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Journal of Chromatography A, 1077 (2005) 80–89
JOURNAL OF CHROMATOGRAPHY A
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Characterisation of natural polysaccharides (plant gums) used as binding media for artistic and historic works by capillary zone electrophoresis

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Received 18 February 2005; received in revised form 8 April 2005; accepted 19 April 2005

Abstract

The monosaccharide constituents of plant gums were separated by capillary electrophoresis at pH 12.1 and detected with indirect UV absorbance. The plant gums investigated were gum arabic, gum acacia, gum tragacanth, cherry gum and locust bean gum (carob gum). The monosaccharides obtained after hydrolysis with 2 M trifluoroacetic acid and lyophilisation of the hydrolysate were arabinose, galactose, mannose, rhamnose, xylose, fucose, and glucose, and the two sugar acids galacturonic and glucuronic acid, in accordance with the literature. They were separated in a background electrolyte consisting of NaOH to adjust the pH, 20 mM 2,6-pyridinedicarboxylic acid as chromophore for detection and 0.5 mM cetyltrimethylammonium bromide as additive to reverse the electroosmotic flow. Based on their electropherograms, the plant gums could be identified by their typical composition (depicted in a decision scheme) as follows: a peak of glucuronic acid, together with that of rhamnose, is indicative for gum arabic. Peaks of galacturonic acid and fucose point to gum tragacanth. Locust bean gum shows a major peak for mannose (with the concomitant galactose peak in ratio 4–1), whereas a glucuronic acid and a mannose peak together with a prominent arabinose peak indicates cherry gum. The method was applied to identify the plant gums in samples like watercolours and in several paint layers like gum tempera or those with egg white or drying oils as additives. Artificial aging experiments of thin layers of gum arabic on paper or glass carried out with UV-A radiation (366 nm) did not result in changes of the saccharide patterns, in contrast to the simultaneously conducted aging of a drying oil layer.

Keywords: Binding media; Natural binders; Plant gums; Glues; Polysaccharides; Sugars; Uronic acids; Museum objects; Paint; Paint layers; Artistic; Historic works; Capillary electrophoresis

1. Introduction

Plant gums consist of polysaccharides, which are exuded by many trees and plants when their barks are wounded [1]; they are materials of high molecular weight, which are soluble in water, or which can be at least dispersed therein. Gum arabic and gum tragacanth are not only the most important representatives of this family in the field of artistic and historic works [2], they are also of broader economic relevance [3] and therefore produced in rather large quantities. Of interest for the topic of the present investigation is the application of such natural gums since the second century B.C. as binding media for pigments in Egyptians ointments for mummification, their use in mural paintings in Christian catacombs [2,4], in paintings on silk [4], and in manuscript illumination in the middle ages [2,5]. However, the major area of application lies in watercolours. In the agro-alimentary, cosmetic and pharmaceutical industries plant gums are also used, mainly as thickening agents or emulsion stabilisers.

The gums have in common that they consist of complex, highly branched polysaccharides composed of natural sugars and hexuronic acid monomers [1]. The manner in which these units are linked together is generally only partly known, how-

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doi:10.1016/j.chroma.2005.04.075

ever, a reliable classification may be achieved on the basis of their monosaccharide composition after stoichiometric cleavage of the glycosidic bonds. The number of possible sugars the plants gums consist of is rather limited: L-arabinose (Ara), D-galactose (Gal), D-mannose (Man), L-rhamnose (Rham), D-xylose (Xyl), L-fucose (Fuc), and D-glucose (Glu). Additionally, there are two sugar acids, D-galacturonic (GalAc) and D-glucuronic acid (GluAc) [1].

Various techniques have been developed for the identification of plant gums in artistic or historic works [6] (most of them developed for analytical purposes in food chemistry). Detection by infrared (IR) spectroscopy [7] has limited use since many of the spectra overlap, but chromatographic techniques have proved very useful for the analysis of historic and artistic works. In early stages of development, paper chromatography was applied, but it has the disadvantages of a long development time, poor resolution, and a limited detectibility. Although through the application of thin-layer chromatography to art samples some interesting results have been obtained [8], gas chromatography (GC) is probably the most widely used technique nowadays [9–13], although additional steps are required to obtain the volatile derivatives necessary. Furthermore, techniques like high-pressure liquid chromatography (HPLC) [14] and mass spectrometry (MS), as well as combinations of the methods mentioned earlier like GC plus IR spectrometry [12] or GC–MS [9,10] have been utilized recently in this field. We have applied GC–MS for the identification of plant gums and animal glues following the hydrolysis and group-specific separation of amino acids and sugars [9].

Sugar monomers have already been determined by capillary electrophoresis (CE) [15–28] but mainly for food analyses, e.g. for the determination of carbohydrates in food samples like fruit juices, yoghurt, rice flour and sake. For this goal, different separation systems have been developed. They utilise either direct detection after derivatisation to obtain UV-absorbing species [29,30], or determine the underivatised sugars through complexation using direct UV detection [15,25–28]. By complexation of the saccharides with borate-based electrolytes a (negative) charge necessary for electrophoresis is imparted. The separation and detection based on chelation with Cu(II) was described also [31].

As sugars are very weak acids they ionise only at strongly alkaline pH (pH > 12). At this pH, the carbohydrates are present as negatively charged alcoholates. This property was exploited by several research groups for separation, followed by the subsequent indirect UV, conductivity [18], or electrochemical detection [32]. CE with indirect UV detection is still an area of development to overcome the difficulty encountered with the analysis of carbohydrates that lack detection centres in their structures. A CE method using 2,6-pyridinedicarboxylic acid (PDC) as the chromophoric background electrolyte (BGE) was described by Soga and Imaizumi [33] at a high pH. By adding cetyltrimethylammonium bromide (CTAB) to the running electrolyte, the EOF was reversed from cathodic to anodic and co-electroosmotic separation conditions were achieved. It is the goal of the present work to adapt this system for the determination and characterisation of sugar monomers and uronic acids contained in different plant gums, matrices that were not investigated by CE so far. The paper deals with those gums that are relevant for museum objects (artistic and historic works), namely with gum arabic (gum acacia) and gum tragacanth, further with cherry and locust gum. With the present methodology, the plant gums are simply hydrolysed and analysed by CE, and due to the use of indirect UV detection no derivatisation of the sugars has to be performed. We do not consider gums of minor importance in this field like karaya gum, ghatti gum, guar gum, etc. [2]. It is also not our intention to distinguish the different species, e.g. the large number of acacia gums which are commercially available from different provenance. We concentrate primarily on the identification of the gum family which is of primary interest for the restorer and conservator, and do not consider aspects of chemical taxonomy.

