DNA coatings on cotton fabrics: Effect of molecular size and pH on flame retardancy

UNIVERSITÀ DEGLI STUDI DI TORINO

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ABSTRACT: Deoxyribonucleic acid (DNA) has been mostly considered as a carrier of genetic information and gene expression. Recently, we have demonstrated that this biomacromolecule is very effective in providing cellulosic fabrics with flame retardant features, thanks to its intrinsic intumescent-like behaviour. Here, we have investigated the role played by different parameters on the overall flame retardant features: DNA molecular size, pH of the DNA aqueous solutions, and number of impregnations. For this purpose, herring DNA, at three different molecular sizes, low (100-200 base pairs, bp), medium (400-800 bp) and high (2000-10000 bp), has been selected as model biomacromolecule; its effect as fire protective coating on cotton fabrics has been assessed in terms of resistance to a flame application (through horizontal flame spread tests) and to an irradiative heat flux of 35kW/m^2 (by cone calorimetry). Furthermore, thermogravimetric analyses have been exploited for evaluating thermal and thermo-oxidative stability of the treated fabrics.

The results clearly indicate that, despite a similar composition, the coatings containing low molecular size DNA exert a superior fire protection on the fabric substrate, as clearly indicated by horizontal flame spread and cone calorimetry tests. Multiple impregnation treatment turned out to be more performing than the single one, being equal the final add-on achieved. More specifically, the use of multiple impregnations at pH=4 and 8 allowed achieving self-extinction for 86 and 74% of the tested samples; in addition, 45 and 25% reductions of total heat release and heat release rate peak under 35 kW/m^2 have been found with respect to untreated cotton. These findings may be attributed to themorphology of the low molecular size DNA coating, which shows a greater penetration into the microfibrillar surfaces of the component fibres within the fabric and to its higher thermal stability in air.

KEYWORDS: biomacromolecules; DNA; cotton; green flame retardants; coatings.

1. INTRODUCTION

In the scientific literature, almost all the works related to DNA consider this molecule in its main role as a carrier of genetic information and gene expression; on the basis of this task, DNA has been investigated in very different areas, ranging from medical to forensic field and archaeological science. In particular, it is possible to mention some different applications like DNA-based drugs, recombinant DNA for the creation of industrial microorganisms, DNA for biosensors development, DNA application in the environmental monitoring, and so on [1-5].

Commercially-available DNA can be extracted from different sources (i.e. fish testes or sperm, calf thymus, mouse, human placenta, bacteria) with different purity, affecting, in most cases, the costs.

DNA from herring sperm and testes is commonly applied for biomolecular researches and numerous scientific papers describe the interaction of biologically active small molecules with DNA by non-covalent bonds [6]. Although these studies are of great importance in many areas, the most relevant application refers to the development of anti-tumor drugs ranging from metal ions to dyes like phenothiazinium or curcumin [7-11]. Most of the cited works regard DNA from herring sperm probably because, as reported by Jaffer et al. [12], it is one of the few model DNAs intensely studied to understand drug-DNA binding and it is used as a blocking agent in prehybridization and hybridization procedures to minimize non-specific binding of hybridization probe to membranes and *in situ*.

In the present work, DNA is considered as a polymer as such, consisting of two long chains of nitrogen-containing bases, adenine (A), guanine (G), cytosine (C) and thymine (T), allocated in the inner portion of the skeleton, with backbones made of five-carbon sugars (deoxyribose units) and phosphate groups, placed outside. More precisely, this polynucleotide is exploited, without any genetic implication, as a polymer able to form coatings on cotton fabrics and thus to confer peculiar flame retardant features to cellulosic substrates. This is an outcome of a recent investigation based on the selection of effective biomacromolecules, suitable as environmentally sustainable flame

retardant systems for cellulosic substrates and of the discovery that a DNA coating, applied to these substrates, allows achieving even the self-extinction of cotton when a methane flame is applied.

In this context, relatively high amounts of DNA, as compared to its aforementioned applications in biomolecular researches, are required: for this reason, DNA from herring sperm and testes is preferred.

As a matter of fact, in previous reports, we have clearly demonstrated that this biomacromolecule is very effective in conferring flame retardant features to cellulosics [13, 14]. In particular, in the first pioneering work [13]we have proved that the only application of low molecular size DNA from herring sperm to cotton fabrics (with a final 19 wt.-% dry add-on) by an impregnation/exhaustion method allows enhancing the thermal stability and flame retardant properties of cotton fabrics. Indeed, the flammability tests in horizontal configuration clearly indicated that after two applications of a methane flame for 3 s, the DNA-treated cotton fabrics did not burn at all; in addition, when exposed to an irradiative heat flux of 35 kW/m^2,the fabrics did not ignite [14]. Finally, a 28% LOI value was achieved for the treated fabrics as opposed to 18% of the untreated cotton.

Pursuing this research, the same impregnation method was used for investigating the effect of different DNA add-ons (namely, 5, 10 and 19 wt.-%): the obtained results indicated that10 wt.-% was the minimum DNA add-on value necessary to provide self-extinction to cotton when subjected to horizontal flame spread tests [14].

DNA was then combined with chitosan for building-up Layer by Layer assemblies on cotton fabrics [15]. More specifically, 5, 10 and 20 DNA/chitosan bi-layers were applied to cotton fabrics reaching a total dry mass gain of 2,5, 7 and 14wt.-%, respectively: despite the very thin morphology of the deposited assemblies, the 20 bi-layer coatings were capable of reaching the self-extinction of cotton during horizontal flammability tests, increasing the limit oxygen index up to 24% and reducing the heat release rate by 40% during cone calorimetry tests (35 kW/m^2).

All the aforementioned findings can be referred to the atomic composition and chemical structure of DNA, which acts as an "all-in-one" intumescent coating [16, 17], hence providing the fabric substrate with flame retardant features. From an overall point of view, intumescent coatings represent a suitable way for conferring fire resistance to different substrates, like metals, plastics, foams and textiles. As clearly reported in the literature, the efficiency of commonly used intumescent flame retardant systems derives from the presence in their chemical structure of a carbon source, an acid donor and a blowing agent: this allows interrupting the self-sustained combustion [18]. The DNA macromolecules naturally contains all these three components[19]: the phosphate groups, involved in phosphodiester bonds, are the phosphoric acid donors, the deoxyribose sugars are carbon sources and blowing agents together with the purines and pyrimidines bases which, under heating [16, 17], may release ammonia within 180-230°C, during the pre-ignition step upon flame application, due to the presence of nitrogen in their molecule.

The present work is aimed at thoroughly investigating the effect of DNA molecular size on cotton combustion. Using herring DNA, three different molecular sizes have been used for treating cotton fabrics, exploiting either single or multiple impregnations, and achieving the same final add-on (8 wt.-%). In addition, the effect of pH solutions value below (4.0) and above (8.0) the DNA isoelectric point (pI) has been examined. The overall flame retardant features of the treated fabrics have been evaluated by horizontal flame spread and cone calorimetry tests.

2. MATERIALS AND METHODS

2.1 Materials

Cotton (COT, 220 g/m^2) was purchased from FratelliBallesioS.r.l. (Torino, Italy) and cut into squares of 100 X 100 mm^2.

DNA from herring sperm (#D3159, size range 100-200 bp) and DNA from herring testes (Type XIV sodium salt, #D6898, size range 3000-10000 bp) were purchased from Sigma-Aldrich S.r.l. (Milano, Italy) and stored at 4°C before use.

2.2 Preparation and characterization of DNA solutions

Solutions of herring sperm DNA (coded as HS-DNA) at two different concentrations (namely, 2.5 and 6% w/v) were prepared by slowly dissolving the powder in distilled water under magnetic stirring (300 rpm) for 2h at room temperature. For both concentrations, three different pH values (4, 7, and 8) were chosen in order to test the behaviour of DNA below and above its isoelectric point (pI = 5) [19, 20]. The pH was adjusted with NaOH.

DNA from herring testes (coded as HT-DNA) was slowly dissolved in distilled water under low magnetic stirring (50 rpm) overnight; two solutions (0.5 and 2.5% w/v) were prepared, without pH adjustment (pH=8).

