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Mechanical properties of edible biofilm as a substrate for printed electronics

Goran Stojanović, Milica Pojić, Sanja Kojić, Aleksandra Mišan, Dragana Vasiljević

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Applied Physics A

Mechanical properties of edible biofilm as a substrate for printed electronics

--Manuscript Draft--

Manuscript Number:	APYA-D-19-00740R1
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Abstract:	Edible Electronics offers an alternative to invasive approaches in conventional medicine and provides novel ways of monitoring patient health and attaining point-of-care diagnostics. For further development of this emerging area, it is necessary to develop new biodegradable and eco-friendly materials as well as to determine their properties. This paper presents the process of biofilm preparation using pea protein isolate with the addition of apple pomace extract. Microstructural and morphological properties of this biofilm were performed. Additionally, mechanical characterization of the biofilm was conducted using nanoindentation at four different temperatures; 27 °C, 50 °C, 70 °C and 100 °C. The studied biofilm had lower mechanical flexibility with increasing temperature due to evaporation of liquids from the biofilm. The solubility of the biofilm at these four temperatures was also analysed. Exposing biofilms to higher temperatures reduced their solubility, as they formed strong, compact networks under these conditions. Mechanical characteristics such as hardness index and Young's module at elevated temperatures are very important parameters for determining the suitability of this edible biofilm as a substrate in bioresorbable and edible electronics.
Response to Reviewers:	Manuscript ID: APYA-D-19-00740 Full Title: Mechanical properties of edible biofilm as a substrate for printed electronics REVIEWER1 COMMENTS and AUTHORS' RESPONSES: Reviewer's comment 1: The manuscript titled ((Mechanical properties of edible biofilm as a substrate for printed electronics)), report on the synthesis of edible biofilm and structure surface topography and morphology at different temperatures. The work is with high novelty moderate quality, therefore I suggest to be accepted after moderate revisions. The main notes are: 1. In the abstract, the main results need to be mention briefly, where the author(s) well explained the procedure without any referring to the results.

Authors' response 1: We totally agree with this comment. In the revised version of this manuscript the following sentences has been added in the Abstract:

"The studied biofilm had lower mechanical flexibility with increasing temperature due to evaporation of liquids from the biofilm. The solubility of the biofilm at these four temperatures was also analysed. Exposing biofilms to higher temperatures reduced their solubility, as they formed strong, compact networks under these conditions."

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Reviewer's comment 3: In the characterization methods, the name and model of the 3D Optical Profilometer has to be mention.
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"Surface structure of samples was analysed using 3D Optical Profilometer (Huvitz BioImager® HRM-300) with Huvitz microscope equipped with Panasis software."

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and also for Figure 3 (please see Page 4 of revised version of this manuscript):
"SEM was used to complement the results obtained by optical profilometer, providing a more detailed analysis of the biofilm structure. SEM micrographs revealed that biofilm sample exposed to the highest temperature in this study (100 C) had a higher density of protein aggregates. The reduced distance between protein aggregates results in stronger connections between them and a chain-like formation, with consequent higher mechanical hardness, compared with the other specimens. It is important to note that there was no requirement for special preparation of biofilm for SEM recording, which facilitates rapid recording and analysis of biofilms and provides the potential for high-throughput screens."

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"Figure 5 presents hardness and Young's module of analysed biofilm at four temperatures as a special part of nanoindentation testing."

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Dear Editor, Dear Reviewers,

We authors are very appreciative of and agree with reviewers' comments and suggestions. We have actioned the comments as instructed by the reviewers, please see details below.

Please note that changes to the manuscript appear highlighted in yellow throughout the manuscript.

Thank you very much, once again for giving us opportunity to improve quality of our manuscript.

Yours faithfully

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We tried to improve the quality of this paper and we will be grateful if the reviewer will try to re-consider decision and to evaluate the revised version of this manuscript.

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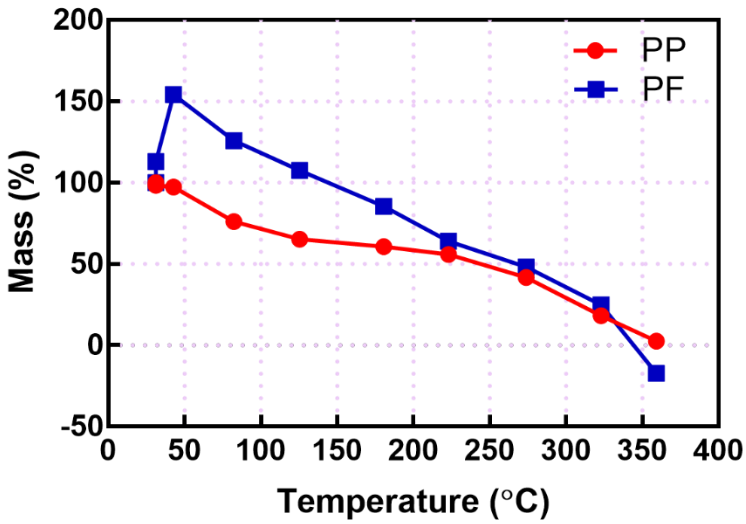

Fig. 6 TGA curves of pea protein isolate (PP) and the biofilm (PF)"

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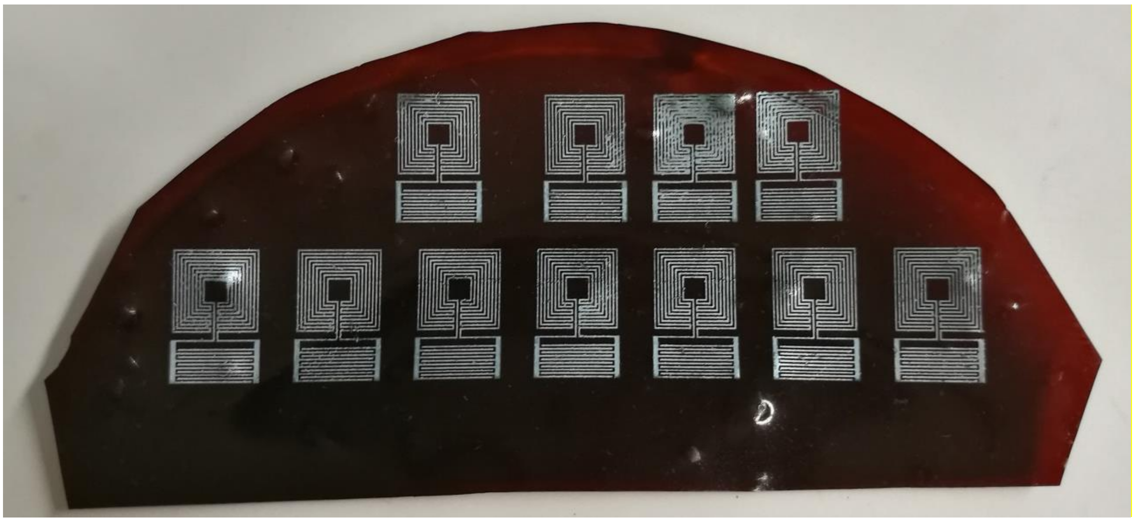
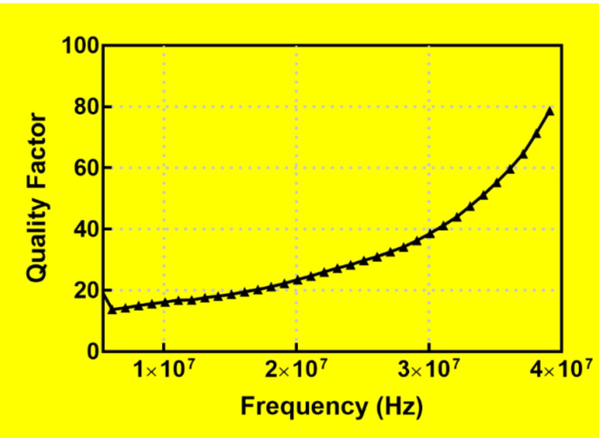
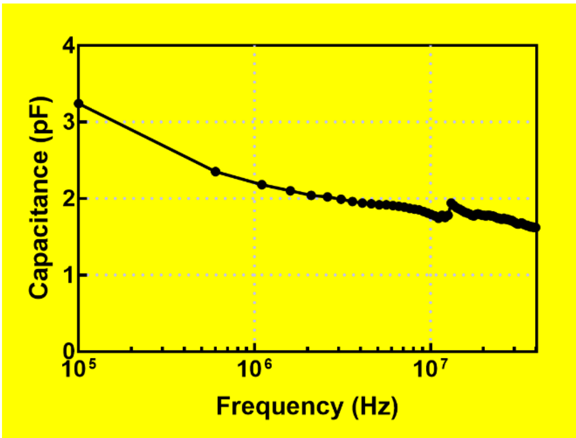


Fig. 7 Inductive and capacitive part inkjet printed on edible biofilm





(a) (b)

Fig. 7 Measured (a) Quality factor as a function of frequency, (b) Capacitance as a function of frequency, for LC structure on edible biofilm

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Mechanical properties of edible biofilm as a substrate for printed electronics

Goran Stojanović[1], Milica Pojić[2], Sanja Kojić[1], Aleksandra Mišan[2], Dragana Vasiljević[1]

[1] Faculty of Technical Sciences, University of Novi Sad, Novi Sad, Serbia

[2] Institute of Food Technology, University of Novi Sad, Novi Sad, Serbia

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ORCID ID: 0000-0003-2098-189X

Abstract. Edible Electronics offers an alternative to invasive approaches in conventional medicine and provides novel ways of monitoring patient health and attaining point-of-care diagnostics. For further development of this emerging area, it is necessary to develop new biodegradable and eco-friendly materials as well as to determine their properties. This paper presents the process of biofilm preparation using pea protein isolate with the addition of apple pomace extract. Microstructural and morphological properties of this biofilm were performed. Additionally, mechanical characterization of the biofilm was conducted using nanoindentation at four different temperatures; 27 °C, 50 °C, 70 °C and 100 °C. The studied biofilm had lower mechanical flexibility with increasing temperature due to evaporation of liquids from the biofilm. The solubility of the biofilm at these four temperatures was also analysed. Exposing biofilms to higher temperatures reduced their solubility, as they formed strong, compact networks under these conditions. Mechanical characteristics such as hardness index and Young's module at elevated temperatures are very important parameters for determining the suitability of this edible biofilm as a substrate in bioresorbable and edible electronics.