2. Experimental

2.1. Materials

All materials and reagents used were of analytical reagent grade. L-Arabinose, D-galactose, D-glucose, L-rhamnose, D-glucuronic acid, as well as 2,6-pyridinedicarboxylic acid and cetyltrimethylammonium bromide, both used for the BGE, were obtained from Sigma–Aldrich Chemie (Steinheim, Germany). D-Galacturonic acid, D-mannose, L-fucose, D-xylose and trifluoroacetic acid (used as hydrolysis agent) were purchased from Fluka Chemie (Buchs, Switzerland). In all cases, double-distilled water was used for the preparation of solutions. The gum samples were either from the collection of binding media at the Department of Conservation and Restoration, Academy of Fine Arts, Vienna, Austria, or they were purchased from specialised traders. For each gum species, at least three samples from different sources were collected and analysed.

2.2. Instrumentation

Most measurements with capillary electrophoresis were carried out with a Capel 105 instrument (Lumex Ltd., St. Petersburg, Russia) equipped with a UV–vis detector and a liquid cooling system. The results from the ageing experiments were obtained by a ^{3D}CE apparatus (Agilent Technologies, Palo Alto, CA, USA). With both instruments uncoated fused-silica capillaries (Supelco, Bellefonte, PA, USA) of 36.0 cm total length (effective length 28.0 cm; 50 μm I.D.) were used. The capillary was thermostated to 20 °C. The electrolyte solution consisted of 20 mM PDC as chromophor and 0.5 mM CTAB to reverse the direction of the EOF. The pH of the BGE was adjusted to 12.1 by titration with 3 M NaOH.

The voltage was set to −20 kV, the resulting current was about 80 μA. Detection was carried out monitoring indirect UV absorbance, the signal wavelength was set to 254 nm. In this way, the carbohydrate zones were recorded as negative peaks.

Prior to first use, the capillary was flushed with 0.1 M HCl, bidistilled water, 1 M NaOH, and again with bidistilled water for five minutes each. After this procedure, the capillary was flushed with the separation electrolyte for 10 min. Before each injection, the capillary was conditioned with the separation electrolyte for 4 min. Sample was injected with 30 mbar for 5 s.

Artificial aging was carried out for thin layers of gum arabic and linseed oil placed on glass and paper (gum) or glass (oil). The layers were irradiated by a UV lamp (model Fluotest Forte, Atlas, Linsengericht, Germany) with 180 W at 366 nm (UV-A), which was placed at ambient conditions (27 °C temperature measured at the vicinity of the layer, average humidity 20%) at a distance of 10 cm in parallel to the layers. Irradiation was carried out for 142 h, and sample aliquots were taken after different times and analysed by CE.

2.3. Sample preparation

2.3.1. Hydrolysis procedure

Plant gum or starch samples (amounts between 0.2 and 4 mg) were taken up in 2 M trifluoroacetic acid (TFA) (100 μl TFA/0.2 mg sample) and hydrolysed in capped glass vials for 2 h at 110 °C [8]. Optimum hydrolysis time is dependent on a balance between the rate of release of hydrolysable polysaccharides and the degradation of monosaccharides that occurs during prolonged treatment under the experimental conditions [34].

After hydrolysis, the samples were frozen and then lyophilised over night at 200 mT and −56 °C. Then, 0.3 ml of the separation electrolyte was added to the dried samples, ultrasonicated for 1 min, the solution centrifuged for 2 min at 13 000 × *g* in a tabletop centrifuge (Eppendorf, model 5415D, Hamburg, Germany), and capillary electrophoresis analysis was carried out with the supernatant.

The oils samples were saponified prior to CE as described [40].

3. Results and discussion

3.1. Separation of monosaccharides

As pointed out in the introduction, CE has been applied for the separation of the alcoholates at high pH without complexing agent in order to analyse the sugars of interest mainly in food industry. In the present work, we used a similar approach for the separation of the monosaccharides obtained after the hydrolysis of the plant gums. According to the literature [1], these monosaccharides are L-arabinose, D-galactose, D-mannose, L-rhamnose, D-xylose, L-fucose, and D-glucose. Additionally, there are two sugar acids, D-galacturonic and D-glucuronic acid. It is obvious that we are not interested in the separation of the enantiomers of the individual monosaccharides, and therefore we did not select chiral BGEs.

In a first step, the separability of the pure monosaccharides was investigated by varying the pH of the BGE between 11.7 and 12.7 in steps of 0.2 units. It turned out that the most favourable conditions for the analysis of the present analytes are the same as those described by Soga and Heiger [35] for partly other monosaccharides, namely a BGE at pH 12.1. The resulting electropherogram (recorded with indirect UV detection, with 2,6-pyridinedicarboxylic acid as chromophore) obtained with reversed EOF upon addition of CTAB is shown in Fig. 1. It can be seen that the nine analytes migrate in eight peaks. The two uronic acids GluAc and GalAc have the highest total mobility, followed by Man and Xyl, which are separated only partially, and by Rham. Ara co-migrates with Glu, and Gal and Fuc elute then. It will be shown below that the resulting electrophoretic peak pattern allows for identification of the plant gums, although the pair Man/Xyl is

