archaeometry

*Archaeometry* **53**, 4 (2011) 830–841 doi: 10.1111/j.1475-4754.2010.00577.x

# CONSTRUCTION OF THE KHOJA ZAYNUDDIN MOSQUE: USE OF ANIMAL GLUE MODIFIED WITH URINE*

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*As part of an ongoing project at the World Heritage Site of Bukhara, we investigated the glue used in the construction of the Khoja Zaynuddin mosque. Analysis by a range of techniques confirmed that it consisted of a collagen-based glue. However, the glue contained many non-protein constituents. The presence of lipid material suggests that the glue was produced by a relatively unsophisticated process. More surprisingly, various marker compounds of urine were found. Study of the viscosity of a mixture of a modern collagen-based glue and urea showed that the presence of urea at a concentration typical of human urine reduced the viscosity of the glue, thus improving its spreading characteristics and enabling its use at a lower temperature. Whilst the advantages of adding urea to glue were known to craftsmen in Europe and America in the 19th century, it would appear that the builders of Bukhara were aware of the technique, using less pure components, at a significantly earlier date.*

*KEYWORDS:* BUKHARA, KHOJA ZAYNUDDIN MOSQUE, *MUQARNAS*, GLUE, COLLAGEN, URINE

## INTRODUCTION

Bukhara, in Uzbekistan, is a city of great historical and cultural significance. Its position on the Silk Road made it a major cultural and trading centre throughout its history. The city centre is a rare example of a large urban structure in which extensive areas of residential quarters have been preserved in a 'traditional' state; that is, with constructions dating largely to pre-industrial periods. The city of Bukhara comprises a great number of historical monuments, including mosques, mausolea and madrasas, dating from the 10th century AD onwards. Both factors have induced the protection of the Old City of Bukhara as a UNESCO world cultural heritage site (Gangler *et al*. 2004). The Khoja Zaynuddin mosque (constructed *c*. AD 1540) and its surrounding district are part of an ongoing research project in which the methods of construction are being investigated (Badr and Tupev 2010). While the main body of the building is built of brick, significant parts, especially the porticos, are wooden constructions. Particularly notable is the decoration with *muqarnas*, complex corbelled and overhanging structures, used as decoration of column capitals, cornices and small domes (Fig. 1).

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Figure 1 *Detail of the Khoja Zainuddin Mosque: (a) the ceiling of the portico, with cornice* muqarnas*; (b) the ceiling of the portico, with central* muqarnas*; (c) a section of the portico* muqarnas *showing the presence of glue; (d) a close-up image of the glue used to construct the portico.*

In the architecture of the Islamic Middle East, the origin of *muqarnas* can be traced back to the 10th–11th centuries, when transitional zones of brick domes in Iran and Central Asia were first designed with overhanging corbels and cells. The geometry of these elements was further refined during the following centuries, and *muqarnas* became one of the most frequently used decorative elements in Islamic architecture (Necipoglu 1995; Ettinghausen *et al*. 2001). Geographically, it spread across the whole region between Central Asia and North Africa, between Anatolia and Yemen. Its use was extended from domes and niches to cornices, capitals and other forms, and all kinds of materials were used. The geometry of *muqarnas* designs has been subject to architectural documentation and art-historical interpretation. The geometrical layout is normally based on interlocking polygons on the level of the ground plan, while the elevation follows a relatively schematic sequence of cells and corbels that can be modelled in an algorithm (Notkin 1995; Dold-Samplonius and Harmsen 2005). In the case of Khoja Zaynuddin, stucco *muqarnas* are an important element of architectural decoration on the interior, where two large half-domes, the squinches, and a circular cornice below the central cupola of the prayer hall are adorned in this manner. On the exterior porticos, wooden *muqarnas*, today partially lost, formed the main body of the column capitals, cornices outlining the wooden ceilings, and domes forming the centrepiece of ceiling panels. These structures have been held together using only glue and small dowels, without the use of nails or clamps, for about 500 years.

As can be seen from Figure 1, parts of the structure are in need of restoration and the objective of this project was to determine the nature of the glue to aid in the eventual conservation of the mosque. Small samples of the glue were removed and subjected to analysis to determine its chemical composition and possible biological source.