In order to obtain herring testes DNA having a reduced molecular size (300-800 bp), a 2.5% w/v solution (pH=8) was sonicated in an ice bath with an XL-2020 sonicator (Misonix) equipped with a Misonixmicrotip probe 419 (3.2-mm tip diameter; 16.5 cm length) at 550 W. The obtained DNA has been coded as FHT-DNA.

Aliquots of the different DNA (5 μg each) were analysed by gel electrophoresis on 0.8% w/vagarose gels (Sigma-Aldrich S.r.l. Milano, Italy) at 5 V/cm for 2 h in 1 x Tris-acetate-ethylenediaminetetraacetic acid (TAE) buffer containing 0.5 μg/ml of ethidium bromide. Gels pictures were taken using a Gel Doc XR+ Imaging System (BioRad). 100 bp (BioRad) and 1 kb (Thermo Scientific) molecular rulers were run with DNA samples to estimate lengths.

2.3 Impregnation of cotton fabrics

Cotton squares were immersed for 5 min in the DNA solutions at room temperature. During the impregnation step, a rocker-shaker was used to keep the solutions moving. Depending on the DNA type and its concentration in the aqueous solution, both single and multiple impregnation

procedures were adopted for achieving the desired final dry add-on value. After each impregnation step, the fabrics were dried in an oven at 105°C for 30 min.

The total dry add-on on cotton samples (AO%) was determined by weighing each sample before (W_i) and after impregnation with the solution and the subsequent drying (W_f), using an analytical balance (Scaltec) (accuracy: $\pm 10^{-4}$ g).The DNA uptake was calculated according to the following equation (Eq. 1):

$$AO\,\% = \frac{W_f - W_i}{W_f} \cdot 100 \qquad \text{(Eq. 1)}$$

Hereafter, we will code the prepared samples as follows: HS (HT or FHT)-DNA-pH *X*_*Y*I, which indicates the fabric treated with HS-DNA, HT-DNA or FHT-DNA at a specific pH (*X*=4, 7 or 8) where *Y* is the number of impregnations (namely, SI or MI for single or multiple impregnations, respectively).

2.4 Characterization techniques

The surface morphology of the DNA-treated samples was studied using a LEO-1450VP Scanning Electron Microscope (beam voltages: 5 kV for fabrics). Fabric pieces (5x5 mm^2) were cut and pinned up to conductive adhesive tapes and gold-metallized. The concentrations of P element in the commercial DNA (DNA from herring sperm, 100-200 bp; DNA from herring testes, 2000-10000 bp), as well as on the fabrics treated with the different DNA solutions (HS-DNA solution 2.5% w/v at pH 8; HT-DNA solution 0.5%w/v; sonicated herring testes DNA solution 0.5%w/v) were determined by performing ICP-MS tests. These measurements were carried out with a ICAP-Q apparatus (Thermo Fisher, USA). The DNA (20 mg) or the DNA-treated fabrics (100 mg) were dissolved into 80 ml of HNO_3/$HClO_4$ aqueous solution (4:1 molar ratio) at 50°C, then cooled down at room temperature; the obtained solution was added of bidistilled water up to a final volume of

100 mL and then diluted in order to reach a concentration suitable for the tests (between 100 and 1000 ppb). The apparatus was previously calibrated with a multielement standard for phosphorus.

The resistance to a flame application of untreated and treated cotton fabrics was assessed by flammability tests in horizontal configuration. These tests were carried out by applying a methane flame (25 mm length) for 3 s on the short side of the samples (50 x 100 mm^2), which were clamped by a U-shaped metallic frame 45° tilted with respect to the plane containing the frame.

3 tests were performed for all the samples that did not exhibit self-extinction, while 6 tests were carried out for all the others. Total burning time, char length, total burning rate after the flame application (calculated as the ratio between the char length and total burning rate) and final residue were evaluated. The flammability tests aim to mimic the procedure described in the ASTM D4804 standard, commonly employed for thin films, although the specimen size is different (50 x 200 mm^2 in the ASTM D4804 standard).

The resistance to a heat flux of square fabric samples (100 x 100mm^2) was investigated using cone calorimetry (Fire Testing Technology). The measurements were carried out under a 35kW/m^2 irradiative heat flux in horizontal configuration, following the procedure described elsewhere [21]. Such parameters as Time To Ignition (TTI, s), Flame Out time (FO, s), Total Heat Release (THR, MJ/m^2g) and peak of Heat Release Rate (PHRR, kW/m^2 g) were measured. The last two parameters were normalised as a function of initial mass due to the significant difference between untreated and treated fabrics. The fire performance index (FPI, s m^2 g/kW) was also calculated as TTI to pkHRR ratio. This parameter was taken in consideration in order to establish a sort of ranking of most efficient coating in terms of DNA type (namely, length or molecular weight), pH conditions and impregnation number. Indeed, as it is well known [22], the higher the FPI, the better are the flame retardant performances.

The experiments were repeated three times for each material investigated to ensure reproducible and significant data; the experimental error was ±5%. Prior to combustion tests, all the specimens were conditioned at 23±1°C, for 48h at 50% relative humidity in a climatic chamber.

Finally, the thermal stability of untreated and treated fabrics was evaluated by thermogravimetric (TG) analyses from 50 to 800°C with a heating rate of 10°C/min, both in nitrogen and in air (60mL/min for both the atmospheres). To this aim, a TAQ500 thermogravimetric balance was used, placing the samples in open alumina pans (10mg). The experimental error was 0.5% on weight and 1°C on temperature.

3. RESULTS AND DISCUSSION

3.1 Characterization of DNAs

The molecular size of herring DNAs used in this study was characterized by agarose gel elecrophoresis (Fig. 1). DNAs were: 1) commercial DNA from herring testes type XIV (HT-DNA) that proved to be composed of a mixture of fragments mostly ranging from 2000 to 10000 bp; 2) native herring testes DNA fragmented by sonication to a size of 400-800 bp (FHT-DNA) to investigate the influence of a reduced molecular length on flame retardancy characteristics of the same DNA; and 3) commercial herring sperm DNA (HS-DNA) that, among the types of DNA used in this work, has the lowest molecular size being in the form of a mixture of short DNA fragments ranging from 100 to 200 bp.

3.2 Morphology of the treated fabrics: influence of DNA molecular size

As shown in Fig. 2, after impregnation with DNA solutions, the surface of the fabrics becomes smoother as compared with the untreated counterparts. Furthermore, the DNA coatings deposited on the fabrics, being equal its final add-on (8%), seem to affect the final morphology of the fibre surface depending on the average molecular size of the used DNA. More precisely, the HS-DNA coating seems to have the greatest penetration capability into the microfibrillar surfaces of the component fibres within the fabrics (Fig. 2B); while, the coatings obtained by impregnating the fabrics with either HT-DNA (Fig. 2C) or FHT-DNA (Fig. 2D) seem not only to cover the fibres, but also to fill the interstices in between them.

3.3 Flame retardancy and thermal stability of cotton treated with HS-DNA: effect of pH

As mentioned in the Experimental Part, HS-DNA solutions at 2.5%w/v concentration were prepared at pH of 4, 7, and 8. At these pH values, the DNA powder can be easily dissolved in distilled water; thus, it was possible to investigate the effect of pH values above and below the DNA isoelectric point value (pI = 5) on its flame retardant activity. In order to obtain comparable samples, all the cotton specimens were impregnated up to a final AO% value of 8% achieved through an equal number of consecutive impregnations (i.e.6), for all the above-mentioned solutions.

