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Gas Chromatographic Analysis of Organic Residues from Vessels found in the Period 4 Pithos Building at Kissonerga-Mosphilia

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SUMMARY

A preliminary investigation into the use of pottery vessels recovered from a pithos building at the site of Kissonerga-Mosphilia, Cyprus was undertaken using gas chromatography (GC). Twenty-four sherds, identified by ware and type, were analysed. Evidence of lipid and particularly wax residues were found on the inside and outside of most potsherds examined. →

BACKGROUND

The excavations at Kissonerga-Mosphilia are part of the Lemba Archaeological Project, run by the University of Edinburgh, which has been on-going since 1983. The site at Mosphilia formed part of the chalcolithic Erimi culture which dates from around 3800-2500 BC but evidence from the ceramics found so far indicate that the site was occupied for much longer, from about 4000-2300 B.C., making it the first to show continuity between the Late Neolithic and the Early Bronze Age (Peltenburg 1990?).

A particularly interesting find on this site was made in Period 4, Building 3, dated to the mid-third millennium BC. Like other buildings on this site it is circular (9m dia.) with a central hearth but in contrast to these other buildings, there was an extraordinary amount of ceramic debris found covering the floor at the Northern end. It was also evident that the hearth had not been used for its original purpose and was instead choked with unfired clay fragments. The excellent ceramics preservation was due to the dry environment and the building being destroyed suddenly by fire causing it to collapse inwards. Although this event was physically severely damaging, the buildings' ceramic contents remained undisturbed until their excavation.

The ceramic debris has since been classified into 4 categories, in line with other ceramics on the site: Coarse Ware (CW); Coarse Painted Ware (CPW); Spalled Ware (SW); and Red and Black Stroke Burnished Ware (RBSB). These are then further classified by vessel type, eight of which have been identified within building 3 (appendix 1). The vast majority of the vessels were identified as CPW, many of which were of the same type, designated no. 6, called pithio. These pithio are very large storage vessels and so Building 3 has been interpreted as

(not listed in ref)

a storage room or pithos. About 20 smaller vessels of various types and wares were also found; some were probably for storage, others might have been scoops. These conditions offer a unique chance to study their ceramics and to learn not only about the different types of vessel but their use and contents as well.

Study of the floor of the building showed that it was designed to hold 11 pithio in two rows along a wall, but approximately 40 pithio were found, the majority being located around the hearth. The most likely explanation would be that this arrangement was temporary. Initial calculations of vessel diameter and floor space clearly demonstrated that these vessels would have needed to be stacked. This would suggest that they were empty because if they had been filled, access to the contents of upper level vessels, or any at the back, would have been extremely difficult. It is also doubtful if the lower level of vessels would hold the extra weight of stacked full vessels (Peltenburg 1990).

Routine flotation carried out over the whole area yielded only a few pistachio seeds, giving little in the way of clues as to the possible contents of the vessels (Peltenburg 1990). Visual examination of the vessel sherds did not reveal any obvious residue, nor any charred material as might be expected after a fire. A selection of sherds were analysed by gas chromatography to examine any organic residue which may have been trapped within the pottery matrix (appendix 2). Studies have shown that because unglazed ceramics are porous, they absorb their liquid organic contents (Evershed et al, 1990). After initial degradation, such as oxidation, lipids appear to remain relatively inert for an indeterminate number of years in the right conditions, notably those which are anaerobic, which inhibit microbial-induced degradation. Although lipids are known to survive in water-logged conditions, hydrolysis ultimately leads to the breakdown of the original triacylglycerols to their individual free fatty acid components making identification of the source much more difficult (ref). The dry conditions at Kissonerga-Mosphilia greatly reduced the effects of hydrolysis and therefore were particularly favourable for organic residue preservation. + red

METHOD

All the sherds had been cleaned with a brush by the excavators but some had also been washed (details not available). The sherds had been wrapped in newspaper and stored in cardboard boxes prior to analysis. To minimise further contamination from handling, plastic gloves were worn while sampling the sherds. Cotton gloves would be a better alternative and are suggested for future work. This was done with a dental drill (Diprofil) fitted with a flat diamond file. Samples weighing between 300mg and 500mg each were taken from three parts of a sherd; two separate areas of the inner surface and the third from the outer surface. The approximate area sampled was 4mm^2 (squared) x 0.5mm depth. Areas which had been glued or numbered were avoided to prevent interference from adhesive or varnish. The basic morphology of the sherd was not disrupted by this sampling technique to enable future vessel reconstruction.