Keywords: biofilm, nanoindentation, solubility, edible electronics

1 Introduction

Electronic waste or E-waste is considered to be the fastest growing component of solid waste, which can cause serious environmental and ecological problems [1]. Bioresorbable electronics or food-based electronics is a new and innovative concept featuring electronic devices that can be degraded or dissolved into the surrounding environment naturally. Food-based electronic devices can also be consumed and processed through the digestive system. They can provide promising solutions for continuous monitoring of physiological parameters of the human body [2]. Flexible, biodegradable electronics offer many advantages compared with their implantable counterparts [3] that may improve the diagnosis [4] and treatment of pathologies ranging from gastro-intestinal infections to diabetes. These advantages are: (a) reduced infection risk; (b) rapid, pain-free and cost-effective deployment and (c) no threats to health and ecosystems [5], [6]. Progress in ingestible electronics may benefit from the development of biodegradable electronic materials derived from nature [7] or food-based materials [8] as substrates in flexible and printed electronics. For instance, potato and crab shells by embedding conductive carbon nano tubes were used for creation of transparent substrate [1], tattoo-paper was used as a platform for integration of electronics onto food [9], graphene was formed on substrates such as food or paper [10], xanthan gum matrix with solid lipid nanoparticles was used for preparation of edible films [11], etc. Edible films are thin, flexible (sometimes also stretchable) structures based on natural materials and they are under specific focus of scientific and industrial community [12]. Mechanical characteristics of edible films are very important from their application point of view in electronics, food industry, packaging, etc. Biofilm created from cassava starch and soy protein were mechanically characterized in [13], the mechanical properties of whey protein emulsion films were analysed in [14] and quinoa protein-chitosan-sunflower oil edible film in [15]. Apart from substrate, mechanical characterization was performed on robust conducting hydrogel from edible materials [16]. Nanoindentation technique can be applied for determination of mechanical characteristics of different materials. However, it is very rear to find in open literature nanoindentation tests for edible films, on different temperatures [17].

In this work, a method for processing biodegradable and mechanically flexible film, which can be used for substrates in printed, edible electronics, is presented. The edible biofilm was prepared from peas and apple extract, which are inexpensive, biodegradable raw materials. Structural, morphological, mechanical and solubility tests of the edible films were performed. These tests were conducted during biofilm exposure to four different temperatures (27 °C, 50 °C, 70 °C and 100 °C) to simulate the sintering of ink on biofilm during edible electronics manufacture. To illustrate the rigorous conditions the biofilm must withstand, after inkjet printing on flexible substrates, sintering is performed at temperatures around 100 °C for 30 minutes.

2 Materials and methods

2.1 Biofilm preparation

Commercially available pea protein isolate (80% protein content) (Pulsin, UK) was suspended in distilled water at level of 8% (w/w) with the addition of 15% (w/w) of apple pomace extract. Dried apple pomace obtained as by-product from juice industry was used for the extraction with 80% (v/v) ethanol in the ratio 1:5 (w/v), 15 minutes ultrasound bath treatment at the frequency of 45 kHz and the temperature of 50 °C to obtain apple pomace extract rich in plant phenolic compounds (total phenolic content of 16.6 mg/ml of gallic acid equivalents per g of dry extract) [18]. pH value of the suspension was adjusted with 1M NaOH to be in the range 8-9. The solution was heated in water bath at 90 °C for 30 minutes after which glycerol as a plastizer was added at level of 50% (w/w) on protein basis. 15 ml of the resulting solution was casted in Petri dishes taking care that air bubbles were not present. The films were dried at 60 °C and 50% relative humidity for 24 h and peeled off for testing.

2.2 Characterization methods

The morphology of analysed samples was characterized by Scanning Electron Microscopy (SEM), Hitachi TM3030. The surface structure of samples was analysed using 3D Optical Profilometer (Huvitz BioImager® HRM-300) with Huvitz microscope equipped with Panasis software. The Nanoindenter G200, equipped with the Berkovich diamond indenter with a face angle of 65.2°, was used for mechanical characterization based on the nanoindentation method. The biofilm was exposed to elevated temperatures in a standard laboratory oven. Thermogravimetric analyzer LECO 7010 was used for the analysis of the thermal stability of the biofilm and pea protein isolate which was used for the biofilm preparation. Ceramic crucibles were used to set up the samples. Degradation of the samples was monitored under a nitrogen atmosphere with a flow rate of 20 cm^3 min^{-1}, with a heating rate 10 °C min^{-1}, in a temperature range of from 20 to 650 °C. Mass of the sample was around 5 mg. Solubility testing of the biofilm in water was performed as described in [19].

3 Results and Discussion

3.1 Edible film visual appearance

A photograph of the prepared biofilm can be seen in Fig. 1 (a) at room temperature (27 °C). After exposing this edible film to elevated temperatures (50 °C, 70 °C and 100 °C) for 30 minutes this biofilm became darker (Fig. 1 (b)) and exhibited reduced mechanical flexibility. The biofilm was made from pea proteins, to which the plasticizer was added - in our case glycerol, which is hydrophilic. The role of glycerol is to attract and retain water molecules, and thereby provide elasticity, improve the biofilm's flexibility, and reduce its toughness. At the higher temperatures applied, the water was no longer retained by the plasticizer, and evaporated. During the heating process, a reaction of non-enzymatic darkening, known as Maillard's reaction or glycation, occurred between sugars from the apple extract and amino acids from the protein. Covalent bonds are formed between free amine groups in amino acids and carbonyl groups from reducing sugars, resulting in the darkening of the film (as can be seen in Fig. 1(b) and Fig. 2(d))

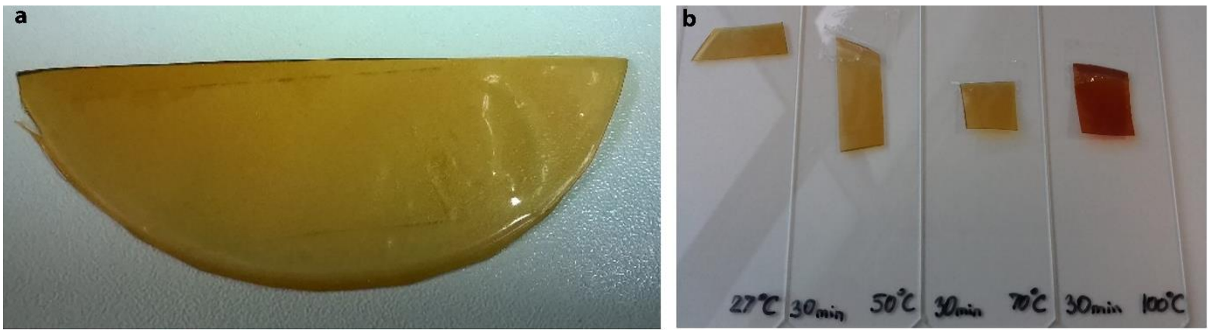

Fig. 1 (a) Visual appearance of the prepared edible film immediately after processing, (b) edible films after exposure at elevated temperatures for 30 minutes.

3.2 Surface roughness

The surface morphology of substrates plays a very important role in the quality of inkjet printing of functional inks on edible film. Indeed, their suitability to be used as a substrate material is determined by this characteristic. The surface roughness was investigated by optical profilometer and results are shown in Fig. 2. As can be seen, the surface topography is relatively smooth and continuous with average roughness around 15 nm. Additionally, the darkening colour of the biofilm at elevated temperatures is also clearly visible in Fig. 2 (from (a) to (d)). From Fig. 2, it can be seen that biofilm texture was changed at elevated temperatures, for example, Fig. 2(d) shows that the distance between protein aggregates is reduced. This will reduce the evenness of the substrate surface, which will influence the printing and functionality of the fabricated electronic components.

