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# Interactions between inorganic pigments and rabbit glue in reference paint reconstructions

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# Interactions between inorganic pigments and rabbit glue in reference paint reconstructions

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## Abstract

We characterised the thermal degradation of rabbit glue, a collagen-based protinaceous material used as a paint binder in paintings. Paint reconstructions of the glue on its own or mixed with, azurite ($Cu_3(CO_3)_2(OH)_2$), calcium carbonate ($CaCO_3$), hematite ($Fe_2O_3{\cdot}nH_2O$) and red lead ($Pb_3O_4$) were analysed using a thermo-analytical approach. This method enabled us to investigate the interactions between the glue and pigments before and after artificial indoor light ageing. The study was carried out using thermogravimetry analysis and differential scanning calorimetry. The results highlighted that all the inorganic pigments interact with rabbit glue, thus decreasing the thermal stability of the binder. Light ageing further decreased the thermostability of pigmented paint replicas suggesting a moderate increase in the rate of the degradation.

**Keywords** rabbit glue, azurite, calcium carbonate, hematite and red lead, thermogravimetric analysis, differential scanning calorimetry.

## Introduction

Proteinaceous materials have been used as paint media to disperse and apply pigments since the Bronze Age [1]. Animal glue, egg and milk or casein are the most common proteinaceous binders used as painting media [2], although many other protein-containing materials have been found as media in polychrome artistic objects, including garlic [3] and animal blood [4, 5]. The characterization of paint binders in general, and proteinaceous ones in particular, is complex due to the sample size, the high inorganic content, the degradation phenomena undergone with time, the simultaneous presence of other organic materials, etc. [2]. Fluorescence spectroscopy [6], Raman spectroscopy [7, 8], infrared spectroscopy [9], immunological techniques [10-12], gas chromatography mass spectrometry [2, 13, 14], MS-based proteomic approaches [15-18] are the most common approaches. The main difficulties arising when analysing protein-containing samples relate to the fact that changes take place in the course of ageing, which are influenced by the pigments present [2, 10, 16, 19-24]. A loss in solubility [2], changes in the molecular profiles [16], and analytical interferences have been observed [19]. The chemical reasons for these phenomena are still unknown, despite their importance in designing the analytical procedures and data interpretation models required.

This study is the part of a research project carried out on pigmented paint reconstructions using proteinaceous binders aimed at clarifying how these binders age also as a consequence of interactions taking place with pigments. Our previous research focused on the thermal degradation of paint reconstructions containing ovalbumin and casein both pigmented and

unpigmented, analysed fresh and artificially aged [23, 24]. In this study we focus on animal glue, specifically rabbit glue, and its interactions with the inorganic pigments commonly used in paintings.

Collagen-based animal glues have been widely used in artworks, serving as adhesives, binding media for pigments and consolidants for organic and inorganic materials. They are natural polymers obtained by the extraction and partial hydrolysis of the protein constituent collagen of animal hides and bones. Collagen in its natural state is a triple helix protein. It is water insoluble and must be conditioned to be solubilised. The conversion of collagen into soluble glue involves breaking the intra- and intermolecular polypeptide bonds through the use of acid or alkali and heat. The collagen–glue transition is a stepwise process involving the melting of the trihelical network to an amorphous form, followed by the sequential hydrolysis of various types of covalent bonds [25]. In glue aqueous solutions, the polypeptide chains take up random mainly linear configurations [26].

We prepared paint reconstructions of the glue binder on its own and mixed with four inorganic pigments (azurite ($Cu_3(CO_3)_2(OH)_2$), calcium carbonate ($CaCO_3$), hematite ($Fe_2O_3 \cdot n H_2O$) and red lead ($Pb_3O_4$)) on a glass support. The paint reconstructions were then characterized by thermogravimetry (TG) and differential scanning calorimetry (DSC). TGA and DSC were performed on solid-state samples before and after artificial ageing in a Solarbox (indoor light ageing). These techniques have been successfully used in the field of cultural heritage for a wide range of different materials [23-24, 27-32] and were thus chosen to investigate the modifications undergone by rabbit glue due to the effects of the pigments and artificial ageing.

## Experimental

### Materials

Paint reconstructions were prepared using rabbit glue (53921) purchased from Bresciani srl (Milan, Italy) on its own or mixed with azurite($Cu_3(CO_3)_2(OH)_2$), calcium carbonate ($CaCO_3$), hematite ($Fe_2O_3$) and red lead ($Pb_3O_4$). The glue was dissolved in water and heated in a bain-marie until a clear/fluid solution was obtained. The pigment was mixed with the fluid binder in proportions that produced a paintable paste. The paint and the pure glue were then applied with a brush on microscope glass slides. A set made up of each type of pigment/protein replica and of pure glue was analysed before and after artificial ageing in the Solarbox (see next section) and then stored at room temperature in the laboratory.

