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### ► To cite this version:

Alexander Efimov, Christophe Dagallier, Céline Frochot, Bauyrzhan Myrzakhmetov, Philippe Arnoux, et al.. LASU: An efficient and stable phthalocyanine dye with tolerable safety profile for self-disinfecting anti-COVID textiles activated by ambient light. *Photodiagnosis and Photodynamic Therapy*, 2024, 45, pp.103978. 10.1016/j.pdpdt.2024.103978 . hal-04530902

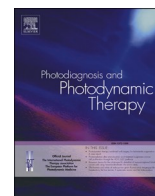
**HAL Id: hal-04530902**

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Submitted on 7 Nov 2024

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## LASU: An efficient and stable phthalocyanine dye with tolerable safety profile for self-disinfecting anti-COVID textiles activated by ambient light

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### ARTICLE INFO

#### Keywords:

Photodynamic antimicrobial chemotherapy

Phthalocyanine

Antiviral

Self-disinfecting textile

Photodynamic therapy

### ABSTRACT

**Background:** Recent COVID crisis has demonstrated that modern society urgently needs an accessible protection against mass infections, especially viruses, as the new strains are appearing at an ever-increasing pace and cause severe harm to the population and the world economy.

**Methods:** We have developed an efficient phthalocyanine photosensitizer LASU, that is suitable for dyeing textiles and allows to prepare reusable self-disinfecting fabrics with strong antiviral properties. The safety profile of LASU was evaluated in accredited laboratories by several in vitro assays according to the OECD-guidelines.

**Results:** The textiles impregnated with LASU phthalocyanine showed a significant antiviral photodynamic effect even under moderate indoor and outdoor light. The dye did not show any genotoxic potential in human lymphocyte micronucleus assay. It showed a possible indication for eye irritation in human EpiOcular™ model and was phototoxic when tested in mouse BALB/c 3T3 cell test in the presence and absence of UVA-irradiation.

**Conclusion:** Novel phthalocyanine-dyed textiles are suitable for general use as self-disinfecting antiviral barriers and materials in hospitals, households, and public places. The safety profile of LASU is the phototoxic effect which is related to LASU's mode of action.

### 1. Introduction

Viruses and microbes will continue to pose a global risk to human health and economy. COVID crisis has shown that large scale quarantines and shutdowns are detrimental to the societal wellbeing. Initially, the sole protection available against Covid infection were face masks and hand hygiene. The issue with the face masks is that they need to be replaced after every few hours of use. Additionally, they merely act as a barrier filter against the invading pathogen, instead of inactivating it. Unreliable quality and issues of supply chain's disruption during a pandemic is another problem associated with the use of face masks. In places like schools or hospitals, it would be beneficial to have self-disinfecting surfaces, barriers and cloths in addition to a common manual disinfection. Simple calculations show that wearing a self-

disinfecting mask that inactivates viruses within 30 min lifetime is as protective as using a new mask every hour. Therefore, there is a huge yet unmet demand for self-disinfecting textiles and filters, which would be equally effective against current and – most importantly – future pandemics.

In this work we aimed to show that a textile dyed with a phthalocyanine photosensitizer could provide a self-disinfecting surface which would be efficient against corona viruses under daylight or conventional indoor light. The tetracationic zinc phthalocyanine compound LASU (Fig. 1) recently developed by us was employed in this work. It was already demonstrated, that LASU molecule has a strong antibacterial and antifungal properties under weak artificial white light when deposited on cellulose support [1–3]. In the present work we studied the antiviral efficiency of a phthalocyanine-impregnated cotton fabrics. We

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<https://doi.org/10.1016/j.pdpdt.2024.103978>

Received 31 October 2023; Received in revised form 15 January 2024; Accepted 16 January 2024