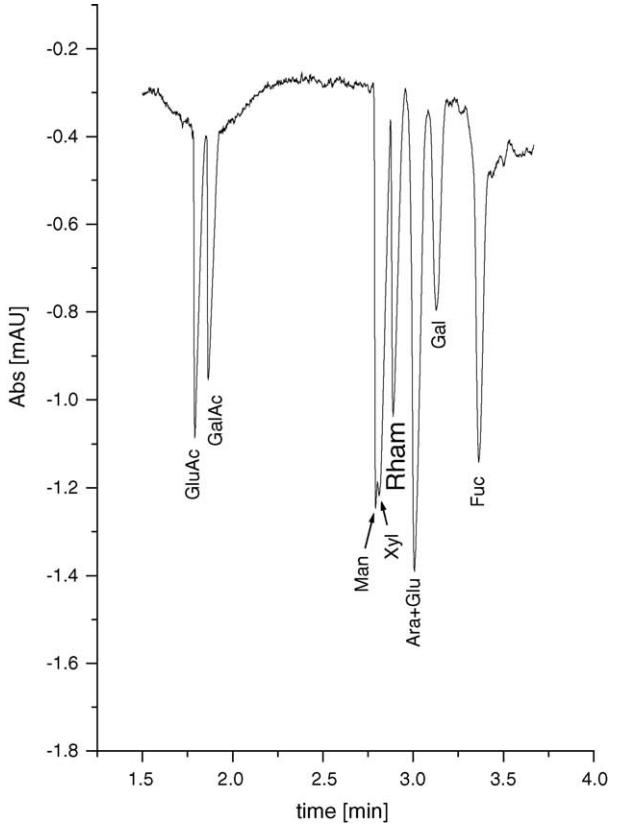

Fig. 1. Electropherogram of nine monosaccharide standards at pH 12.1 with indirect UV detection and reversed electroosmotic flow: D-galacturonic acid (GalAc), D-glucuronic acid (GluAc), L-arabinose (Ara), D-galactose (Gal), D-mannose (Man), L-rhamnose (Rham), D-xylose (Xyl), L-fucose (Fuc), D-glucose (Glu). Concentrations of analyte standards 3 mM each. Conditions: fused-silica capillaries of 36.0 cm total length (effective length 28.0 cm; 50 μm I.D.). BGE: 20 mM PDC, pH 12.1 (adjusted with NaOH), 0.5 mM CTAB. Voltage −20 kV, thermostating temperature 20.0 °C. Injection 150 mbar s.

only incompletely resolved, and Ara and Glu are unresolved at all.

3.2. Gum hydrolysates

Hydrolysis is the elementary part in the preparation of the polymeric samples containing neutral sugars and uronic acids. The cleavage procedure was carried out according to ref. [8]. This simple and rapid hydrolysis method for polysaccharides uses 2 M TFA and was applied to all samples. The use of TFA has the considerable advantage over some mineral acids of being sufficiently volatile to permit its removal simply by freeze drying (no neutralization is needed after hydrolysis and prior to CE in this way). In addition, relatively mild action is applied to sugars and hydrolysis residue, and a high yield is obtained although the procedure is rather short. The major products of TFA hydrolysis of gum polysaccharides are free monomers; however, a loss of monosaccharide may occur due to esterification during glycosidic bond breaking. Furthermore, in the case of GluAc, possible lactone-formation during hydrolysis can cause a problem. Formation of glucofurano-6,3-lactone was clearly indicated especially in the analysis of cherry gum samples. In this case the peak of GluAc decreased with increasing reaction time, and concomitantly an additional peak appeared in the electropherogram (not shown). For GalAc, on the other hand, lactone-formation is precluded due to structural features.

In Fig. 2 the electropherograms obtained after hydrolysis of the different plant gums are shown. Although the excess of TFA is removed by lyophilisation, a (negative) system peak at about 1.5 min (and sometimes a smaller, positive system peak at about 2.2 min) is observed in the hydrolysed samples.

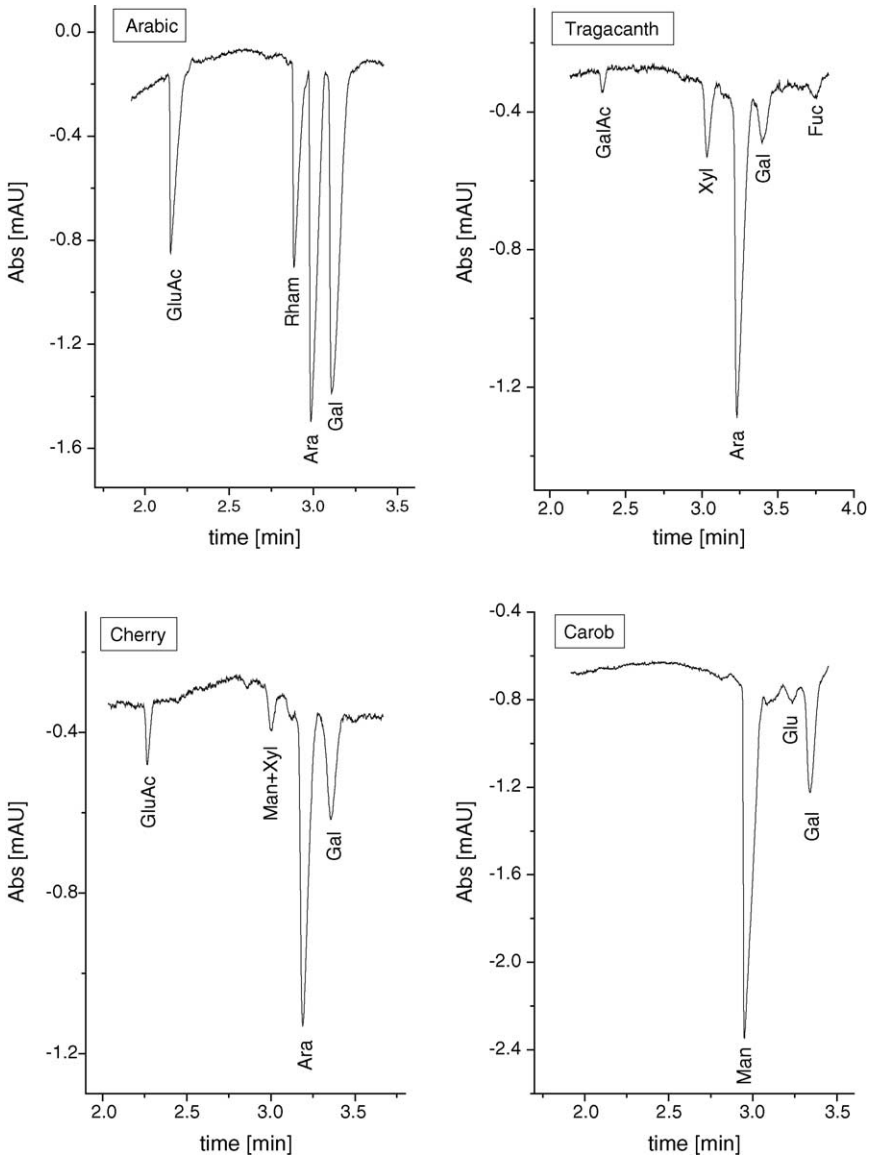

Fig. 2. Electropherograms of gum arabic, gum tragacanth, cherry gum and carob bean gum, respectively, after hydrolysis. Conditions and abbreviations as in Fig. 1. Sample treatment see text.