## Apparatus and methods

**Thermogravimetry.** A TA Instruments Thermobalance model Q5000IR equipped with an FTIR (Agilent Technologies) spectrophotometer Cary 640 model for evolved gas analysis (EGA) was used. TG measurements were performed at a rate of 10 °C/min, from 40 °C to 900 °C under air flow (25 ml/min). The amount of sample in each TG measurement varied between 2 and 4 mg. TG-FTIR measurements were performed at a rate of 20 °C/min, from 40 °C to 900 °C under nitrogen or air flow (90 ml/min), in the range 600-3000 $cm^{-1}$ with a 4 $cm^{-1}$ width slit. A background spectrum was taken before each analysis in order to zero the signal in the gas cell and to eliminate the contribution due to the amount of ambient water and carbon dioxide. The amount of sample in each TG-FTIR measurement varied between 4 and 10 mg.

**Differential Scanning Calorimetry**. A Perkin-Elmer differential scanning calorimeter Pyris Diamond was used. Solid samples (4-8 mg) were sealed in aluminium pans and scanned from 150 °C to 550 °C at 5 °C/min under air flow. Empty pans were used as references. The calorimeter was calibrated with indium as a standard.

**Solarbox**. The Solarbox (1500e RH, Erichsen, Germany) was used to artificially age the paint replicas. The exposure conditions were 720 h at 25 °C, 50 % relative humidity (RH) and irradiance 550 $W/m^2$. A Soda-lime glass UV filter was used to simulate indoor exposure. Irradiation uniformity was ensured by a parabolic reflector chamber with the xenon lamp in the focus.

## Results and discussion

### Characterization of the paint replica with pure glue

The thermo-oxidative decomposition of rabbit glue samples occurs according to two main degradation steps, as already found for other proteinaceous paint materials [23, 24] and collagen from bovine Achilles tendon [33]. Figure 1 shows a comparison between the degradation curves and the corresponding derivative curves recorded by TG for the unaged and aged animal glue paint replica, and Table 1 summarises the experimental temperatures and the percentage weight loss of the thermal degradation steps obtained.

In addition to water loss (a mass loss of about 10% below 100 °C), Fig. 1 highlights a step in the 250-350°C range, and one around 540°C, due to protein decomposition. The step at 250-350°C is characterized by a broad DTG peak that is significantly lower than the one at 540°C. This suggests that the maximum rate of mass loss is lower at lower temperatures because the degradation products are formed more slowly. The higher rate of mass loss above 500°C is due

to the easier oxidation with rapid formation and release of volatile compounds, as expected for the complete combustion process. By analysing the DTG curves, it is clear that both the degradation bands are constituted by the overlapping of multiple unresolved peaks, most likely related to different degradation pathways. At 600°C almost all the sample is decomposed, although a small residue remains (2-5%), most likely due to inorganic material.

The TG curves of the glue recorded in the nitrogen atmosphere showed a single pyrolytic decomposition (DTG peak at 325°C) with a final residue around 20 %.

The gaseous species evolved by thermal degradation of the animal glue were analysed in TG-FTIR experiments both in air and in nitrogen atmospheres. Figure 2 shows the FTIR spectrum of the gas evolved at 325°C under nitrogen. In air we observed almost the same degradation products although the bands are overwhelmed by the predominant signal due to $CO_2$ (data not shown). $H_2O$, $CO_2$, $NH_3$, HNCO can be identified in the FTIR spectrum in Fig. 2 together with molecules containing aliphatic chains (aliphatic C-H stretching bands at 2968-2883 $cm^{-1}$; aliphatic C-H bending bands at 1414-1326 $cm^{-1}$) and carbonyls (C=O stretching broad band at 1690-1730 $cm^{-1}$ presenting a maximum at 1710 $cm^{-1}$). Similar results have been reported for the thermal decomposition of collagen [33] and casein [34] with the exception of C=O stretching bands. It is possible that the carbonyl stretching vibrations observed in Fig. 2 can be attributed to the radical cleavage of peptide bonds leading to the formation of volatiles amides and free carboxylic acids.

Figure 3 shows the DSC curves in air flow for the unaged and aged rabbit glue unpigmented paint reconstruction. The two plots have very similar shapes with a series of exothermic bands due to the decomposition of the proteinaceous binder. The first broad and slightly exothermic band (200-350°C) matches the first TG decomposition step. However, in the temperature range 425-550°C, where the complete combustion occurs, the DSC thermogram reveals three distinct peaks, whereas the DTG plot shows a narrower unresolved signal in the range (475-525 °C). The first band is due to the overlapping of a pyrolytic process and oxidative degradation due to the oxidation of fragments of the peptidic backbone, while the second band is due to complete combustion [24, 34]. The thermal stability of rabbit glue is not significantly influenced by artificial ageing, unlike what has been observed for ovalbumin and casein [24].