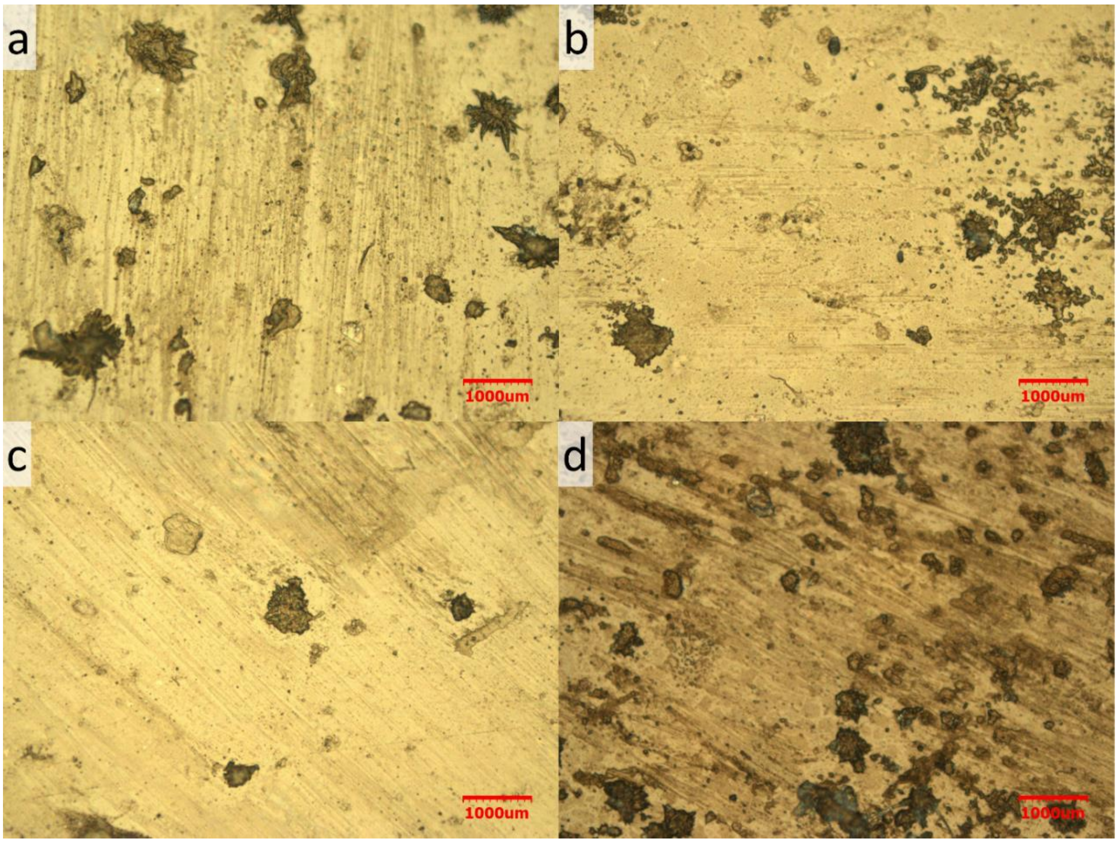

Fig. 2 Topographic images from profilometer of biofilm at (a) 27 °C, (b) 50 °C, (c) 70 °C, and (d) 100 °C

3.3 SEM micrographs

SEM micrographs of edible biofilm are presented in Fig. 3. In SEM images protein aggregates are clearly visible. SEM was used to complement the results obtained by optical profilometer, providing a more detailed analysis of the biofilm structure. SEM micrographs revealed that biofilm sample exposed to the highest temperature in this study (100 °C) had a higher density of protein aggregates. The reduced distance between protein aggregates results in stronger connections between them and a chain-like formation, with consequent higher mechanical hardness, compared with the other specimens. It is important to note that there was no requirement for special preparation of biofilm for SEM recording, which facilitates rapid recording and analysis of biofilms and provides the potential for high-throughput screens.

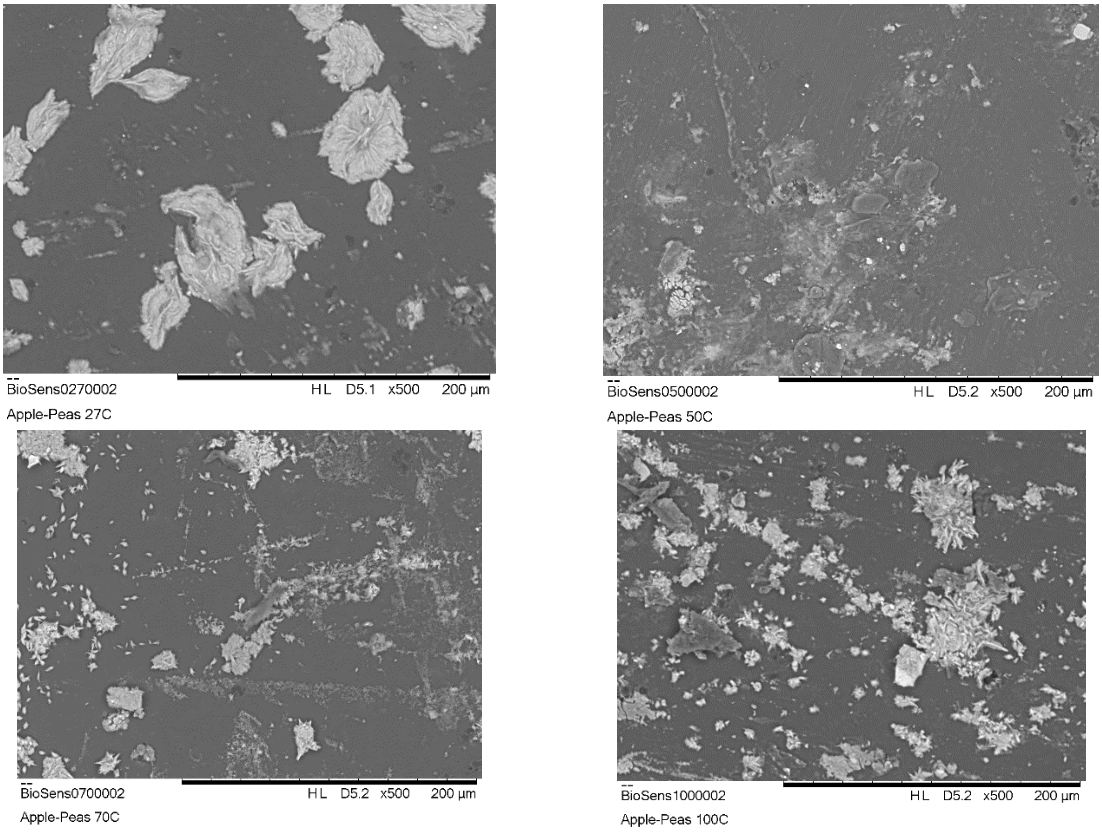

Fig. 3 SEM microimages of analysed biofilm at four different temperatures

3.4 Nanoindentation tests

Nanoindentation has been used to provide relevant information about mechanical properties of edible films as well as data about their hardness or Young's modulus. We performed nanoindentation tests on the biofilm after 30 minutes exposure to (a) room temperature, (b) 50 °C, (c) 70 °C, and (d) 100 °C. The experimental results obtained are illustrated in Fig. 4. For each case, the ten measurements conducted plus average curves are presented in Fig. 4.

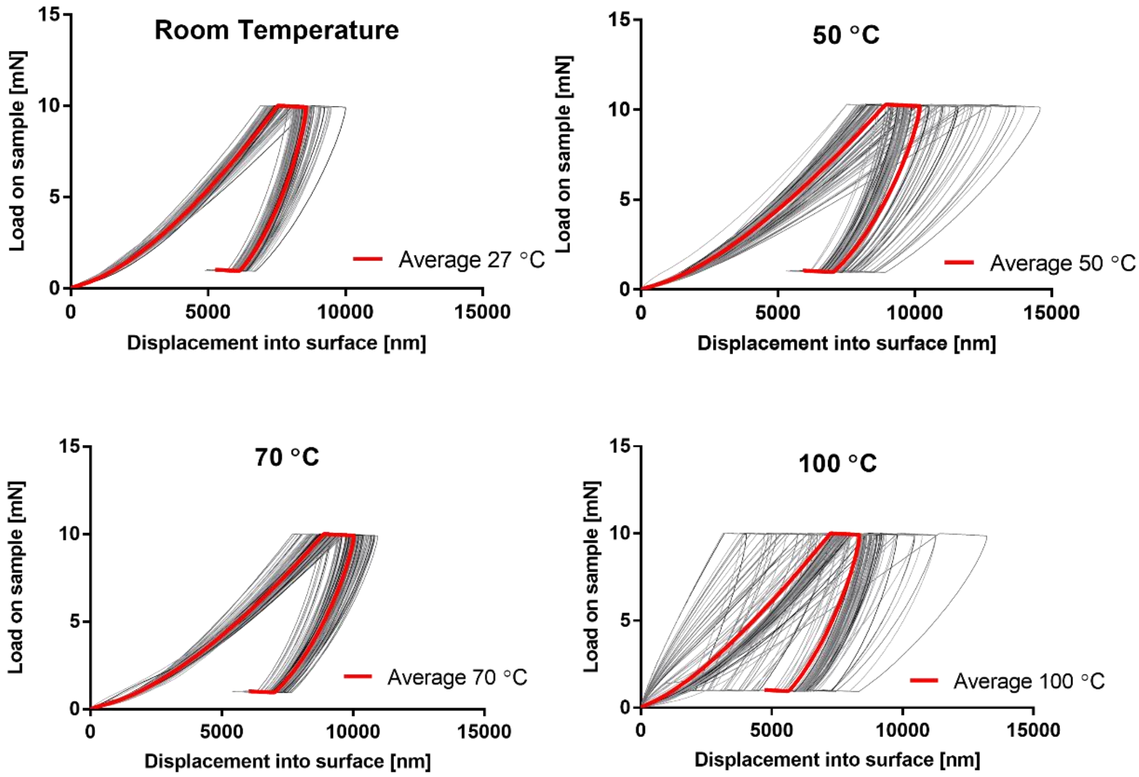

Fig. 4 Nanoindentation tests of Load-displacement curves of analysed biofilm at four different temperatures