Lipids were extracted from each powdered sample by dissolving it in 5ml 2:1 chloroform:methanol, adding $50\mu\text{l}$ 1mg/ml n-tetratriacontane in cyclohexane as an internal standard. After 15 minutes in an ultrasonic bath, followed by centrifuging, the supernatant was decanted and the solvent removed by vacuum rotary evaporation. The residue was reconstituted with $200\mu\text{l}$ cyclohexane and $100\mu\text{l}$ were stored at -20°C for potential further analysis. To monitor contamination, a reagent blank sample was included in each batch of 5 samples extracted during sample preparation and analysed at the same time. Before being injected into the GC, each sample was derivatised with $50\mu\text{l}$ (BSTFA + 1% TMCS).

The gas chromatograph (Hewlett Packard 5890 series II) was equipped with a cool on-column injector and flame ionisation detector. The oven was programmed to start 50°C for 2mins before ramping to 350°C at $10^\circ\text{C}/\text{min}$ and holding this for 10mins. Two cross linked methyl/silicone capillary columns were tried. Column 1 (12m x 0.22mm i.d., $0.10\mu\text{m}$ film thickness) gives good separation of total lipids. Column 2 (10m x 0.25mm i.d. $0.15\mu\text{m}$ film thickness) was used solely because wax references had been separated with it in previous analysis. Although the chromatographic parameters of this column were very similar to those used for the total lipids, it was thought worthwhile to use. $1\mu\text{l}$ of each sample was injected into the GC using a silica needle syringe and each sample run at least twice. Because site evidence for what the vessels contained was negligible, (analysis focused on evidence of total lipids (TL) and later waxes.) A total lipid reference of a select number of pure free fatty acids, mono-, di- and tri- acylglycerols and sterols were used to profile the sherd samples (appendix 3). A limited range of natural waxes were also available for comparison.

RESULTS

The majority of blank samples analysed not only contained the internal standard n-tetratriacontane but also an additional unidentified peak with an approximate retention time (t_r) of 19.5mins (fig 1). The amount of this component varied from sample to sample, ranging from 0% to over 69%. This peak was also present in a number of sherd samples and has consequently been assigned to contamination from an unknown source, presumably picked up during sample preparation.

Analysis with column 1 revealed a number of distinct chromatogram profiles amongst the sherd samples. Profiles 1 and 2 featured in the majority of the samples. Typical chromatograms are shown on figures 2 - 10.

- Profile 1** A cluster of peaks eluting with $12\text{min} < t_r < 18\text{min}$ (fig 2). Major peaks appear to elute at the same time but the peak areas vary significantly between samples, quantification is necessary before direct comparisons can be made. A variation of this profile displays a significant peak at approximate t_r 13.6mins (1a, fig 3). An underlying 'bump' often appears, this has been attributed to low volatility components from the previous run.
- Profile 2** A series of smaller peaks with $19\text{min} < t_r < 26\text{min}$ either evenly spaced (2a, fig 4) or irregular (2b, fig 5).
- Profile 3** A group of peaks with $32\text{min} < t_r < 37\text{mins}$ seen in all three samples from one sherd (fig 6). This profile was more distinct with column 2 (fig 7).
- Profile 4** A number of compact peaks eluting $19\text{min} < t_r < 21\text{min}$ (fig 8). It was not always consistent between each run. Unfortunately the interference peak co-eluted with one of these components making it disproportionately large.
- Profile 5** This appears to be very different but profiles 1 and 2 are still evident but less consistent in peak numbers. There were also additional peaks representing large quantities of additional components not present in any of the other sherds (fig 9).

The following table based on vessel type and ware, summaries the findings. In the majority of cases, the inside and outside surface profiles compared favourably. Assignment of profiles has been as consistent as possible but is subjective.

CPW/mono, type 5

Sherd	Position	Profiles
13	Below Rim	1, 2a, 4

The basic profile was evident but profile 4 dominated and overlapped profile 2. This type of vessel was similar to the common pithio but it was smaller and had a flat base, rather than a curved one.

CPW/mono, type 6

Sherds	Position	Profiles
1	Body	1, 2a
2	Body	2a
3	Base	1, 2a
7	Body	1, 2a
8	Body	1, 1a, 2b
9	Body	1, 2a
12	Body, Near Rim	1, 2b, 4
14	Body	1, 2a
15	Lower Body	1, 2a, 4
16	Rim	1, 2a, 4
20	Body	1, 2a, 4
24	Body	1, 2a

These sherds are the pithio vessels. The quantities of the components of both profile 1 and 2a varied ~~to~~ a little. The distinct profile found in sherd 13 shows up again in 12, to a lesser extent in 15 and 16 and small amounts are also detected in 20 and 24. This trend appears to coincide with vessels with little or no evidence of a profile 2a.