Available online 17 January 2024

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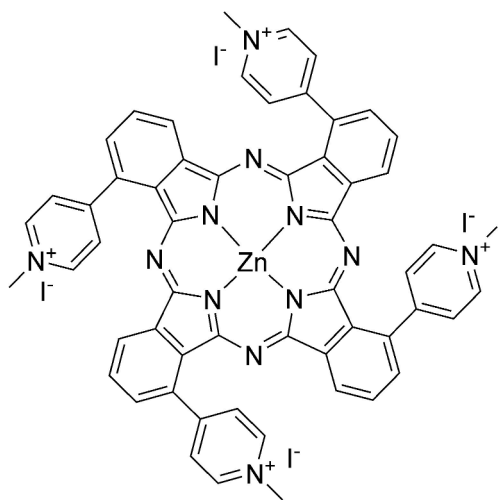


Fig. 1. LASU phthalocyanine.

suggest, that because of the combined antiviral, antibacterial and antifungal effect these textiles can be used to produce self-disinfecting face masks and cloths.

## 2. Materials and methods

Synthesis of LASU phthalocyanine (Fig. 1) is described elsewhere [1].

### 2.1. Photophysical properties

Absorption spectra were measured with Shimadzu UV-3600 UV-visible dual-beam spectrophotometer. The fluorescence spectra were measured with Fluorolog FL3-222 spectrofluorimeter (Horiba Jobin Yvon, Longjumeau, France) equipped with a 450 W xenon arc lamp, a thermostatically controlled cell holder compartment (25 °C), an R928 UV-visible photomultiplier tube (HAMAMATSU Japan) and a liquid nitrogen-cooled InGaAs infrared detector (DSS-16A020L Electro-Optical System Inc, Phoenixville, PA, USA). The excitation beam is separated by a SPEX double grating monochromator (1200 lines/mm blase at 330 nm). Fluorescence was measured by the UV-visible detector via the SPEX dual-array emission monochromator (1200 lines/mm blased at 500 nm). Singlet oxygen generation was measured by the infrared detector via the SPEX dual-array emission monochromator (600 lines/mm blased at 1 μm).

The spectral analysis of the dye in the tissue was done from the back scattered light by the sample using the Kubelka-Munk equation.

$$\frac{K}{S} = \frac{(1 - R_{\infty})^2}{2R_{\infty}}$$

Where  $K$  is the absorption probability of the light over a thickness  $dx$ .  $S$  is the backscattering probability of the light over a thickness  $dx$ .  $R_{\infty}$  is the fraction of the incident light that is back scattered by an infinitely thick sample, four layers of tissue in our case. The white sample of the fabric was used as a reference of a non-absorbing sample.

The setup for spectral and quantitative analysis of the fabrics is shown in Figure S1 of Supporting information. The incident light source was Maglite 3 lamp with a Krypton bulb. The light was analyzed by an Ocean Optics spectrograph Maya. The incident light source was Maglite 3 lamp with a Krypton bulb. The light was analyzed at 45° by an Ocean Optics spectrograph Maya.

### 2.2. Staining and stability of textiles

In a typical staining procedure, pieces of mercerized and unsized cotton tissue, weight 20 g each, were dipped in 1.23 L of a water solution of LASU at a concentration of 14 μmol/L for 5 min. The tissues were then dried and ironed using an electric iron. During the staining procedure, the dye solution was gradually losing its color, upon a successive soaking of every new cotton sample unless completely colorless water was left behind. The dye load on the cotton was therefore calculated as 199 mg/m<sup>2</sup> or 1.53 mg/g of the fabric.

The color of each tissue was assessed using Minolta CR-200B Handheld Chroma Meter. The device had an 8 mm diameter measuring area and diffuse illumination, and 0° viewing angle geometry. The CIELAB color space was used for describing the colors. It is referred as  $L^*a^*b^*$ , and was defined by the International Commission on Illumination (CIE) in 1976. The color is expressed as three values:  $L^*$  for perceptual lightness and  $a^*$  and  $b^*$  for the four unique colors of human vision: red, green, blue and yellow (RGBY). CIELAB was intended as a perceptually uniform space, where a given numerical change corresponds to a similar perceived change in color. It operates with the parameters Delta  $L^*$ , Delta  $a^*$ , Delta  $b^*$  and Delta  $E$ . Delta  $E$  is the global variation of color and is determined by the following equation.

$$\Delta E = \sqrt{\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2}}$$

For each cotton sample, the parameters  $L^*$ ,  $a^*$ ,  $b^*$  were measured. The data and a sample of the color of fabric is shown in Supporting information in Table 1 and Fig. S2. Colorimetry was used to determine the reproducibility in the staining of each textile with LASU, also before and after the washing tests.