## MATERIALS AND METHODS

### *Materials*

Two of the glue samples, H1 (~200 mg) and F1 (~100 mg) were of particular interest. Both samples showed some light and some dark surfaces. Under magnification, it was noted that the dark surfaces were ill-defined and similar in appearance to the exposed glue surfaces (Fig. 1). In contrast, the lighter surfaces were yellow, similar in colour to pearl glue, and showed clear indentations of tool marks or graining from the wood. We conclude that the lighter surfaces were in close contact with the wood until recently, whilst the exposed darker surfaces possibly have some form of microbial contamination.

The pearl glue was purchased from Liberon Ltd.

### *Methods*

*Nuclear magnetic resonance* Samples of Bukhara glue (F1), pearl glue and casein were suspended in $D_2O$, ultrasonicated for ~30 min, and transferred to the NMR tubes. NMR spectra were recorded at 25°C on a JEOL Alpha500 spectrometer (500 MHz for $^1$H) by using an inverse probe head with field gradient coils (HMQC spectra) or a multinuclear probe head (one-dimensional $^1$H- and $^{13}$C-spectra). The spectral data are shown in Figure 2 ($^1$H-,$^{13}$C-HMQC spectra): 512 data points in $t_2$, zero filled to 1024, 256 increments in $t_1$, zero filled to 512, 128 (a), 128 (b) and 256 (c), respectively, frequency width 4452 Hz ($f_2$) and 23 474 Hz ($f_1$), exponential window in $t_2$ (BF = 5.0) and Gaussian window in $t_1$ (CBF = −10, CGF = 40). One-dimensional spectra ($^1$H and $^{13}$C) are referenced to external acetone in $D_2O$ (2.09 ppm and 28.10 ppm, respectively). The measuring times (see Fig. 2) are as follows: (a) 19.3 h, (b) 19.3 h and (c) 38.5 h.

*Bulk isotope analysis* Samples were dried and weighed into tin capsules and C and N isotope analyses performed on a Europa 20-20 mass spectrometer fitted with a Roboprep combustion unit. The samples were determined in duplicate and the results averaged.

*HPLC analysis* A small amount of lipid-extracted sample (2.2 mg) was hydrolysed in 6 M HCl for 18 h at 110°C and subsequently evaporated to dryness. Hydrolysate was rehydrated in 0.01 M HCl, containing 0.01 mM l-homo-arginine as an internal standard, and 0.77 mM $NaN_3$ to inhibit bacterial growth. The sample was derivatized with isobutyryl-l-cysteine and *o*-phthaldialdehyde, separated using rHPLC (Hypersil BDS 5 µm, 250 × 5 mm) and detected by fluorescence (230/445 nm) (Penkman *et al*. 2008).

### *MALDI-TOF/TOF*

Warm water protocol Fragments of the protein residue of extracted glue sample H1 (<1 mg) were incubated for 1 h at 65°C in 50 mM of ammonium bicarbonate (pH 8.0) in a polypropylene Eppendorf tube. Samples were briefly centrifuged and the supernatant collected; the extraction