Table I summarizes the flammability data (tests performed in horizontal configuration) of cotton fabrics impregnated with HS-DNA at pH of 4, 7, or 8. Referring to pH=7 (HS-DNA pH7_MI sample), none of the tested specimens showed self-extinction, although a significant enhancement of theflame retardancy was obtained, as compared with untreated cotton. Indeed, the residue % at pH=7 was quite considerable, higher than 60%, while the total burning rate was substantially comparable with that obtained at pH=4 or pH=8 (HS-DNA pH4_MI and HS-DNA pH8_MI samples). Higher levels of flame retardancy were achieved for these two last pH values: in both cases, it was possible to reach the flame-out (Fig. 3) for approximately 80% of the tested samples (86 and 74% for HS-DNA pH4_MI and HS-DNA pH8_MI samples, respectively). Total burning time, char length and, consequently, total burning rate were quite similar, while the residue % was higher for the samples impregnated with a DNA solution at pH=4 (HS-DNA pH4_MI sample in Fig. 4).These results confirm the char-former character of DNA for cotton fabrics [13, 14] and agree with their thermal stability in air observed in thermogravimetry (Fig. 5). Certainly, this type of DNA, regardless of the solution pH, anticipates the degradation (indeed, all curves are shifted toward lower temperatures), but at the same time promotes the char formation, as well evident in Fig. 5 (50% at 325°C for HS-DNA pH4_MI, 45% at 335°C for both HS-DNA pH7_MI and HS-DNA pH8_MI *vs*. 20% at 355°C for cotton).

On the basis of these results, next, we decided to reduce impregnation times necessary to reach the desired AO% (8%), exploiting a single impregnation step by using DNA solutions at a higher

concentration. For this purpose, 6% w/v HS-DNA solutions were prepared at pH=4, 7, and 8. Then, cotton fabrics were impregnated once with these solutions to obtain AO=8% and tested for their flammability. The final add-on has been quantified not only weighing the samples, but also by thermogravimetry in air; Fig. 6 reports TG and dTG curves of HS-DNA pH7_MI and HS-DNA pH7_SI in air as an example of the different pH conditions and corresponding samples.

As shown in Table I, the flame-out was achieved only for the fabrics treated at pH=4 (HS-DNA pH4_SI, for 33% of the tested samples), thus indicating that although it reaches the same level of AO%, DNA coating deposited on the fabrics by repeated impregnations, is substantially more effective in protecting cotton as compared with a single impregnation deposition (*compare* data in Table I of HS-DNA pH4_MI and HS-DNA pH4_SI samples). Furthermore, for pH=7 and 8, apart from the absence of self-extinction, the residue %was lower than that obtained with all the other coatings (Fig. 4, *compare* HS-DNA pH7_MI with HS-DNA pH7_SI and HS-DNA pH8_MI with HS-DNA pH8_SI).

From an overall point of view, the shorter total burning time values observed for HS-DNA pH4_MI and HS-DNA pH8_MI can be ascribed to the DNA effect that promotes the self-extinction of cotton; conversely, in all the other samples, with the only exception of HS-DNA pH4_SI (which shows self-extinction for only 33% of the tested specimens), the total burning time is longer as compared to untreated cotton, even if in the adopted experimental conditions, the biomacromolecule is less efficient in slowing down/stopping the flame propagation.

Pursuing our research, the flame resistance of these samples to an irradiative heat flux of 35 kW/m^2 has been tested by using cone calorimetry. Table II summarises the collected data. Referring to samples treated with multiple impregnations (namely, HS-DNA pH *X*_MI samples), it is possible to observe that cotton TTI and FO are significantly reduced, keeping however a comparable overall combustion duration. This finding was expected as already observed in a previous work [14]. Indeed, the all-in-one intumescent character of DNA anticipates cotton cellulose dehydration at lower times (and temperatures), promoting the char formation instead the release of volatile species

[16], according to thermogravimetry results (Fig. 6). Meanwhile, the formed char is capable of protecting the surrounding polymer, reducing the amount of combustible species and consequently the total heat release, as well visible comparing THR values of these sample with that of untreated cotton (-45, -36 and -36% for HS-DNA pH4_MI,HS-DNA pH7_MI and HS-DNA pH8_MI, respectively), and increasing the final residue. In addition, the combustion kinetics is also remarkably reduced, as demonstrated by PHRR values reported in Table II (-25, -47 and -40% for HS-DNA pH4_MI,HS-DNA pH7_MI and HS-DNA pH8_MI, respectively). Comparing the effect of the different pH values on the basis of FPI values, it is noteworthy that the best performances have been achieved with HS-DNA pH7_MI sample, which, conversely, turned out to be the worst formulation under horizontal flame spread tests. Indeed, the higher the FPI, the better are the flame retardant performances (0.92 *vs*. 0.35, 0.76 and 0.51 s m^2 g/kW for HS-DNA pH7_MI, HS-DNA pH4_MI, HS-DNA pH8_MI, and untreated cotton, respectively)[22].

This finding can be ascribed to the different heating rates of the two tests employed here, which regard two different fire scenarios. In horizontal flame spread tests, a very small circular part of sample (usually, 20mm of diameter) is exposed to 900°C in few seconds (approximately 3 s); conversely, in cone calorimetry, a larger sample (100 x 100 mm^2) reaches the ignition temperature (350°C for cotton [23]) in 27 s (Table II). Sometimes the results from these tests can be in disagreement, as a certain flame retardant system can be very efficient when a flame is directly applied on a fabric surface, but not so effective when it has to protect the same fabric under an irradiative heat flux, as already demonstrated by our group [24].

Reducing the impregnation number (*see* HS-DNA pH *X*_SI samples in Table II), once again TTI, FO,THR (-40, -27 and -9% for HS-DNA pH4_SI, HS-DNA pH7_SI and HS-DNA pH8_SI, respectively) and PHRR values (-16, -27 and -16%) were significantly reduced. However, the FPI value of these samples was lower than that of untreated cotton.

3.4 Flame retardancy and thermal stability of cotton treated with DNA: effect of molecular size

To test the effect of DNA molecular size on flame retardancy, a second set of experiments was performed with HT-DNA consisting of fragments with length between 2000 and 10000 bp (Fig. 1).HT-DNA solutions were prepared without pH adjustment (pH=8) and the high viscosity of 2.5% w/v solutions did not allow to carry out reliable impregnations. Therefore, it was necessary to reduce the HT-DNA concentration to 0.5% w/v and, as a consequence of this low concentration, multiple impregnations had to be performed with HT-DNA to achieve the desired AO=8%.

From the thermal point of view, this sample behaves similarly to HS-DNA counterpart, as well visible comparing TG and dTG curves reported in Fig. 7, promoting the char formation of cotton. However, this effect is slightly less pronounced (45 vs. 50% for HT-DNA pH8_MI and HS-DNA pH8_MI, respectively). The only difference has been observed during the char oxidation at high temperatures, since cotton T_{max} passed from 465 to 510°C, for HS-DNA pH8_MI and HT-DNA pH8_MI, respectively, as pointed out in Fig. 7. In spite of this, the final residue is almost equal.

As shown in Table III, although the self-extinction was not reached in any sample and the residues % were not very high (35%, Fig. 4), HT-DNA was able to significantly reduce cotton total burning rate (1.04 vs. 1.50 mm/s).

In order to assess the effect of the molecular size on the flame retardancy properties of the same DNA, FHT-DNA solutions were prepared without pH adjustment (pH=8) and at two different concentrations, i.e. 0.5 and 2.5% w/v, required for multiple or single impregnation, respectively. A 2.5% FHT-DNA concentration could be employed because of its relatively low viscosity compared to HT-DNA. In both cases, the final AO=8% was reached and the same thermal stability already observed in air for HT-DNA has been registered for FHT-DNA (Fig. 8).

As reported in Table III, FHT-DNA samples burnt completely when exposed to the methane flame. Furthermore, no differences between the fabrics subjected to single or multiple impregnations were observed, as clearly evidenced by the comparable total burning rate values and residues % (Fig. 4).

The burning behaviour of FHT-DNA was found in between that of the other tested DNAs: more specifically, the burning rate of FHT-DNA was lower than that of low molecular weight HS-DNA, while the residues % were higher than those of native HT-DNA.

The effect of the molecular size of tested DNAs on flame retardancy can be further inferred by comparing the final residues of the different flammability tests. As shown in Fig. 4, HS-DNA at pH= 4 and 8 (multiple impregnations) showed the best fire performance with high residues % and self-extinction for about 80% of the tested samples.

In comparison with HS-DNA, the effect of HT-DNA on flame retardancy was significantly lower: this behaviour could be ascribed to the fact that long DNA fragments not only cover the cotton fibres, but also fill the interstices, irrespective of the adopted multiple impregnations. This finding is further supported by the results obtained with FHT-DNA (400-800 bp). Indeed, as shown in Fig. 4, the residues of cotton impregnated with FHT-DNA, irrespective of the number of impregnations, are lower than those obtained with HS-DNA, even though the molecular sizes of both DNAs are in the same order of magnitude.