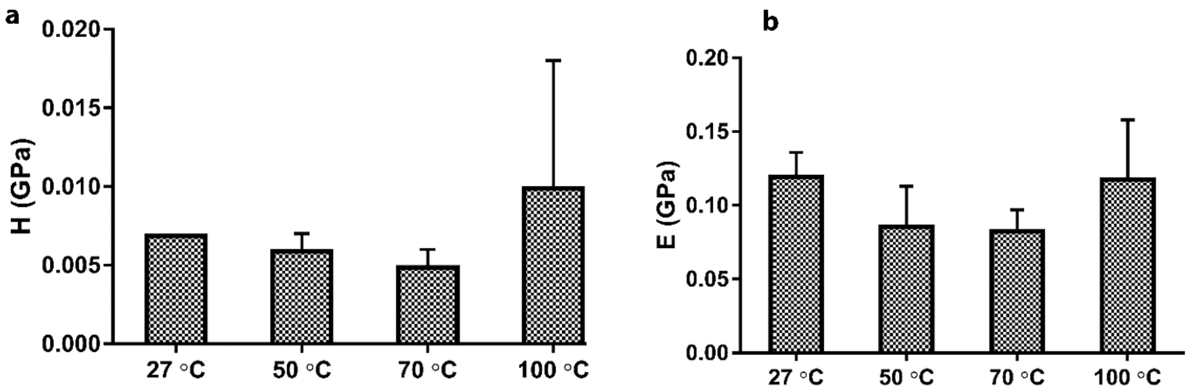

Fig. 5 (a) Hardness of biofilm at different temperatures, (b) Young's module of biofilm at different temperatures

It can be concluded (Fig. 4) that exposure to elevated temperature causes physical and chemical changes in edible film and consequently structural changes in the biofilms, resulting in variation of its mechanical properties. Figure 5 presents hardness and Young's module of analysed biofilm at four temperatures as a special part of nanoindentation testing. The harder biofilm structure was obtained at higher temperature, which is a consequence of evaporation of liquid part of the film (moisture content). This will result in less mechanical flexibility of the substrate at higher temperatures.

3.5 Solubility of biofilm

Water solubility of the biofilm samples was tested as it is relevant for its intended purpose. The results are presented in Table 1.

Table 1 Solubility of analysed biofilm samples at different temperatures

Temperature (°C)	27	50	70	100
Solubility (%)	33.52	33.04	31.03	27.69

The electronic component manufacturing process unavoidably includes the exposure of biofilm to elevated temperatures, required for sintering the printed silver ink, This reduces the solubility of the biofilms due to the formation of a strong and compact network. High temperature treatment thus increased film cohesion, resulting in stronger, more condensed films that are less soluble in water.

3.6 Thermal stability of the biofilm

Thermogravimetric analysis (TGA) was performed to investigate the thermal stability of the biofilm sample and to compare it with the stability of protein powder which was used for the film preparation. As displayed in Fig. 6, the weight loss (%) of the protein powder was higher than that of the biofilm, where the first stage of weight loss occurred before 100 °C and is probably associated with the loss of free and bound water, while further loss was probably caused by thermal decomposition of the protein backbone [20]. As far as the biofilm is concerned, initial weight gain before 42.73 °C reflected the absorption capacity of the film. Further increase in the temperature was followed by weight loss. Chen *et al.* reported in [21] that hydrocolloid samples have a minor weight loss under 100 °C, perhaps due to moisture loss of the film, and a major weight loss from 250 to 400 °C, due to thermal decomposition. According to the same authors, the weight loss may also be attributed to the decomposition of glycerol, used as a plasticizer in the film composition.

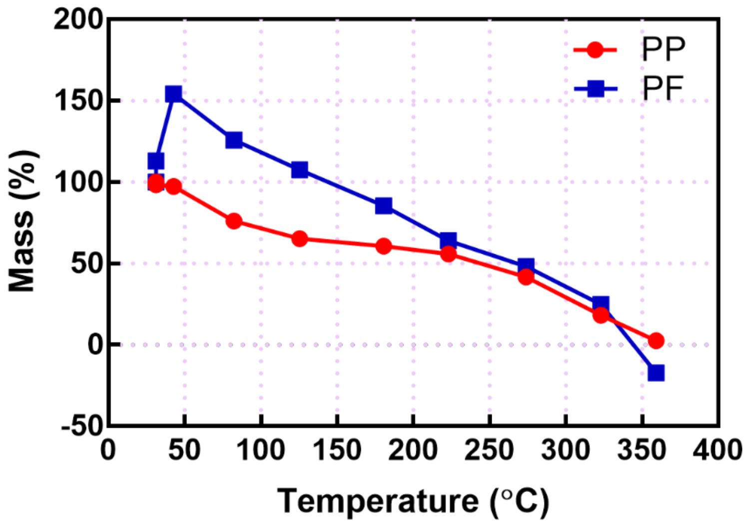

Fig. 6 TGA curves of pea protein isolate (PP) and the biofilm (PF)

In order to demonstrate the application of the presented biofilm, we designed an inductor-capacitor (LC) structure in one metal layer, a design typically used in remote sensing. A deposition material printer Dimatix DMP-3000 was used to print silver ink on edible biofilm and fabricated LC structures can be seen in Fig. 7. The width of the conductive segments was 200 μm, whereas the gap between the segments was 100 μm. The edible substrate was heated in the oven at 100 °C, for 45 minutes, to evaporate solvent from silver ink and to obtain solid conductive traces. It can be seen in Fig. 7, that the colour of the substrate is the same as in Fig. 1(b) at 100 °C. It is important to note that a safe dose of silver is 350 μg per day for a 70 kg person. The amount of silver used to manufacture the proposed LC structure was around 5 μg, which is just 1.4% of the safe daily dose. We have therefore demonstrated the successful manufacture of edible LC sensor structures. To demonstrate electronic functionality, measurements of electrical parameters were performed using an Impedance Analyzer HP4194A and results for Quality factor (Q-factor) and Capacitance are presented in Fig. 8.

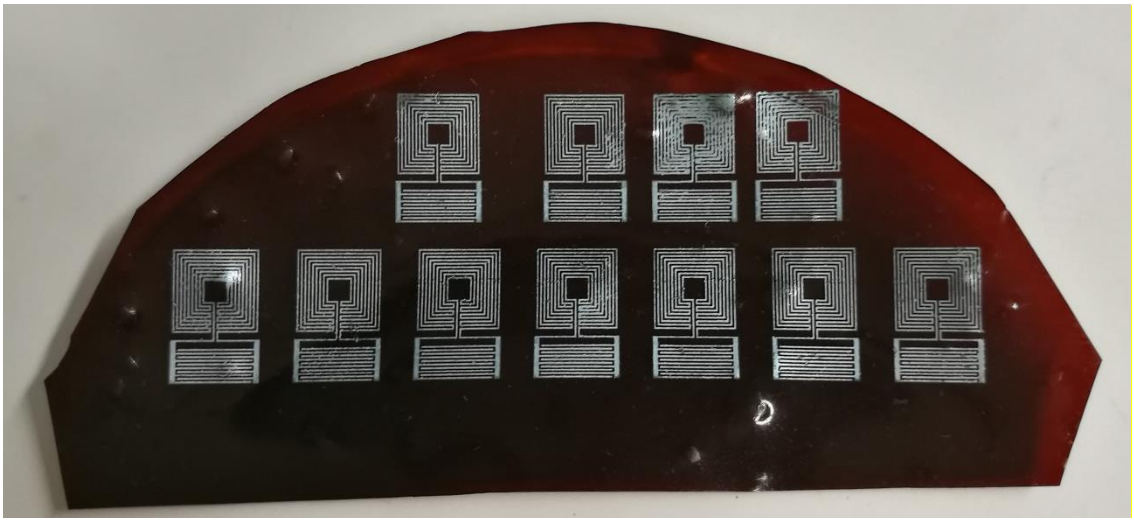

Fig. 7 Inductive and capacitive part inkjet printed on edible biofilm

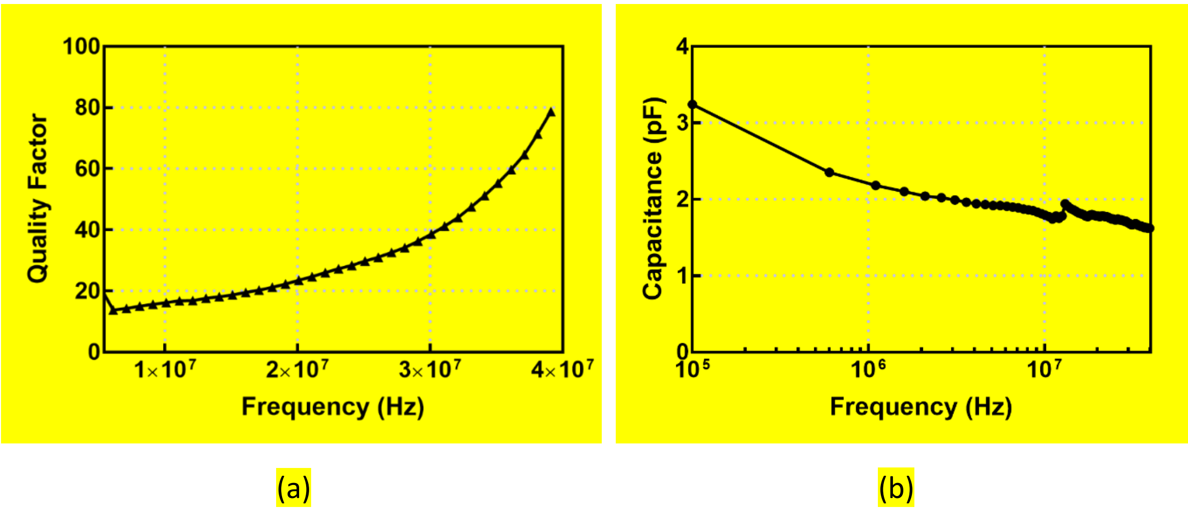

Fig. 8 Measured (a) Quality factor as a function of frequency, (b) Capacitance as a function of frequency, for LC structure on edible biofilm

The main contributions of this study can be summarized as follows: (1) an edible biofilm of this composition was processed for the first time; (2) comprehensive mechanical testing of this biofilm was performed; (3) edible biofim was used as a substrate for the fabrication of an LC electronic resonant circuit, which can be used in remote sensing applications.

