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QUANTITATIVE DETERMINATION OF THE DEGREE OF CAPPING AND STRUCTURAL INVESTIGATION OF OCTADECYLSILANE PHASES BY MEANS OF PYROLYSIS-GAS CHROMATOGRAPHY

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SUMMARY

Pyrolysis-gas chromatography has been used for the quantitative determination of the degree of capping of chemically bonded phases for reversed-phase high-performance liquid chromatography. The method has been applied to the evaluation of different capping procedures and for settling the capping extent of octadecylsilane phases. In addition, the influence of starting materials and method of preparation on the properties of octadecylsilane phases have been studied by means of pyrolysis-gas chromatography and other methods.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (HPLC) constitutes the major technique of analytical LC separations^{1,2}. In this technique chemically bonded silica, especially octadecylsilyl (ODS) bonded silica², is used to a great extent.

Because of the lack of methods for chemical characterization, reversed-phase HPLC is still a relatively undefined separation method³. In a previous paper⁴ pyrolysis gas chromatography (Py GC) was shown to be a valuable tool for the investigation of the structure of chemically bonded stationary phases (CBSPs). Two aspects of that work gave impetus to further studies, namely post-silanization or capping and the variation of the properties of ODS phases with differing starting materials and preparative conditions.

For di- and trifunctional ODS reagents one or two unreacted groups per molecule of silanizing agent, respectively, can be left, these lead to the formation of additional silanol groups after hydrolysis. Silanol groups can give rise to mixed retention mechanisms and result in unfavourable effects on, for example, column stability, retention reproducibility and peak symmetry^{2,5}. However by capping with, for example, trimethylchlorosilane (TMCS) the undesirable effects of silanol groups can be reduced^{2,5}. Py-GC was previously used to ascertain qualitatively whether or not an ODS phase was capped⁴. In the present work a Py-GC method has been developed for the quantitative determination of capping groups, *i.e.* trimethylsilyl (TMS)

groups. We are not aware of any method for this kind of quantitative analysis having been published previously.

The ODS silica can be derived either from mono-, di- or trifunctional octadecylchlorosilanes and octadecylalkoxysilanes or from cyclic polymers. Polymerization, which can take place only for di- and trifunctional silanizing agents, has been assumed to occur during hydrous conditions^{3,6,7}. However, if the reaction with silica is carried out under anhydrous conditions, polymerization is largely prevented. For a well defined CBSP, monofunctional silanizing agents are recommended, although such reagents might possess a lower reactivity^{2,8,9}. We have investigated the properties of several ODS phases, prepared under different conditions, by means of Py-GC, elemental analysis and chromatographic characterization in order to establish the nature of the CBSPs.

EXPERIMENTAL

Pyrolysis conditions

The pyrolyser used was a CDS 150 Pyroprobe (Chemical Data Systems, Oxford, PA, U.S.A.) with a probe of the platinum-coil type. A quartz tube with a sample holder was inserted into the coil. A detailed description of apparatus and procedure is given elsewhere⁴. Optimal pyrolysis conditions for the postsilanization study were found to be a dial setting of 850°C, 5 sec, and for the study of ODS phases 750°C, 10 sec. The interface temperature was set at 250°C.

Gas chromatography

A Varian 2400 gas chromatograph with a 1.9 mm I.D. stainless-steel column was used. The carrier gas was nitrogen at a flow-rate of 30 ml/min. A flame-ionization detector was used at 290°C and the hydrogen and air flow-rates were 30 and 240 ml/min, respectively. The injector temperature was 330°C.

Two GC systems were used: (A) a 1-m Chromosorb 102 column with isothermal conditions at 40°C. After each run the column was kept at 250°C for 15 min in order to elute high-boiling compounds. The other system (B) consisted of a 2.4-m column packed with 3% OV-1 on Chromosorb W HP (100/120 mesh) with temperature programming from 100 to 270°C at 20°C/min.

Liquid chromatography

The apparatus, column tubing, column packing, fittings and procedure have been described elsewhere¹⁰. For calculation of the theoretical plate number (N) the formula $N_s = 5.54(t_r/W_{1,2})^2$ was used. The asymmetry factor A_s was calculated according to ref. 11.