Then, to further clarify the role of the molecular size of different DNAs, the phosphorus content of HS-DNA and HT-DNA, as well as that of the fabrics coated (AO=8%) with three different solutions of DNAs (HS-DNA 2.5%, HT-DNA 0.5% and FHT-DNA 0.5%) was determined by means of ICP-MS analyses. The data are collected in Table IV. Since phosphorus is a key component in intumescent flame retardants, ICP-MS was exploited for assessing any possible differences in phosphorous content due to the presence of impurities (e.g. proteins). ICP-MS measurements demonstrated that: i) HS-DNA and HT-DNA have the same phosphorous content (around 8%), and ii) the concentration of this element in the coated fabrics, irrespective of the molecular size of the employed DNA, is the same (0.5%). This result thus indicates that the best flame retardant properties of HS-DNA could not be ascribed to a higher phosphorus content. On the other hand, this behaviour can be explained according to their different thermal behaviour in air, as demonstrated by the TG curves of the two samples reported in Fig. 9. Indeed, the maximum

efficiency of DNA as flame retardant occurs only with the right degradation sequence of its intumescent components. DNA is believed to decompose upon heating [16], producing an intumescent effect that is responsible for the formation of a multicellular, foamed, thermally insulating material at a relatively low temperature (120-250°C). If this does not occur, its flame retardant feature may not work in a very proper way. Comparing the TG curves reported in Fig. 9, it is possible to observe that within 120 and 250°C, HS-DNA and HT-DNA lose weight in a different way: indeed, the former has proven to be more stable than the latter, as evidenced by the residues at 150°C reported in Fig. 9 (95 vs. 90% for HS-DNA and HT-DNA, respectively). Probably, the char formed by HS-DNA is more coherent and thermally stable than the other generated by HT-DNA: indeed, at 800°C the final residue left by HS-DNA is significantly higher than that of HT-DNA (35 vs. 30% for HS-DNA and HT-DNA, respectively).

Some other hypotheses must be considered to explain the different flame retardant performances of the three DNA-based coatings on cotton. In particular, it could be reasonable to consider the differences in the morphology of the coated fabrics, as assessed by SEM analyses (Fig. 2): indeed, unlike HS-DNA coatings, which surround and coat the cotton fibres, HT-DNA and FHT-DNA coatings fill the fabric interstices, too. Thus, for these latter, the DNA coating surrounding the fibres is probably thinner and less effective during the intumescent process triggered by the flame application. This may limit the formation of a thermally stable and fire-proof char. In addition, the HS-DNA coating may have a greater penetration into the microfibrillar surfaces of the component fibres within the fabrics, due to its low molecular size.

HS-DNA and HT-DNA exhibited an analogous behaviour to that observed when exposed to a flame application upon an irradiative heat flux, as well visible in Table V, where the combustion data obtained by cone calorimetry are presented. Although HT-DNA has proven to be efficient for reducing cotton THR and PHRR (-18 and -27%, respectively) and for increasing its final residue, its action is less effective as compared with that of HS-DNA (-36 and -40%). Indeed, the resulting FPI

of HT-DNA pH8_MI is lower than those of both untreated cotton and HS-DNA pH8_MI. The worst protection level has been observed when HT-DNA is further fragmented (FHT-DNA pH8_MI sample); this sample showed the lowest THR and PHRR reductions (-9 and -22%), as well as the lowest FPI value (0.32 s m^2/kW).

4. CONCLUSIONS

In the present study the effects of different parameters of cotton fabric impregnation with herring DNA have been defined in order to obtain the best flame retardant properties.

Differences in the morphologies of the fabrics after the treatments with low or high molecular size DNAs have been found: indeed, larger DNAs were found not only to cover the fibres but also to fill the interstices in between.

The flame retardant performances significantly changed according to the DNA molecular size, pH of the impregnation solution and number of impregnations.

Notwithstanding that flame retardant properties have been achieved for all the DNA-treated fabrics, the best flammability results were obtained with multiple fabric impregnations with low molecular size DNA solutions: indeed, these treatments showed the highest residues after flammability tests and self-extinction for about the 86% and 74% of the tested samples, for pH=4 and 8 respectively. These performances have been ascribed to the lowest molecular size of the HS-DNA coating that shows a greater penetration into the microfibrillar surfaces of the component fibres within the fabrics.

On the other hand, under an irradiative heat flux of 35 kW/m^2, with multiple impregnations, regardless of the used pH conditions, all the samples have exhibited a remarkable enhancement of cotton flame retardant properties, mainly regarding the reduction of total heat release and heat release rate peak. In this context, the highest protection in terms of fire performance index has been achieved with the HS-DNA solution at pH=7, although the highest total heat release decrease was found for the counterpart at pH=4.

Acknowledgements

The authors would like to thank European COST Action "Sustainable flame retardancy for textiles and related materials based on nanoparticles substituting conventional chemicals -FLARETEX MP1105. Mr. Fabio Cuttica and Mr. Alessandro Di Blasio are also acknowledged for performing the cone calorimetry tests and SEM measurements, respectively.

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Table I. Combustion data of cotton fabrics coated with HS-DNA obtained by horizontal flame spread tests at different pH conditions.

Sample	Total burning time (s)	Char length (mm)	Total burning rate (mm/s)	Residue (%)	Self-extinction (%)
Cotton	66	-	1.50	-	NO
HS-DNA pH4_MI	57	68	1.20	76	YES (86)
HS-DNA pH7_MI	81	100	1.24	68	NO
HS-DNA pH8_MI	52	68	1.30	60	YES (74)
HS-DNA pH4_SI	76	89	1.17	65	YES (33)
HS-DNA pH7_SI	79	100	1.27	46	NO
HS-DNA pH8_SI	74	100	1.36	39	NO

Table II. Combustion data of cotton fabrics coated with HS-DNA obtained by cone calorimetry.

Sample	TTI (s)	FO (s)	Combustion duration (s)	THR (MJ/m^2 g) (reduction, %)	PHRR (kW/m^2 g) (reduction, %)	FPI ($s\ m^2$ g/kW)	Residue (%)
Cotton	27	60	33	1.1	53.0	0.51	1
HS-DNA pH4_MI	14	40	26	0.6 (-45)	39.9 (-25)	0.35	8
HS-DNA pH7_MI	26	48	22	0.7 (-36)	28.2 (-47)	0.92	7
HS-DNA pH8_MI	24	49	25	0.7 (-36)	31.7 (-40)	0.76	6
HS-DNA pH4_SI	13	42	29	0.9 (-40)	44.5 (-16)	0.41	7
HS-DNA pH7_SI	20	49	29	0.8 (-27)	38.6 (-27)	0.34	6
HS-DNA pH8_SI	16	42	26	1.0 (-9)	44.7 (-16)	0.36	6

*The experimental error was ±5%.

Table III. Combustion data of cotton fabrics coated with different weight DNA obtained by horizontal flame spread tests.

Sample	Total burning time (s)	Char length (mm)	Total burning rate (mm/s)	Residue (%)	Self-extinction
Cotton	66	-	1.50	-	NO
HT-DNA pH8_MI	98	100	1.04	35	NO
FHT-DNA pH8_MI	88	100	1.14	46	NO
FHT-DNA pH8_SI	91	100	1.10	49	NO

Table IV. Phosphorus content as determined by ICP-MS.

Sample	P content [%]
HS-DNA	7.9
HT-DNA	8.0
HS-DNA pH 8_MI	0.51
HT-DNA pH 8_MI	0.49
FHT-DNA pH 8_MI	0.49

Table V. Combustion data of cotton fabrics coated with HS-DNA, HT-DNA and FHT-DNA obtained by cone calorimetry.

Sample	TTI (s)	FO (s)	Combustion duration (s)	THR (MJ/m^2 g) (reduction, %)	PHRR (kW/m^2 g) (reduction, %)	FPI ($s\ m^2$ g/kW)	Residue (%)
Cotton	27	60	33	1.1	53.0	0.51	1
HS-DNA pH8_MI	24	49	25	0.7 (-36)	31.7 (-40)	0.76	6
HT-DNA pH8_MI	18	48	30	0.9 (-18)	38.5 (-27)	0.47	6
FHT-DNA pH8_MI	14	43	29	1.0 (-9)	43.4 (-22)	0.32	7

*The experimental error was ±5%.