4 Conclusions

The benefits of using food-based edible electronic devices over traditional build-to-last are significant, particularly considering the impact on the health and wellbeing of human beings and the positive effect related to the protection of our environment. In this emerging field of electronics, it is very important to procure suitable substrates made from edible materials. The biofilm presented in this work, constituted from pea protein isolate with the addition of apple pomace extract, is one such material. To print electrical circuits on the edible biofilm, the inkjet fabrication process is usually applied. After functional ink printing, it is necessary to perform sintering, which is done at elevated temperature. Accordingly, we studied the performance of the biofilm at four temperatures, 25, 50, 70 and 100 degrees Celsius. A comprehensive morphological analysis of this biofilm has been performed. The mechanical characteristics at these temperatures have been determined and it can be concluded that biofilm becomes mechanically harder at higher temperatures. This is associated with a decrease in solubility (from 33.52% to 27.69%) and a small decrease in surface evenness, though a functional LC circuit was successfully printed using this biofilm as a substrate.

Acknowledgements

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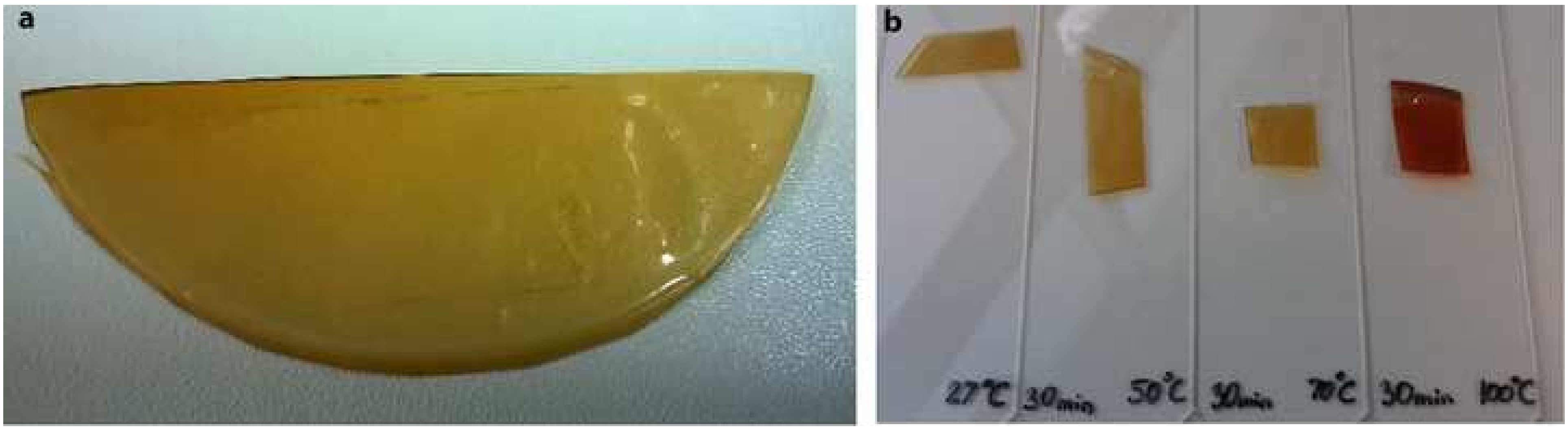
Fig1



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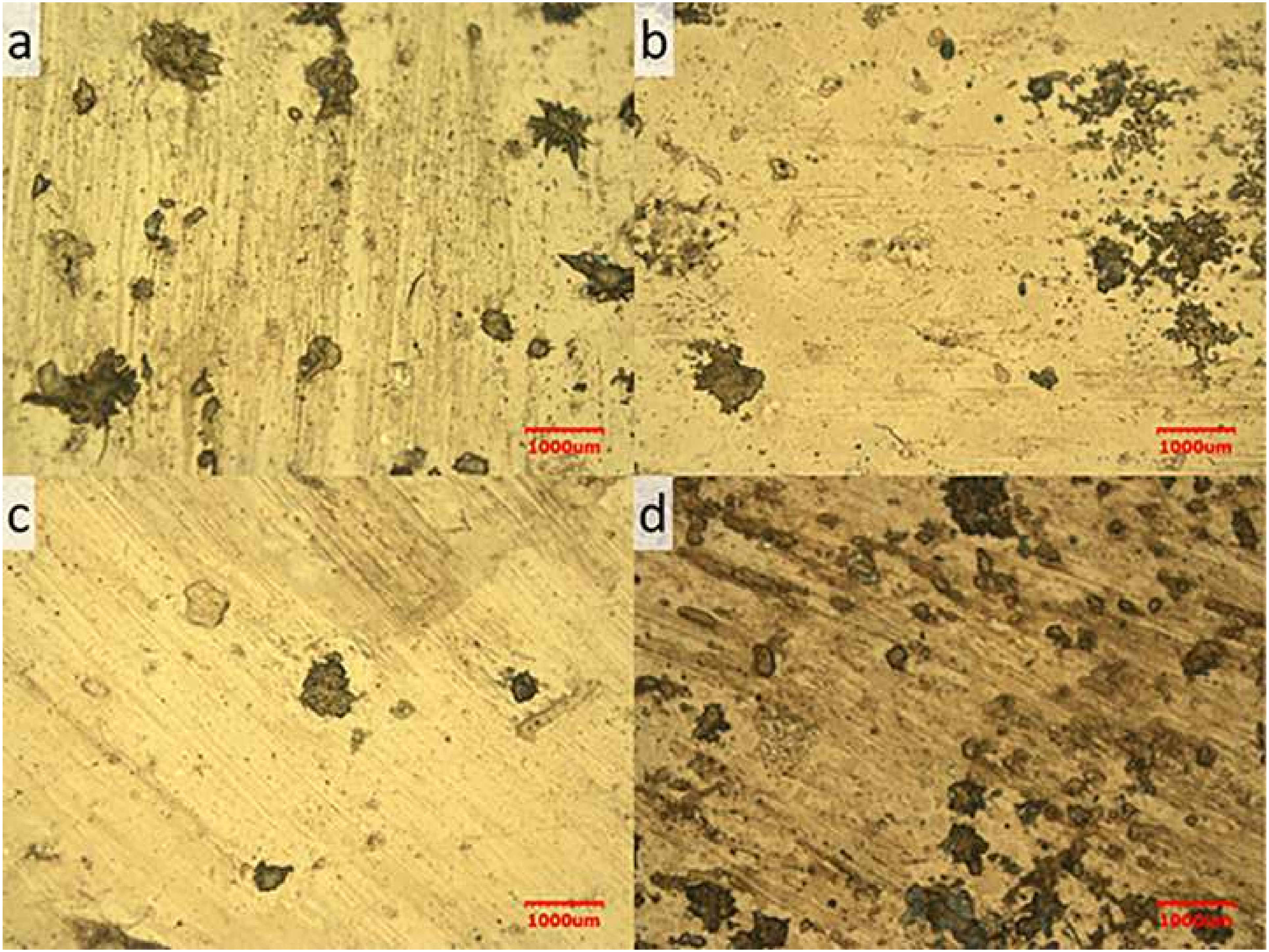

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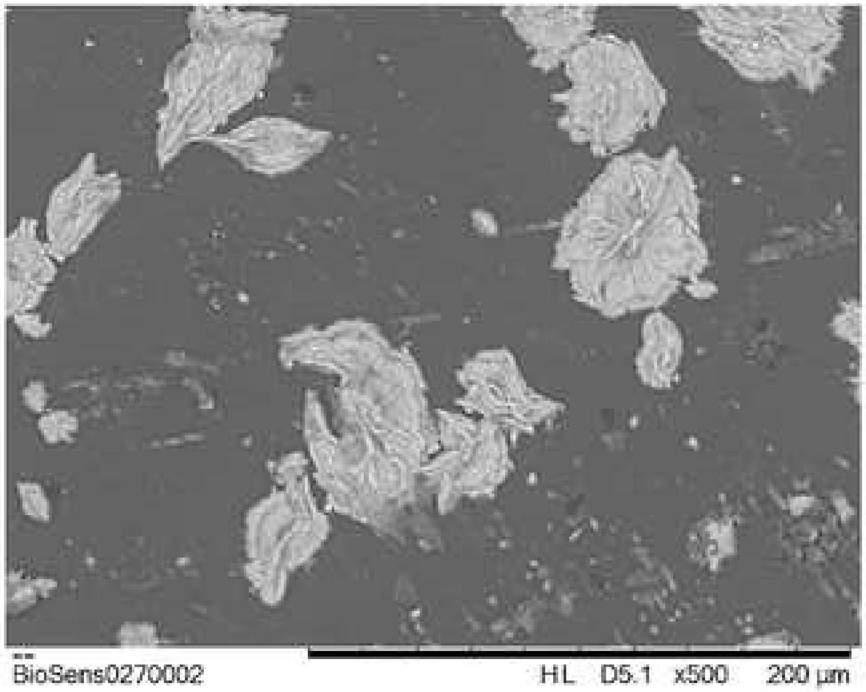