Chemicals

Octadecyldimethylchlorosilane (ODMCS) and octadecylmethyldichlorosilane (OMDCS) were purchased from Magnus Scientific (Sandbach, Great Britain). Octadecyltrichlorosilane (OTCS), trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) were purchased from E. Merck (Darmstadt, G.F.R.) and trimethylsilylimidazole (TMSI) from Fluka (Buchs, Switzerland). The silica gel used to prepare the bonded phases was LiChrosorb Si-100, 5 μ m (E. Merck) from the same batch (F

1643, No. 1 in Table III) except for one experiment in the ODS phase study, where a different batch (F 3213, No. 2 in Table III) was used.

Preparation and capping of CBSPs

The bonding procedure used to prepare the different CBSPs was basically that described by Hemetsberger *et al.*¹² but with some variation of the reaction conditions. If nothing else is stated, the bonding procedure was carried out by adding 90 ml of toluene (sodium-dried), 8 mmol of chlorosilane and 5 ml of pyridine (potassium hydroxide-dried) to 4 g of silica (dried over night at 220°C in air). The mixture was refluxed for 6 h under dry nitrogen and continually stirred with a glass rod during the reaction. A funnel with a sintered-glass bottom was used for the washing of the CBSP with successive batches of toluene, chloroform, methanol, methanol water, acetone and diethyl ether.

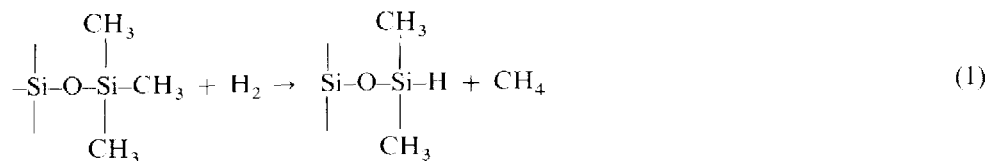
The capping of the ODS phase was carried out in the same way as the preparation of CBSP itself, except that 0.5 g of ODS silica was dried at 100°C in air over night and 1 ml of TMCS, 20 ml of toluene and 1 ml of pyridine were added to the reaction vessel. In two other capping procedures 0.5 ml of TMCS and 1 ml of HMDS or 0.5 ml of TMCS and 1 ml of TMSI were used as silanizing agents together with 1 ml of pyridine and 20 ml of toluene. A blank was prepared using the same procedure, except that the silanizing agent was omitted. All samples were dried at 120°C and 3 mmHg for 30 min prior to pyrolysis or elemental analysis.

For the ODS phase study, using method I (see Table III) the CBSP was prepared under normal dry conditions¹². In the second method (II), 150 µl of water was added to the reaction vessel after 2 h and another 150 µl of water after 4 h. The total refluxing time was 6 h, as for method I. In the third method (III) the silica was not dried but kept in contact with saturated solution of lithium chloride overnight. After that, the same procedure as for method I was used.

RESULTS AND DISCUSSION

Postsilanization (capping)

The production of methane was utilized for determining the number of capping groups, *i.e.* TMS groups. A TMS group bonded to silica is degraded upon pyrolysis according to eqn. 1.



Hydrogen is normally formed in the pyrolysis reaction¹³. However, methane is also produced on pyrolysis of the C₁₈ chain in the ODS phase. The production of ethylene was used as a measure of the number of C₁₈ groups. It is known^{8,14} that thermal degradation of alkylsilyl-bonded silica also yields alkylsiloxanes. However, these compounds could not be detected with the GC method used.

For the quantitative determination of the degree of capping, a calibration

graph was constructed. Uncapped ODS silica, obtained from OTCS and silica according to method I, was mixed with TMS silica, obtained by reaction of TMCS with silica using the same method, and the mixture was then pyrolyzed. If K is the ratio of the methane (C_1) to ethylene (C_2) peak heights in the sample and x is the weight percentage of TMS silica, the following relationship is obtained:

$$K = \frac{(1-x)a + xa'}{(1-x)b} \quad (2)$$

where a and b represent the peak heights of C_1 and C_2 , respectively, per gram of ODS silica and a' represents the peak height of methane per gram of TMS silica. The production of ethylene from TMS silica is negligible. If $K_0 = a/b$, eqn. 2 becomes

$$K = K_0 + \frac{x}{1-x} \cdot \frac{a'}{b} \quad (3)$$

Eqn. 3 is applied to "trifunctional" ODS silica in Fig. 1 and to "difunctional" ODS silica in Fig. 2. As the structures of the standards and capped ODS silica are not exactly the same, some uncertainty exists. The deviation of the lowest point from the linear relationship for difunctional ODS silica in Fig. 2 is unexpected.

