculated from carbon content was introduced. Table III summarizes the results. It is immediately seen that the reproducibility at equal reaction times is not very good, although the same batch of silica was used. Therefore, great care is needed with respect to conclusions. The complexity of both bonding and opening reactions obviously determines these differences and this effect adds to the well known divergence of properties [21,22] of apparently identical bonded phases based on different silica matrices. The entirely different behaviour of various diol silicas described in the literature [11] is then easily understood. Comparing average values of X , Y and Z in experiments under vacuum and at ordinary pressure a small decrease in these parameters is observed and may be interpreted as a result of enhanced methyl ether formation. No relative increase in the epoxide loss with time is the first satisfactory result; the other is the increase in the total amount of hydroxy groups per ligand with time as expressed by parameter Z . X values again

indicate that the opening reaction is accompanied by significant glycol ether formation. Sample 12 therefore represents the best epoxy-modified reactive silica with surface coverage of $2.4 \mu\text{mol/m}^2$ of epoxide groups. After the epoxide opening, $4.8 \mu\text{mol/m}^2$ of hydroxy groups (75% of them being diol groups) are obtained. In terms of theoretical epoxide content (calculated from carbon content), 57% undergo bonding and opening to form diol groupings and the remainder give only some isolated hydroxy groups. Fig. 1 shows that using this optimized diol packing a correct SEC behaviour of poly-(ethylene glycol)s is achieved in water as mobile phase.

To evaluate the effect of cyclic oligosiloxane formation in the bonding reaction, the reactions were performed with GPTMS prehydrolysed for given periods of time before silica was added (Table IV). Indeed, cyclization takes place and the cyclic products are able to be attached to the silica surface, as follows from the increased carbon content with prehydrolysis for 3 h. The epoxide loss compares well with Table III and the same holds for X . A small decrease in Y and

TABLE III
OPTIMIZATION OF GPTMS-SILICA REACTION IN WATER AT pH 8.5

X and Y as in Table I; $Z = \text{total OH } (\mu\text{mol/m}^2) / \text{epoxide } (\mu\text{mol/m}^2)$ ligand calculated from carbon content; reaction described under Experimental; 24-26°C; samples 1-6 under vacuum.

Sample No.	Duration (h)	Elemental analysis		Titration			Epoxide loss (%)	X	Y	Z
		Carbon (%)	Ligand ($\mu\text{mol/m}^2$)	Epoxide ($\mu\text{mol/m}^2$)	<i>vic.</i> -OH ($\mu\text{mol/m}^2$)	Total OH ($\mu\text{mol/m}^2$)				
1	1	7.11	2.06	1.79	2.90	3.15	12.5	1.60	1.40	1.52
2	1	7.16	2.06	1.58	2.65	2.75	23.1	1.66	1.28	1.33
3	1	6.79	1.96	1.56	2.12	2.42	20.3	1.36	1.09	1.23
4	1	7.43	2.15	1.75	2.62	2.94	18.1	1.50	1.22	1.37
5	1	6.56	1.90	1.58	2.17	2.52	16.0	1.36	1.14	1.33
Average 1-5	-	7.01	2.02	1.65	2.50	2.75	18.0	1.50	1.23	1.36
6	0.33	5.87	1.69	1.50	2.10	2.29	11.8	1.41	1.25	1.36
7	1	6.55	1.90	1.58	2.17	2.38	16.0	1.36	1.14	1.25
8	1	7.48	2.17	1.54	2.10	2.38	28.2	1.35	0.97	1.10
9	1	6.11	1.77	1.50	1.87	2.42	14.6	1.23	1.06	1.36
Average 7-9	-	6.71	1.94	1.54	2.04	2.40	19.6	1.31	1.06	1.24
10	20	9.30	2.69	2.17	3.12	4.08	19.8	1.44	1.16	1.52
11	96	9.95	2.87	2.21	2.92	4.15	23.5	1.32	1.02	1.44
12	192	10.81	3.12	2.42	3.58	4.81	22.9	1.48	1.15	1.54