This is not unusual in indirect UV or conductivity detection. However, the pattern of the monosaccharides can clearly be recorded.

Gum arabic, which is also known as gum acacia, or as Turkey, Indian, Senegal or Sudan gum, depending on its origin, is probably the most common gum found in the area of museum objects. It is produced from a large number of trees belonging to the *Acacia* species. It has been applied since antiquity, e.g. in ancient Egypt, as adhesive. Beside other monosaccharides gum arabic contains sugar acids. In the electropherogram of the gum arabic sample (Fig. 2) we can distinguish the peaks of GluAc, Rham, Ara and Gal, which is in agreement with the description of the plant composition in the literature [1]. The same pattern is found also in the electropherogram from gum acacia (not shown), which is clear due to the similar origin of this gum from *Acacia* species. It was, by the way, demonstrated that there are no obvious trends in the chemical composition with age or source of Acacia senegal [36]. This finding should, on the other hand, not be generalised as, e.g. the composition of the gum exuded by Acacia karroo depends on its African locations [37].

Gum tragacanth is also used since ancient times; it was already described by Theophrastus in the third century B.C. [38,39]. It originates from *Astragalus* species growing in near East, especially from Astralagus gummifer Labillardiere, a tree from the Leguminosae family. As its harvest is more cumbersome compared to gum arabic it was not used so widely. Its electrophoretic pattern (Fig. 2) differs from that of gum arabic. The peak of GalAc – present only in tragacanth – together with those of Xyl, Ara/Glu, Gal and Fuc are seen, also in accordance with the composition described in the literature [1].

Cherry gum is produced from *Prunus cerasus*. In this material GluAc is found in the electropherogram (Fig. 2), together with Man/Xyl, Ara and Gal. An additional peak between Man/Xyl and Ara seemingly does not stem from a monosaccharide.

Carob or locust bean gum is a product from the kernels of the beans of the carob tree (*Ceratonia siliqua* L.), which grows in the Mediterranean area. Like guar and tara gum it belongs to galactomannans, carbohydrates that are produced by the many Leguminosea in the endosperms of the seeds as a reserve. Even if not commonly used (e.g. as an adhesive for pigments) it has some relevance for the present topic, because it is an additive in the manufacture of paper [1] and might thus be found when analysing artwork on paper. Carob gum mainly consists of Man and Gal, which is in agreement with the electropherogram of its hydrolysate, see Fig. 2.

Identification of the plant gums based on these electrophoretic patterns needs some comments. Although the peaks of Ara and Glu are not resolved, Glu is normally not expected to be present in gum samples of high quality—traces of starch and cellulose are only present in low quality gums, and therefore Glu peaks on the trace level are present only in such samples. However, Glu could originate from paint grounds. In the case of locust bean gum, it can be assumed from the molecular composition that only mannose and galactose are present (in proportion of 4:1), and the peak in the corresponding electropherogram stems rather from Man, not from Xyl. On the contrary, according to the literature, in gum tragacanth only Xyl should be contained, but no Man. The peak can be related thus to the former sugar. In the case of cherry gum, both, Man and Xyl are present in the heteropolysaccharide, but no separation of the two components can be achieved and therefore their proportion is not determined.

The histogram depicting the relative sugar composition of the gums is given in Fig. 3. In the present work, this composition is simply related to the areas of the sugar peaks in the electropherogram without further calibration with individual response factors. The compositions are given as the result of measurements of three different and independently collected

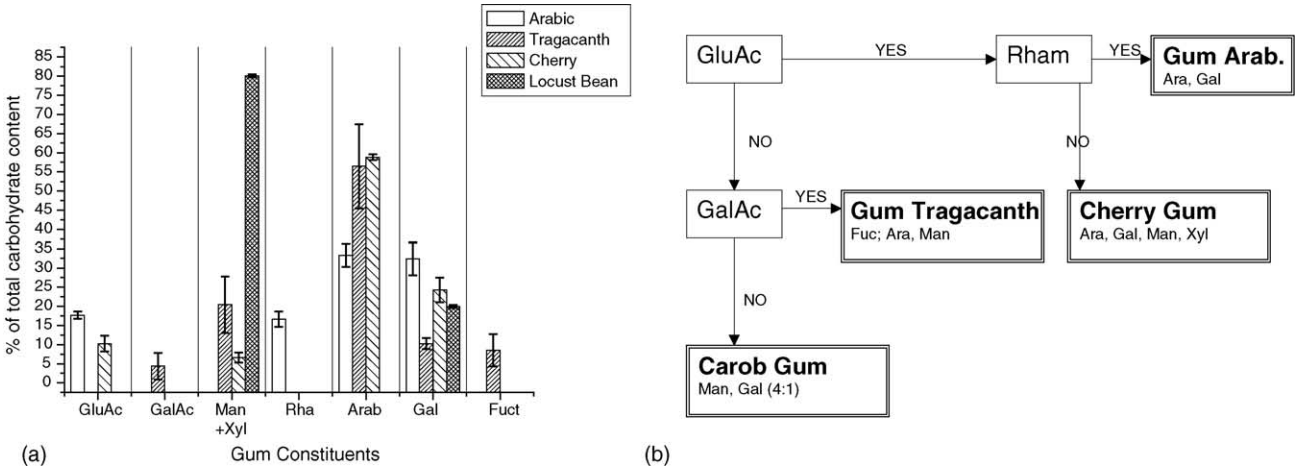

Fig. 3. (a) Histogram depicting the monosaccharide composition of the plant gums. Percentage is derived from the electrophoretic peak area of the individual sugars related to the sum of the peak areas. Bars indicate the lowest and the highest content of the sugar in the samples. Results from three individual gum samples of different origin. (b) Decision scheme for the identification of the plant gums according to the monosaccharide content.

products for each gum in order to take also the variability of the samples into account. The bars in the histograms indicate the lowest and the highest concentrations, respectively, measured in the three samples of each monosaccharide species. By the aid of this histogram a decision scheme can be constructed, which can serve for the identification of the gums according to their monomer sugar constituents, similarly to the above given discussion: identification of gum arabic is based on GluAc and on Rham, as only this gum contains these monosaccharides. Gum tragacanth is the only gum containing both GalAc and Fuc. GluAc and Ara, the latter being the main constituent, are indicative for cherry gum (Gal is present as well). Locust bean gum has a large concentration of Man/Xyl and Gal, the concentration of the latter being around one-fourth of the former; no uronic acids are present in this gum.