The elemental analysis results gave a carbon content of 2.87% for the TMS silica used as the standard. This carbon content corresponds to 0.797 mmol TMS groups per gram of TMS silica. The number of TMS groups (n) expressed as mmol per gram of capped ODS silica is given by eqn. 4.

$$n = \frac{y}{1+y} \cdot 0.797 \quad (4)$$

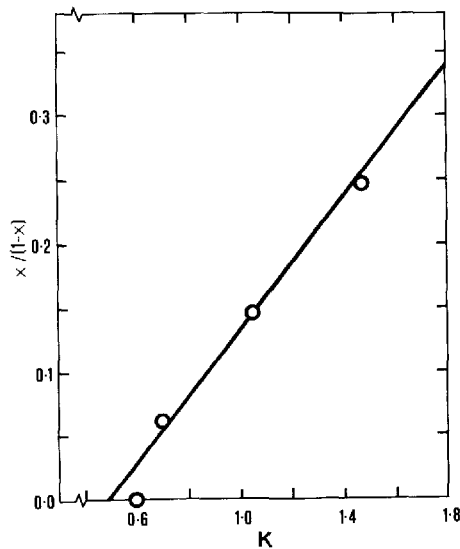
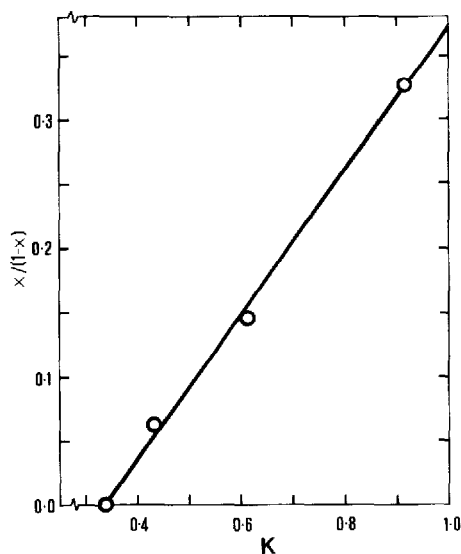


Fig. 1. The weight ratio of TMS silica to trifunctional ODS silica $[x/(1-x)]$ plotted vs. the peak height ratio C_1/C_2 (K) for pyrolysed samples.

Fig. 2. The weight ratio of TMS silica to difunctional ODS silica $[x/(1-x)]$ plotted vs. the peak height ratio C_1/C_2 (K) for pyrolysed samples.

TABLE I

INVESTIGATION OF CAPPING EFFICIENCY OF DIFFERENT CAPPING REAGENTS FOR ODS PHASES PREPARED FROM OTCS

Capping reagent	Peak height ratio, C_1/C_2^*	TMS per g (μmol)
Blank	0.34	0
TMCS + pyridine	0.77	157
TMCS + HMDS + pyridine	0.66	123
TMCS + TMSI + pyridine	0.67	126

* The variation in the peak height ratio for the same sample was smaller than 4%.

The value of $y = x/(1 - x)$ is obtained from Fig. 1 or 2 by reading the y value corresponding to the measured K value of the sample.

A study of the efficiency of different capping methods was made for "trifunctional" ODS phases prepared from OTCS and the results are shown in Table I and Fig. 3. It can be seen that TMCS in the presence of pyridine is the most efficient of the tested silanizing agents under the conditions used.

