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Ion chromatography characterization of polysaccharides in ancient wall paintings

Maria Perla Colombini[a,*], Alessio Ceccarini[b], Alessia Carmignani[b]

[a]*Dipartimento di Scienze dell'Ambiente e del Territorio, University of Milano-Bicocca, P.za Scienza 1, Milan 20126, Italy*
[b]*Dipartimento di Chimica e Chimica Industriale, University of Pisa, Via Risorgimento 35, Pisa, Italy*

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Abstract

An analytical procedure for the characterisation of polysaccharides and the identification of plant gums in old polychrome samples is described. The procedure is based on hydrolysis with 2 *M* trifluoroacetic acid assisted by microwaves (20 min, 120 °C, 500 W), clean-up of the hydrolysate by an ion-exchange resin, and analysis by high-performance anion-exchange chromatography with pulsed amperometric detection. Using this method the hydrolysis time was reduced to 20 min and the chromatographic separation of seven monosaccharides (fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose) and two uronic acids (galacturonic and glucuronic) was achieved in 40 min. The whole analytical procedure allows sugar determination in plant gums at picomole levels, with an average recovery of 72% with an RSD of 8% as tested on arabic gum. The analytical procedure was tested with several raw gums, watercolour samples and reference painting specimens prepared according to old recipes at the Opificio delle Pietre Dure of Florence (Italian Ministry of Cultural Heritage, Italy). All the data collected expressed in relative sugar percentage contents were submitted to principal components analysis for gum identification: five groups were spatially separated and this enabled the identification of arabic, tragacanth, karaya, cherry+ghatty, and guar+locust bean gum. Wall painting samples from Macedonian tombs (Greece) of the 4th–3rd Centuries B.C., processed by the suggested method, showed the presence of a complex paint media mainly consisting of tragacanth and fruit tree gums. Moreover, starch had probably been added to plaster as highlighted by the presence of a huge amount of glucose.

Keywords: Art analysis; Paints; Polysaccharides

1. Introduction

Since ancient times, organic media of mainly vegetable origin were commonly used both as glues or as binders for pigments in wall paintings, easel paintings and in illuminated manuscripts [1]. Important examples are honey, starch and plant gums which are exudates of several species of plants. Tragacanth, arabic and fruit tree gum (gum mainly from cherry, apricot and plum trees) were used the most as a medium in ancient Greek paintings, in watercolour paintings and often in mixtures with proteinaceous matter or oil [2] to obtain particular colours.

*Corresponding author. Tel.: +39-2-6448-2817; fax: +39-2-6448-2890.
E-mail address: mariaperla.colombini@unimib.it (M.P. Colombini).

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Chemically, plant gums are polysaccharides consisting of pentose, methylpentose, hexose and uronic acids joined together. Each natural gum presents a variable distribution in mean molecular mass depending on the species of the plant, while the composition of the constituent sugar percentages remains reasonably constant. Their chemical characterisation is a subject of sustained interest to experts in chemistry who are asked to give reliable results for the diagnosis of a work of art and information useful for its restoration. Indeed, not only is it difficult to identify plant gums and other polysaccharide-based matter in heterogeneous painting microsamples (generally containing less than 100 μg of organic material), but also the presence of degradation products from the original material and the presence of proteinaceous binders may affect the determination of sugars. Thus, due to their chemical complexity, the polysaccharide identification in paintings requires the application of very sensitive analytical procedures. Though FT-IR [3] and Raman microscopy [2,4] offer the possibility of analysing the object under study without sampling, as in the case of illuminated manuscript analysis, or with a sample of just a few microns, thus reducing the damage on the artefact, they have a limited use in distinguishing the various polysaccharide materials. This is due to the strict similarities between spectra of different gums. Gas-chromatographic techniques coupled with mass spectrometry [5–7] or other specific detectors [8] are well suited to the analysis of polysaccharide binders, provided that a preliminary depolymerization step to break the glycoside linkage and free the sugars, and a derivatisation step of the released monosaccharides are appropriately performed. These procedures give detailed information. However, it is not a simple matter to interpret the obtained highly complex chromatograms where multiple peaks for each monosaccharide (up to five products) are present due to tautomerism, and an irreproducible quantification may occur. Only recently, a GC–MS procedure for plant gum characterization using a derivation step giving a single peak for each monosaccharide, was proposed [9]. The method seems valuable but the determination of uronic acids requires a second different derivatisation step which entails a long analysis. Pyrolysis-gas chromatographic techniques [10] allow minimal sample manipulation, since the hydrolysis step is not necessary but they still suffer from interferences due to the matrix composition.