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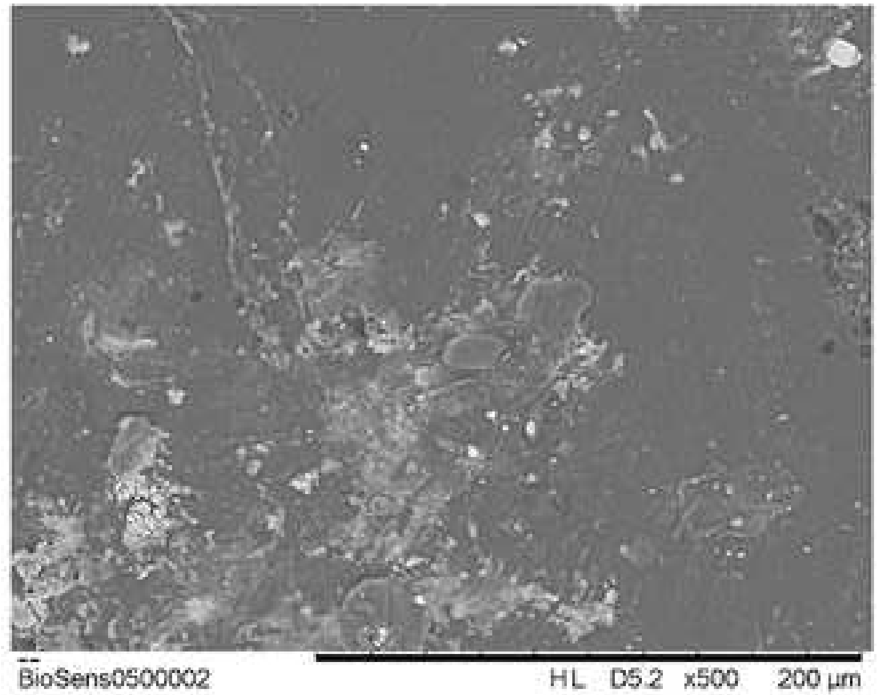

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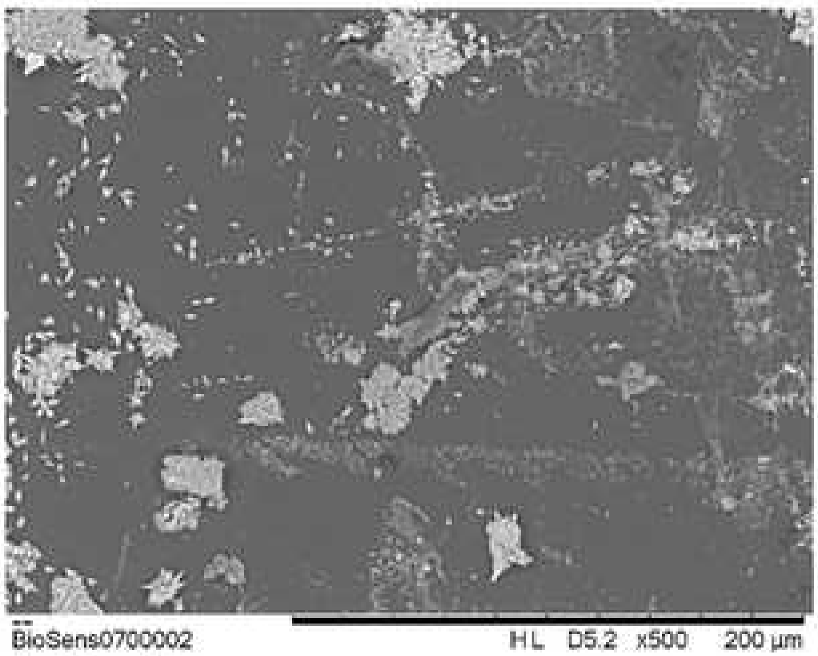

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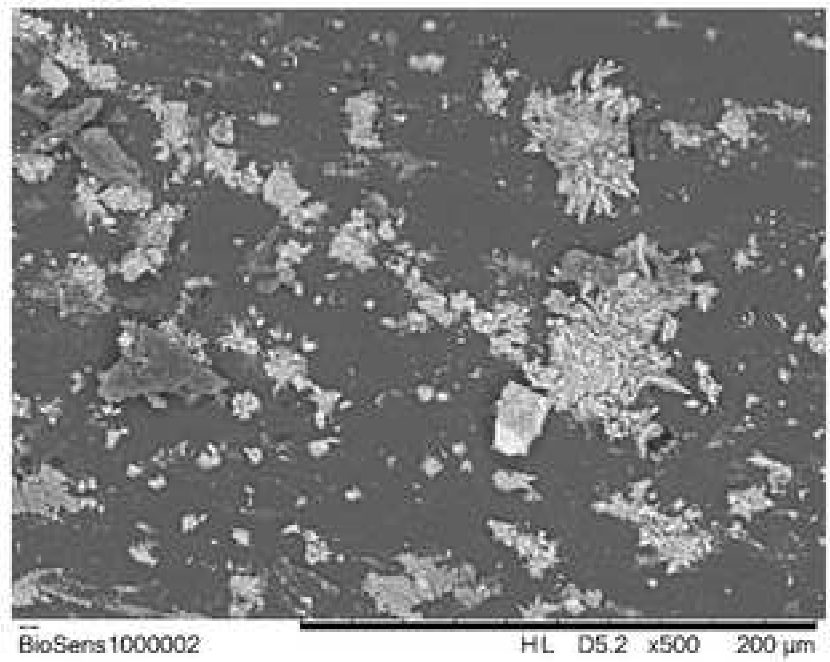

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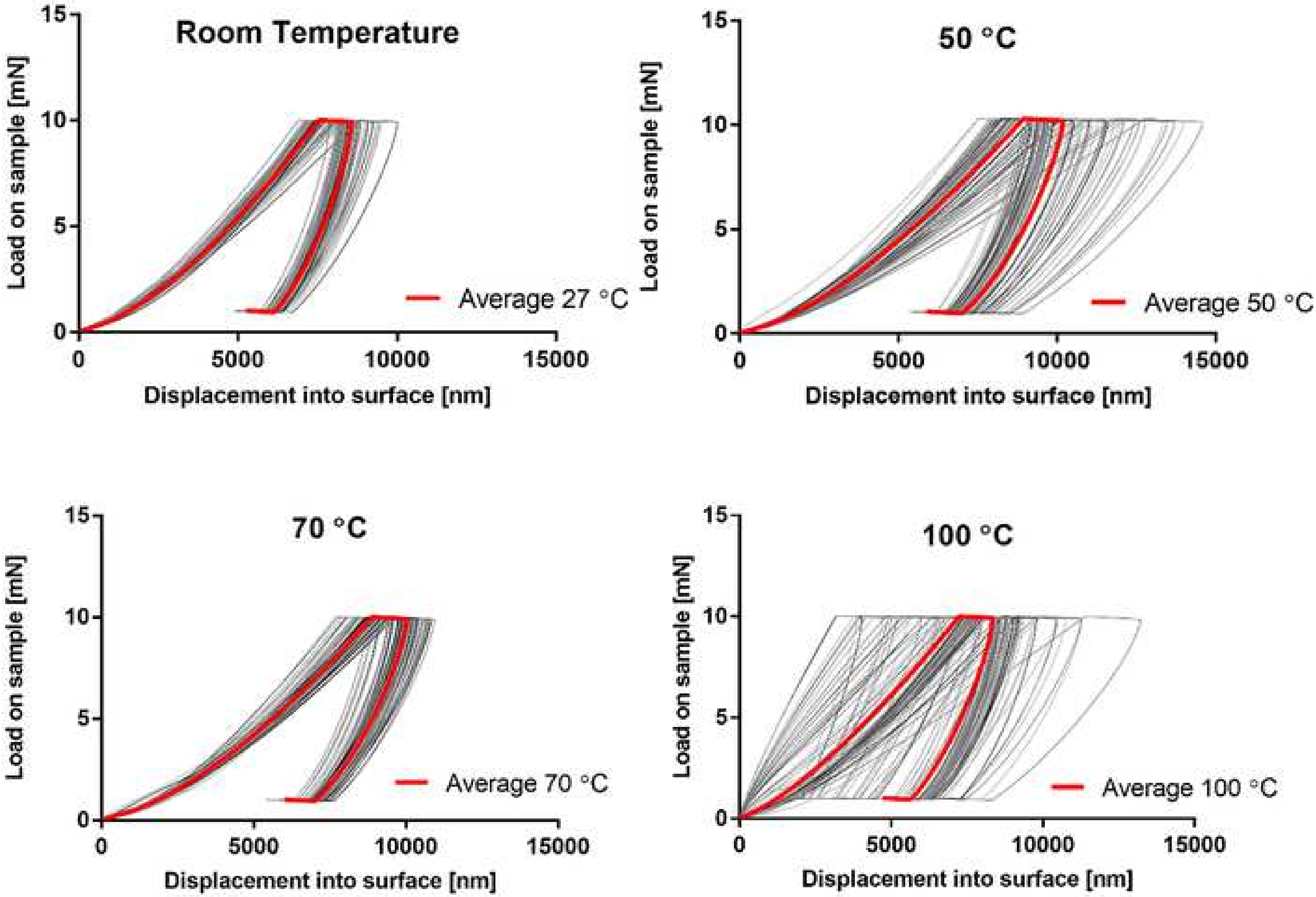
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70 °C
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Fig5