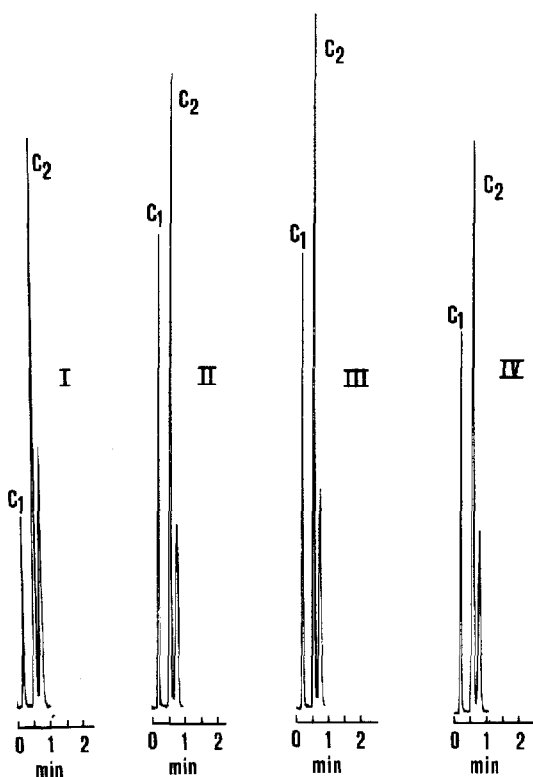


Fig. 3. Pyrograms of ODS phases prepared from OTCS and different capping reagents (see Table I). I = Uncapped; II = capped with TMCS; III = capped with TMCS + HMDS; IV = capped with TMCS + TMSI. GC: system A.

TABLE II
CAPPING OF ODS PHASES WITH THE TMCS (PYRIDINE) SILANIZING AGENT

Starting material used for preparation of ODS phase	Peak height ratio, C_1/C_2	TMS per g (μmol)	C (%)
OTCS (uncapped)	0.34	0	16.7
OTCS (capped)	0.77	157	16.9
OMDCS (uncapped)	0.60	0	17.2
OMDCS (capped)	0.84	67	16.3
ODMCS (uncapped)	1.26	0	16.8
ODMCS (capped)	1.00	0	14.5

The capping efficiency of TMCS for ODS phases prepared from chlorosilanes of varying functionalities was also investigated (Table II). For uncapped ODS silica made from OTCS, OMDCS and ODMCS, there is an increase in the C_1/C_2 peak height ratios from 0.34 to 1.26, which can be attributed to the increasing number of methyl groups (0, 1 or 2) attached to the silicon atom holding the C_{18} chain. The yield of methane from OTCS-treated silica originates from the degradation of the C_{18} hydrocarbon chain, which of course contributes also to the methane production in the other cases.

It has been stated that capping with TMCS of "fully covered" ODMCS-treated silica is unnecessary⁸. Our results confirm this statement. Capping with TMCS would increase the carbon content as well as the C_1/C_2 ratio, while in fact the reverse results are obtained. This can only be explained by a loss of methyl groups from the $C_{18}(\text{CH}_3)_2\text{Si}$ group during capping. Splitting of methyl and alkyl groups from silicon has been reported¹⁵ in the presence of hydrogen chloride and catalytic amounts of aluminium chloride. A similar reaction might take place here, although the conditions used in capping are somewhat different, *viz.* the reaction is carried out in the presence of pyridine. Aluminium, together with some 30 additional trace elements, has been shown¹⁶ to be present in silica gel used for HPLC.

On TMS capping of silica containing $C_{18}\text{CH}_3\text{Si}$ and $C_{18}\text{Si}$ groups bound to silica, the C_1/C_2 ratio increases. For the carbon content there is a decrease in $C_{18}\text{CH}_3\text{Si}$ and a slight increase in $C_{18}\text{Si}$, while an increase would have been expected in both cases. The decrease in carbon content on capping of $C_{18}\text{CH}_3\text{Si}$ silica would seem to indicate that C_{18} groups, and possibly also CH_3 groups, are lost (see above). On the basis of the number of TMS groups and the carbon content, it can be calculated that for $C_{18}\text{Si}$ silica approximately one silanol group per five C_{18} groups is capped, while for $C_{18}\text{CH}_3\text{Si}$ silica the figure is approximately one silanol group per eleven C_{18} groups. There is reason to believe that the capped silanol groups are situated at the C_{18} silicon atom.

