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Two archaeometric methods for cellulosic textile finds using enzymatic test

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Abstract
A biosensoristic approach to the problem of dating cellulosic finds is reported. Two methods are proposed both based on the intramolecular modifications of the cellulose molecule resulting in increased degrees of carboxylation and of methylation with age. By calibration with referred samples it is possible to date unknown materials. The first method uses carboxylic group as immobilizing agent of enzyme molecules the amount of which is evaluated by the reaction catalysed by the enzyme. The second adopts a demethylating enzyme as focal component of the sensor measuring the concentration of a marker compound obtained by the methylation of a target molecule due to the methyl groups removed by the enzyme from the sampled cellulose.

Keywords: dating, enzyme, textiles, cellulosic finds, biosensor

1. INTRODUCTION

Our research consisted in designing, setting up and applying two methods for the scientific dating of textiles of archaeological and historical interest. The work must be considered as a possible future way of dating cellulosic finds, even if being at the present at a not complete assessment and definition.

The first method is based on an enzymatic biosensor, previously designed and applied by us to the dating of wood finds [1] and extended here to the case of cellulosic textiles. The principle of the method is that the number of carboxylic groups in cellulosic material increases with the passage of time, due especially to the intramolecular oxidation, so that it is proportional to age. As the presence of these groups allows an enzyme to be covalently bound to the cellulosic textile, it follows that the immobilised enzymatic activity is higher, as older is the sample. This activity can be evaluated by the analysis of the enzymatic reaction (for instance if enzyme is glucose oxidase the final product is gluconic acid to produce which oxygen is consumed).

The second method is based on SAMT (S-adenosylmethionine-transmethylase), an enzyme isolated from *Penicillium chrysogenum*. It was experimentally found that the alkylation degree of β-D-glucosidic monomers of cellulose (more particularly the presence of methyl and carboxyl groups in cellulosic textiles) increases with sample age. It was also observed that demethylation, catalysed by the SAMT enzyme, in the presence of SAM (S-adenosylmethionine), is more pronounced as older are the textiles. This process leads to the breakdown of SAM into adenosine and homocysteine. If the amount of adenosine generated by the reaction is determined, it is found to be proportional to the age of the sample.

2. SAMPLES

The cellulosic textile samples consisted of linen and rope, dating respectively to the 8^{th}, 14^{th} and 16^{th} centuries, kindly supplied by Moscow University, Turkish Museums and private collections, together with linen samples from more recent periods (20^{th} and 21^{st} century).

3. METHODS

3.1 Biosensor method

The method is based on the correlation between cellulose carboxylation, which progresses with time, and the ability to bonding between the carboxyl groups produced and an enzyme. Quantities of enzyme bonded may be evaluated by the electrode's response to oxygen, whenever the enzyme, as in this case, belongs to the oxidase group. To achieve this, an enzyme [2], glucose oxidase, which catalyses the reaction:

$$C_6H_{12}O_6 + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} C_6H_{12}O_7 + H_20_2 \quad (1)$$

is immobilized on the cellulosic material being tested.

Therefore, if glucose is added in excess to the solution in which the electrode containing the cellulose sample with the immobilized enzyme is dipped, reaction (1) will take place.

The O_2 variation with the time (that means O_2 consumption rate) due to reaction 1 detected by the transducer (Clark electrode), is proportional to the immobilised enzyme

activity, which itself correlates with the number of carboxyl groups on the cellulosic material and thus with its age.

To this purpose, 8 mm disks were cut out of the linen samples. These disks were then weighed and placed in small beakers, where they were subjected to enzymatic immobilization treatment using carbodiimmide [3].