FIGURES CAPTIONS

Figure 1. Characterization of the molecular sizes of DNAs. Lanes: 1, molecular mass markers, 1 kb ladder; 2, HT_DNA; 3, FHT_DNA; 4, HS_DNA; 5, molecular mass markers, 100 bp ladder.

Figure 2. SEM micrographs at different magnifications of neat cotton (A), cotton treated with HS_DNA (B), cotton treated with HT_DNA (C), and cotton treated with FHT_DNA (D). For all the treated fabrics, the final add-on (8%) was achieved by using multiple impregnations in the DNA solutions.

Figure 3. Residues after flammability tests (horizontal configuration) of untreated cotton (a.), HS-DNA pH4_MI (b.), and HS-DNA pH8_MI (c.).

Figure 4. Residues of HS-DNA, HT-DNA and FHT-DNA after flammability tests as a function of different pH values.

Figure 5. TG and dTG curves of untreated cotton, HS-DNA pH4_MI, HS-DNA pH7_MI and HS-DNA pH8_MI in air.

Figure 6. TG and dTG curves of HS-DNA pH7_MI and HS-DNA pH7_SI in air.

Figure 7. TG and dTG curves of HS-DNA pH8_MI and HT-DNA pH8_MI in air.

Figure 8. TG and dTG curves of HS-DNA pH8_MI, HT-DNA pH8_MI and FHT-DNA pH8_MI in air.

Figure 9. TG curves of HS-DNA and HT-DNA in air.

Figure 1

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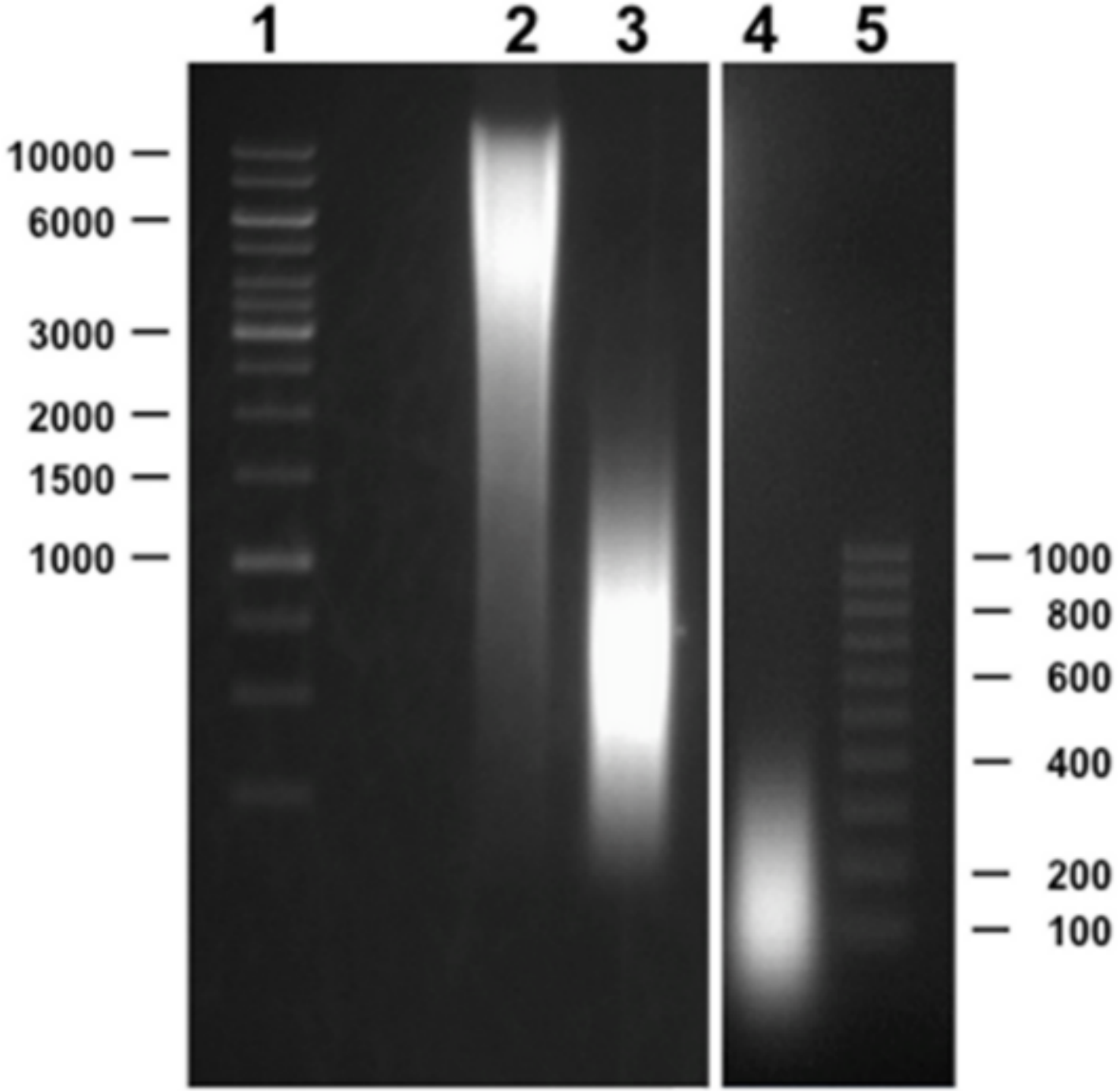

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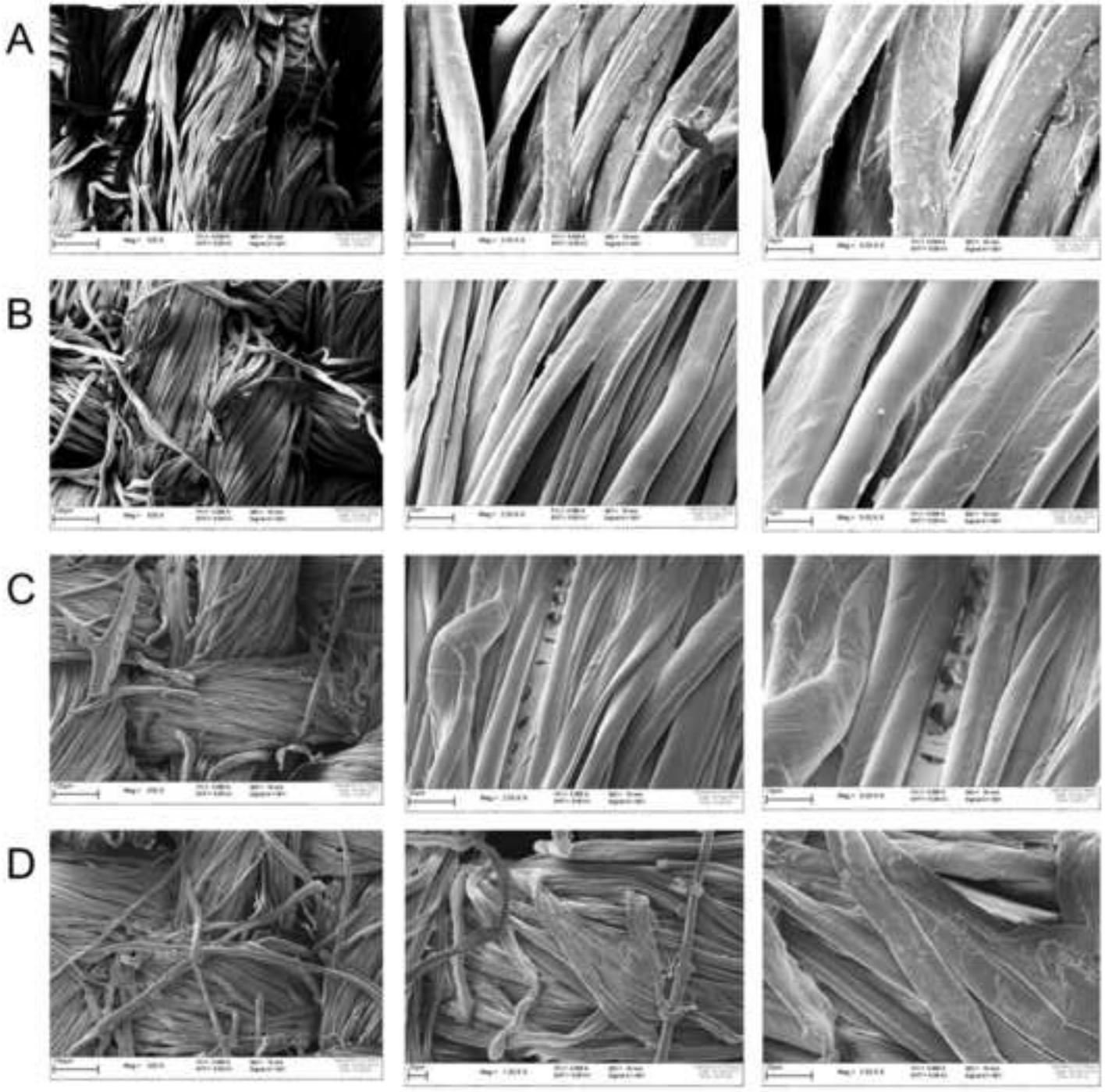