3.3. Identification of plant gums in mixtures with other binders

In many media applied in artefacts plant gums are mixed with other binders like animal glues or drying oils. It is therefore a relevant problem whether the present method is suited for the identification of the monosaccharides in the hydrolysates of such mixtures. We have prepared a number of samples with these binders admixed in different proportions, either with one, or with both vehicles. The mixtures were then subjected to hydrolysis and preparation as described in Section 2. Electropherograms from the hydrolysates of the ternary mixtures of gum arabic with a drying oil and casein as animal glue are shown in Fig. 4. They are obtained from samples composed from the same proportion of gum and either linseed oil or casein, and the 10-fold excess of the third binder. It can be seen that in none of the samples interferences caused by the other binders are observed; in all cases the identification of the gum based on the monosaccharide pattern is clearly possible. The same results were obtained from the binary mixtures (not shown). It should be mentioned that for mixtures of different gums the records of the monosaccharides are the result of the proportional superposition of the individual gum constituents. However, this is not a specific

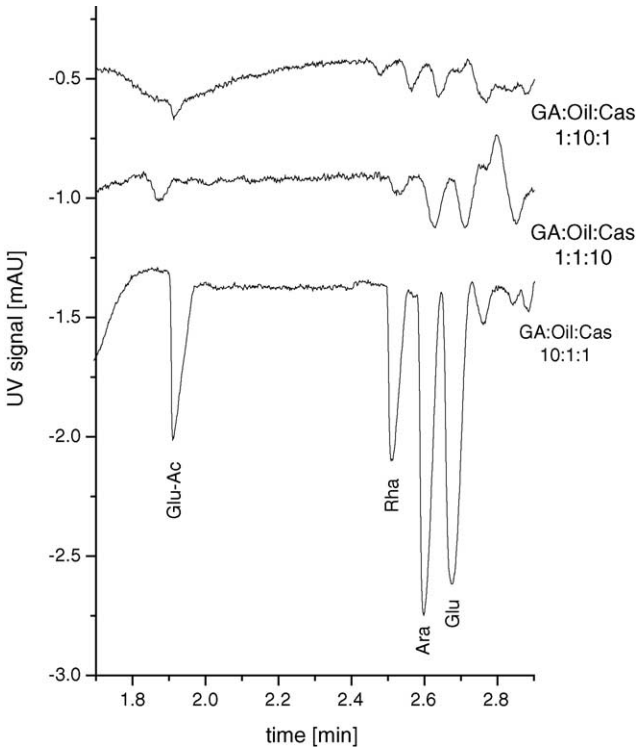

Fig. 4. Electropherograms of mixtures of gum arabic (GA) with casein (Cas) and linseed oil (Oil) in different proportions (all in w:w:w). The mixtures were hydrolysed and prepared as described in Section 2. Conditions and abbreviations as in Fig. 1.

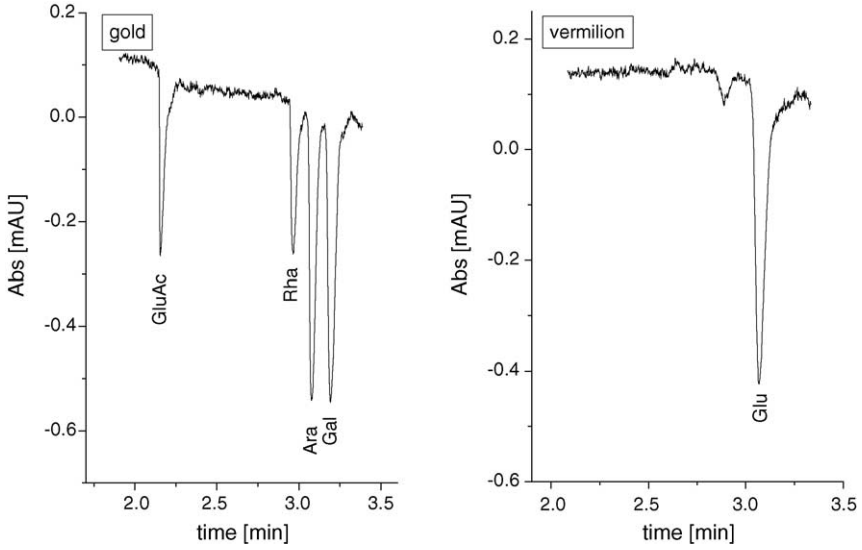

Fig. 5. Electropherograms of a watercolour containing gold powder (left) or vermilion (right), respectively, as pigment. Conditions and abbreviations as in Fig. 1. Sample treatment see text.

restriction to the present method, it is similar to other techniques like the chromatographic ones.

3.4. Identification of plant gums in paint material

We have tried to identify the plant gums in different objects: in a number of commercially available recent watercolours, and in several paint layers from the collection of the Department of Conservation and Restoration of the Academy of Fine Arts, Vienna, with defined history and composition. In Fig. 5 two electropherograms are given from two different watercolours. The left electropherogram is from a watercolour containing gold as pigment. This pigment was selected because of its importance in medieval illumination. The electropherogram clearly allows the identification of gum arabic as binding medium, with GluAc, Rha, Ara and Gal as constituents (compare with Fig. 2, and the histogram in Fig. 3). The right electropherogram in Fig. 5 is from a watercolour with vermilion (mercury (II) sulfide) as pigment. Although being unreactive with other pigments, due to its sulphide group it might not be chemically so inert like, e.g. oxidic pigments. Interestingly all peaks except that from Glu are missing here, meaning that none of the plant gums has been applied as binder. The large peak of Glu gives an indication that starch (or modified starch like dextranes) was used. The information obtained from the manufacturer of this watercolour after our analysis indeed confirmed that modified starch together with a synthetic binder, polyvinylalcohol, instead of natural plant gum was used here.