The cellulose sample with the immobilized glucose oxidase was then sandwiched between the gas-permeable membrane of the Clark electrode and nylon netting which was then secured to the electrode cap using a rubber O-ring. After assembly, the biosensor was connected to the power supply and the measuring device and was then ready for use. It was then immersed in a beaker containing 10 mL of acetate buffer 0.1 mol/L at a pH = 5.1. The electrode was allowed to be stabilized for about 10 min in these conditions till to constant signal; this state was indicated by a plateau on the recorder. A micropipette was then used to add 1.0 mL of glucose 1 mol/L, that is, a strong excess of substrate. The addition of substrate led to a diminution of the dissolved oxygen concentration as a result of reaction (1). As soon as the glucose was added a chronometer was started and amounts of oxygen (in ppm, i.e. parts per million) were recorded every 10 sec. At the same time, a recorder connected to the potentiometer plotted the corresponding curve. The signal was then allowed to reach a stationary state, for which a time of about 8 min was necessary.

Using pre-referenced samples and plotting the oxygen consumption rate (due to the enzymatic reaction) versus the sample age, it was possible to record a calibration curve for which the age of unknown samples can be determined.

3.2 SAMT method

It is known that the structure of ancient textiles contains chemically modified β-D-glucose residues. In particular, it was found that cellulose alkylation increases with textile age [4].The method used to date the cellulosic material consists in allowing the previously washed and air dried sample to react with a solution containing the enzyme SAMT (S-adenosylmethionine-transmethylase), which catalyses the demethylation reaction in presence of SAM (S-adenosylmethionine) and THFA (tetraidropholic acid), a cofactor that, by forming a complex, THFA-CH_3, facilitates the subsequent demethylation of the cellulose. The older the ancient textile the more methyl groups it contains.

Therefore, in presence of the enzyme SAMT and under these reaction conditions, the textile sample is demethylated, that is, the SAM ↔ SAH equilibrium is shifted towards the SAH (S-adenosylhomocysteine) [5]. The latter is then broken down into adenosine and homocysteine.

$$\text{cellulosic material} \xrightarrow{\text{SAMT+SAM+THFA}} \text{demethylated cellulosic material + adenosine + homocysteine}$$

The amount of adenosine thus released will be higher for the older textiles and lower for the younger ones. The adenosine released is determined either by the thin layer chromatography (TLC), using ammonia/acetic acid /acetone/ n-butanol/chloroform (3:2:5:4:1) (v/v) as eluent and densitometric measurement (TLC-UV at λ = 254 nm) or better by the high – performance liquid chromatography (HPLC), which allows amounts of nmol order to be measured [4]. Therefore, using referenced, that is, dated samples, it was possible to plot an archeometric curve showing the quantity of adenosine released, expressed in (μmol/g of dry material) 10^{-3}, versus the age of the sample expressed in years.

The operating conditions used for the HPLC measurements are shown in table 1.

TABLE 1. Conditions of HPLC analysis of adenosine

Analytes	Adenosine (ADO), SAM and SAH
Column	Supelcosil LCI8 (25 cm x 4.5 mm)
Mobile phase	20% MeOH, 80% solution: 20 mmol/L sodium phosphate buffer (pH=5.5), 1mmol/L EDTA, 3% MeOH
Flow rate	1 mL/min
Detection	UV λ = 254 nm

4. RESULTS AND DISCUSSION

Results obtained with the biosensor method

By applying the (glucose oxidase) biosensor to textile cellulosic materials experimental values were obtained that allowed to construct the archeometric curve shown in fig. 1.