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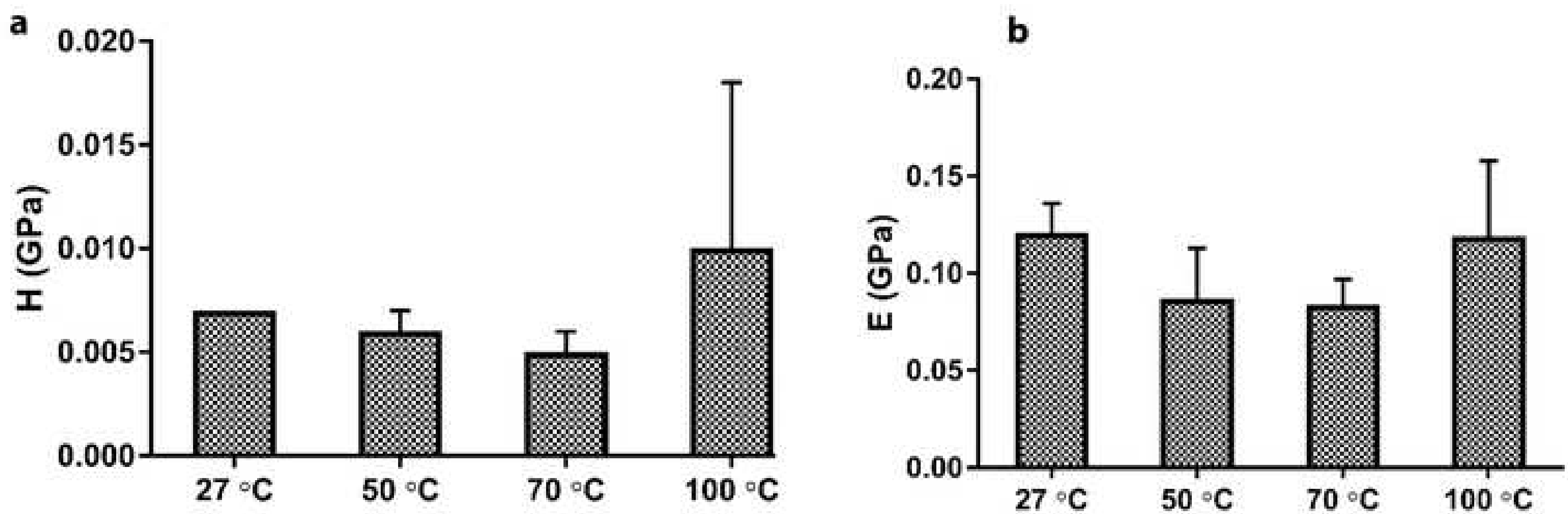

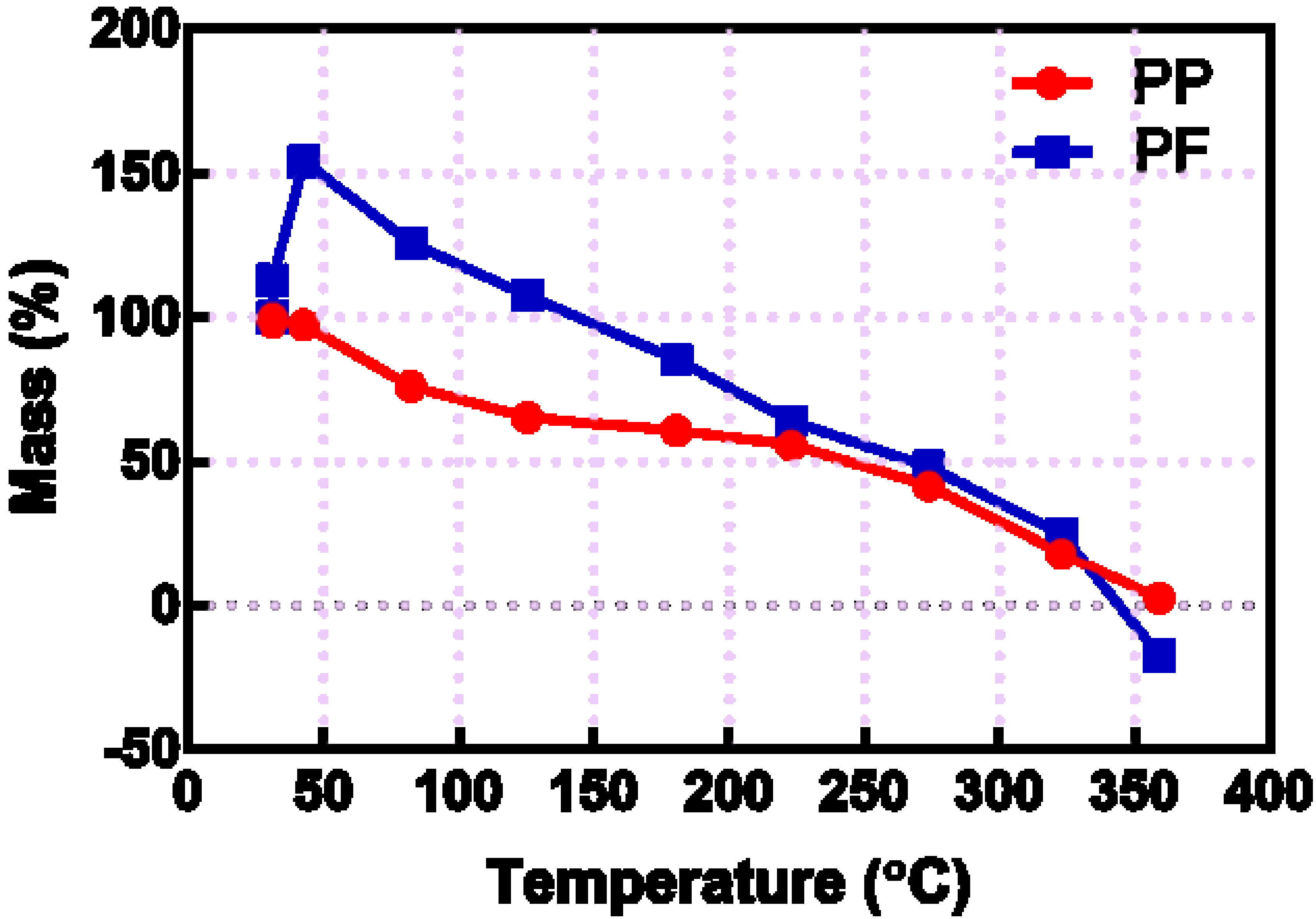

200
150
100
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Mass (%)
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350
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Temperature (°C)
PP
PF