Generally, the analytical procedures proposed in the literature require a hydrolysis step in an aqueous environment. Using of a liquid chromatographic method which employs an aqueous mobile phase without a derivatisation step would thus seem to be the most convenient and direct method for monosaccharides analysis. The biochemistry literature offers a lot of valuable approaches for sugar determination such as those based on anion-exchange chromatography [11,12]. The latter seem suitable for the separation of carbohydrates in painting samples since they allow a minimal sample pretreatment and show appropriate detection limits.

This paper describes an analytical procedure for the characterisation of polysaccharide binders aimed at optimising the hydrolysis step, minimising microsample manipulation and reducing chromatogram complexity. The procedure is based on hydrolysis with trifluoroacetic acid assisted by microwaves and determination of sugars by high-performance anion-exchange chromatography (HPAEC) coupled with pulsed amperometry (PAD) using gold working electrodes. A clean-up step, which consists of passing the hydrolysate through a small home-made column packed with a cation-exchange resin, allows us to retain pigments, amino acids or peptides eventually present in the sample, thus giving us the chance to identify these compounds by another analytical procedure [13] and to elute only saccharides. Raw plant gums, commercial watercolours and reference paint samples obtained from the Laboratory of Opificio delle Pietre Dure in Florence (Italian Ministry of the Cultural Heritage) were employed to test and calibrate the overall procedure. The chemometric technique principal components analysis (PCA) [14,15] applied on the relative percentage contents of seven monosaccharides and two uronic acids allows the identification of the polysaccharide binders present in the sample. Finally, the results obtained for the binder characterisation of some samples from funerary wall paintings in Macedonian tombs (4th–3rd Centuries B.C.) are discussed.

2. Experimental

2.1. Chemicals and reagents

D-(+)-Galactose 99%, L-(−)-fucose 99%, L-(+)-arabinose 99%, L-(−)-rhamnose monohydrate 99%, L-(−)-mannose 99%, D-(+)-xylose 99%, D-glucuronic acid sodium salt monohydrate 99%, D-(+)-glucose 99.5%, D-galacturonic acid monohydrate 98% were from Sigma (Milan, Italy). Acetic acid HPLC-grade 99–100% and 50% NaOH standard solution were from Baker (J.T. Baker Italia, Milan, Italy). The trifluoroacetic acid 99% was from Fluka (Milan, Italy). Sodium azide was from Carlo Erba (Milan, Italy). Amino acid standard solution of collagen hydrolysate (12.5 μmol/ml of proline and hydroxyproline, and 2.5 μmol/ml in 0.1 *M* HCl of all the other amino acids) and Dowex 50W-X8, cross-linkage 8% resin in hydrogen form, 100–200 mesh and 5 mequiv./g were supplied by Sigma. Double-distilled water (Carlo Erba) was treated in the Elgastat UHQ System to produce ultra-high quality (UHQ) pure water (18 MΩ cm at 25 °C). The water was sparged with helium before eluent preparation.

Stock solutions of monosaccharides with concentrations ranging from 200 to 400 ppm were prepared in pure water containing 0.1% of sodium azide to prevent microbial growth, and were stored at 4 °C. Working standard solutions were prepared daily from stock solutions.