Influence of materials and conditions and structural investigation of the ODS phases

In order to ascertain to what extent the reagent, the silica and the preparation procedure influence the structure and properties of the CBSP, the experiments summarized in Table III were made. Three methods of preparation were used, *viz.* refluxing of silanizing agent with dry silica under dry conditions for 6 h (method I), reflux-

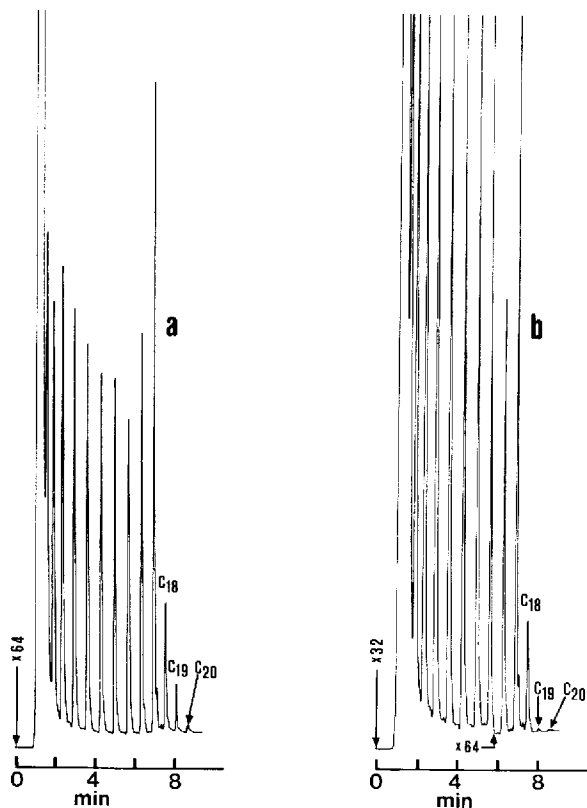


Fig. 4. Pyrograms of two CBSPs, a and b, prepared from silica and two different batches of OTCS. GC: system B (see Results and discussion).

ing as above but with the addition of water after 2 and 4 h, respectively (method II), and refluxing of moist silica with silanizing agent for 6 h (method III). The dry ODS phase was analysed in several ways: it was pyrolysed and the C_{17}/C_{18} alkene ratio determined, the carbon content was assayed by elemental analysis and, in order to characterize the phase chromatographically, the retention time (t_r), asymmetry factor (A_s) and plate number (N) were established.

The first group of experiments concerns silica treated with OTCS in the three manners described above. It can be seen that there is a distinct difference between the C_{17}/C_{18} ratios of the phases obtained under dry (I) and wet (II and III) conditions. At the same time the carbon content changes, being highest for the wet cases. The increase in carbon content means that the number of C_{18} groups per unit area of the silica surface increases. From this fact and from the increase in retention time it is concluded that methods II and III produce a polymeric multilayer CBSP structure. In view of this, the higher plate number for the wet-method phases is somewhat unexpected.

The change in the C_{17}/C_{18} ratio on going from the dry (I) to the wet procedures (II and III) depends on the ease with which the C_{18} group is split from the silicon atom. Accordingly, the results signify a decrease in the bond strength of the C_{18} -Si

TABLE III
PREPARATION OF ODS PHASES USING DIFFERENT MATERIALS AND METHODS

Series of experiment	Starting material used for preparation of ODS phase	Method of preparation	Silica batch No.	Peak height ratio C_{17}/C_{18} *	C (%)	Plate number, N	Retention volume (ml)**	Asymmetry factor, A_s **
1	OTCS	I	1	4.4	16.6	6900	8.0	2.0
		II	1	2.9	17.1	7800	8.8	1.5
		III	1	2.6	19.9	7800	8.7	1.8
2	OTCS	I	2	1.1	16.0	7100	7.7	1.6
		II	2	0.8	17.4	6900	8.4	1.6
		I***	2	5.5	16.0	6000	7.1	1.3
		II***	2	5.0	17.0	—	—	—
3	OMDCS	I	1	2.3	17.2	6500	8.8	1.0
		II	1	—	15.3	—	—	—
		III	1	2.8	11.7	4800	6.6	2.0
4	ODMCS	I	1	1.0	16.8	7700	8.3	1.4

* The precision of the peak height ratio expressed as relative standard deviation was 2.3% ($n = 17$).