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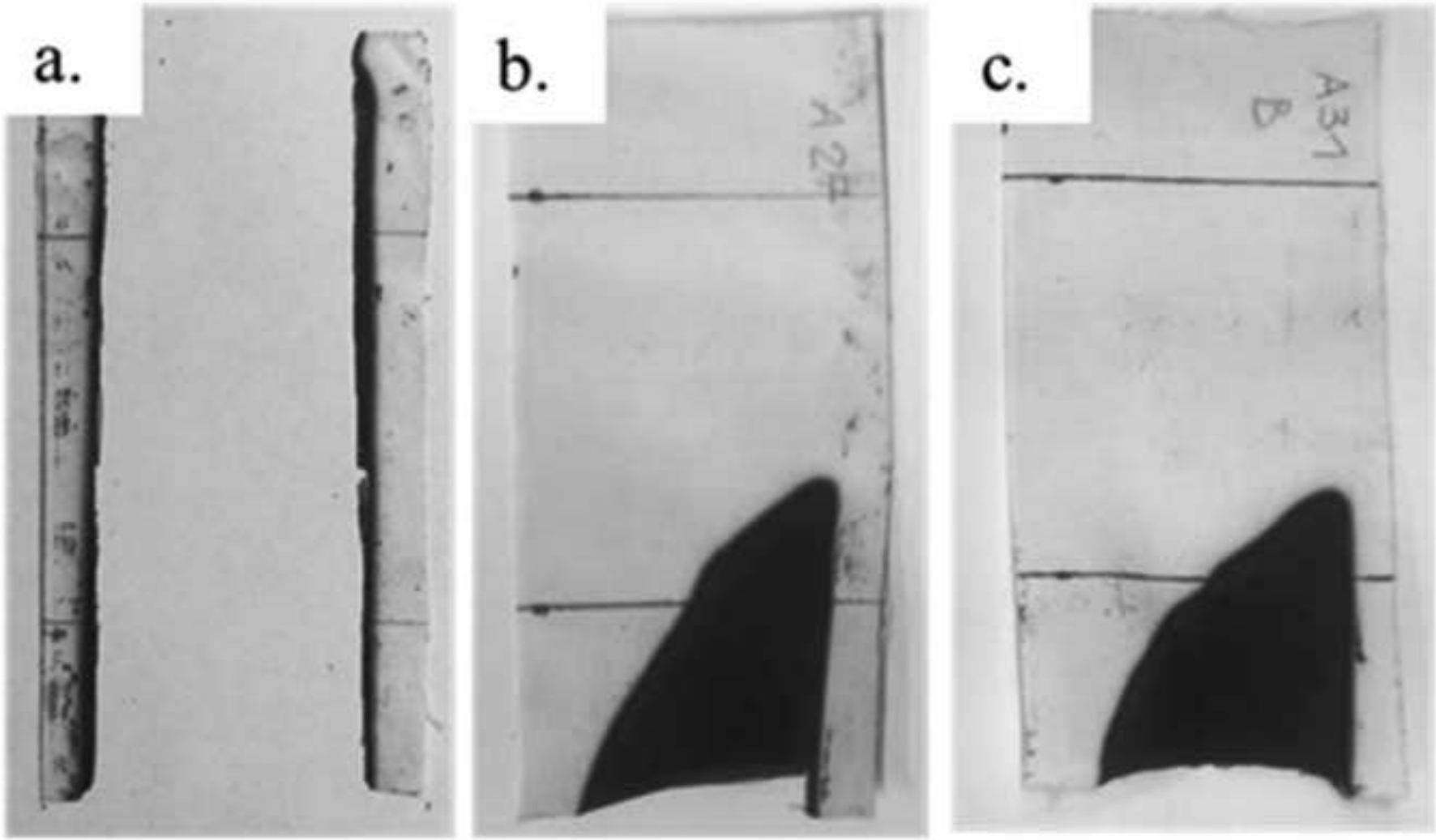

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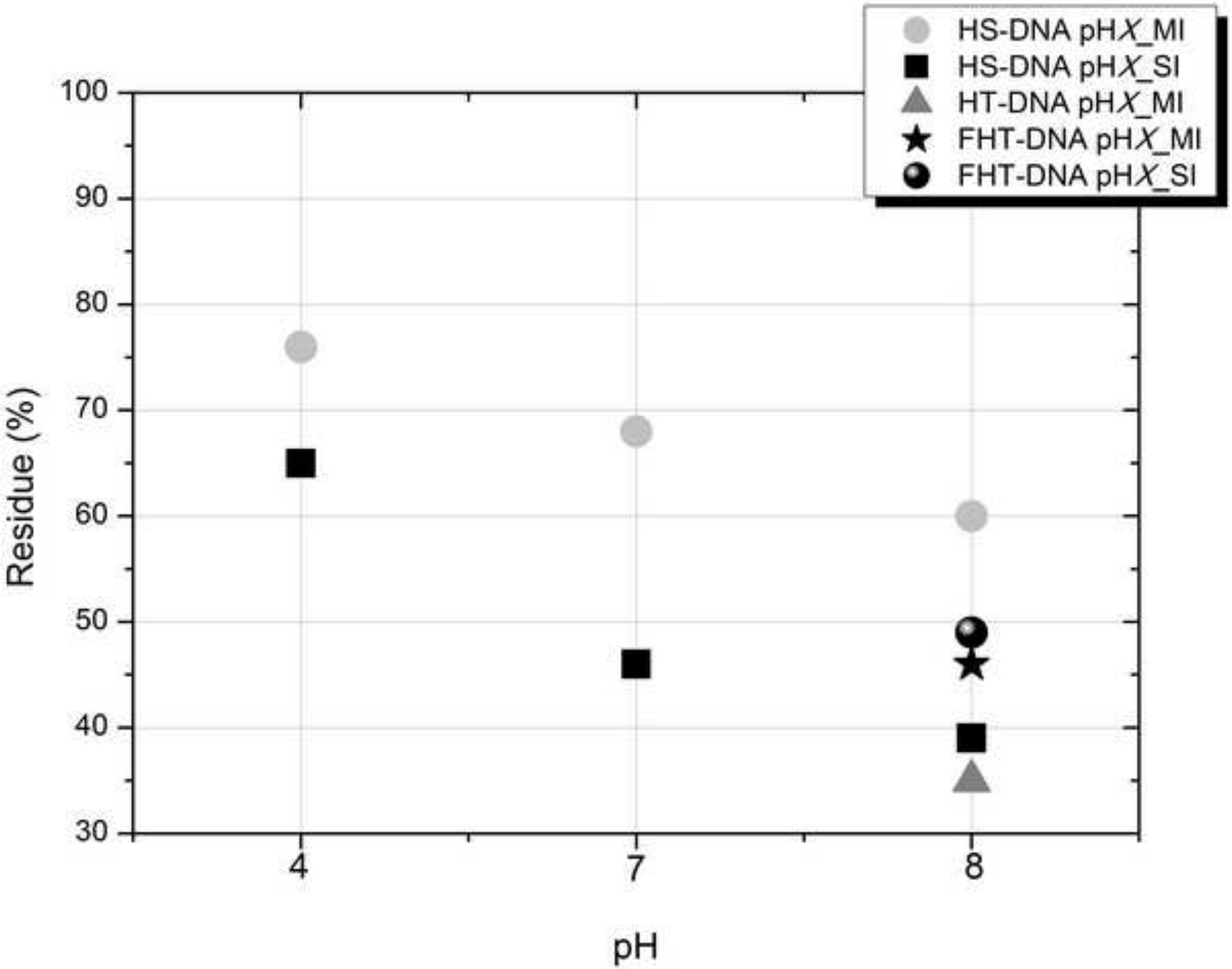