2.2. Raw materials

Arabic, karaya, ghatti, guar, locust bean and tragacanth gums were purchased from Sigma. Arabic, tragacanth, were also purchased from Zecchi (Florence, Italy), cherry, plum and peach gums were kindly provided by the Opificio delle Pietre Dure.

2.3. Painting samples

Watercolours were supplied by Maimeri (Florence, Italy). They contained arabic gum with pigments cadmium sulphide (yellow) WCY, chromium oxide (green) WCG, iron oxide (red) WCR and cobalt aluminate (cobalt blue) WCB. Reference painting specimens of gums layered on glass tiles (C1, C2, C3, C4) were provided by Opificio delle Pietre Dure, Florence (Italy). They were prepared in 1975 following old recipes and were stored in the dark: C1 and C2 contain arabic gum and tragacanth gum, respectively; C3 contains a mixture 1:1 (w/w) of arabic gum and casein; C4 contains a mixture 1:1 (w/w) of arabic gum and animal glue.

2.4. Ancient wall painting samples

The main features of the ancient samples collected from wall paintings of Macedonian tombs (Greece) 4th–3rd Centuries B.C. are summarised in Table 1. The tombs were discovered within the last 20 years and have not been restored. The samples carefully

Table 1
Characteristics of the ancient wall painting samples

Origin of samples	Sample name	Mass (mg)	Colour
Aineia III tomb	M1	7 and 4	Red
	M5	7, 10 and 4	Gold
	M2	8	Pale green
Aghios Athanassios tomb	AGM4	3 and 7	Dark red
	AG.AT10	10 and 6	Blue
	AG.AT17	9 and 11	Pale blue
	AG.AT61	15 and 8	Blue
Finikas tomb	F4	15	Dark red

collected by Dr. Hariclia Brecoulaky (Wiener Laboratory of the American School of Classical Studies, Athens, Greece) were in the form of fragments where the plaster was the main component of the sample. Preliminary FT-IR analysis of the samples showed weak peak absorptions related to the presence of polysaccharide and proteinaceous binders.

2.5. Apparatus and chromatographic conditions

A microwave oven MLS MEGA Milestone (FKV-Milestone, Sorisole, Italy) was used to assist the sample hydrolysis. The system used for the chromatographic separation of monosaccharides consisted of a Dionex chromatographic pump (Dionex, Sunnyvale, CA, USA) model 4000i, a Dionex pulsed amperometric detector equipped with an Au electrode, a Dionex CarboPac guard (50×4 mm) and a Dionex CarboPac PA1 column (250×4 mm).

The eluents used were: A, 19 m*M* NaOH; B, 75 m*M* NaOH+150 m*M* CH_3COONa; C, 200 m*M* NaOH. The eluents were prepared by diluting a stock solution of 50% NaOH. Eluent B was prepared by partial neutralization of a NaOH solution with acetic acid. The gradient profile used for the chromatographic separation was: 100% eluent A up to 18 min then from 100% eluent A to 100% eluent B in 12 min, hold eluent for 10 min, up to 100% eluent C in 1 min, hold for 8 min and from 100% C to 100% A in 1 min.

The potential–time waveform used with a PAD system, with a Ag–AgCl reference electrode, was: 0.05 V up to 0.5 s; 0.6 V from 0.5 to 0.6 s; −0.6 V from 0.6 to 1.01 s; 0.05 V from 1.01 to 2 s. In order to enhance the baseline stability and the response, a 300 m*M* NaOH solution, at a flow-rate of 0.35 ml min^{-1} was added to the eluent at the column outlet.