CPW/mono, type 23

Sherd	Position	Profile
6	Neck Base	1, 2a

This is the only example of a collared jar. Profile 1 was prominent with a trace of profile 2.

CPW/mono, type 30

Sherd	Position	Profile
11	Body	1, 1a, 2a

This sherd has been identified as a similar type to sherd 21, a saucer, but analysis has shown them to be different. Profile 2a is only a trace.

CW/mono, type 6

Sherd	Position	Profile
22	Body	1, 1a, 2a,

This sherd is physically very similar to the pithio (CPW/mono, type 6) and displays the same organic profile.

CW/massive, type 30?

Sherd	Position	Profile
21	Body	1, 2b(a?), 3

It was impossible to tell the inside from the outside of this sherd because one side was covered in soil. This meant that the sampling strategy was a little different. 21A was taken from one side and included surface deposits: 21C, from directly below 21A was a sample of ceramic material only. 21B was taken from the other side and included some soil. Profile 3 was dominant for 21B, less prominent for 21A and even less for 21C.

RBSB, type 2

Sherd	Position	Profile
10	Rim	1, 1a, 2a, 5
19	Lower Body	1, 1a, 2a, 5

These two examples of hemibowls demonstrated a very distinct profile not displayed in the other sherds. This profile was also found on the outside of the sherds.

RBSB, type 17

Sherd	Position	Profile
23	Rim	1, 1a, 2a

Although the same ware as sherds 10 and 19, this sherd comes from a spouted bowl. There is only a slight trace of profile 2a.

SW, type 7

Sherd	Position	Profile
4	Lower Body	1, 1a, 2a
17	Body	1, 2a
18	Body	1, 1a, 2(?)

Despite being the only sherds analysed from what have been identified as flasks, the profiles are very similar to the majority of other sherds analysed. Sherd 18 has an extra 'bump' in the chromatogram covering much of the range where the profile 2 would be expected. This profile is quite distinct in sherds 4 and 17.

SW, type 20

Sherd	Position	Profile
5	Unknown	1, (1a?), 2a

This sherd is from a much simpler storage vessel than that represented by sherds 4, 17 and 18 and could well have had a different contents. Profile 2 appears to be more prominent in this sherd but can not be compared to the other sherds until quantified.

have been
the results

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The residues from the outside of each sherd were very similar. Profile 1 is of a fairly consistent size, sherds 4, 11, 12 and 14 all have profile 1a. Profile 2a is fairly consistent as well. This is all backed up by the second set of tests which, because there appeared to be little difference between them, was only carried out on a selection of the sherds, 6, 14 and 18. 10 and 19 were also analysed because their profiles were similar to their inside ones.

Some sherds appeared to have been a brush-painted surface on both sides, most notably on no. 20. X-ray fluorescence spectroscopy was used to compare the red inorganic compounds on the surface of sherds ?????? with the pottery body to see if there was a noticeable difference which might indicate a painted finish.

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The dominating element was found to be iron, present in similar quantities in both the body matrix and the surface (figure 10).

* Get details from Phyllis about the instrument settings.

DISCUSSION

The chromatograms for both sides of sherds 10 and 19 (Hemibowl) match each other (example, figure 9), and are very similar to natural beeswax analysed by Evershed et al. (Evershed, 1993) with the same GC parameters (figure 11). Ideally a sample of natural beeswax should have been analysed in this work to account for small differences in column behaviour, but lack of time precluded this. However, it seems fairly conclusive that these sherds were part of pottery which had been in contact with natural beeswax, although it would be prudent to confirm this finding. Other significant peaks, distinct from the wax profile, were found in these samples and indicate at least one other, as yet unidentified, residue.

Beeswax is composed of relatively inert hydrocarbons, saturated free fatty acids and several wax esters (figure 12), giving it good chemical stability with little variance between different sources (Tulloch, 1972). This accounts for its frequent occurrence in historical and archaeological artifacts, supported by it being readily available in abundance to many cultures (Needham and Evans, 1987). Beeswax would have been ~~have~~ suitable for water-proofing porous pottery intended for storage or transport of liquids (Rice 1987), which could explain its presence on sherds 10 and 19.