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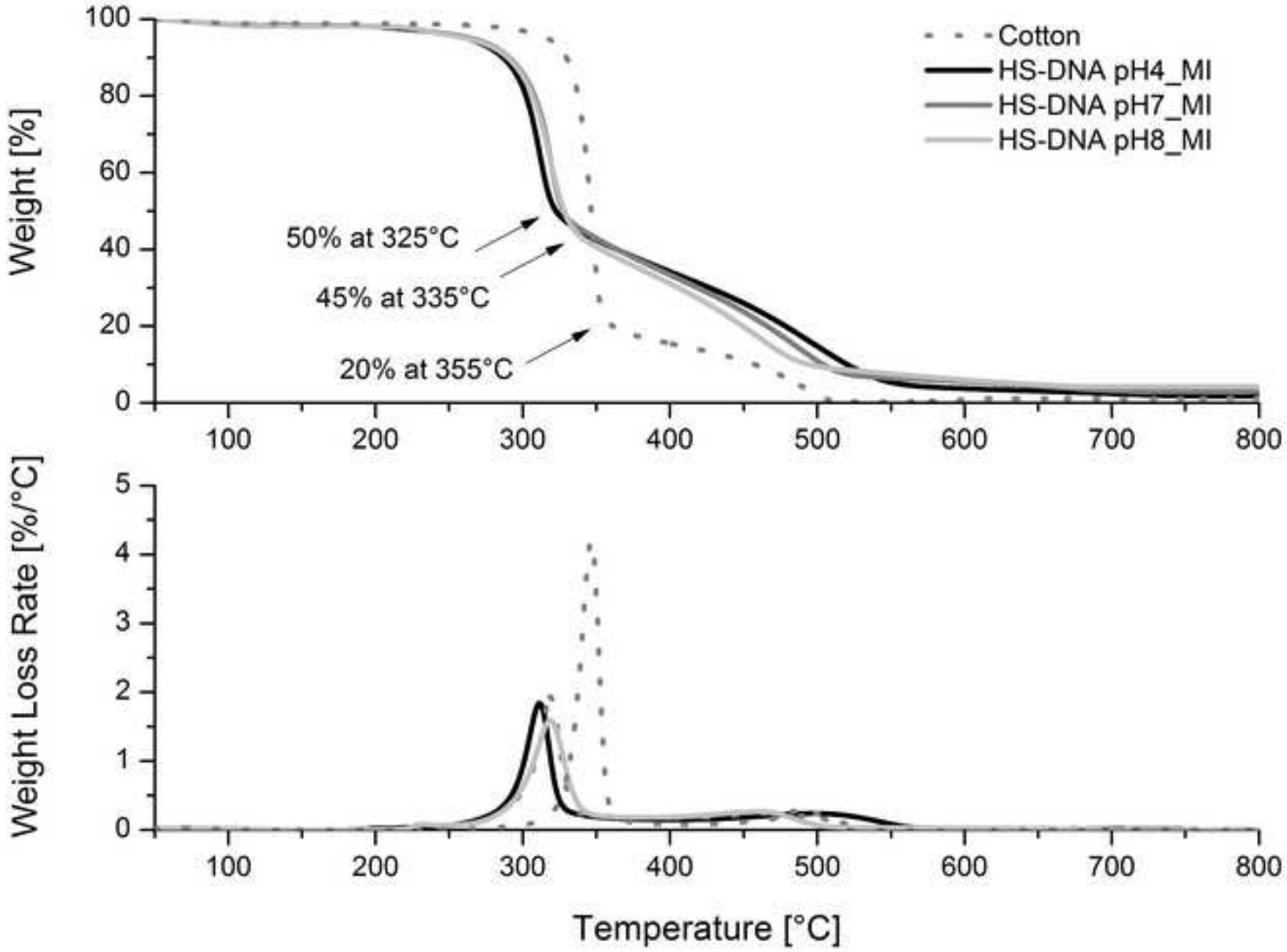

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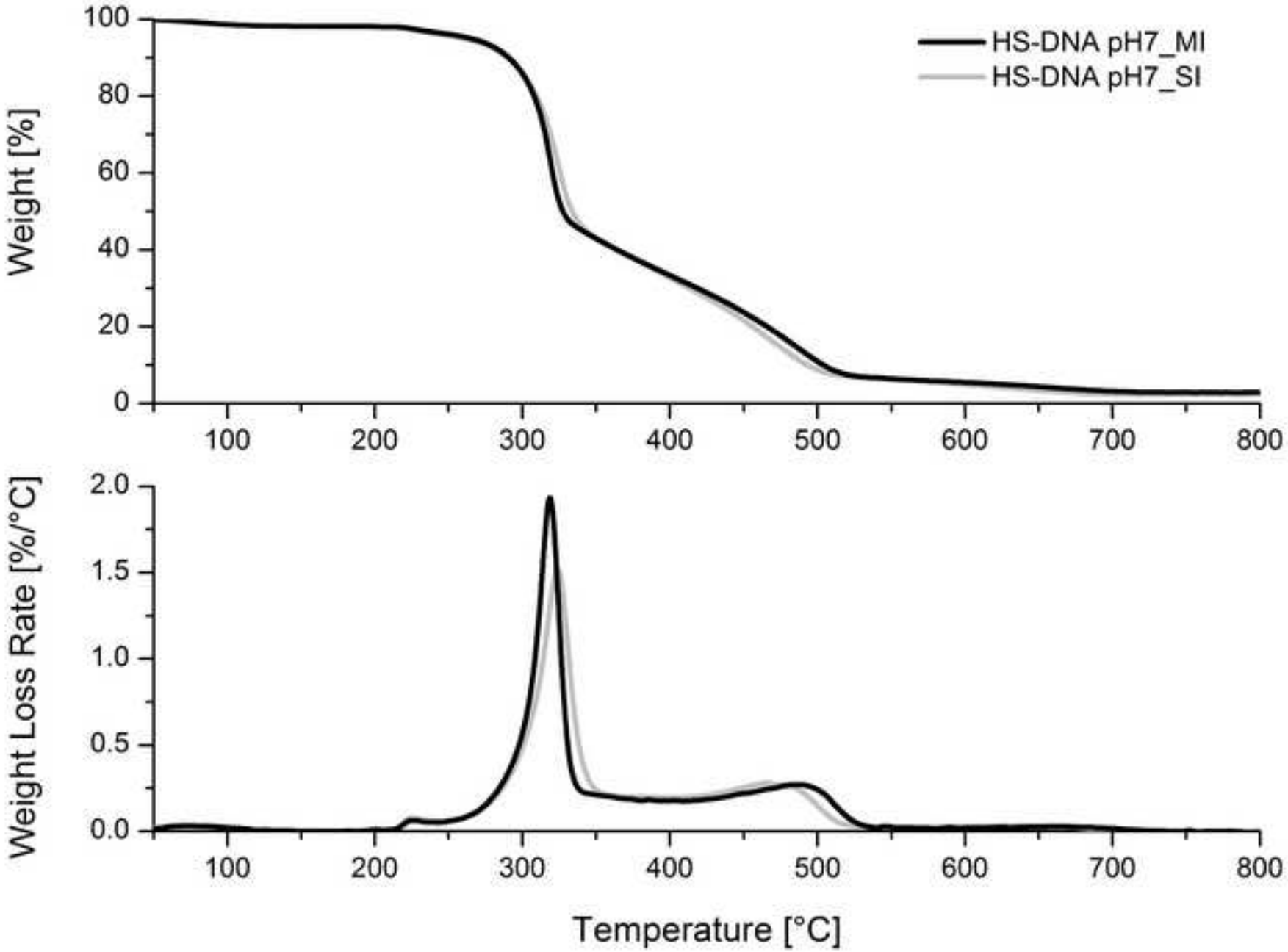