2.6. Analytical procedure

A weighed amount of painting sample was admixed with 500 μl of 2 *M* trifluoroacetic acid in closed vials of PTFE and subjected to acid hydrolysis assisted by microwaves using the following conditions: 20 min at 120 °C and 500 W. The hydrolysate was filtered with a PTFE membrane, and was passed through a glass column (5×50 mm) packed with a strong cation-exchange resin (250 mg of Dowex AG 50W-X8 in hydrogen form). The resin was conditioned with a solution of 2 *M* HCl and washed with water until it became neutral. Once the hydrolysate was loaded on the column, the elution of anions and non-ionic species was performed with 4 ml of 0.02 *M* HCl. The eluate was dried under a gentle N_2 stream, and the residue dissolved in 100 μl of water. Then, 25 μl of the solution were injected into the ion chromatography column. This procedure allows the determination of fucose (Fuc), rhamnose (Rham), arabinose (Ara), galactose (Gal), glucose (Glc), mannose (Man), xylose (Xyl), galacturonic (GalUA) and glucuronic (GlcUA) acids as their oxoanions, whose capacity factors and reproducibility are reported in Table 2. Particular care was taken to ensure a carbonate-free alkaline mobile phase flowing through the column.

Carbohydrates are detected by measuring the electrical current generated by their oxidation at the Au electrode. Since the oxidation products may poison the electrode surface [16], the Au surface has to be cleaned and restored by a series of short positive and negative potential pulses. Linear calibration curves of sugar standards were obtained in the concentration range 0.03–20 ng/μl. Since xylose and mannose chromatographic peaks are partially overlapped, the quantification was performed measuring peak heights instead of areas. The detection limit was in the range 0.01–0.03 ng/μl for all carbohydrates. The percent content of all sugars was obtained by rationing the content of each monosaccharide in the final solution, expressed in μg, to the total content of the weighed gum when known or to the total content of all sugar present in the sample.

Table 2
Capacity factors and reproducibility as obtained on five replicates of a standard sugar solution in the concentration range of 0.14–0.33 μg/g

Carbohydrate	Capacity factor	RSD (%)
Fuc	2.9	0.8
Rham	5.6	0.7
Ara	6.6	0.6
Gal	8.9	0.7
Glc	9.8	1.0
Man	10.4	0.7
Xyl	10.9	0.8
GalUA	28.4	0.3
GlcUA	29.7	0.4

3. Results and discussion

HPAEC–PAD using polymer-based stationary phases with alkaline eluent is a powerful tool for the separation and determination of carbohydrates, as shown in Fig. 1 for a standard solution containing seven monosaccharides (fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose) and two uronic acids (galacturonic and glucuronic). The poor resolution between xylose and mannose peaks, which is the best separation obtainable with these chromatographic conditions, does not cause quantitative problems. Moreover, for the gum characterisation these monosaccharides are simultaneously present only in fruit tree gums. This procedure allows us to achieve low detection limits (about 0.1 ng for each sugar) comparable or even better than those obtained in GC methods, but with the advantage of not requiring a derivatisation step and the absence of multiple peaks deriving from sugar tautomeric forms. Epimerization was not observed as proved by using standard sugar solutions. Moreover, the glucuron-6,3-lactone does not interfere with glucuronic acid because the strong alkaline eluent provides a complete saponification of the lactone form giving the sodium salt of glucuronic acid [17].

3.1. Optimisation of the acid hydrolysis procedure

To test and optimise the acid hydrolysis procedure raw arabic gum was chosen for several reasons: it is completely water soluble, it is nearly 98% pure, it contains one aldopentose (ara), one aldohesose (gal), one 6-deoxyaldohesose (rha) and one uronic acid

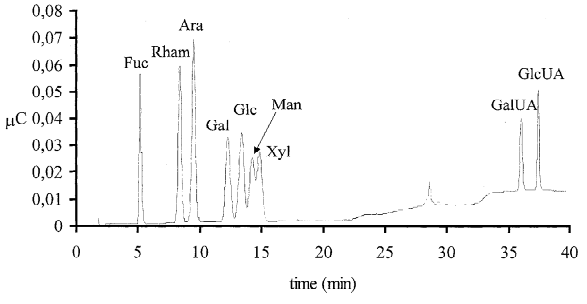

Fig. 1. Chromatogram of a standard solution of seven monosaccharides and two uronic acids in the concentration range 0.14–0.33 µg/g.