For the study of the photobleaching of the dye, the light source was a LED white Sundesk 3 (Dayvia, Lille, France). The illuminance at the surface of the textile was controlled with the Lux meter Model LM 120 from Amprobe and adjusted to be 36,000 Lux, corresponding to the light power density of 16 W/m<sup>2</sup> at 700 nm (See SI, pages 6–9).

The stability against leaching was estimated by washing the dyed cotton samples in a consumer grade laundry machine using a 2 h cycle at 50 °C with addition of a 50 ml solution of a liquid consumer detergent “Le Chat l’expert” by Henkel, containing benzenesulfonic acid mono-10-13-alkyl derivatives and cellulase, amylase and mannanase as the surface active ingredients.

### 2.3. Hazard tests

The possible hazards of LASU phthalocyanine dye were studied on a battery of toxicity tests including neutral red uptake (NRU), cytotoxicity assay using mouse BALB/c 3T3 fibroblasts [4]; skin irritation using human EpiDerm™ tissue [5]; ocular irritation using human EpiOcular™ tissue (Eurofins report STUGC19AA2231-1, 2020); phototoxicity assay using mouse BALB/c 3T3 fibroblasts (Eurofins report STUGC19AA2231-2, 2020); micronucleus assay in human lymphocytes with/without metabolic activation (Eurofins report STUGC19AA2231-3, 2020). The cytotoxicity and skin irritation assays were performed in FICAM, The Finnish Centre for Alternative Methods, Tampere University, and the ocular irritation, phototoxicity and micronucleus assays were performed by the commercial contract research organization, Eurofins laboratories in Munich, Germany. All tests were performed according to the OECD Good Laboratory Guidance

Table 1

The effect of LASU phthalocyanine dye on the viability (given as% as compared to negative control) of EpiDerm™ tissues after 1 hr exposures and 2-day recovery, and the classification to irritant or non-irritant, accordingly.

Test item	Viability%	Classification
solid LASU powder	84,08	non-irritant
LASU-IC20	95,03	non-irritant

[6].

Cytotoxicity studies were performed using BALB/c 3T3 Neutral Red uptake cytotoxicity assay. The study was performed according to OECD GD 129, 2010, an internationally validated and scientifically accepted test method for studying acute basal cytotoxicity. Eight different concentrations of LASU phthalocyanine ranging from 0.0012 to 3.8 mg/ml were tested. Sodium dodecyl sulfate (SDS) (concentrations 4.7–201 µg/ml) was used as a positive control and cell culture medium as a negative control. The BALB/c 3T3 cells were exposed to the LASU, SDS and vehicle control for 48 h. After the exposure, NRU assay was performed to assess the number of viable cells. Washed cell cultures were incubated with 25 µg/ml Neutral red (NR) solution for 3 h. The intracellular NR accumulated by the living cells was then released with acetic acid and the absorption at 540 nm was measured using a Tecan Spark multimode reader. The results were given as dose-response curve with IC<sub>50</sub>.