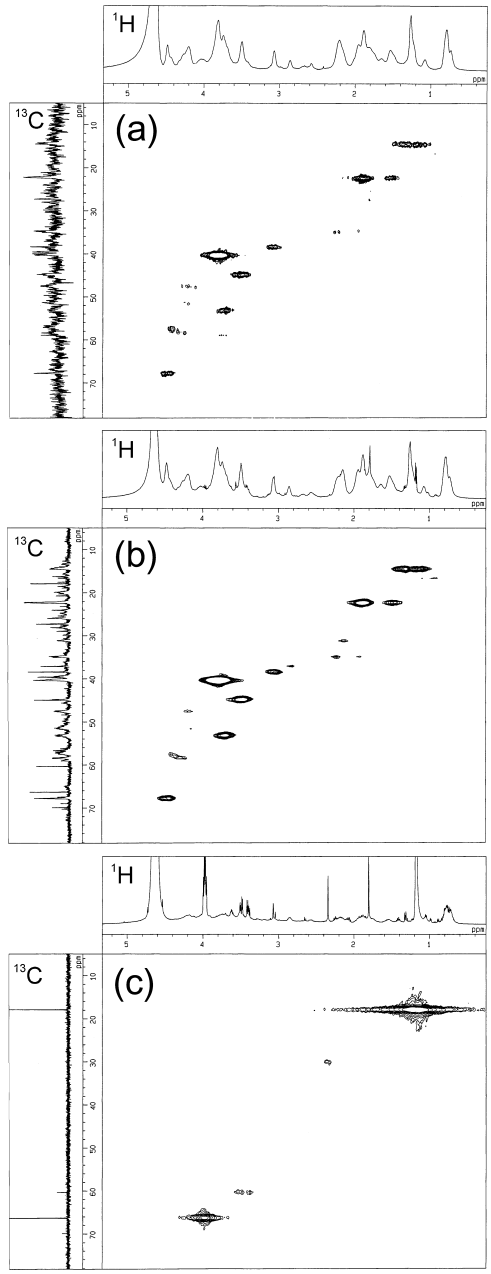


Figure 2 *HQMC spectra of glue samples from (a) Bukhara (F1), (b) authentic pearl glue and (c) casein.*

was repeated, and again supernatant was collected. Both extracts were incubated overnight (<18 h) with 1 µl of 1 µg µl$^{-1}$ sequencing-grade modified porcine trypsin (Promega) at 37°C. The tryptic digest was filtered over C18 resin (Millipore, Varian, Porvair) to desalt and concentrate the peptides by washing with 0.1% TFA. The peptides were eluted in a final volume of 10 µl of 50%

Table 1 *Proportions of components used to make up the experimental glues*

| *Pearl glue (g)* | *Water (g)* | *Urea (g)* | *Urea/water (%)* | *Urea/glue (%)* |
|---|---|---|---|---|
| 15 | 25 | 0 | 0 | 0 |
| 15 | 25 | 0.5 | 2 | 3.3 |
| 15 | 25 | 1.0 | 4 | 6.7 |

ACN / 0.1% TFA (v/v). 1 µl of eluate was mixed on a ground steel plate with 1 µl α-cyano-4-hydroxycinnamic acid matrix solution (1% in 50% ACN / 0.1% TFA (v/v/v)) and dried to air. Each sample was analysed in triplicate and matched to a calibrant of six known peptides, using a calibrated Ultraflex III (Bruker Daltonics) MALDI-TOF/TOF instrument in reflector mode to measure the mass to charge ratios (*m/z*) of trypsinated fragments. Obtained spectra were analysed using flexAnalysis software v. 3.0 (Bruker Daltonics). Indexed peptides were identified manually from MS spectra (1). Only the second extract was suitable for subsequent MS/MS analysis, obtained through collision-induced dissociation of selected peptides with the highest peak intensity. Product ion spectra were matched through a MASCOT search to find fragments within the series that determine *de novo* sequences.

*Gas chromatography – mass spectrometry*

Lipid extraction The samples were extracted by ultrasonication (2 × 15 min) in 10 ml dichloromethane (DCM) and methanol (2/1 v/v). Following centrifugation, the solvent was removed using disposable Pasteur pipettes and dried under nitrogen. Aliquots were trimethylsilylated using *N,O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (BSTFA; 50 µl) at 60°C for 20 min before being dried under nitrogen and redissolved in 100 µl of DCM prior to gas chromatography – mass spectrometry.

Analysis Analysis was carried out by combined gas chromatography – mass spectrometry (GC–MS) using an Agilent 7890A Series GC connected to an 5975C Inert XL mass-selective detector. The splitless injector and interface were maintained at 300°C and 340°C, respectively. Helium was the carrier gas at constant inlet pressure. The temperature of the oven was programmed from 50°C (2 min) to 350°C (10 min) at 10°C $min^{-1}$. The GC was fitted with a 15 m × 0.25 mm, 0.25µm HP-5ms 5% phenyl-methylpolysiloxane phase fused silica column. The column was directly inserted into the ion source, where electron impact (EI) spectra were obtained at 70 eV with a full scan from *m/z* 50 to 800.