In Fig. 6 an example is shown from a paint layer of Prussian Blue – iron (III) hexacyanoferrat (II) – as pigment on paper. This paint layer is about 1 year old; it was prepared at the Academy of Fine Arts. The binder was a mixture of 1 part gum Arabic, 0.5 part sugar, 10 drops of bile, and 1 part pigment. For analysis, the sample (paper of about 1 cm^2 in area coated with the typical thin film of paint layer) was treated with boiling water for 10 min, the solid matrix removed by centrifugation, the supernatant hydrolysed, lyophilised, redissolved and injected. In the resulting electropherogram the peaks from GluAc, Rham, Ara and Gal can be distinguished, pointing to the presence of gum arabic, in accordance with the description of the object. We can conclude from this result that neither the present pigment obstructs the identification of the binding medium, nor is the presence of the other additives a hindrance for the proper identification of the gum. The absence of Glu, originating from the sugar added to the paint layer according to the recipe is not fully clear for us. For getting an impression about the amount of sample needed for identification an electropherogram obtained from a 0.2 mg sample of gum arabic is given on the right panel of Fig. 6. From this electropherogram it can be concluded that the method seems sensitive enough to fulfil the demands on sample consumption and size typical for the present topic.

A more complex example is a 3 years old gum tempera layer, applied on paper, consisting of gum arabic and linseed oil (in about equal proportions). From the paper the layer material (about 10 mg in total) was scratched off and this sample was extracted with boiling water for 10 min. The aqueous extract was processed after removal of the residual paper matrix by centrifugation, and hydrolysed as described. The resulting electropherogram is shown in Fig. 7(left). The presence of GluAc, Rham, Ara and Gal can clearly be seen, indicating gum arabic as sugar constituent of the paint layer (from the composition, it can not be differentiated from acacia gum). It can be followed that the oil constituents do not impede here the identification of the gum, as no additional peaks occur in the relevant migration time range. It should be mentioned

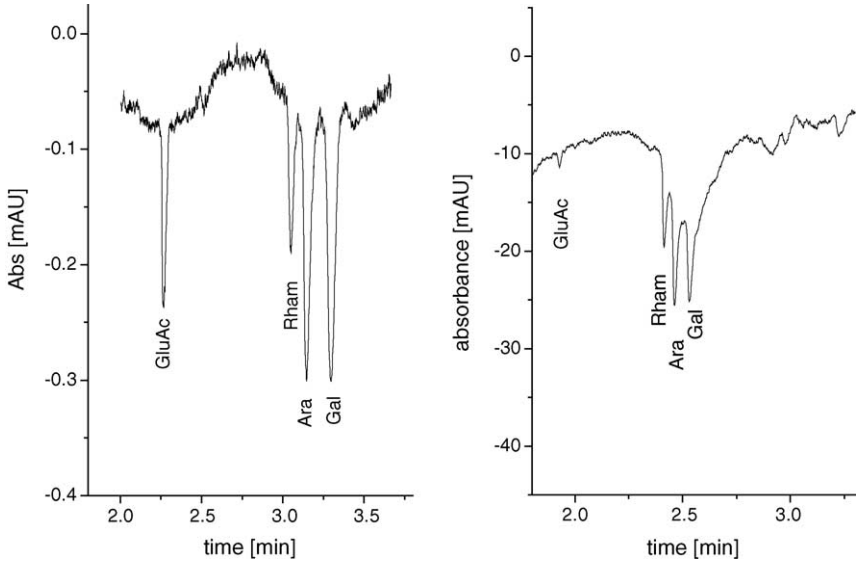

Fig. 6. Left panel: Electropherogram of a paint layer of Prussian Blue as pigment on paper with gum arabic as binding media. Right panel: For comparison the electropherogram of a 0.2 mg sample of gum arabic is shown. Conditions and abbreviations as in Fig. 1. Sample treatment see text.

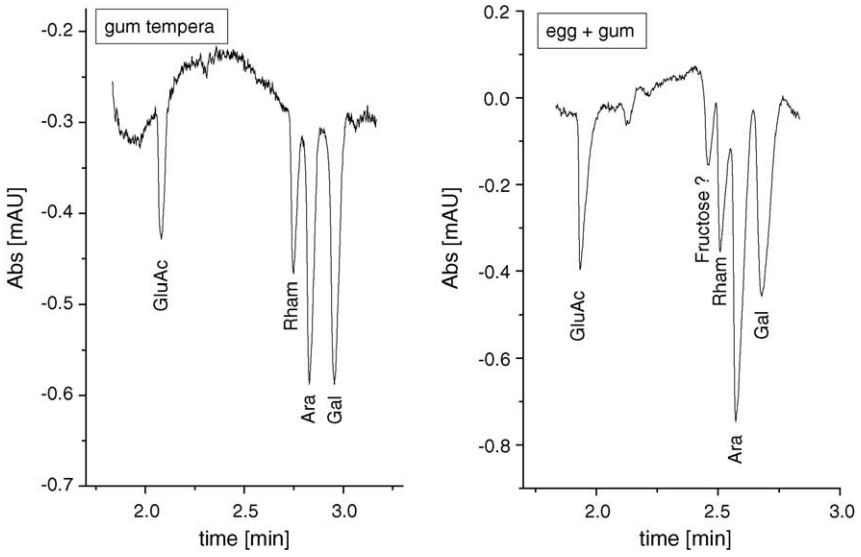

Fig. 7. Electropherograms of paint layers of gum tempera containing both gum arabic and linseed oil (left) and egg-white and gum arabic (right) as binding media. Conditions and abbreviations as in Fig. 1. Sample treatment see text.

that the same sample processed without boiling water gives the sugar peaks only at a very low intensity (not shown). This boiling step to extract the sugars from the paper matrix can thus be considered as essential.

When trying to analyse a paint layer consisting of egg white together with a gum, one encounters the problem that, according to literature, a considerable amount of sugars, namely glucose, fructose and galactose are also constituents of egg white. They are present at about 15 wt.% relative to the proteins [2,5]. Therefore the monomer sugar units of egg white should superimpose those from the gum. We have analysed such a paint layer, with the resulting electropherogram given in Fig. 7, right panel. Most likely the peak emerging before Rham is from fructose stemming from egg white; it has the same migration time and appears at this position in the electropherogram, which was confirmed by addition of a standard. However, it can be concluded that the identification of gum arabic in the sample is still possible, especially based on the presence of GluAc, which is not constituent of egg white.