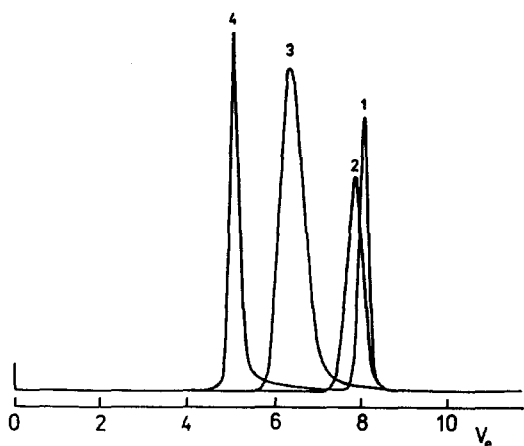


Fig. 1. SEC behaviour of poly(ethylene glycol)s on optimized diol packing with water as mobile phase. Injection of 100 μ l of (1) 0.05% ethylene glycol, (2) 0.05% PEG 200, (3) 0.1% PEG 2000, (4) 0.05% PEG 20000. Packing No. 12 (Table III); flow-rate, 1.92 ml/min; refractive index detection.

Z might indicate slightly higher formation of glycol ethers in solution during prehydrolysis for 3 h.

Bonding reactions of GPTMS in organic solvents are seldom found in the literature [23,24] and, as a rule, the reaction conditions are not well described. To obtain a full picture, the bonding reaction was performed in toluene (common solvent for anhydrous silane bonding [25]) at 110 and 26°C (Table V). An unexpected result was obtained: most of the epoxide groups disappeared even at ambient temperature and very low conversion to hydroxy groups was

obtained in comparison with the optimum conditions in water. The total hydroxy conversion (Z) is approximately constant in all instances and only a slightly increased yield of vicinal hydroxyls (Y) is found at 26°C. Alumina acidified with 5% acetic acid is known to be an efficient catalyst in oxirane opening reactions [9]; the acidic silica surface may exhibit the same effect especially under these conditions. The oxirane opening may be initiated by released methanol or hydration water present on the silica surface, and extensive polyaddition may follow that can be terminated by another methanol or hydration water molecule. Diol-bonded silica prepared in CCl_4 at 60°C has been examined by C and Si NMR spectroscopy [23] and no methoxy groups were observed; this might indicate the participation of hydration water. Such a comparison requires caution; the bonding reaction mechanism may depend strongly on the selection of the organic solvent. For instance, we observed no surface bonding if dioxane was used as a reaction solvent. Anyway, the low content of hydroxy groups obtained especially at 110°C indicates rather an "ether"-bonded phase structure described many years ago [26].

CONCLUSIONS

Bonding of 3-glycidyoxypropyltrimethoxysilane to silica in water should be performed at pH 8.5 and ambient temperature to obtain a high yield of epoxide activation. The corresponding diol phase obtained after the oxirane opening

TABLE IV

REACTION OF SILICA WITH PREHYDROLYSED GPTMS IN WATER AT pH 8.5

X, Y, and Z as in Table III; duration 1 h; 24-26°C.

Prehydrolysis time (h)	Elemental analysis		Titration			Epoxide loss (%)	X	Y	Z
	Carbon (%)	Ligand ($\mu\text{mol}/\text{m}^2$)	Epoxide ($\mu\text{mol}/\text{m}^2$)	vic.-OH ($\mu\text{mol}/\text{m}^2$)	Total OH ($\mu\text{mol}/\text{m}^2$)				
0.25	6.94	2.00	1.60	1.98	2.60	19.7	1.23	0.99	1.30
0.50	5.71	1.65	1.40	1.79	2.67	16.0	1.28	1.09	1.62
0.50	6.30	1.81	1.54	1.92	2.58	15.3	1.24	1.06	1.42
3.00	9.29	2.69	2.06	2.44	3.44	23.5	1.18	0.91	1.28

TABLE V
GPTMS-SILICA BONDING REACTION IN TOLUENE

X, Y and Z as in Table III.