*Preparation of glue samples for viscosity studies* Samples were prepared as shown in Table 1. Water, glue and urea were mixed in glass bottles and allowed to stand overnight. The bottles were then placed in a boiling water bath and the temperature of the glue raised to 60°C with occasional stirring until dispersed. The bottles were then sealed and allowed to cool.

*Viscosity determination* Viscosity measurements were made using an Anton-Paar MCR501 shear rheometer using a 50 mm diameter 1° cone and plate geometry with a central gap of 0.049 mm. Frequency sweeps were made in oscillatory mode within the shear rate range of 50 $s^{-1}$

to 500 $s^{-1}$. Tests were carried out on the base pearl glue and samples with 2% and 4% urea loadings and at fixed temperatures of 40, 50 and 60°C.

## RESULTS AND DISCUSSION

Animal-based protein glues have been used since antiquity (Edwards 2001). For example, in the Dead Sea area, collagen-based glues have been isolated from the inside and outside of baskets dating from *c*. 8000 years ago (Connan *et al*. 1999). Collagen has been shown to have a variety of uses in ancient Egypt (Lucas and Harris 1962). There are several reports of collagen being used to fix wooden artefacts from the period dating 2500 to 1500 BC, one of the most convincing being from the mortuary temple of Queen Hatshepsut (Lucas 1927). In contrast to its use in the Middle East for thousands of years, specialized glue factories were not established in Europe until the end of the 17th century, and in America until the early 19th century (Edwards 2001).

The glue is made from the inedible parts of animals, particularly bones, hides, tendons and cartilage. Typically, the collagen is treated with lime, the pH is adjusted with mineral acid and then it is boiled. As a result, the three polypeptide strands of collagen unravel, yielding a crude form of gelatin. The liquid is then evaporated and cooled when it sets to a solid mass. In modern times, it has been produced as 'pearl glue', named after its physical form. Typically, the glue is derived from large animals such as horses, cows or sheep, although rabbit hide glue and fish glue have an assortment of specialist uses. Collagen glue remained the glue of choice in woodworking until the advent of modern synthetic glues (Edwards 2001).

The only other protein-based glue used extensively on wood is casein, derived from milk. The glue only became commercially available in Switzerland in the 19th century and found its main use in building wooden aircraft frames, where its greater water-resistance over collagen glue was advantageous. However, there are reports that a crude form of casein was used in the Middle Ages in Europe (Anon. 1967).

The infra-red spectra of crude H1 and F1 are identical ($\nu_{max}$ at 3316, 2961, 1657, 1643, 1547, 1451, 1409, 1337 and 1240 $cm^{-1}$) and identical to that of a reference of rabbit hide glue. The $^{1}H$ NMR spectrum of sample F1 is identical to that of pearl glue. Particularly striking evidence is provided by the $^{1}H$-, $^{13}C$-correlation spectra, recorded by using the HMQC (heteronuclear multiple quantum coherence) sequence (Claridge 1999). The spectrum of F1 is essentially identical to that of modern pearl glue and very different from that of a casein-based glue (Fig. 2).

Whilst the spectra strongly suggest the use of collagen glue, carbon:nitrogen analysis of H1, and F1 and pearl glue gave differing C:N ratios (Table 2). However, extraction of the samples with organic solvent removed between 10 and 20% of the solid, whilst pearl glue was unaffected by extraction. It was then found that the insoluble protein fraction of H1 had a C:N ratio

Table 2 *Carbon:nitrogen analysis of glue samples*

| *Sample* | *C:N ratio (mol $mol^{-1}$)* | |
|---|---|---|
| | *Initial* | *Extracted residue* |
| H1 | 4.10 | 3.26 |
| F1 | 3.58 | – |
| Pearl glue | 3.29 | – |