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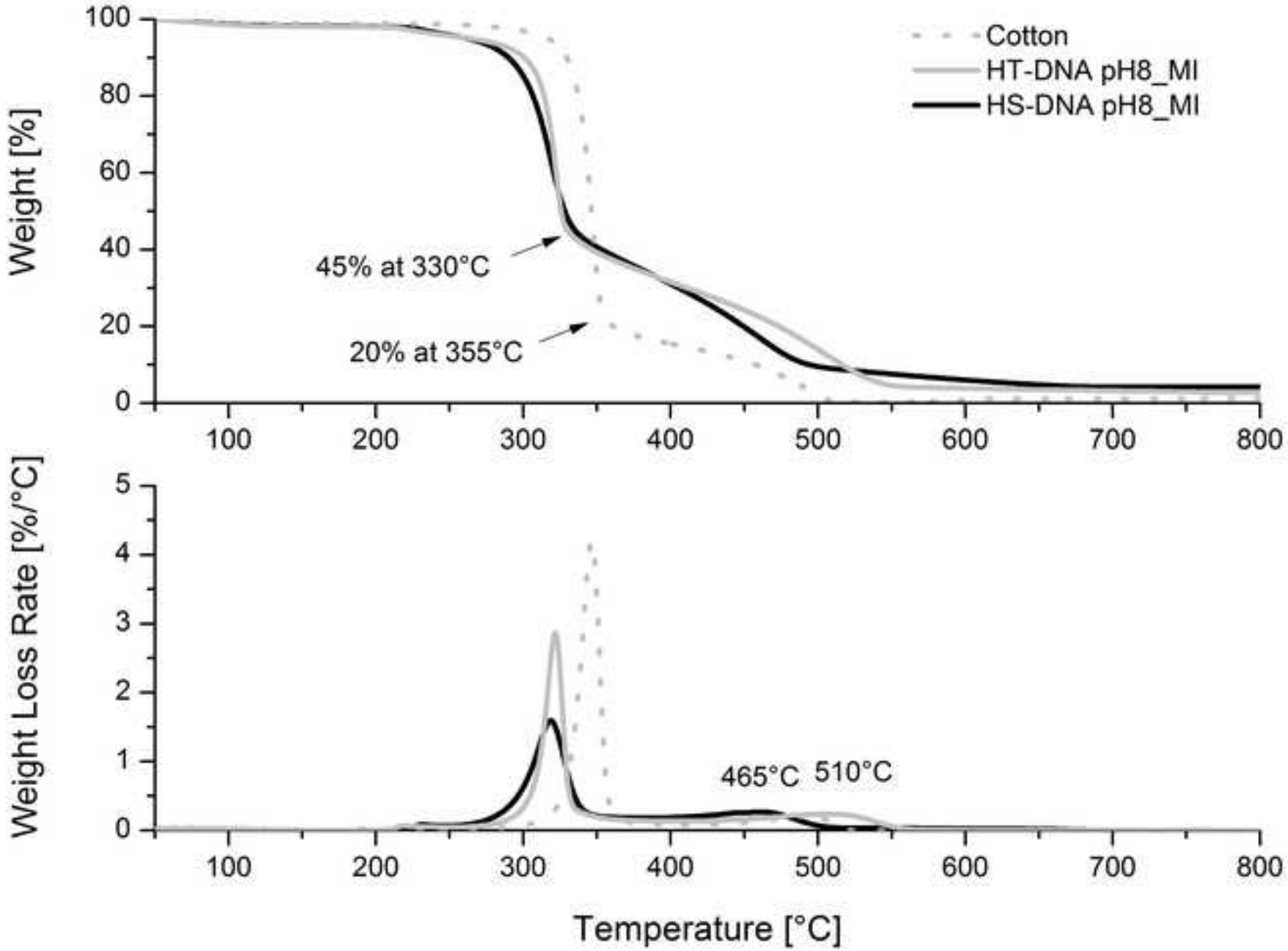

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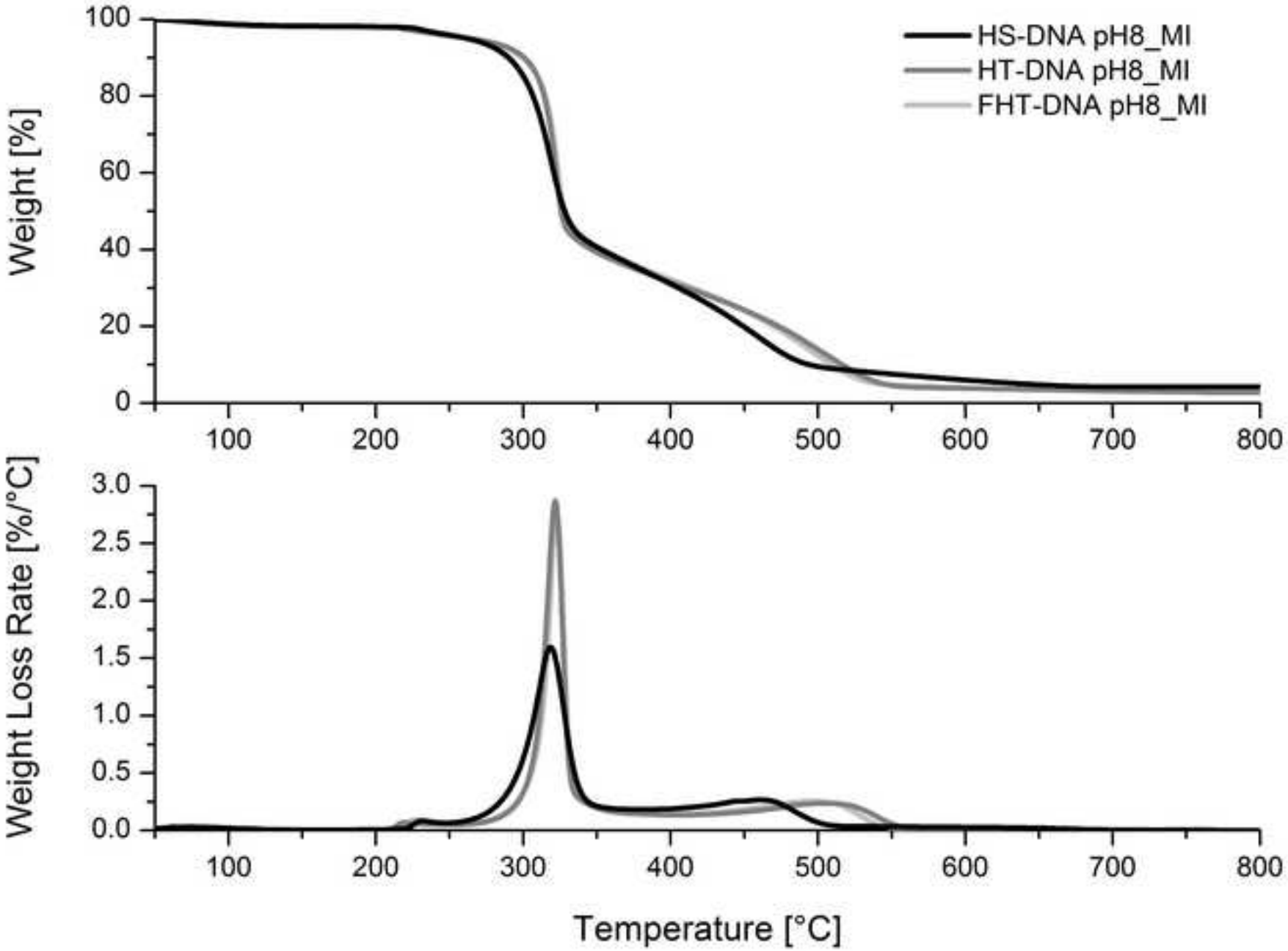

Figure 9

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