3.5. Artificial aging by UV irradiation

As we have characterised the monosaccharide composition of samples not older than 3 years, we investigated the possible change in composition over time by artificial ageing under drastic conditions. For this purpose we exposed layers of gum arabic to UV-A light using a UV lamp of 180 W power. The layers were coated on paper and on a glass plate, respectively, and placed at a distance of 10 cm from the lamp. Simultaneously an according layer of linseed oil (a drying oil) was exposed to the same conditions. After certain time intervals, sample aliquots were taken from the layer and analysed. The result for the gum on paper can be seen in Fig. 8 for a continuous irradiation period of 6 days. Actually no change in the monosaccharide pattern is observed for all 21 sample aliquots when compared to the initial pattern ($t = 0$). This result indicates that there is no variation of the chemical composition of the gum even under these crude conditions.

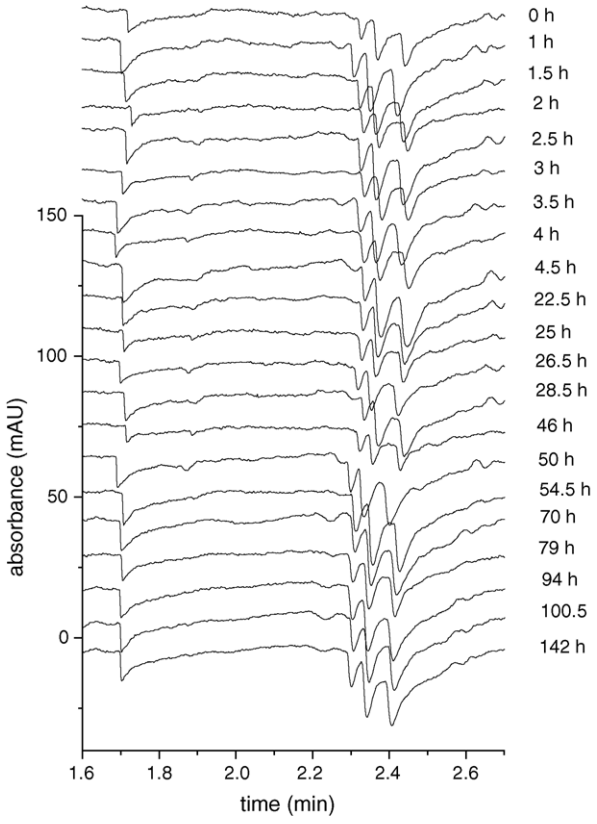

Fig. 8. Ageing of gum arabic: electropherograms of 21 aliquots of a gum layer coated on paper and exposed to UV-A radiation continuously for 6 days. After the time intervals indicated at the different traces sample was taken and analysed (conditions as in Fig. 1).

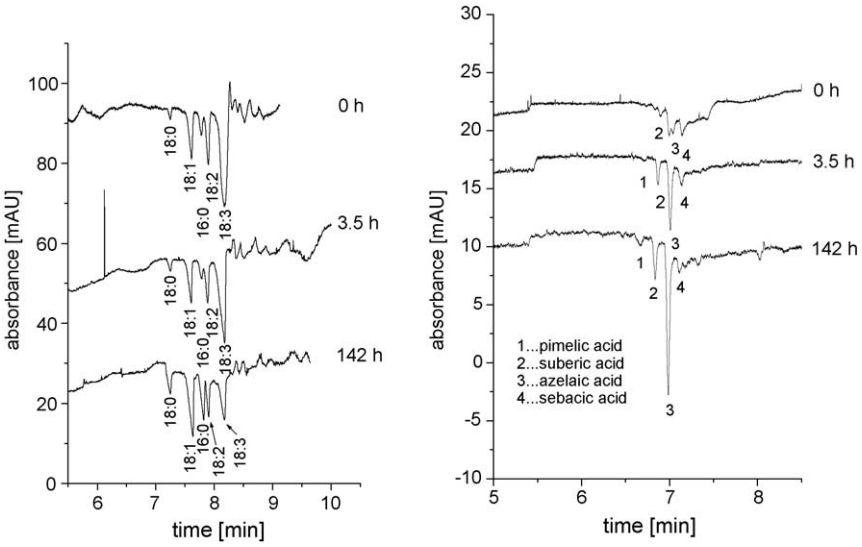

Fig. 9. Ageing of drying oil: electropherograms from linseed before and after irradiation with UV-A under the same conditions as the gum arabic (Fig. 8). Left panel: Pattern of the fatty acids stearic acid (18:0), oleic acid (18:1), palmitic acid (16:0), linoleic acid (18:2), linolenic acid (18:3) at the begin (0 h), after 3.5 and 142 h of exposure to UV-A. BGE: phosphate buffer, pH 6.86, 15 mM, 4 mM sodium dodecylbenzensulfonate, 10 mM Brij 35, 2% (v/v) 1-octanol and 45% (v/v) acetonitrile. Right panel: Pattern of the dicarboxylic acids formed upon oxidative degradation of unsaturated the fatty acids. The oils samples were saponified prior to CE as described [40]. BGE: 10 mM salicylic acid, 20 mM L-histidine, pH 5.85; 0.2 mM cetyltrimethylammonium bromide added to reverse the electroosmotic flow. Indirect detection at 224 nm wavelength. Other conditions as in Fig. 1. BGEs according to ref. [40].

The same result was found for the gum layer on glass (not shown).

For comparison the effect of the irradiation is shown for a layer of drying oil (on a glass plate) exposed to the same condition. These oil samples were saponified and treated as described in a previous paper [40]. The initial composition of the linseed oil (Fig. 9, left panel, 0 h) changes insofar, as the large peak of the highly unsaturated linolenic acid (18:3) relative to those of the saturated palmitic (16:0) and stearic acid (18:0) decreases with time of exposure. Such a (relative) decrease is also observed for the other unsaturated acids (18:2 and 18:1, whereas in lesser extent. This is caused by the degradation of these acids, which leads to oxidative polymerisation ("drying") on the one hand, and to the cleavage of the acid under formation of shorter chain dicarboxylic acids, on the other hand [2]. Indeed the increase in the content on pimelic, suberic, azelaic and sebacic acid (the dicarboxylic acids with 7–10 C-atoms) with the concomitantly decreasing peaks of 18:3 can be followed from the electropherograms shown in Fig. 9, right panel.