Skin irritation was tested in compliance with OECD TG 439 using human 3D-reconstructed EpiDerm™ tissues provided by MatTek Corporation. EpiDerm™ tissues are highly differentiated keratinocyte cultures which closely mimic the biochemical and physiological properties of the upper parts of the human skin, i.e. the epidermis. Skin irritation is evaluated by the production of reversible damage to the skin, i.e. EpiDerm™ tissues. In the test, EpiDerm™ tissues, grown in air-liquid interface on tissue inserts, were topically exposed to LASU phthalocyanine solid powder and IC<sub>20</sub> concentration of dissolved LASU (calculated from the cytotoxicity test). 5 % SDS was the positive control and Dulbecco's phosphate buffered saline (DPBS) was the negative control. The exposure time was 60 min, after which the inserts were thoroughly washed, and the incubation was continued with fresh cell culture medium for two more days to allow recovery. Tissue viability was then determined by employing the MTT assay. EpiDerm™ tissues were incubated with a 1 mg/ml MTT solution for three hours, during which viable cells metabolized MTT to formazan. Formed formazan was released from the cells with 2-propanol. The resulting absorbances were read at 570 nm using a Tecan Spark multimode reader. When the absorbance of the test substance is compared to the absorbance of the vehicle control, a classification can be given as to whether the test substance is an irritant or not.

The eye irritation assay was performed using commercially available test method, EpiOcular™ Human Tissue Model (MatTek), that consists of normal, human derived epidermal keratinocytes and mimics the histological, morphological, biochemical, and physiological properties of the human normal epithelium. The test identifies substances causing irritation and serious damage to the eye. In the test, the ability of test substance to induce cytotoxicity in a reconstructed human cornea-like epithelium (RhCE) tissue construct after topical application, expressed as the reduction of mitochondrial dehydrogenase activity (MTT assay), is measure [7]. The exposure time is 6 h followed by 18 h post-treatment period. The results are compared with the concurrent negative controls. Three independent tests were performed. The ocular irritation potential of LASU was assessed from the relative mean tissue viabilities compared to the negative control tissue concurrently treated with Aqua dest.

The *in vitro* 3T3 NRU phototoxicity test was performed using cytotoxicity assay with BALB/c 3T3 mouse fibroblast cell line according to the relevant guidelines e.g. OECD No. 432 [8]. In this test, the phototoxicity potential of LASU was measured by comparing the cytotoxicity of the test substance when tested in the presence and absence of exposure to a non-cytotoxic dose of UVA light. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of the vital dye neutral red a day after the treatment [9]. Relative cell viability, expressed as percentage of untreated controls, was calculated for all eight concentrations of the LASU. To predict phototoxicity potential, the concentrations responses obtained in the presence and absence of UVA-irradiation were compared at the concentration inhibiting cell viability by 50 % in comparison with the untreated controls. Based on this, the PIF (photo-irritation factor) is calculated. Obtained data was analyzed using the Phototox 2.0 Software.

The potential of LASU to induce micronucleus was tested *in vitro* in human lymphocytes with/without metabolic activation according to the OECD TG No. 487 [10]. The test measured whether LASU is a potential carcinogen via genotoxic clastogenic (both numerical and structural) mechanism. The metabolic activation system used was phenobarbital/β-naphthoflavone induce rat liver S-9 fraction. LASU was diluted in cell culture medium (RPMI medium). The exposures used were without metabolic activation 4 and 44 h and with metabolic activation 4 h. The statistical significance was tested using Nonparametric  $\chi^2$  Test.

#### 2.4. Antiviral activity tests

The studies were done at the Unité de Sécurité Microbiologique à Pasteur Lille (France). The antiviral activity of LASU treated textiles was examined against SARS-CoV 227 virus under light illumination according to the standard ISO 18184:2019. The studies were done on 5 × 5 cm pieces of the cotton tissues stained with LASU as described above and containing 199 mg/m<sup>2</sup> or 1.53 mg/g of LASU phthalocyanine. Two types of fabrics were used for the tests: a) stained and not bleached; b) stained and pre-bleached for 6 h under halogen lamp with color temperature 2900°K, illuminance 36 000 Lux (power density 64 W/m<sup>2</sup> or the total light dose of 140 J/cm<sup>2</sup> at 700 nm, see Supporting Information pages 6–9). During the antiviral experiments, the white light illumination was performed with a Sundesk 3 (Dayvia, Lille, France). The illuminance was controlled with a luxmeter Model LM 120 from Amprobe and was adjusted to be 590 Lux, which corresponds to the light power density of 270 mW/m<sup>2</sup> at 700 nm (See SI, pages 6–9). The 5 × 5 cm stained cotton tissues were inoculated with 50 µL of a 1.10<sup>7</sup> DICT 50/mL virus solution. The dry tissues were exposed to 590 Lux for 30 min (light dose 49 mJ/cm<sup>2</sup> at 700 nm). The surviving viruses of HCoV229E after the exposure of the fabric were extracted and concentrated. The solution was used to contaminate Huh-7 cell cultures for 6 days. The number of contaminating viruses were counted from dilutions until their absence.