A previous study on the thermal decomposition of bovine tendon collagen [33] showed similar results, although the decomposition steps take place at higher temperature and the DSC peaks have a more simple shape. The thermo-oxidation of collagen-based materials shows clear differences due to the different source of the collagen as well as to different degree of hydrolysis related to the different treatments that the protein undergoes in order to be solubilised for the glue binder preparation.

**Table 1** Experimental temperatures and percentage weight loss of thermal degradation steps of unaged and aged pure glue and pigmented glue in the temperature range 250-750 °C.

| Step No. | Temperature of the step (wt. loss ) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Glue** | **Aged Glue** | **Glue/ $Cu_3(CO_3)_2(OH)_2$** | **Aged Glue/ $Cu_3(CO_3)_2(OH)_2$** | **Glue/ $CaCO_3$** | **Aged Glue/ $CaCO_3$** | **Glue/ $Fe_2O_3$** | **Aged Glue/ $Fe_2O_3$** | **Glue/ $Pb_3O_4$** | **Aged Glue/ $Pb_3O_4$** |
| **1** | 298°C (45%)[a] | | 299°C (38%)[a] | | 299°C (30%)[a] | 305°C (33%) [a] | | | 295°C (29%) | 296°C (31%) |
| **2** | 339°C (45%)[a] | 315°C (45%)[a] | 326°C (38%)[a] | 335°C (39%) | | 323°C (33%) [a] | 320°C (19%) | 324°C (18%) | | |
| **3** | 539°C (42%) | 528°C (40%)[b] | 500°C (27%) | 494°C (27%) | 499°C (27%) | 505°C (28%) | 429°C (20%)[b] | 427°C (19%)[b] | 494°C (28%) | 490°C (30%) |
| **4** | | 544°C (40%)[b] | | | | | 464°C (20%)[b] | 470°C (19%)[b] | | |
| **5*** | | | | | 648°C (12%) | 635°C (11%) | | | 708°C (1.2%) | 709°C (2.2%) |

* Degradation step due to unreacted pigment; [a] Total weight loss in the range 200-400°C; [b] Total weight loss in the range 400-500°C.

**Characterization of the pigmented paint replicas**

The thermal behaviour of unaged and aged unpigmented rabbit glue replicas is compared in Fig. 4 (DSC curves) and in Fig. 5 (DTG curves) with freshly prepared or aged pigmented paint reconstructions. Both techniques prove that the pigments interact with the glue, leading to an overall decrease in its thermal stability.

In all DSC curves the first exothermic band (200-350°C) shows a two-step degradation (inserts Fig. 4 a and b). In the case of the unaged unpigmented paint reconstruction, two signals are visible at 280°C and 306 °C, respectively. The presence of a pigment modifies the band profile. This modification is limited in hematite and calcium carbonate paint replicas, whereas a significant shift in the signals to lower temperatures is observed in red lead and azurite paint replicas (insert Fig. 4 a). In all aged replicas, the whole exothermic band is slightly shifted to higher temperatures compared to the band observed in unaged replicas, though maintaining a similar shape.

The main effects of the pigments on the thermal stability of the glue binder observed on the peak at higher temperature, attributed to proteinaceous binder combustion. Both DTG and DSC show that pigments shift this peak to lower temperatures. This indicates that all the pigments, both in the form of salts ($Cu_3(CO_3)_2(OH)_2$ and $CaCO_3$) or oxides ($Pb_3O_4$ and $Fe_2O_3$), interact with glue by decreasing its thermal stability. DTG curves of calcium carbonate paint replica show a peak above 600°C due to the thermal decomposition of free $CaCO_3$ [35]. The decrease thermal stability is particularly evident in the case of the hematite paint replica, which shows the highest decrease in thermostability to the unpigmented glue. This suggests a strong interaction between hematite and glue, probably due to iron's involvement in metal-polypeptide coordination. It has been shown that the iron metal centre can interact with histidine and with the oxygen atoms of proline and hydroxyproline whose content in collagen is particularly high compared to other proteinaceous binders [36]. As has already been observed in the case of red lead/ovalbumin and azurite/casein paint replicas [24], the stronger the binding of the metal centre with nitrogen or oxygen donors of specific amino acid residues, the easier the thermal degradation of the protein. Two concurrent effects are probably responsible for this behaviour: i) the metal-heteroatom coordination weakens the adjacent bonds in the polypeptide backbone and forces the protein into a conformation which is more easily degraded by thermo-oxidative processes and ii) induces a partial disruption of the intermolecular interaction among the polypeptide chains. In most cases adding pigments to glue produces a higher decrease in thermostability than in OVA or casein [24]. As a result, on the basis of the value of the shift of the combustion peak measured for each

proteinaceous binder, we suggest the following order of protein-pigment interaction: glue > casein > OVA.