** Measured for 4-tert.-butylphenol. Mobile phase: methanol-water (70:30), flow-rate, 1 ml/min; column, 200 × 5 mm I.D.

*** After treatment with 0.01 M sodium phosphate (pH = 7.5) methanol (40:60) for 3 days. Each peak height ratio is a mean of five measurements.

bond for the multilayer structures. This can be attributed to a change in the substitution pattern at the C_{18} silicon atom and in the nature of the surrounding siloxane network.

The importance of the quality of the silanizing agent was demonstrated in an experiment with another batch of OTCS (not included in Table III). The resulting ODS silica, obtained by method I from the same silica as before, had a plate number only about half that reported in Table III. Figs. 4a and b show pyrograms of CBSPs prepared from two different batches of silanizing agents. It can be seen that one of the batches contains C_{20} groups while the other is virtually free of them.

In one set of experiments, OTCS was reacted using method I with another batch of silica gel (No. 2) of the same make as above. Surprisingly, a phase with a very low C_{17}/C_{18} ratio (1.1) was obtained, instead of the value of 4.4 given by the previous batch (No. 1). The wet method II produced an ODS phase with an even lower C_{17}/C_{18} ratio, 0.8. This means that the same relative decrease in this ratio resulted when going from method I to method II, as for silica batch no. 1. The same trend is found to exist for the change in carbon content and in retention time. The reason for the low C_{17}/C_{18} ratios is as yet unknown. However, they must be considered to be due to the silica structure, as indicated by the fact that on treatment of the CBSPs with methanol phosphate buffer the ratios increased to more normal values, *viz.* 5.5 and 5.0, respectively. The low C_{17}/C_{18} ratio for the ODS phase from silica batch no. 2 does not impair its chromatographic properties, the plate number lying at the same level as that for the ODS phase from batch no. 1.

Another set of experiments in Table III refers to ODS silica prepared from OMDCS. The properties of the ODS phase obtained under dry conditions do not deviate appreciably from those for the phase made from OTCS, except for the C_{17}/C_{18} ratio which is lower. This is considered to be due to the changed structure pattern at the silicon atom holding the C_{18} group⁴.

However, the wet method III produces an ODS phase with a far lower carbon content, while the C_{17}/C_{18} ratio slightly increases. At the same time the plate number and the retention time decrease by *ca.* 30%. A considerable reduction in the density of $C_{18}(\text{CH}_3)\text{Si}$ groups on the silica surface is indicated. This might be due to cyclosiloxanes, formed by reaction of the OMDCS with the moist silica, which are less prone than chlorosilanes to react with silica under the prevailing conditions¹⁸.

The carbon content obtained for the ODS phase prepared by method II is intermediate between the values for the phases from methods I and III. In this case we started with dry conditions and added small amounts of water after 2 and 4 h and continued the boiling for 4 and 2 h, respectively. Assuming that the silanization reaction is already complete after 2 h¹⁷, the 12% decrease in carbon content must be attributed to removal of CBSP from the silica surface during the boiling procedure following the water additions. It is concluded that the hydrous methods are unsuitable for the preparation of ODS phases from OMDCS.

The last experiment in Table III concerns ODS silica prepared from ODMCS under dry conditions. The only diverging quantity is the C_{17}/C_{18} ratio. The low value obtained is due to the fact that the CBSP contains $C_{18}(\text{CH}_3)_2\text{Si}$ groups bonded to silica instead of $C_{18}\text{CH}_3\text{Si}$ and $C_{18}\text{Si}$, respectively, in the two previous set of experiments⁴.

The change in C_{17}/C_{18} ratio with the number of carbon and oxygen atoms at

C₁₈Si was noted previously⁴ in our paper on the use of Py-GC for the characterization of ODS phases. In the present work, it has been found that the C₁₇/C₁₈ ratio is also dependent on the structures of the silica and the structure of the CBSP. The results collected in Table III accentuate the importance of the functionality and quality of the starting materials used for the preparation of ODS silica as well as of the method of synthesis.

CONCLUSIONS

The Py-GC method developed for the quantitative determination of the degree of capping is useful for the evaluation of different capping procedures and for establishing the capping extent of ODS phases of various kinds.

The results of the investigation of different methods and materials for the preparation of ODS phases accentuate the importance of the functionality and quality of the starting materials and of the procedure for the preparation of the phase.

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