### 3. Results and discussion

#### 3.1. Cotton alone

The photophysical properties of the cotton alone were evaluated. This included the absorption spectrum (Fig. 2), fluorescence emission spectrum (Fig. 3), fluorescence decay profile (Fig. 4) and singlet oxygen emission spectrum after cotton excitation (Fig. 5).

The cotton fabrics absorbed the light below 400 nm. The cotton exhibited bi-exponential decays with lifetimes of 2.0 ns (36 %) and 6.1 ns (64 %).

It should be noted that even the non-dyed cotton demonstrated a

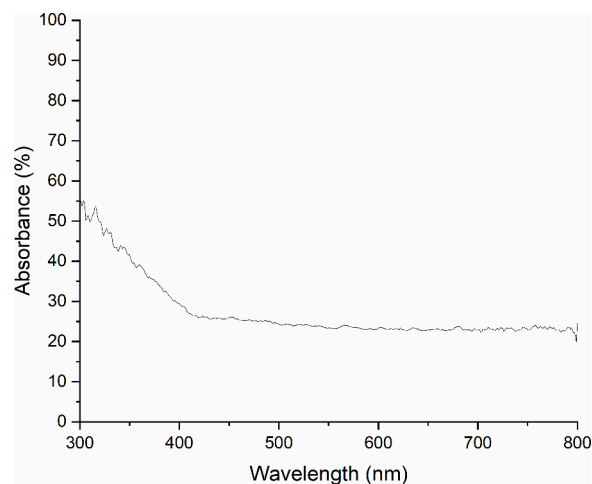


Fig. 2. Cotton absorption spectrum.

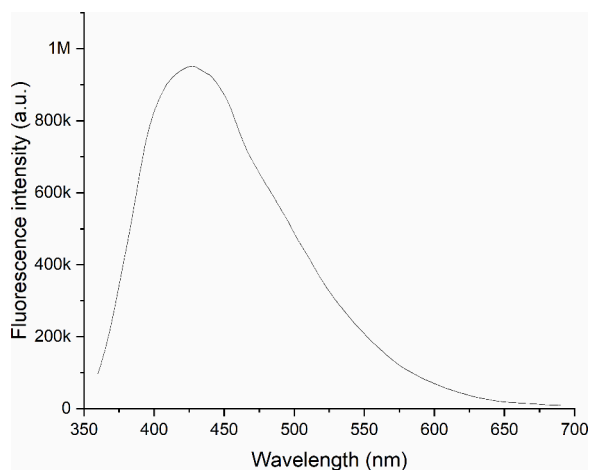


Fig. 3. Cotton emission spectrum upon 350 nm excitation.

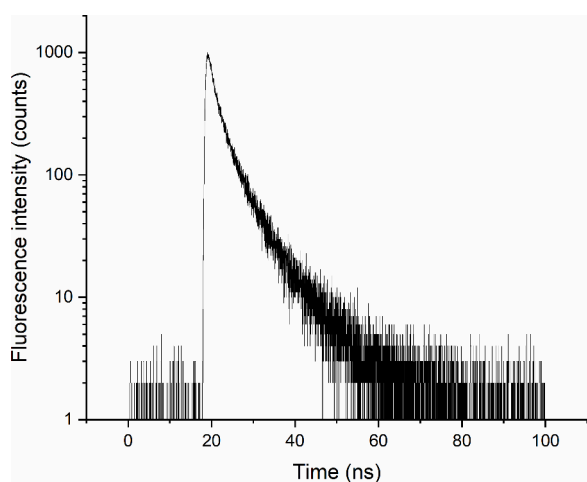


Fig. 4. Fluorescence decay of cotton upon excitation at 408 nm.