The decrease in thermostability of pigmented paint replicas is slightly enhanced with ageing, with the exception of glue/calcium carbonate paint reconstructions. This behaviour is quite similar to that of previously investigated casein based paint replicas [24]. In contrast, the ageing effect on OVA pigmented paint replicas was to increase their thermal stability, and was attributed to OVA cross-linking involving the formation of disulphide bridges and dityrosines. We can therefore rule out the effects of cross-linking on pigmented rabbit glue induced by ageing.

The TG-FTIR spectra of the gaseous species evolved by thermal degradation of the animal glue pigmented paint reconstructions show the same bands as the unpigmented paint reconstructions. This suggests that, although the pigments interact with protein leading to a partial disruption of the protein-protein intermolecular interactions, they do not modify the mechanisms of the protein thermal degradation.

## Conclusions

Despite the extensive work on the characterization of collagen [33, 36, 37], to our knowledge, very little has been published on the effects of the interactions between inorganic pigments and animal glue [21], which is the binder obtained from collagen based materials, such as bones, tendons and other tissues. We investigated the interactions taking place between four commonly used inorganic pigments ($Cu_3(CO_3)_2(OH)_2$, $CaCO_3$, $Pb_3O_4$ and $Fe_2O_3$) and rabbit glue in paint reconstructions, using TG and DSC.

We highlighted that the presence of pigments in all cases induces a decrease in the thermal stability of the proteins. As previously observed for OVA and casein [24], we suggest that pigments act directly on the stability of the protein structure with the following order of protein-pigment interaction: glue > casein > OVA. We believe that this is the result of the direct interaction of the pigment with amino acid functional groups, i.e. by forming coordination complexes, and/or the promotion of oxidative stress, which may lead to a global unfolding rearrangement of the proteins.

We found that $Fe_2O_3$ showed the strongest interaction with rabbit glue, probably due to the ability of iron to form stable complexes with histidine and with the oxygen atoms of proline and hydroxyproline residues, whose content in the collagen is quite high compared to other proteinaceous binders. The glue samples were not very sensitive to artificial light indoor ageing, although pigmented paint replicas slightly decreased their thermostability with ageing, however we can rule out any cross-linking on pigmented glue samples induced by ageing.

## ACKNOWLEDGMENTS

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## Figures Captions

**Fig. 1** Thermogravimetric curve (left axis) and its derivative (right axis) of unaged and aged rabbit glue paint reconstruction performed under air flow at a heating rate of 10°C/min.

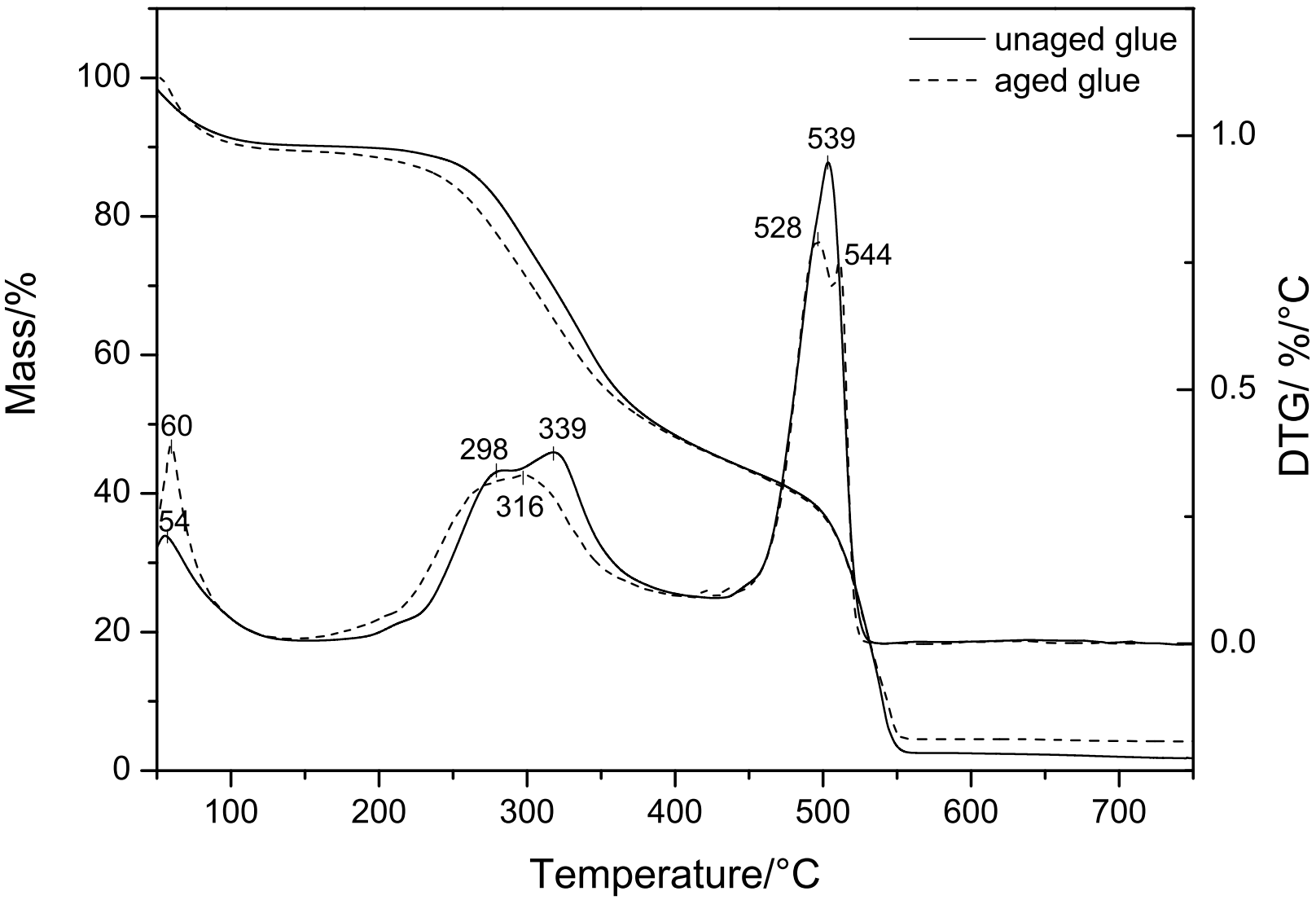
**Fig. 2** FTIR spectrum for rabbit glue thermal degradation at 325°C under nitrogen atmosphere.