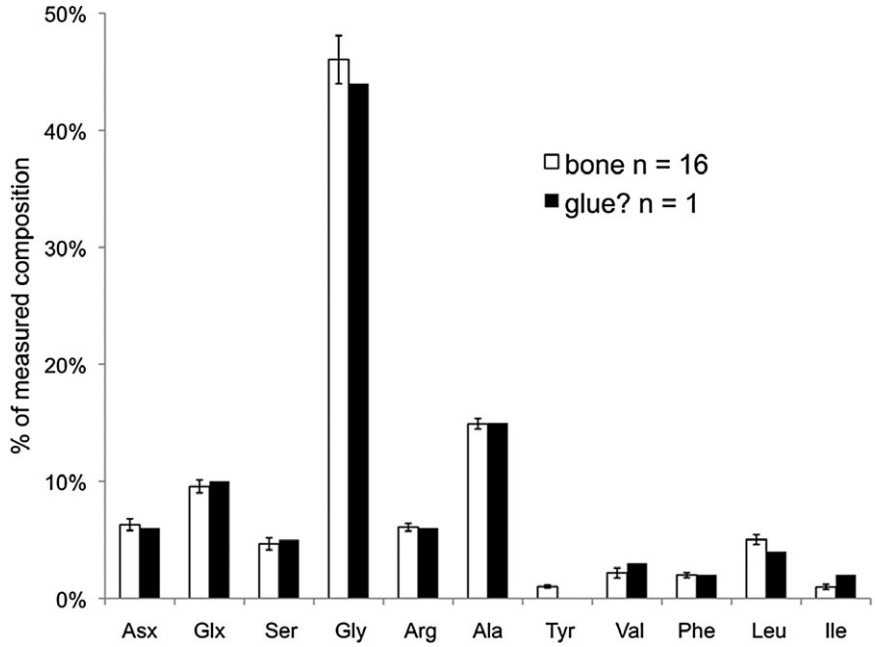


Figure 3 *A quantitative amino-acid analysis obtained by HPLC, comparing the amino-acid composition of a sample of the glue with that of bone collagen (n = 16 analyses).*

(3.26 mol:mol) very similar to that of pearl glue (3.29). Quantitative amino-acid analysis showed a composition similar to bone collagen (Fig. 3). Racemization values of Asx are atypically high when compared with modern processed gelatin, arguably due to the antiquity of the sample. Extrapolation of glycine concentration suggests that 70% of the measured protein is from collagen amino acids.

The Peptide Mass Fingerprint (PMF) spectrum (Fig. 4) contained a number of masses characteristic of collagen as observed in collagen samples used as standards for in-house databases (unpublished). The mass of 1427.7 has previously been identified as a peptide of mammalian collagen in ruminants and horses (Buckley *et al*. 2009). TOF/TOF spectra were of poor quality, but the spectrum for MH+ of 1530.7 is consistent with the COL1A1T80 peptide GEAGPAGPAGPIGPVGAR (horse/donkey); however, the limited quality and range of spectra are not sufficient to rule out other origins. The protein fraction of the glue is, however, confirmed as being collagen-based.

The use of HMQC would thus appear to provide a useful additional and non-destructive technique for the identification of proteins.

The organic fractions of F1 and H1 were subjected to analysis by gas chromatography – mass spectrometry. A typical trace is shown (Fig. 5) and the products found are summarized in Table 3.

As can be seen from Figure 5, all the products present in large quantity contain carbon but no nitrogen, whilst those that contain nitrogen are present in small quantities; hence the significant drop in C:N ratio of H1 on extraction. None of these products were observed in the pearl glue sample.

The presence of the lipid derived materials, particularly the saturated fatty acids, cholesterol and glycerol, are consistent with a mammalian source. The pearl glue sample, however, yielded