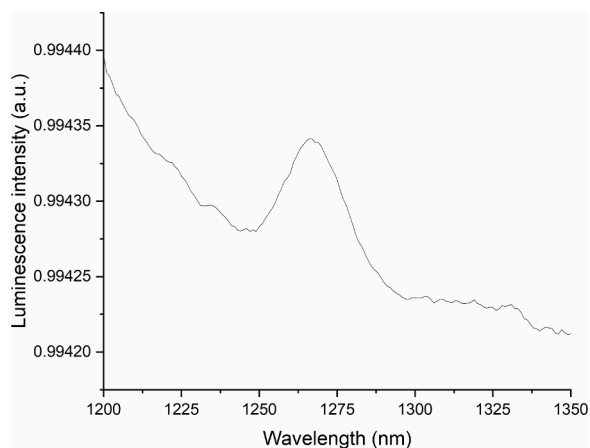


Fig. 5. Singlet oxygen luminescence spectra after illumination of cotton upon excitation at 350 nm.

weak emission singlet oxygen upon light illumination (Fig. 5), which was attributed to the presence of brightening agents probably employed during the yarn manufacturing.

### 3.2. LASU alone

Absorption spectra of LASU phthalocyanine were measured in different solvents (Fig. 6).

LASU presents spectra consistent with phthalocyanines with a B band around 350 nm and two Q bands around 630 and 700 nm. The bands measured in water, D<sub>2</sub>O and toluene were wide and weak, indicating poor solubility and an aggregation of the molecule.

Fluorescence emission was measured in all five solvents and the spectra are presented in Fig. 7.

LASU did not exhibit fluorescence emission in water or toluene, though it emits weakly in D<sub>2</sub>O. It showed two emission bands around 680 nm and 725 nm in ethanol and toluene. Fluorescence quantum yields were calculated using TPP in toluene as a reference and below 0.01 in D<sub>2</sub>O, 0.08 in ethanol and 0.10 in DMF.

The fluorescence emission decays were measured (Fig. 8) as well. In all three solvents where LASU emits, the fluorescence lifetimes were close and consistent with those of phthalocyanines, namely 1.8 ns in DMF and 2.0 ns in ethanol and D<sub>2</sub>O.

Finally, we evaluated the capacity of LASU to produce singlet oxygen upon illumination at 590 nm and the spectra are presented in Fig. 9.

LASU did not generate singlet oxygen in water or toluene. The quantum yield of singlet oxygen was 0.07 in the D<sub>2</sub>O, 0.42 in ethanol and 0.47 in DMF. The singlet oxygen quantum yields were calculated using tetramethylpyridiniumporphyrin in D<sub>2</sub>O with singlet oxygen quantum yield of 0.90 [11] and pyropheophorbide in DMF of 0.52 [12] and in ethanol of 0.53 [13] as references.

### 3.3. LASU-impregnated cotton

The amount of LASU loading was ca. 3 mg/g of cotton. After deposition, the optical spectra of LASU-cotton conjugate were measured (Fig. 10).

The molar extinction coefficient of LASU in methanol at the Q band at 687 nm was  $1.3 \times 10^5$  L/mol/cm. The vibrational satellite bands at 623 nm and 585 nm were also visible. In water, LASU formed aggregates as shown by the new band at 651 nm. In textiles, the valley at 650 nm which corresponded to the absorption of the back scattered light, was not present. The valley between the vibrational bands of the monomer was filled by the aggregates. The monomer Q band was red shifted to 693.5 nm. The valley at 650 nm was however observed on the fluorescence excitation spectrum of a fabric because the aggregates were not fluorescent. The vibrational satellites of the monomer could be clearly