**Fig. 3** DSC curves of unaged and aged rabbit glue paint reconstructions performed under air flow at a heating rate of 10°C/min.

**Fig. 4** DSC curve under air flow of unaged (a) and aged (b) rabbit glue pigmented paint reconstructions.

**Fig. 5** DTG curves under air flow of unaged (a) and aged (b) rabbit glue pigmented paint reconstructions.

Figure 1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544

Figure 2

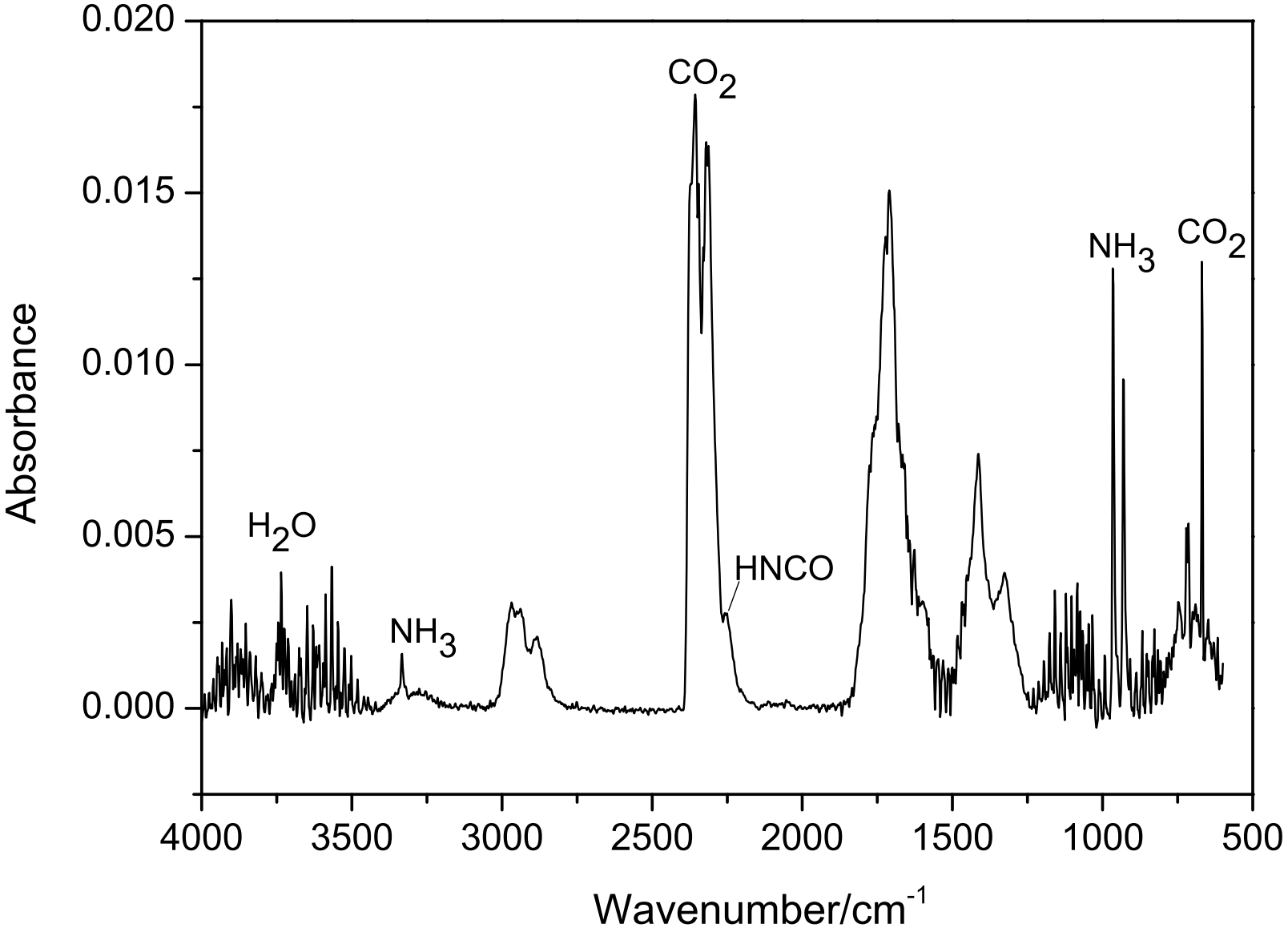

0.020
0.015
0.010
0.005
0.000
Absorbance
$H_2O$
$NH_3$
$CO_2$
HNCO
$NH_3$
$CO_2$
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber/$cm^{-1}$