Chemically treated beeswax, was used in antiquity as an emulsion for wall and picture painting described by Kuhn (1959), a recipe for which is told in accounts by the historian Pliny (Pliny, from Kuhn, 1959). After bleaching, possibly as part of the purification process by exposure to sun light which would break down the components responsible for its yellow to brown colour, may also be to soften it slightly. The wax is heated with an alkali to hydrolyse the wax esters to their long chain free fatty acids and alcohols (figure 13). This saponification converts the wax so that it forms an easily-worked emulsion with water (the most common solvent which would have been available in ancient times) as a medium for pigments and is referred to as punic wax. The chromatogram of saponified beeswax would be lacking in the late-eluting wax ester components (in this instance, those peaks eluting between 26 and 30 min) with a corresponding appearance of significant amounts of palmitic acid as its free acid (palmitic acid accounts for some 60% of total untreated beeswax composition in the wax esters) as shown in figure 14 (*chromatogram of the bleached beeswax reference). Interestingly, profile 2 found for the majority of sherds examined in this work has a similar chromatogram to saponified beeswax, the palmitic acid showing in profile 1, and is very distinct for samples from sherds 1 and 2, type 6 (CPW/mono) and 5 type 20 (SW), to a lesser extent 4 and 17, type 7 (SW) and all outside samples, excluding 10, 11 and 19 which are discussed elsewhere. This could imply that they had been painted with such an emulsion and the pottery does appear to have some type of slip or glaze on both sides. Present knowledge as to whether a slip or glaze was used on this type of pottery is limited (Bolger, 1990). Other sherds from this site are currently being analysed using X-ray diffraction analysis to try to solve this. Preliminary X-ray fluorescence analysis found iron in significant amounts on both the surface and within the matrix. This could suggest a slip but further analysis would be needed. A wax resist for pottery decoration is also another possibility (Rice), although the type of ware from this period in Cyprus is not thought to be decorated. It must be noted that the tentative suggestions assume that a wax is present, but these findings must be confirmed by mass spectrometry or other structural-identification analytical technique before the evidence is conclusive.

* Date
1987

Only samples of the sherd from a saucer (21) displayed a series of peaks where triacylglycerols would be expected to elute (between 32 and 37 min), which was designated profile 3. Assuming that the assignment of significant quantities of palmitic acid, oleic acid and stearic acid to peaks from retention times is correct, this information together suggests the presence of a food lipid. This profile was found to a greater or lesser extent on all samples from this sherd, implying that food residues were present on both sides of the vessel. Further analysis using column 2 indicated a profile very similar to Japan wax standard. Japan wax is a fat derived from the berries of various species of Sumach tree (*Rhus Succedanea*, *Toxicodendron Vernicifluum*, *Annam Urusgi* and *Rhus Sylvestris*) found in India, China and Japan. Although it is unlikely that any of these places would have traded with Cyprus C 2000 BC, it may be possible that similar species of trees were local in the Mediterranean, although plant waxes are not known to be used in Europe until 1990's AD (Kuhn 1959).

Peaks with the same retention times as palmitic, oleic and stearic acids (the most common fatty acids in nature) were identified along with other peaks in the free fatty acid region of the chromatogram in every sample chromatogram. These could be solely from saponified beeswax (if identified correctly), although profile 2a is not always evident, or additional other sources such as food residues. Further analysis is required to confirm this interpretation, but other site evidence must be taken into consideration. Building 3 has been identified as a storage area and would therefore be expected to hold a fairly common product, especially if stored in bulk like the number of pithio suggests. Cereal, wine and olive oil are likely commodities for Cyprus at this time. Dry products, like grain or flour, are extremely unlikely to leave a residue, either absorbed or surface. There is no evidence so far of charred cereal grains from the site, but why should the small range of vessels examined be water-proofed or decorated on both sides if intended for dry contents? Dry contents cannot, however, be dismissed because it could be argued that the vessels were intended for liquids, but were used for something else instead.