Sample	Elemental analysis		Titration			Epoxide loss (%)	X	Y	Z
	Carbon (%)	Ligand ($\mu\text{mol}/\text{m}^2$)	Epoxide ($\mu\text{mol}/\text{m}^2$)	vic.-OH ($\mu\text{mol}/\text{m}^2$)	Total OH ($\mu\text{mol}/\text{m}^2$)				
1 ^a	16.3	4.71	ca. 0	0.29	1.96	ca. 100	—	0.06	0.41
2 ^b	12.7	3.67	0.06	0.38	1.67	98	6.0	0.10	0.45
3 ^c	5.4	1.56	0.17	0.46	0.65	89	2.8	0.29	0.41
4 ^d	5.9	1.69	1.50	2.10	2.29	12	1.41	1.25	1.36

^a 110°C, 15 h.

^b 110°C, 4 h.

^c 26°C, 1 h.

^d Comparable sample in water at pH 8.5.

reaction exhibits the correct SEC behaviour of poly(ethylene glycol)s in water. Neither the silane bonding reaction nor the subsequent oxirane opening gives rise to diol structures only; considerable formation of glycol ethers varies with changing reaction conditions. The bonding reaction in toluene performs much worse; an "ether"- rather than a "glycol"-bonded phase is obtained.

ACKNOWLEDGEMENT

The careful technical assistance of Mrs. J. Nováková is gratefully acknowledged.

REFERENCES

- H.G. Barth and B.E. Boyes, *Anal. Chem.*, 64 (1992) 428R.
- T. Hashimoto, *J. Chromatogr.*, 544 (1991) 249.
- F.E. Regnier and R. Noel, *J. Chromatogr. Sci.*, 14 (1976) 316.
- D.E. Schmidt, Jr., R.W. Giese, D. Conron and B.L. Karger, *Anal. Chem.*, 52 (1980) 177.
- S.H. Chang, K.M. Gooding and F.E. Regnier, *J. Chromatogr.*, 120 (1976) 321.
- D.J. Phillips, B. Bell-Alden, M. Cava, E.R. Grover, W.H. Mandeville, R. Mastico, W. Sawlivich, G. Vella and A. Weston, *J. Chromatogr.*, 536 (1991) 95.
- R.R. Walters, *J. Chromatogr.*, 249 (1982) 19.
- G.R. Bogart, D.E. Leyden, T.M. Wade, W. Schafer and P.W. Carr, *J. Chromatogr.*, 483 (1989) 209.
- Houben-Weil *Methoden der Organischen Chemie*, Band VI/1a, Georg Thieme Stuttgart, 1979, pp. 357-382.
- E.P. Plueddemann, *Silane Coupling Agents*, Plenum Press, New York, 1982.
- E. Pfannkoch, K.C. Lu, F.E. Regnier and H.G. Barth, *J. Chromatogr. Sci.*, 18 (1980) 430.
- P. Roumeolitis and K.K. Unger, *J. Chromatogr.*, 218 (1981) 535.
- H. Engelhardt and D. Mathes, *J. Chromatogr.*, 185 (1979) 305.
- N.D. Cheronis and T.S. Ma, *Organic Functional Group Analysis by Micro and Semimicro Methods*, Wiley, New York, 1964.
- H. Engelhardt and D. Mathes, *J. Chromatogr.*, 142 (1977) 311.
- R.R. Jay, *Anal. Chem.*, 36 (1964) 667.
- J.S. Fritz and G.H. Schenk, *Anal. Chem.*, 32 (1960) 987.
- B. Porsch, *J. Liq. Chromatogr.*, 14 (1991) 71.
- I.D. Lumley, I. Patel and I.C. Cohen, *J. Chromatogr.*, 408 (1987) 115.
- R.W. Stout and J.J. DeStefano, *J. Chromatogr.*, 326 (1985) 63.
- H. Engelhardt and H. Müller, *J. Chromatogr.*, 218 (1981) 395.
- M.P. Henry, *J. Chromatogr.*, 544 (1991) 413.
- E. Bayer, K. Albert, J. Reiners, M. Nieder and D. Müller, *J. Chromatogr.*, 264 (1983) 197.
- G. Szabó, K. Offenmüller and E. Csató, *Anal. Chem.*, 60 (1988) 213.
- H. Engelhardt and P. Orth, *J. Liq. Chromatogr.*, 10 (1987) 1999.
- J.J. Kirkland and J.J. DeStefano, *J. Chromatogr., Sci.*, 8 (1970) 309.