Figure 3

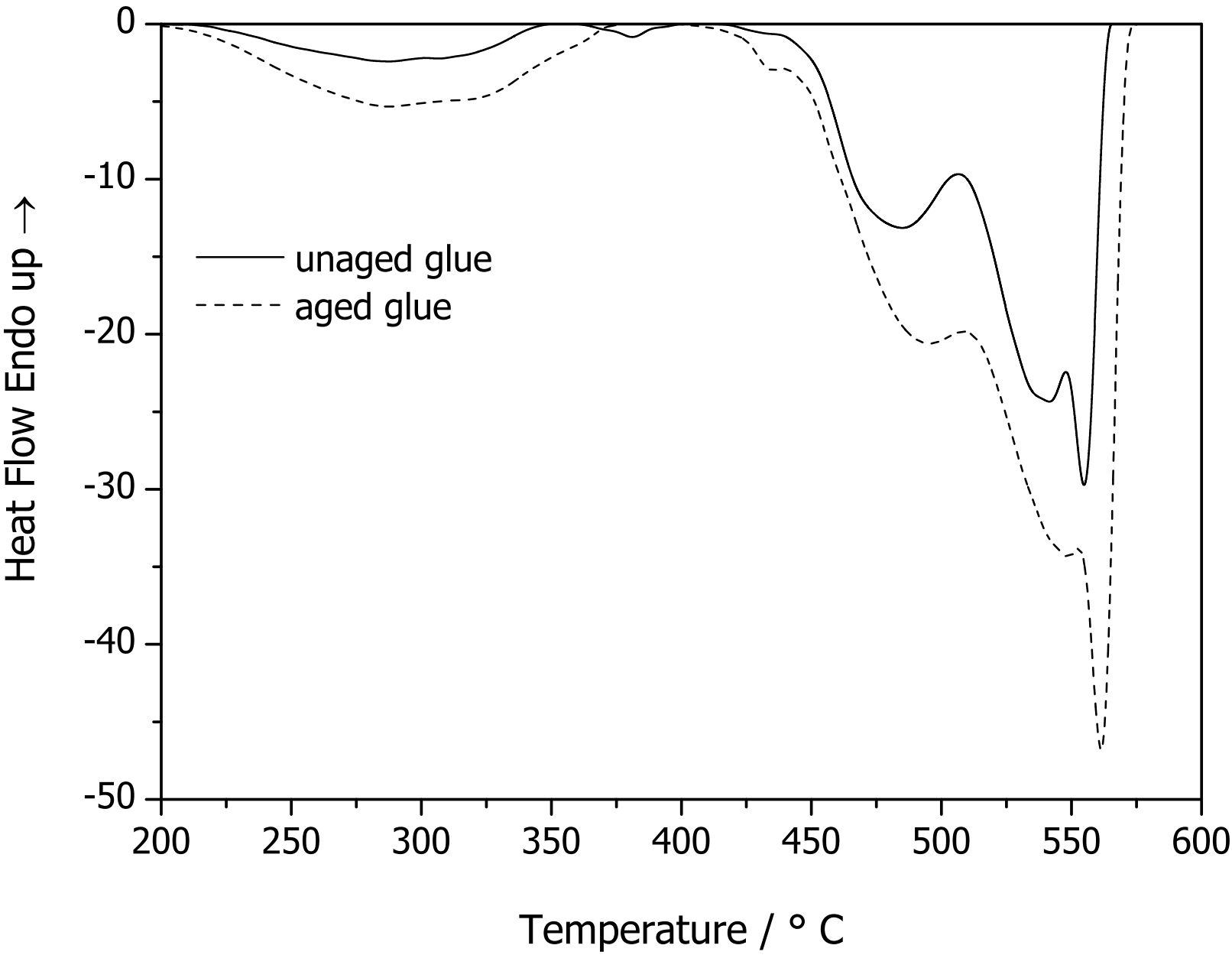

0
-10
-20
-30
-40
-50
Heat Flow Endo up →
unaged glue
aged glue
200
250
300
350
400
450
500
550
600
Temperature / ° C