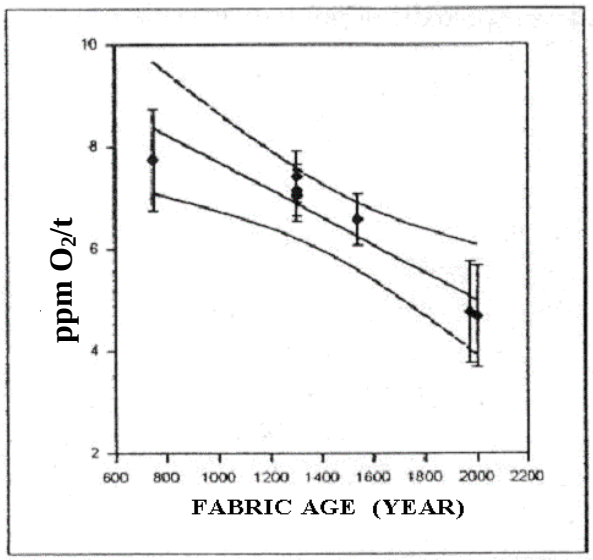

Figure 1 Archeometric curve for the biosensor method.
Equation and confidence limits:
Y = (-0.0027 ± 0.0004) X + (10.4 ± 0.7);
Y = mg/L of O_2/t
X = year r^2 = 0.8868; t = 8.77; (1 –a) = 0.95

Results obtained with the SAMT method

Using dated samples and the SAMT method, experimental values were obtained which could be used to construct an archeometric curve, as shown in fig. 2.

Really, we performed two curves in two different laboratories of our Department.

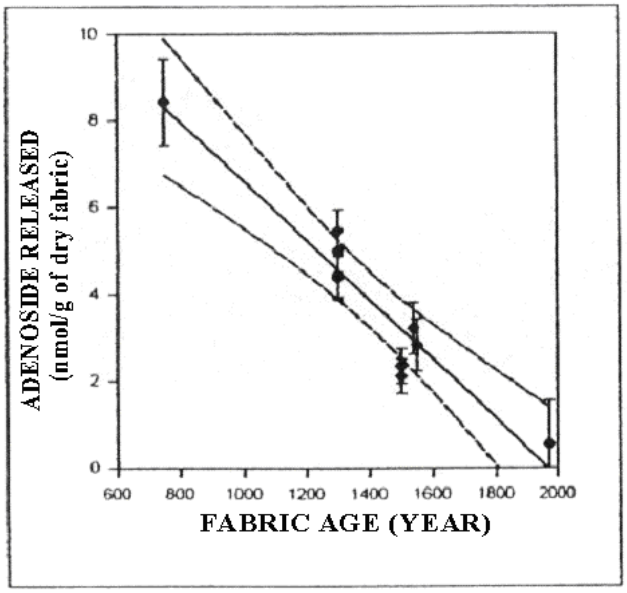

Figure 2 Archeometric curve of SAMT method. Equation and confidence limits:
Y = (-0.00687 ± 0.0007) X + (13.5 ± 1.0);
Y = nmol/g of adenosine;
X = year $r^2 = 0.9222$; t = 14.37; (1 –a) = 0.95

The two curves show fairly good agreement. The slope of the two straight lines are very similar. The r values is slightly higher in the case of the curve obtained by more homogeneous samples. We have also evidance that a positive effect on r is obtained with a purer SAMT enzyme.

It must however be added that sample,T3, colored green deviates from the line. In the hope of identifying the reason for this, photographs were taken under the electron microscope; by comparing this image with that one of the SEM photomicrograph obtained for a sample which instead gave positive result, it seems likely to hypotheses that former is covered by a layer of some unknown substance. Further studies are under way to clarify whether this is the reason for the apparent blocking of the SAMT-catalyzed reaction. In any case, the results confirm the proportionality between the quantity of adenosine released and sample age, which allows ancient textiles to be dated on the basis of the cellulosic material they contain.

5. CONCLUSION

By an original and innovative method based on the enzymatic reaction catalysed by SAMT an archeometric curve was obtained. It can be used to date amount less then normal of ancient textiles of unknown age on the basis of the adenosine released during the reaction. With a reproducibility of about 85%. Also in the case of the biosensor method an archeometric curve was recorded for archaeological textiles which can be used to evaluate unknown ages with a precision of about 90%. Comparison of the sensitivity of the two methods in terms of variation of mole number to be revealed per year shows that the SAMT method is about one hundred thousand times less sensitive than the other. The first method is also faster, simpler and cheaper than the first one, and can be used on smaller amounts of sample.

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