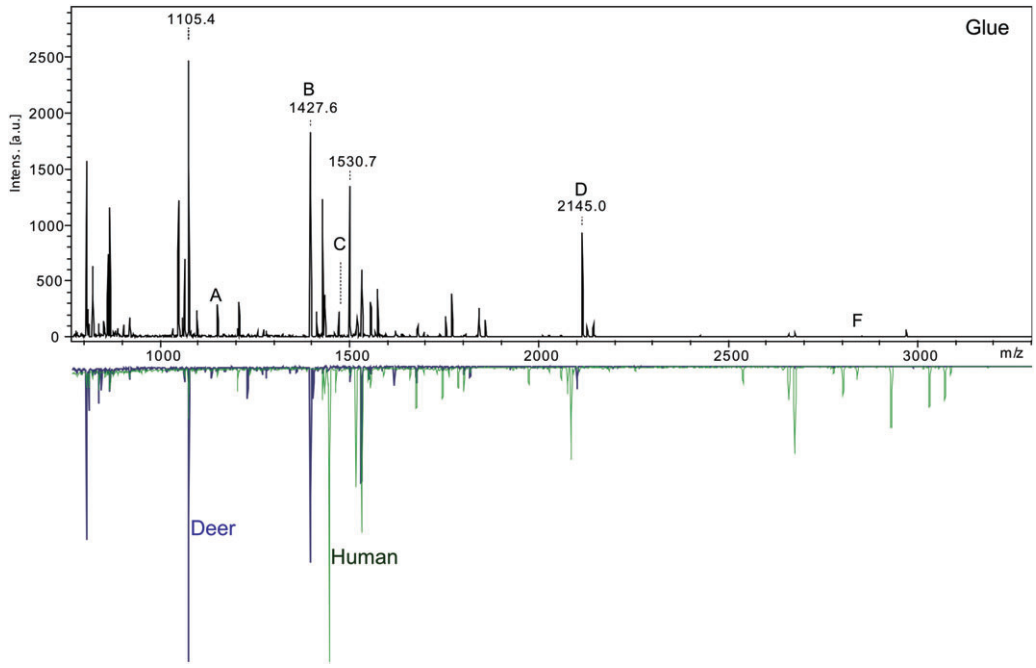


Figure 4 *A Peptide Mass Fingerprint (PMF) spectrum of warm water extracts (indexed peptides identified using the lettering system of Buckley* et al. *2009).*

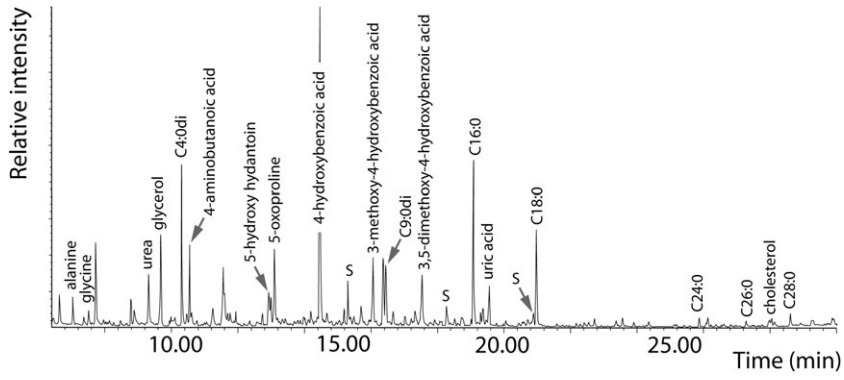


Figure 5 *The total ion chromatogram obtained by GC–MS analysis of the non-protein fraction of the glue.*

no extractable lipid. This suggests that the earlier glue-making process employed in Bukhara did not effectively separate all the lipid from the protein.

4-Hydroxybenzoic acid and its methoxylated derivatives arise from lignin. Biosynthesis of lignin proceeds via *p*-coumaryl alcohol and its methoxylated derivatives coniferyl and sinapyl alcohols (Whetten and Sederoff 1995). Oxidative cleavage of the allylic bonds would lead to the 4-hydroxybenzoic acids found in the glue sample. Extraction of wheat straw with a range of organic solvents yielded a mixture of the oxidation products of the allylic alcohols as well as the