Figure 4

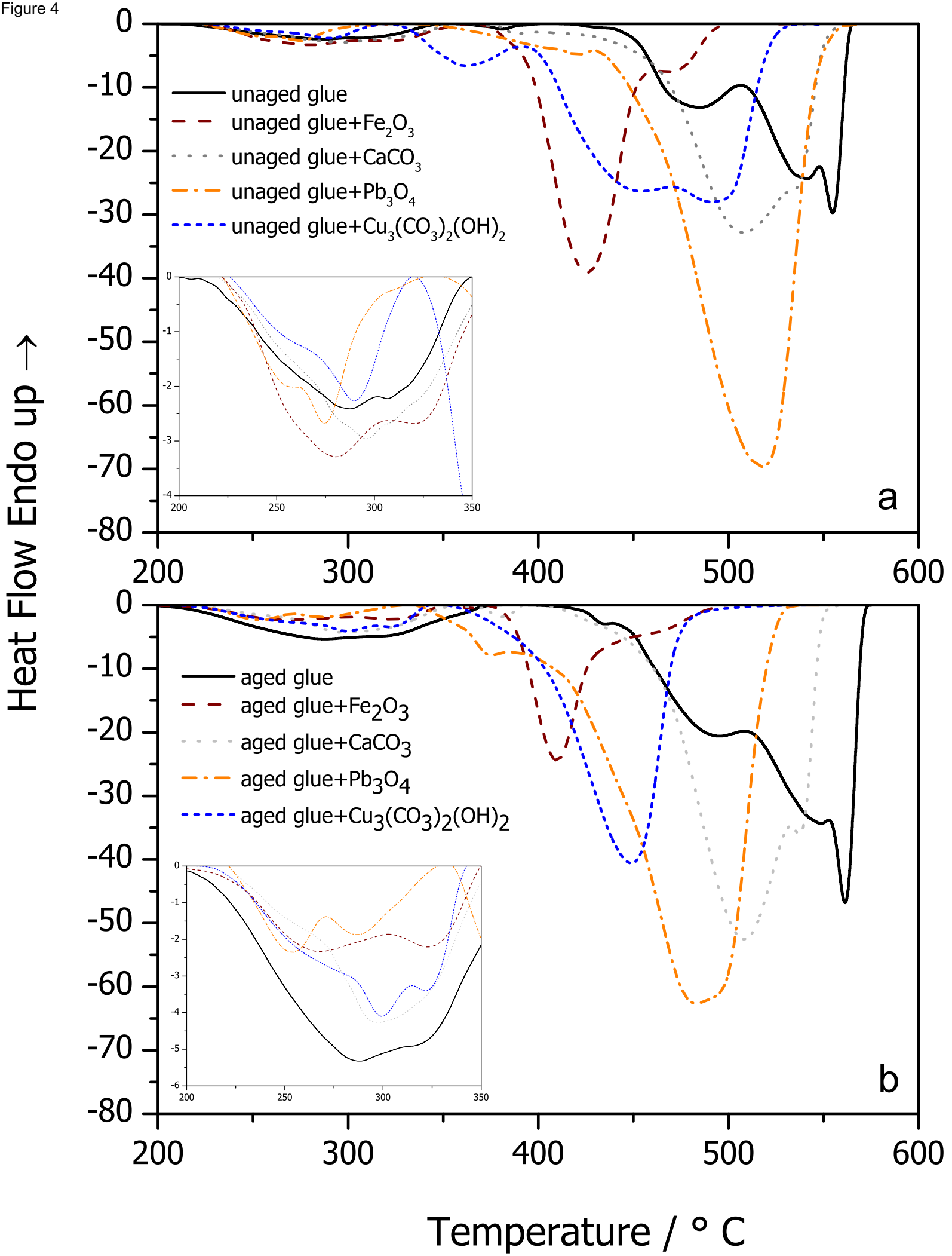
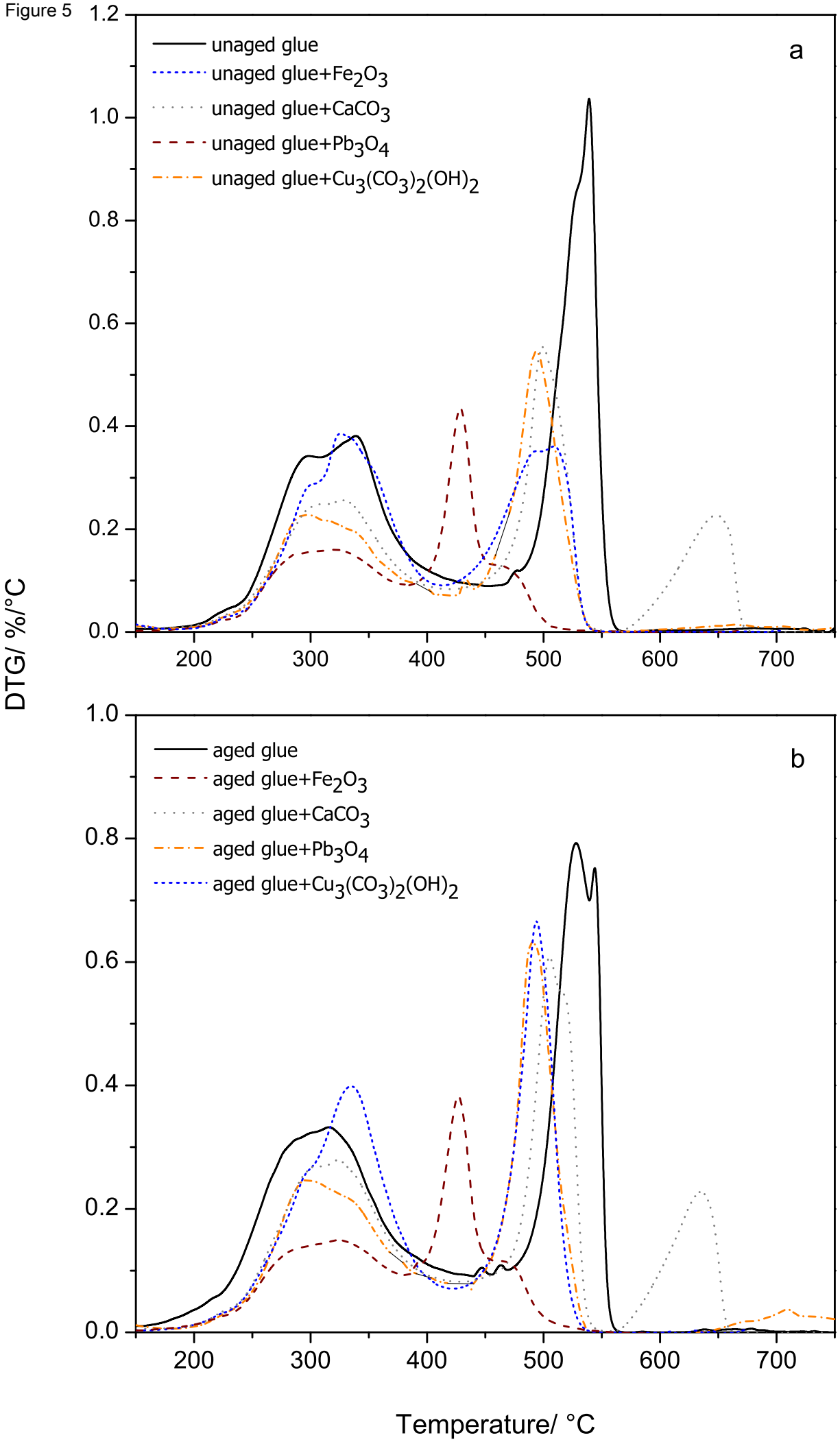

unaged glue
unaged glue+Fe2O3
unaged glue+CaCO3
unaged glue+Pb3O4
unaged glue+Cu3(CO3)2(OH)2
a
aged glue
aged glue+Fe2O3
aged glue+CaCO3
aged glue+Pb3O4
aged glue+Cu3(CO3)2(OH)2
b
Heat Flow Endo up →
Temperature / ° C

Figure 5


a
unaged glue
unaged glue+Fe2O3
unaged glue+CaCO3
unaged glue+Pb3O4
unaged glue+Cu3(CO3)2(OH)2
b
aged glue
aged glue+Fe2O3
aged glue+CaCO3
aged glue+Pb3O4
aged glue+Cu3(CO3)2(OH)2
DTG/ %/°C
Temperature/ °C

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