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# HUMAN SALIVA AS A CLEANING AGENT FOR DIRTY SURFACES

Paula M. S. Romão, Adília M. Alarcão and César A. N. Viana

Abstract—The use of human saliva to clean dirty surfaces has been an intuitive practice for many generations. The authors have established the scientific basis for this practice by means of qualitative tests and chromatographic techniques.  $\alpha$ -amylase was found to be the main constituent responsible for the cleaning power of saliva and therefore amylasic preparations obtained from bread or from microorganisms were tested as saliva substitutes.

#### **1** Introduction

Saliva has long been widely used as a cleaning agent for all kinds of surfaces and has shown good performance, especially on gold-leaf objects [1, 2]. At the Instituto José de Figueiredo (Lisbon, Portugal) it was noticed that some conservators preferred their own saliva to any other solvent for cleaning fragile painted layers on low-fired ceramics (clay objects), painted cork, and weakened gold-leaf surfaces.

Their arguments were that by so doing they obtained cleaner surfaces without damaging them or their supports, as can occur with the cleaning agents usually employed in conservation. In order to obtain scientific support for these statements and possibly a more hygienic substitute, a research project was initiated [3].

# 2 Experimental

# 2.1 Solubility and resistance tests

Solubility and resistance tests [2, 3] were performed on five gilded and polychromed sculptures dating from the eighteenth century, in order to register in a qualitative way the effect of different agents applied on various surfaces, namely saliva, white spirit, 2-methyl heptane (iso-octane), methyl benzene (toluene) and dilute ammonia (see Table 1).

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#### 2.2 Ion-exchange chromatography

Saliva was collected with a plastic syringe and subjected to refrigerated (T = 4°C) centrifuging (10,000g, 50 minutes), and the supernatant was dialyzed against NaCl. 6ml of this solution were applied to a carboxymethylcellulose (MCM, Sigma, pre-swollen, 1meq g<sup>-1</sup>) column (0·7 × 10cm) previously equilibrated in 5mM disodium phosphate and 1mM acetic acid, pH 5·9 [4]. The elute was made up with 0·1M sodium chloride (rate = 10ml h<sup>-1</sup>) and each fraction of 3·8ml was analyzed for protein (OD<sub>280</sub>) and amylase activity [3, 4].

# 2.3 Testing the enzymatic fractions

The solubility and resistance tests described above were carried out with the enzymatic fractions obtained by ion-exchange chromatography, as well as with the following substances:

- 1 Dialyzed (against NaCl) and denatured  $(T = 80^{\circ}C)$  saliva.
- 2 Amylase extracts from the soft part of the bread [5], bakers' yeast, potato [5], and *Bacillus subtilis* [6].
- 3 Albumin, glycine and gelatine, 5% solutions in water.

# 2.4 Thin-layer chromatography

The aim of these experiments was to determine the lipids of the dirt removed by saliva after rubbing the surface of the object with wet cotton-wool.

The dirty swab was washed with dichloroethane and  $10\mu$ l of the resulting solution were applied to a glass slab covered with previously activated silica. The slab was placed vertically into a glass chamber containing the eluent (hexane, diethyl ether and acetic acid, 85:15:1). The eluent was allowed to rise until it reached 1cm below the top and the slab was subjected to resolution in the iodine chamber [7].

Surface	Cleaning agent	Solubility tests
Tempera		
blue	saliva	±
	2-methylheptane	±
	xylene	+
	white spirit	+
brown	saliva	+
	2-methylheptane	±
	xylene	±
	white spirit	±
red	saliva	±
	2-methylheptane	+
	xylene	±
	white spirit	<u>+</u>
white	saliva	+
	2-methylheptane	-
	xylene	$\pm$
	white spirit	+
Oil painting		
black	saliva	+
	2-methylheptane	±
	xylene	<u>+</u>
	white spirit	± ± +
carnation	saliva	+
	2-methylheptane	—
	xylene	_
	white spirit	
green	saliva	+
	2-methylheptane	
	xylene	—
	white spirit	_
red	saliva	+
	2-methylheptane	$\pm$
	xylene	-
	white spirit	±
Gold leaf	saliva	+
	2-methylheptane	<u>+</u>
	xylene	<u>+</u>
	white spirit	+
	2-methylheptane	
	+ xylene (2:1)	+
	2-methylheptane +	
	white spirit (3:1)	土
	ammonia + water	
	(1:1)	-
	(1:3)	_
	(1.5)	_

#### Table 1 Results of the qualitative tests

#### **3** Results and discussion

The results obtained with the qualitative tests (Table 1) confirmed saliva as the 'best' cleaner for the surfaces tested, especially for the gilded ones [8]. Nevertheless, attention must be drawn to the fact that red and blue mat surfaces were slightly attacked, showing that saliva dissolves vermilion and azurite.

The mechanism of the cleaning process with saliva can be described as follows [3]:

- 1 Enzymatic action: some substances are solubilized in water when saliva enzymes catalyze dirt degradation.
- 2 Washing action: as a result of aqueous activity.

The enzymatic action was confirmed by testing the fractions resulting from ion-exchange chromatography. Salivary  $\alpha$ -amylase was separated by this technique and the qualitative results were compared to those obtained with normal, dialyzed and denatured saliva, and also with the amylase extracts listed above.

All the amylase extracts showed similar behaviour which was essentially identical to saliva. Dialysis did not affect saliva cleaning power (dialysis removes phosphate and bicarbonate ions and it only affects saliva buffering power). Thermal denaturing breaks down enzyme activity and so denatured saliva presented a 'negative answer' to the qualitative tests.

The thin-layer chromatography experiments confirmed that fatty acids and phospholipids are the main lipid components of dirt and that they agglutinate organic (mostly proteins) and inorganic residues [9]. That is one of the reasons for saliva having good cleaning power on dirty surfaces: one class of enzymes—lipases—catalyzes degradation of fatty substances and another class—hydrolases—catalyzes degradation of hydrolytic substances [10].

However, one of the hydrolases— $\alpha$ amylase—seems to be principally responsible for the excellent cleaning power of saliva, which may enable the latter to be replaced by an amy-

Solubility tests: '+' = a 'positive' answer for the cleaning agent applied, i.e., no pigment dissolution, good cleaning power and/or no penetration into the paint layer; '-' = the reverse; ' $\pm$ ' = an intermediate situation.

Pigments [8]: blue = azurite,  $2Cu_2CO_3(OH)_2$ ; brown = brown ochre, Fe<sub>2</sub>O<sub>3</sub>; red = vermilion, HgS; white = lead white, Pb<sub>2</sub>CO<sub>3</sub>(OH)<sub>2</sub>; black = charcoal, C; carnation = lead white + ochre; green = malachite, Cu<sub>2</sub>CO<sub>3</sub>(OH)<sub>2</sub>.

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lase preparation [3, 5]—from *Bacillus subtilis*, for instance—or, in some cases, by a gelatine solution (5% in water).

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