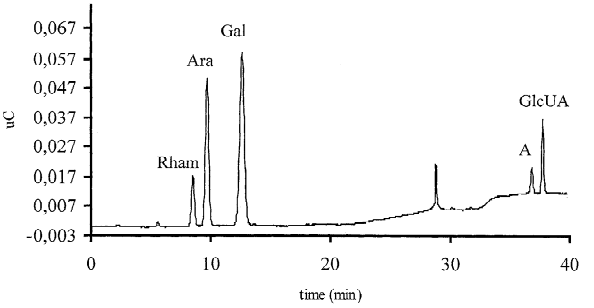

Fig. 2. Chromatogram of an arabic gum sample. The peak A (36.8 min) is identified as an oligosaccharide made up of galactose and glucuronic acid.

(gluAU), thus including the typical glycoside compounds encountered in all plant gums, and finally it is widely used as a painting binder. Fig. 2 shows the typical pattern of arabic gum obtained by the suggested method. The peak with a retention time of 36.8 min (A), which has a capacity factor of 28.8 and does not overlap with uronic acid peaks, seems due to an oligosaccharide constituted by glucuronic acid and galactose [18]. Its area seems related to the capacity of the method to break the glycosidic linkage: the lower the area of A, the higher those of glucuronic acid and galactose. Table 3 gives the results for six replicate measurements of 10 mg samples of arabic gum at various temperatures and a constant reaction time (20 min) and power (500 W). In these experimental conditions the best recovery, with a relative standard deviation of 3%, was achieved with a temperature of 120 °C.

A comparison with the classic acidic hydrolysis (2 *M* trifluoroacetic acid, 120 °C, 1 h) [19] shows that the classic method gives a lower average recovery (62±4%), mainly due to a lower recovery of glucuronic acid (4%) confirmed by a higher area level of the peak A. Both methods exhibit a similar

Table 3
Average percentage recovery (%, w/w) of sugars on six replicate analyses of arabic gum in the acidic hydrolysis assisted by microwaves performed for 20 min at 500 W

T (°C)	Rham	Ara	Gal	GlcUA	Total
110	8.4	20.1	28.0	5.0	62
120	9.8	21.0	32.7	9.0	72
130	6.7	14.0	27.1	7.0	55
140	1.1	2.2	6.0	0.5	9.8

degree of acid decomposition [20] for each carbohydrate, as proven by submitting standard sugar solutions to the two procedures.

The hydrolysis procedure assisted by microwaves was thus tested in the range 0.01–10 mg of arabic gum: the average recovery was 72% with an RSD of 8%. In conclusion, this original procedure reduces the analysis time to 20 min, and allows us to achieve better recoveries since it is more effective in the rupture of glycosidic linkage involving uronic acids.

3.2. Cation-exchange clean up procedure

A cation-exchange clean up procedure prior to the chromatographic analysis was advisable to suppress interferences due to:

1. formation of weak complexes between carbohydrates and some metal ions [21] which come from plaster (mainly $CaCO_3$) and pigments causing shifts in retention times [22]. The interference was clearly evident only for metal concentrations higher than 0.1 M;
2. presence of amino acids and peptides coming from proteinaceous binders which can be separated and determined in HPAEC–PAD [23]. In fact, a standard collagen hydrolysate showed an overlapping of amino acid peaks with that of rhamnose at 8.5 min and that of glucose at 13.5 min.

Replicate measurements on a standard solution of sugars (concentration range 4.3–7.1 μg/g) containing collagen hydrolysate and 0.2 *M* Ca^{2+} at pH 2 (maximum conc. obtainable from a plaster sample), passed through a cation-exchange column showed that the average recovery was 95–100% (RSD 5%) of the initial sugar content. The amino acids and peptides strongly retained on the column may be analysed by applying another analytical procedure [13].