4. Conclusions

CE is found being a suitable method to characterise and identify plant gums used in artistic and historic works. The present paper is part of our investigations to use CE as alternative to chromatographic methods, mainly to GC and GC–MS, which we apply in our lab since about 15 years for the identification of binders [9,41–43]. We have adapted or developed CE methods now for the analysis of drying oils [40], proteinaceous binders like animal glues [44], plant gums. Work was mainly directed so far to a single class of binder, although samples consisting of their mixtures were characterised as well. However, combination of different media in the same object certainly needs more investigation about mutual interferences during analysis including sample pre-treatment and preparation. In this work we have found that no such interferences for plant gums result from the presence of drying oils and animal glues, but it gives a specific rather than a general answer to this important topic [45]. Also of high relevance is the question which of the many different pigments and dyes interfere the identification of the binding media. More detailed work is currently carried out in our lab, which is directed in the moment to ethnological objects; especially these objects often contain undefined mixtures of binders from many different sources, which makes the problem of interferences decisive in practice.

Acknowledgement

The authors thank Lumex Ltd., St. Petersburg, Russia, for instrumental support.

References

[1] J.R. Daniel, R.L. Whistler, A.C.J. Voragen, W. Pilnik, in: B. Elvers, S. Hawkins, W. Russey (Eds.), Ullmann's Encyclopedia of Industrial Chemistry, VCH, Weinheim, 1994, p. 1.
[2] J.S. Mills, R. White, The Organic Chemistry of Museum Objects, Butterworth Heinemann, London, 1994.
[3] F. Berger, Handbuch der Drogenkunde, Wilhelm Maudrich Verlag, Wien, 1964.

[4] H.-P. Schramm, H.B. Historische, Malmaterialien und ihre Identifikation, Akademische Druck- und Verlagsanstalt, Graz, 1988.
[5] M. Doerner, Malmaterial und seine Verwendung im Bilde, Enke, Stuttgart, 1989.
[6] I. Zadrozna, K. Polec-Pawlak, I. Gluch, M.A. Ackacha, M. Mojski, J. Wirowska-Jarosz, M. Jarosz, J. Sep. Sci. 26 (2003) 996.
[7] W.W. Morris, W. Salmon, Anal. Chem. 35 (1963) 600.
[8] B.V. Kharbade, G.P. Joshi, Stud. Conserv. 40 (1995) 93.
[9] U. Schneider, E. Kenndler, Fresenius J. Anal. Chem. 371 (2001) 81.
[10] N.K.M. Sarmini, Diploma thesis, University of Vienna, Vienna, 1996.
[11] P. Mejanelle, J. Bleton, A. Tchapla, S. Goursaud, J. Chromatogr. Libr. 66 (2002) 845.
[12] S.L. Vallance, Analyst 122 (1997) 75R.
[13] V. Pitthard, P. Finch, Chromatographia 53 (2001) S317.
[14] M.P. Colombini, A. Ceccarini, A. Carmignani, J. Chromatogr. A 968 (2002) 79.
[15] M. Ristolainen, J. Chromatogr. A 832 (1999) 203.
[16] Y. Cao, Y. Wang, X. Chen, J. Ye, Food Chem. 86 (2004) 131.
[17] S. Honda, Methods in Molecular Biology, vol. 213, Humana Press, Totowa, NJ, United States, 2003, p. 81.
[18] A.Z. Carvalho, J.A.F. da Silva, C.L. do Lago, Electrophoresis 24 (2003) 2138.
[19] T. Soga, M. Serwe, Food Chem. 69 (2000) 339.
[20] T. Soga, G.A. Ross, J. Chromatogr. A 837 (1999) 231.
[21] Z. El Rassi, Carbohydrate Analysis by Modern Chromatography and Electrophoresis, Elsevier, Amsterdam, 2002.
[22] X. Xu, W.T. Kok, H. Poppe, J. Chromatogr. A 716 (1995) 231.
[23] P.J. Oefner, A.E. Vorndran, E. Grill, C. Huber, G.K. Bonn, Chromatographia 34 (1992) 308.
[24] Y.H. Lee, T.I. Lin, J. Chromatogr. B 681 (1996) 87.
[25] S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann, H.M. Widmer, Anal. Chem. 63 (1991) 1541.
[26] Y. Liu, C. Shu, J.D. Lamb, J. Capillary Electrophor. 4 (1997) 97.
[27] C.R. Noe, B. Lachmann, S. Mollenbeck, P. Richter, F. Zeitschrift, Lebensmittel-Unters. Forsch. A: Food Res. Technol. 208 (1999) 148.
[28] Ph. Schmitt-Kopplin, K. Fischer, D. Fritag, A. Kettrup, J. Chromatogr. A 807 (1998) 89.
[29] D.T. Nguyen, H. Lerch, A. Zemann, G. Bonn, Chromatographia 46 (1997) 113.
[30] K.E. Andersen, C. Bjergegaard, H. Sorensen, J. Agric. Food Chem. 51 (2003) 7234.
[31] A. Bazzanella, K. Bachmann, J. Chromatogr. A 799 (1998) 283.
[32] R.P. Baldwin, J. Chromatogr. Libr. 66 (2002) 947.
[33] T. Soga, M. Imaizumi, Electrophoresis 22 (2001) 3418.
[34] J.F. Kennedy, S.M. Robertson, M. Stacey, Carbohydr. Res. 49 (1976) 243.
[35] T. Soga, D.N. Heiger, Anal. Chem. 72 (2000) 1236.
[36] O.H.M. Idris, P.A. Williams, G.O. Phillips, Food Hydrocolloids 12 (1998) 379.
[37] D.M.W. Anderson, G. Pinto, Bot. J. Linnean Soc. 80 (1980) 85.
[38] D. Verbeken, S. Dierckx, K. Dewettinck, Appl. Microbiol. Biotechnol. 63 (2003) 10.
[39] R.L. Whistler, in: J.N. Bemiller (Ed.), Industrial Gums, Academic Press, San Diego, 1993, p. 318.
[40] I. Surowiec, I. Kaml, E. Kenndler, J. Chromatogr. A 1024 (2004) 245.
[41] E. Kenndler, F. Mairinger, W.P. Bauer, F. Rainer, K. Schmidt-Beiwl, Stud. Conserv. 33 (1988) 115.
[42] E. Kenndler, F. Mairinger, Fresenius J. Anal. Chem. 338 (1990) 635.
[43] E. Kenndler, K. Schmidt-Beiwl, F. Mairinger, M. Pöhm, Fresenius J. Anal. Chem. 342 (1992) 135.
[44] I. Kaml, K. Vcelakova, E. Kenndler, J. Sep. Sci. 27 (2004) 161.
[45] S. Harrison, I. Kaml, F. Rainer, E. Kenndler, J. Sep. Sci., in press.