Table 3 *Molecules identified in the glue samples and their proposed source*

| *Product* | *H1* | *F1* | *Source* |
|---|---|---|---|
| 4-Hydroxybenzoic acid | Yes | Yes | Lignin |
| 3-Methoxy-4-hydroxybenzoic acid | Yes | Yes | Lignin |
| 3,5-Dimethoxy-4-hydroxybenzoic acid | Yes | Yes | Lignin |
| Glycerol | Yes | Yes | Lipid |
| Cholesterol | Yes | Yes | Lipid |
| Butan-1,4-dioic acid | Yes | Yes | Lipid |
| Nonan-1,9-dioic acid | Yes | No | Lipid |
| Straight chain acids: | | | |
| C14:0 | No | Yes | Lipid |
| C16:0 | Yes | Yes | Lipid |
| C18:0 | Yes | Yes | Lipid |
| C24:0 | Yes | Yes | Lipid |
| C26:0 | Yes | Yes | Lipid |
| C28:0 | Yes | Yes | Lipid |
| Urea | Yes | Yes | Urine |
| 5-Hydroxyhydantoin | Yes | Yes | Urine |
| 5-Oxoproline | Yes | Yes | Urine |
| Uric acid | Yes | No | Urine |
| Alanine | Yes | No | Uncertain |
| Glycine | Yes | No | Uncertain |
| 4-Aminobutanoic acid | Yes | No | Uncertain |
| Various sugars | Yes | Yes | Uncertain |

Table 4 *Mass spectral data for the urine components in the glue*

| *Compound* | *Molecular ion ($M^{+\cdot}$)* | *Base peak* |
|---|---|---|
| Uric acid (*tetrakis*-TMS) | *m/z* 456 | *m/z* 73 |
| Urea (*bis*-TMS) | *m/z* 204 | *m/z* 147 |
| 5-Hydroxyhydantoin (*tris*-TMS) | [M-15] 317 | *m/z* 73 |
| 5-Oxoproline (*bis*-TMS) | *m/z* 273 | *m/z* 156 |

three hydroxybenzoic acids detected in the glue (Lawther *et al*. 1996). We can thus conclude that the hydroxyacids are lignin-derived.

Some components, such as amino acids and sugars, could have arisen from more than one of the components in the original mixture, so their source must be a matter of speculation. Much more interesting is the presence of components from urine in the samples: not just urea, and uric acid (in one sample), but the minor components 5-oxoproline (Meister 1978) and 5-hydroxyhydantoin (Wiseman and Halliwell 1996). The mass spectral data for the urine components are shown in Table 4.

The two samples were chosen from different locations within the mosque complex and one sample was taken from the interior of a *muqarnas*. This eliminates the possibility of contamination of the glue by animals or birds. We thus postulate that urine was deliberately added to the glue by the builders during its preparation.

When collagen glue is made up, it is allowed to soak in water overnight, during which time it swells. The weight of water used is about 1.8 times the weight of glue. It is then heated in a double pot, where the outer pot holds water close to boiling. This ensures that the glue in the inner pot is maintained at ~60°C. This is essential, as the glue must be applied hot. As the temperature falls, the glue's viscosity increases and its efficiency drops. At about 40°C, the glue solidifies and becomes unworkable (heating readily reverses the process). The glue sets in a two-part process, first during the cooling process, and second during drying over a 12–24 h period as water is lost by evaporation, but mostly by absorption into the wood. The glue retains moisture and can be converted back to liquid even after a century or more (Edwards 2001).

It should be noted that all the molecules extracted from the collagen contain polar groups (hydroxyl, amino or carboxyl) that are capable of hydrogen bonding to proteins. Furthermore, the lignin-derived materials must have diffused into the protein from the wood. It would be reasonable to assume that some diffusion took place in the opposite direction. Some molecules, most notably urea, are water soluble and may well have been partially absorbed into the wood during drying. Conversely, there are no acylglycerols present in the protein, only free fatty acids and glycerol. There are possible explanations for this. During the preparation of the glue, the lipids would separate from the aqueous phase. However, conversion of collagen to gelatin involves treatment with alkali, then with acid, followed by prolonged boiling. This could result in partial hydrolysis, with the production of free fatty acids. These may bond to the protein rather than separate with the acylglycerols. Also, hydrolysis of any acylglycerol present may have taken place over time. Intact acylglycerols cannot form hydrogen bonds to protein and so may tend to diffuse into the wood. It is clear that a meaningful quantitative estimation of the various components in the mix would require analysis not only of the glue, but of the neighbouring wood fraction. Wood samples from the *muqarnas* were not available to us.