3.3. Analysis of raw gums

The overall procedure was checked by analysing almost six replicate samples of the available raw plant gums; the results are reported in Table 4.

The sugar profiles of the analysed gums resemble those reported in the literature [24], even though the natural compositional variability of the plant gums may induce a 15% change in sugar compositions. The recovery for each gum varies between 36% and 76% with an RSD lower than 10%. The lower recovery found for karaya, tragacanth and cherry gums may be connected above all with their low water solubility.

3.4. Analysis of painting samples

Replicate measurements on watercolour and reference painting samples gave the typical profile of the contained gum and highlighted the absence of interference from anions and cations. Table 5 reports the average relative percentage contents of monosaccharides and uronic acids found for six replicate analyses of samples.

The recovery for each gum in the reference samples was very close to the expected one. It was lower in the presence of proteins, probably due to the

Table 4
Relative percentage content of sugars, average percentage recovery and relative standard deviation on six replicate analyses of raw plant gums

Plant gum	Fuc	Rham	Ara	Gal	Glc	Man	Xyl	GalUA	GlcUA	Recovery (%, w/w)	RSD (%)
Arabic		13.4	29.5	45.0					12.0	73	3.4
Karaya		30.6		41.9				22.2	6.2	36	8.5
Ghatti		3.5	47.0	36.0		2.9	t[a]		11.0	66	7.7
Guar			3.1	34.0		63.0				73	2.4
Locust bean			1.6	19.0		79.0				76	3.7
Tragacanth	7.7	3.3	31.0	9.6	11.8		16.0	17.0	3.7	46	6.5
Cherry		2.7	35.0	37.0		5.0	6.6		13.0	63	4.5

[a] t, traces.

Table 5
Average relative percentage content of sugars on six replicate analyses of watercolours and reference painting samples

Sample	Fuc	Rham	Ara	Gal	Glc	Man	Xyl	GalUA	GluUA
WCY		13.5	30.0	47.0					9.5
WCG		13.1	31.1	46.0					9.8
WCR		13.0	32.1	44.3					10.7
WCB		13.3	31.0	45.9					9.0
C1		13.2	29.1	48.0					12.0
C2	3.4	2.8	47.9	11.0	10.2		9.5	15.0	0.7
C3		13.2	29.1	48.1					10.1
C4		13.1	27.5	48.2					12.1

Maillard reaction. However, the recovery might be influenced also by the formation of humins due to the reaction between monosaccharides and amino acids—reactions which can occur in these experimental conditions.

3.5. Identification of gums

Due to the natural compositional variability, the identification of a gum has to be based on the overall glycoside profile. For these purposes the application of PCA on the relative sugar percentage contents (content of each sugar ratioed to the total sum of sugars determined) is a valuable tool which allows us to group objects into clusters according to similarities between original variables. Glucose was not selected as an original variable since it may derive from other materials simultaneously used in paintings with gums such as honey (made by glucose and fructose) or starch (made only by glucose). Fig. 3 shows the three-dimensional score plot of relative sugar percentages of raw gums, watercolours and reference painting samples which takes into account the 93.5% of the total data variance. Five clusters are spatially separated, in particular:

(i) arabic, tragacanth and karaya gums are well separated since they have quite a different chemical composition;
(ii) guar and locust bean gums are quite separate but since the two gums are constituted by the same monosaccharides and their amounts may vary considerably depending on the geographical area of the origin of gums, they may be considered as a sole cluster;
(iii) fruit tree and ghatti gum clusters overlap: their identification is based on the relative amounts of xylose. This is present in trace in ghatti gum and at about 7% in tree gum.