Wine is unlikely to have been stored in the wide-necked pithio, but even so would be expected leave some kind of sticky residue on the base sherds. Not necessarily though, wine is mostly water and alcohol and will therefore evaporate leaving very little identifiable residue making it difficult to identify. It could have been stored in a smaller vessel (eg flask?). Modern Cypriot populations use pithio to store olive oil (Peltenberg, personal communication), and olive oil residues has been identified in a number of cases using GC/MS (Condamin et al 1976) by the presence of palmitic acid, palmoleic acid, oleic acid, linoleic acid and stearic acid, although these are the basic composition of a number of foodstuffs besides olive oil. It is possible to work it out by ratio (Condamin, 1976) but because the samples analysed were small and each component degrades at a different and unknown rate, also different sources produce different ratios, there is also the fact that the component found may have more than one source. On studying the shape of each vessel analysed, types 7 (sherds 4, 17, 18), 20 (sherd 5) and 23 (sherd 6) seem to be the most likely to hold liquid because of their narrow necks. A small number of goats were kept at this time in Cyprus (Croft, 1990), but they are not thought to have been a very important part of the economy. If profile 1 represents fatty acids as suggested above then it is possible that myristic acid and lauric acid were identified in a number of sherds. These are found in milk and may mean that goats were exploited to a small extent for their milk which, although this is unlikely because it would have gone off too quickly. It is more likely to have been produced on smaller scale. It is possible that milk was used to seal the inside surface of new vessels (Rice 1987), but this has not been explored.

Chromatograms for sherds 5?, 6?, 12, 13, 15, 16, 20? and 22? have a distinct profile (no. 4)(fig 8). No theories about its origin have been made. *

Quantification of the amount of organic residue per sample is necessary to be able to directly compare relative amounts, and this will be part of future work on this material.

The sherds were cleaned with a brush and sometimes with water as well, the use of water was stopped when it was realised that it was found to be causing the edges of the sherds to crumble more rapidly. It is not known which of the sherds analysed here were washed with water but if any were then it may have biased the results. Although lipids are not soluble in water, the water may still have removed any residue (Evershed 1993). An outline of all procedures for the collecting, handling and preparation of sherds is given in Heron (19??). *

The different types of vessel analysed demonstrated a variety in thickness and porosity which may have affected the amount of residue absorbed, as could the consistency of the liquid being stored and the time it was in contact with the vessel. *(The area + depth of sample taken may have affected the results, if most residue remains on surface or sinks into clay)* It has been suggested (Heron, Evershed and Goad 19??, 20) that ceramic material which was unlikely to have had contact with the vessel contents should also be analysed as a 'validation study'. These could include kiln wasters or the handles of the vessels. This would be a check to find if the lipids came from the surrounding soil, tests (Heron 19??) have shown this not to be the case, or as has been suggested through organic matter in the clay or organic temper. *

CONCLUSION

This preliminary gas chromatographic analysis of organic residues from the small selection of early bronze age Cypriot, pithio and other assorted vessel, has indicated that organic materials are present. Natural beeswax was identified on two sherds representing a bowl or scoop, and it would appear that a saponified beeswax is common to the majority of samples from the other sherds. There is also evidence for foodstuffs, but all of these identifications

need to be confirmed by further analysis with a structure-specific technique such as gas chromatography-mass spectrometry or nuclear magnetic resonance spectroscopy.

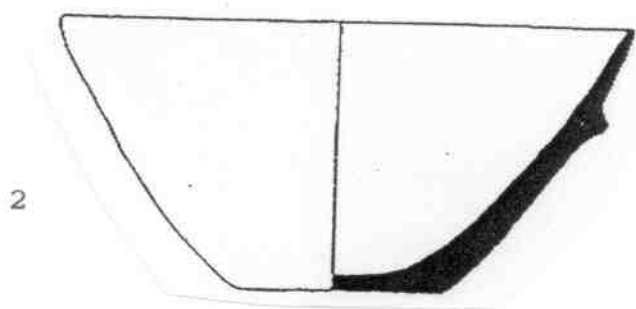
The sherds selected for analysis represent the pottery identified so far. They seem to reflect the quantity of each found, the majority were pithio with one or two examples of each smaller type of vessel. Consequently, it was impossible to say if those analysed were typical examples because in most cases there was nothing to compare the results to. A more logical way to approach it would have been to pick one vessel type or ware and concentrate on variations in these.