The need to apply the glue whilst hot is not a serious problem if the area being glued is small, but becomes a major problem when applying a thin layer of glue over large areas and manoeuvring large objects into place, or in applying veneering. There is evidence that early 19th century European cabinet makers added urea to their glue when applying veneers to large wood surfaces. It is known that adding 5–10% urea by weight extends the gel time and produces a liquid glue at room temperature (Edwards 2001). Addition of urea to glue does not significantly affect the glue strength (Schofield 2007*)*. It has also been shown that addition of urea to dilute gelatin solutions lowers the viscosity of the mixture (Henriques *et al*. 1996). We believe that the use of urea was known to the builders of Bukhara at an earlier date.

The composition of urine varies considerably, even from the same individual, during the course of a day, depending on diet and exercise levels. One study of nine active males showed levels of urea varying from 1.3 to 3.5% during a 7 h period, with an average of about 2% (Moller *et al*. 1928). To test whether this level of urea would be effective, the following experiments were carried out. Three samples of pearl glue were prepared using identical quantities of glue and water. However, to one sample was added urea equivalent to 2% of the water volume and to a second sample, 4%. These concentrations correspond to 3.3 and 6.7% of the glue used. Spreading the glue at low temperatures on to wood indicated quite clearly that this did improve the spreading properties so that the glue could be used at temperatures as low as ~30°C. In a room at 23°C, this approximately doubled the time period in which the glue was usable.

To confirm this observation, accurate viscosity measurements were carried out (Mezger 2006). The results clearly show (Fig. 6) that over the range of temperatures studied and at all shear rates, the viscosity of the glue is significantly reduced by the inclusion of 2% urea. However, increasing

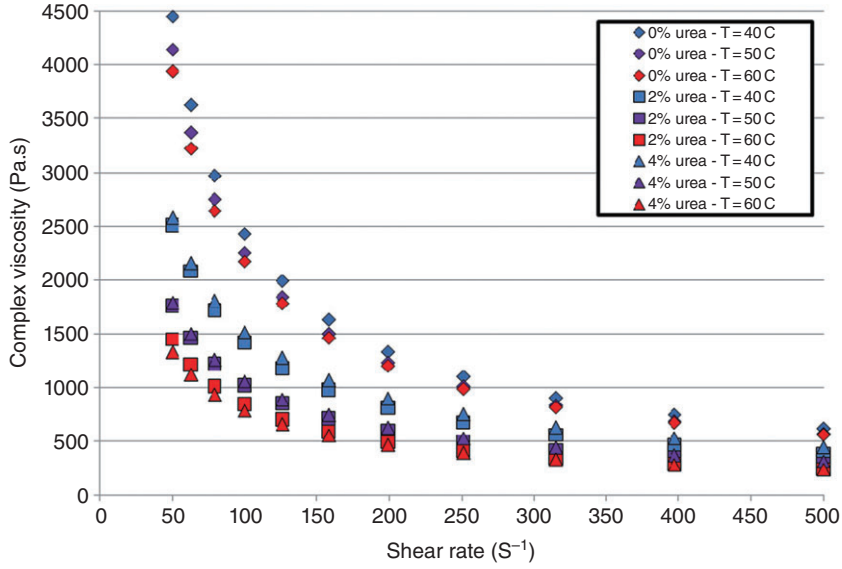


Figure 6 *Complex viscosity versus shear rate data obtained on collagen-based glues containing 0, 2 and 4% urea at temperatures of 40, 50 and 60°C.*

the urea concentration to 4% did not markedly reduce the viscosity further. It can also be seen that the addition of urea makes the glue viscosity four times as sensitive to temperature within the tested range. This confirms that incorporating urine in the glue would improve its performance and could be used as supplied without any need to increase the urea concentration by evaporation.

## CONCLUSIONS

Our research has shown that original construction techniques can be determined, thus aiding the overall goals of the Bukhara project, especially with regard to its eventual restoration. In particular, it demonstrates that the builders not only used an animal-based collagen glue but understood how to modify its viscosity by using urine. The reduction in viscosity would enable the glue to remain spreadable at lower temperatures, thus significantly increasing the time available to glue and assemble relatively large objects. The Bukhara craftsmen had discovered a technique to improve the ease of fixing large objects using crude sources of animal protein and urea at a period in time significantly before it became known in Europe, when industrial-scale manufacturing meant that purer components became available.

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