Using this method, the statistical approach shows that unpigmented, pigmented and reference painting samples are properly located in clusters suggesting that the procedure is not affected by interferences from inorganic and organic cations. In these conditions having constructed a reference data base, a reliable identification of a binder based on plant gum may be achieved.

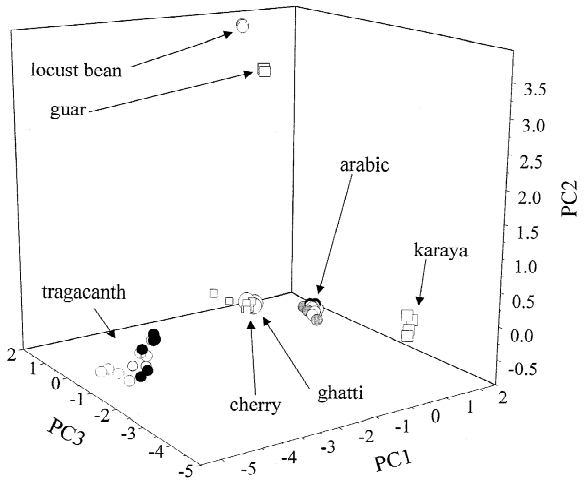

Fig. 3. The score plot of the first three principal components (PC1, PC2 and PC3) obtained from PCA applied to the relative percentage contents of monosaccharides and uronic acids: empty symbols refer to raw gums, grey circles to watercolours and black circles to reference painting samples.

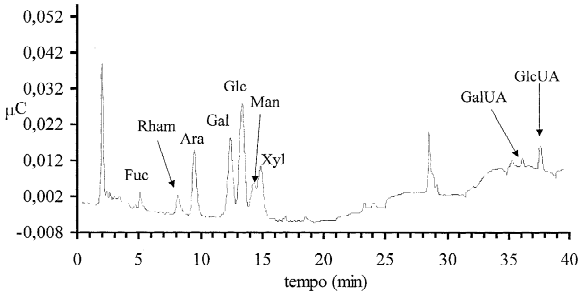

Fig. 4. Chromatogram of a sample (M1) from an ancient funerary wall painting of the Aineia tomb.

3.6. Analysis samples from ancient wall paintings of Macedonian tombs

Fig. 4 shows the chromatogram of a sample (M1) from the tomb of Aineia, nowadays known as Nea Michaniona acropolis (Calchidiki, Greece). The following monosaccharides, which highlight the use of a polysaccharide binder, were identified: fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, galacturonic and glucuronic acid. The sugar profile of all the other samples looks quite like this one; their relative percentages are reported in Table 6. The samples M5 and AG.AT61, practically consisting of plaster, exhibit a glucose content of 51% and 74%, respectively, much higher in respect to that of the other samples (15–38%). In any case, the content of glucose exceeds the expected one (10–12%) for plant gums. This fact may be related to the presence of another polysaccharide substances mainly consisting of glucose, such as honey, starch or cellulosic material, which is added to the plaster in order to improve the adhesion of the paint [1]. Since fructose, which has a capacity factor of 10.1 as tested

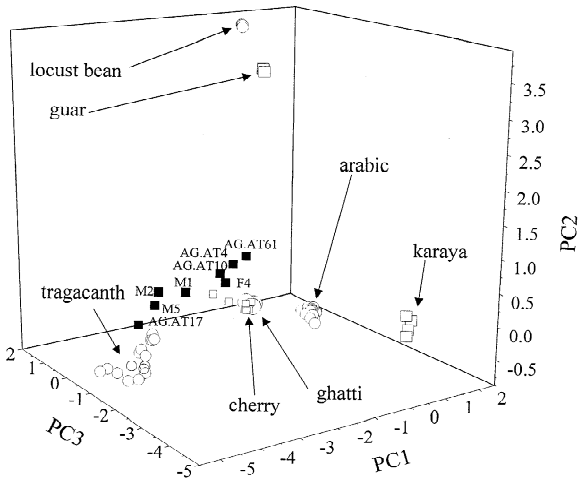

Fig. 5. The score plot of the first three principal components (PC1, PC2 and PC3) obtained from PCA applied to the relative percentage contents of monosaccharides and uronic acids: empty symbols refer to the reference data set and black squares to ancient samples.

on honey samples, seems absent, the presence of honey, which was also used for this purpose, is rather unlikely.