Fig7

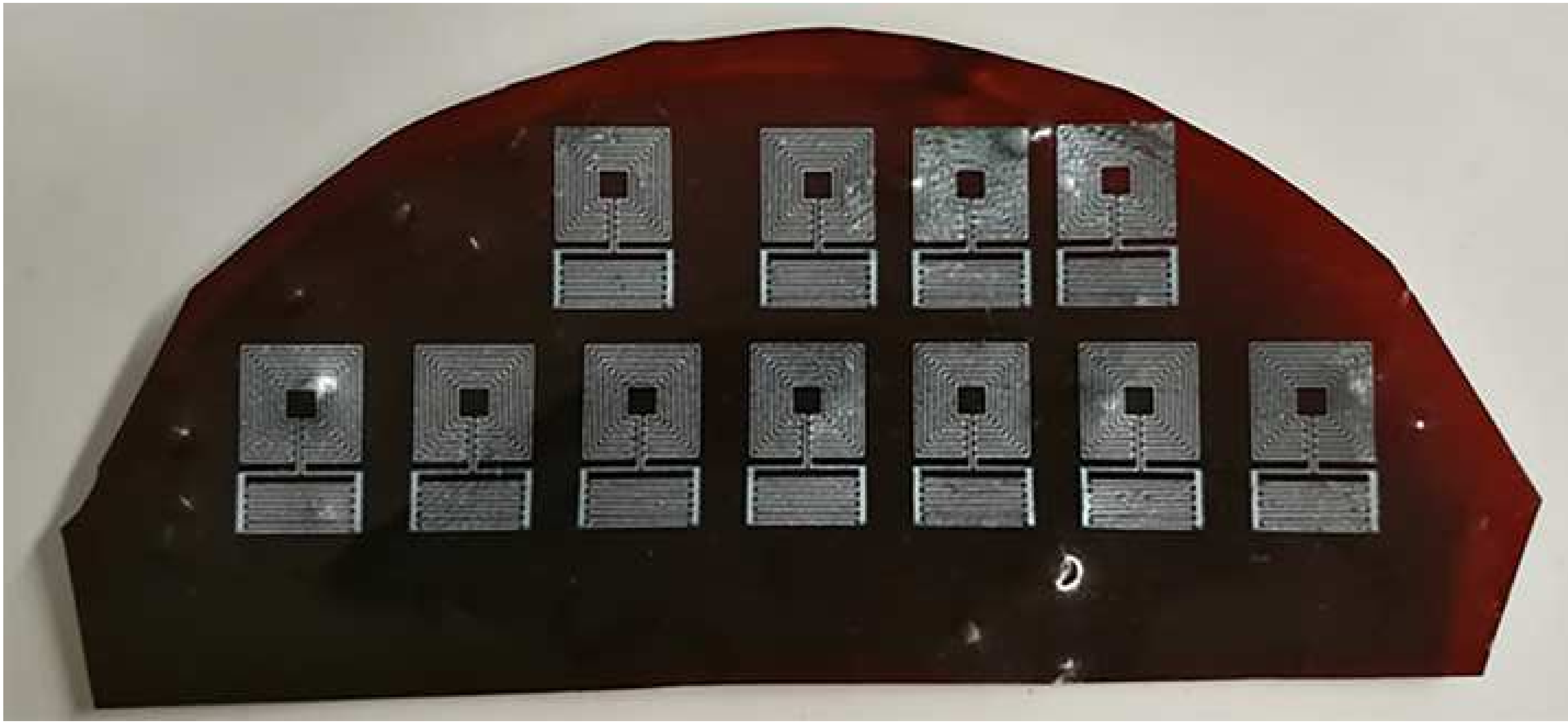

Fig8a

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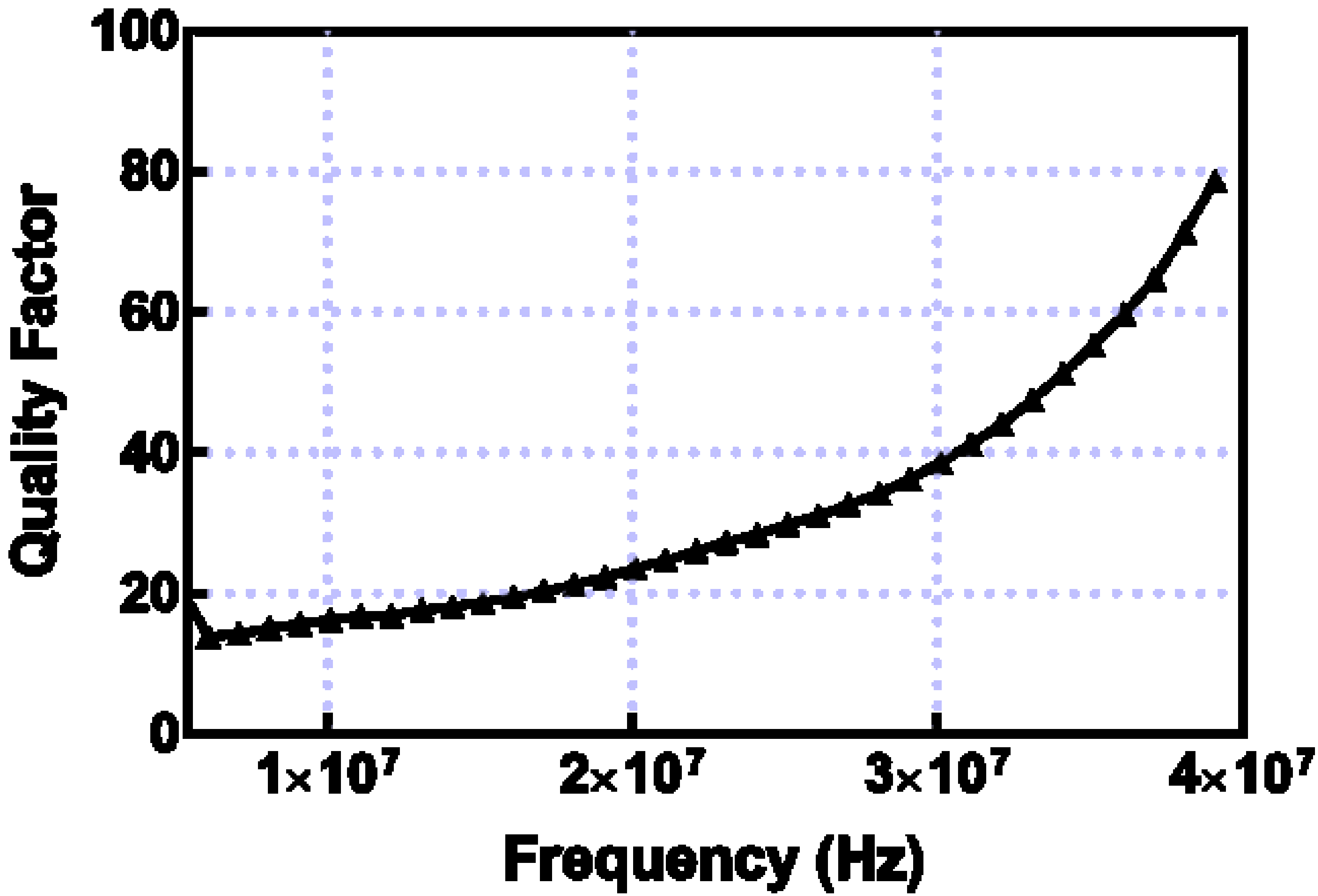

Fig8b

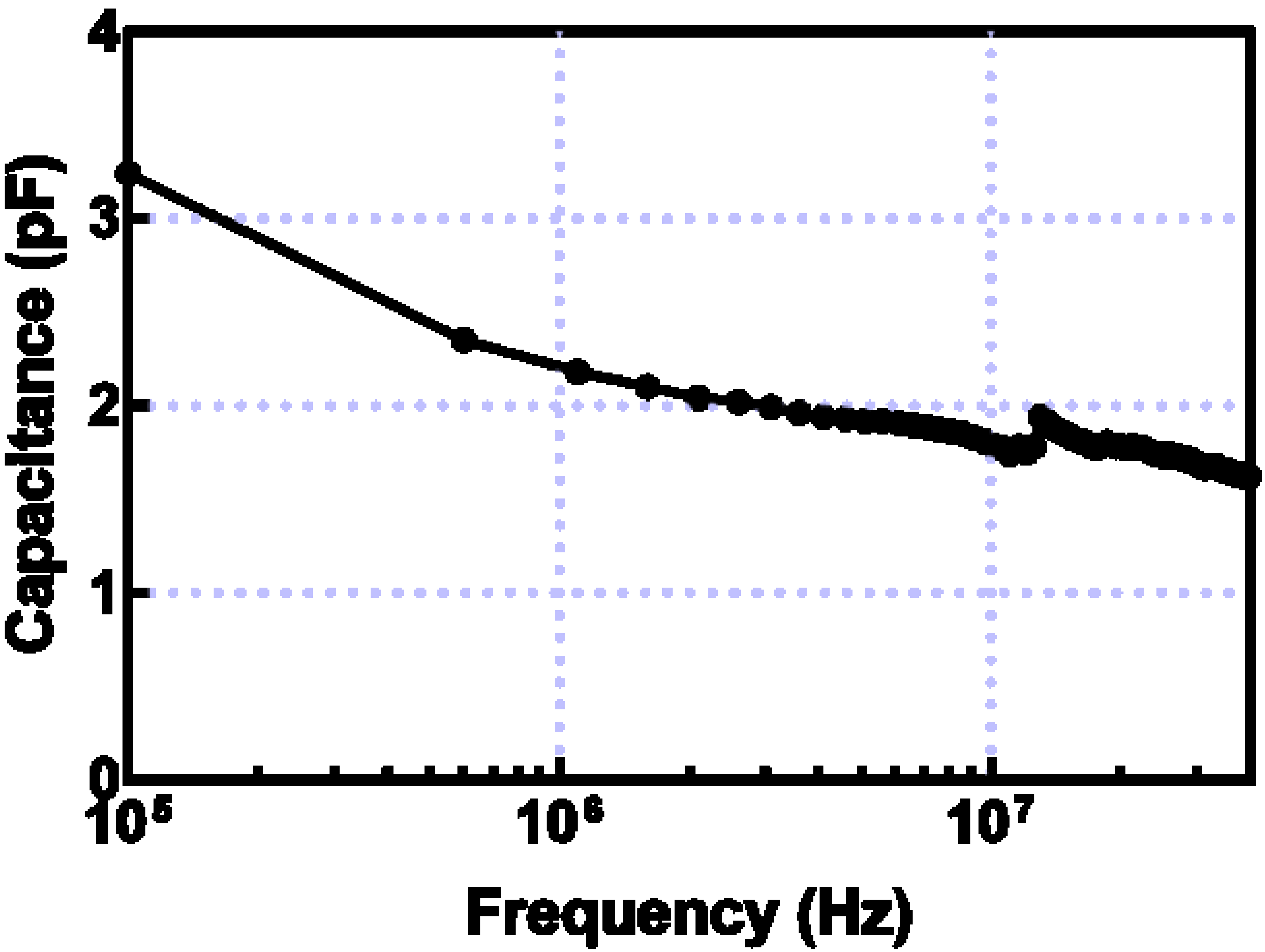

Table1

Table 1 Solubility of analysed biofilm samples at different temperatures

Temperature (°C)	27	50	70	100
Solubility (%)	33.52	33.04	31.03	27.69

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