Further Work

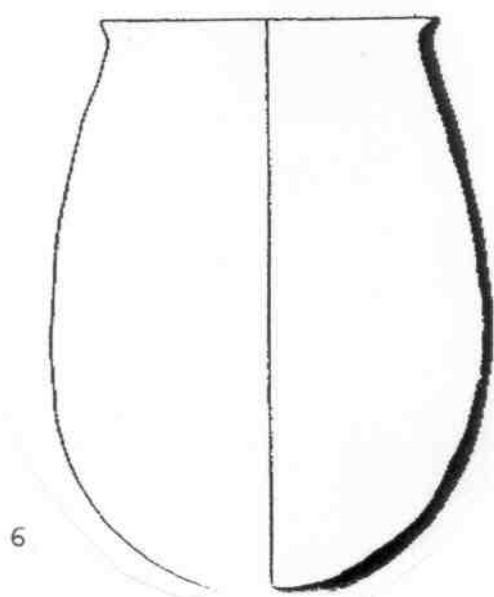
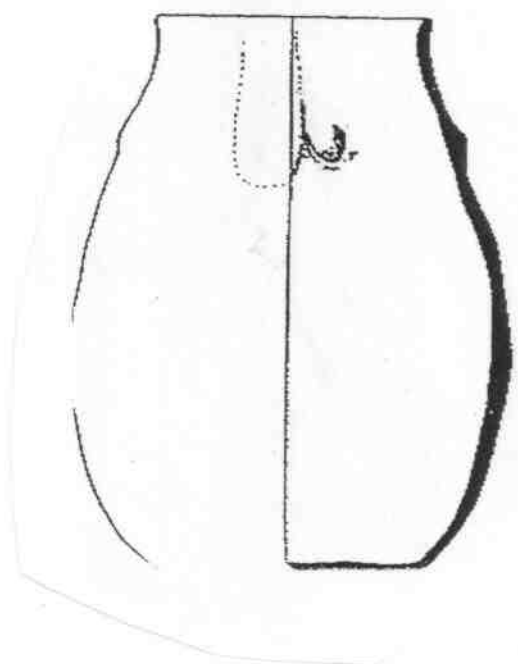
Another possibility would be to test for gums, this has not been done but the presence of vegetation residue may indicate that gums were there, because they are originally derived from trees. (look up gum profiles and what's written about them) They have not shown so far because they are not soluble in cyclohexane but in water and would therefore have to be extracted separately and further analysis carried out to find out if they were present. If some or all of the sherds have been washed then any gums are likely to have been removed.

The inorganic content will be further explored using SEM, it is hoped that this will give more information about the components of the paint or slip layer found on the sherds, maybe even defining which it is and therefore the possibility of the beeswax or punic wax being a paint binder. FTIR will be used to analyse any colour pigments other than iron.

Appendix 1 - Classification of vessel types



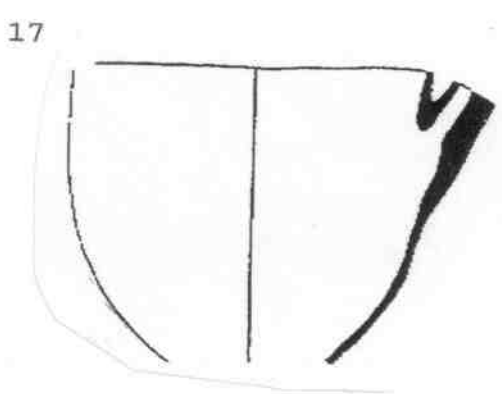
HEMIBOWL



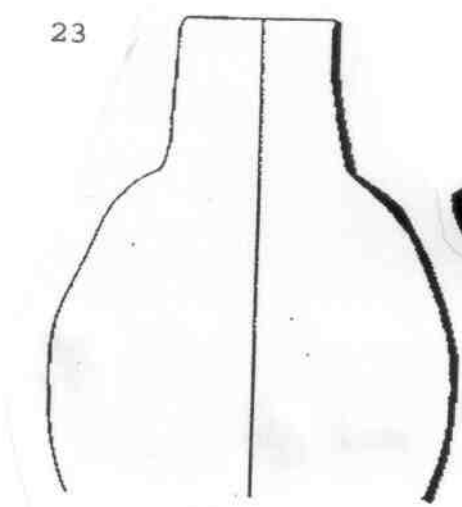
PITHIO



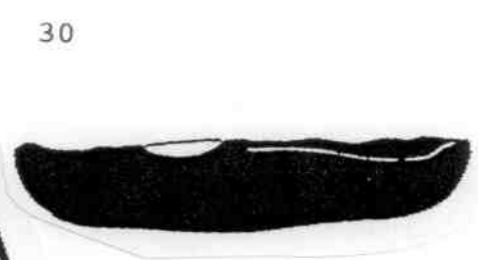
FLASK



SPOUTED BOWL



COLLARED STORAGE JAR



SAUCER

SCALE = 10cm