The simultaneous presence of Fuc, GalUA and GlcUA in practically all the samples suggests the use of tragacanth gum, while that of mannose shows evidence of the addition of another gum in the paint. For a better identification, the data submitted to PCA analysis (Fig. 5) show that:

1. the samples M2, M5 and AG.AT17 belong to the cluster of tragacanth gum;
2. all the other samples are located between the tragacanth gum and fruit-ghatti gum clusters.

Caused by a partially altered sugar distribution due to ageing processes, the positions of the ancient

Table 6
Relative percentage content of sugars in ancient samples

Sample name	Fuc	Rham	Ara	Gal	Glc	Man	Xyl	GalUA	GlcUA
M1	2.3	5.2	12.0	18.6	31.4	11.0	12.7	1.9	4.8
M2	2.1	3.4	9.4	15.1	38.6	11.3	18.7	n.d.	1.3
M5	1.8	3.3	4.4	4.6	74.1	4.2	7.3	n.d.	0.3
AGM4	1.9	5.7	8.9	15.8	38.1	14.8	7.4	1.9	5.5
AG.AT17	0.8	3.4	20.9	14.5	18.5	n.d.	38.1	0.8	2.9
AG.AT10	2.3	3.3	8.3	18.2	37.4	12.8	8.7	2.7	5.9
AG.AT61	2.5	2.3	4.6	15.8	50.9	13.0	2.6	2.2	6.1
F4	1.8	6.0	10.5	17.8	38.1	9.8	10.0	1.9	4.0

samples are slightly shifted toward the upper part of the two clusters. To take into account the degradation processes undergone by organic matter, artificially aged reference painting samples need to be analysed. This data implementation should provide a better tool for gum identification. Basically from all the obtained results, it seems that tragacanth gum and a fruit gum, rather than the Indian ghatti gum which was practically unknown in the 4th–3rd Centuries B.C. in Greece, were used for painting and for the final retouching.

4. Conclusions

The suggested procedure allows the reliable identification of a plant gum at a level of a few micrograms in a painting sample. Acid hydrolysis assisted by microwaves permits us to reduce the hydrolysis time to 20 min. Moreover, the conditions used in the HPAEC–PAD separation, achieved in 40 min, avoid the formation of lactone and tautomeric forms of sugars. Interferences from inorganic and organic cations were suppressed by using an ion-exchange resin in the clean-up step of the hydrolysed, and the whole analytical procedure allows an average recovery of 72% with an RSD of 8% as tested on arabic gum. Even if the overall recovery was far from quantitative, the relative percentage composition of the monosaccharide mixture obtained from the hydrolysis reaction with a good reproducibility, reflects the monosaccharide composition in the gum, thus giving the possibility of identifying a polysaccharide material.

The analysis of a wide range of raw plant gums, watercolours and naturally aged reference painting specimens allows one not only to optimise the analytical procedure, but also to collect data for constructing a data set which is useful for binder identification when a chemometric method such as PCA is applied. The application of the analytical procedure to ancient wall painting samples highlights that the same executive paint technique based on the use of polysaccharide binders to disperse pigments and fix the paint was widely employed in Macedonia during the 4th–3rd Centuries B.C. Starch or cellulosic material in the plaster and tragacanth and fruit gums in the paint characterise this ancient tempera technique. It should be highlighted that these findings agree with the only piece of scientific information on pre-Hellenistic art available from the literature [25] reporting the use of arabic gum in the painted decorations of the Eurydice Tomb.

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