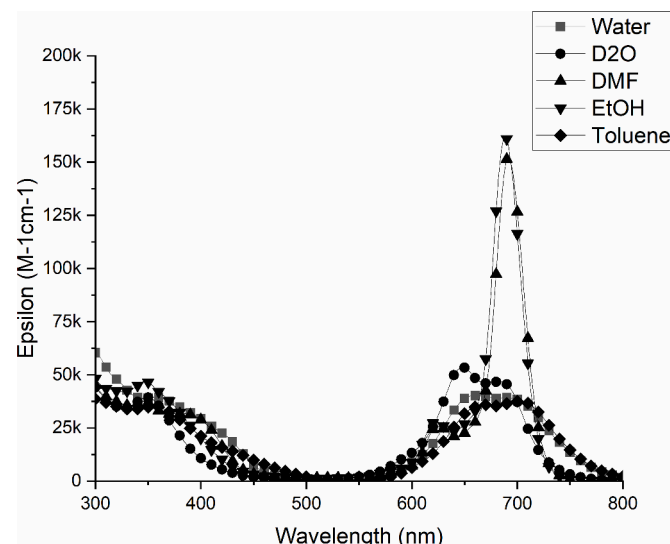


Fig. 6. Absorption spectra of LASU in water, D<sub>2</sub>O, DMF, EtOH and toluene.

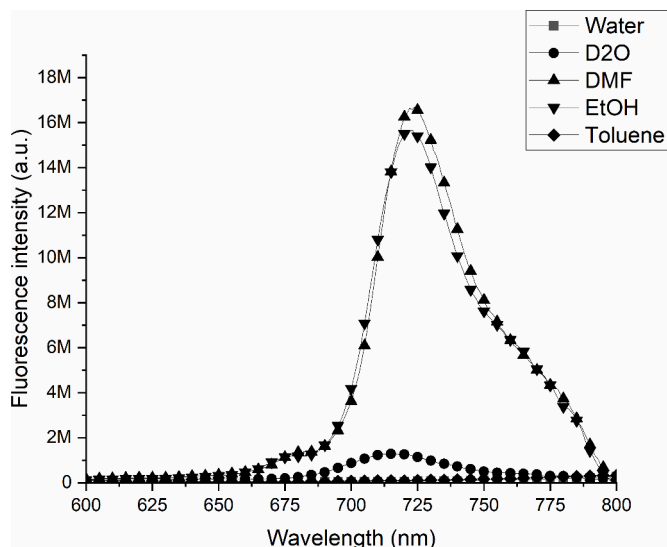


Fig. 7. Fluorescence emission spectra of LASU in water, D<sub>2</sub>O, DMF, EtOH and toluene.

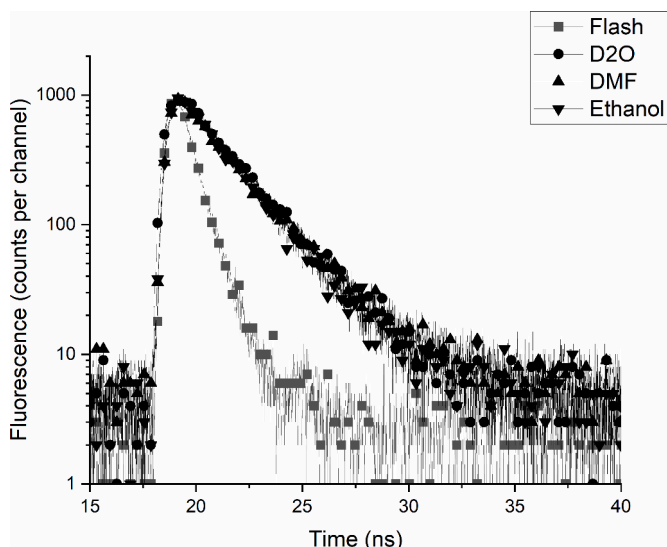


Fig. 8. Fluorescence decays of LASU in water, D<sub>2</sub>O, DMF, EtOH and toluene.

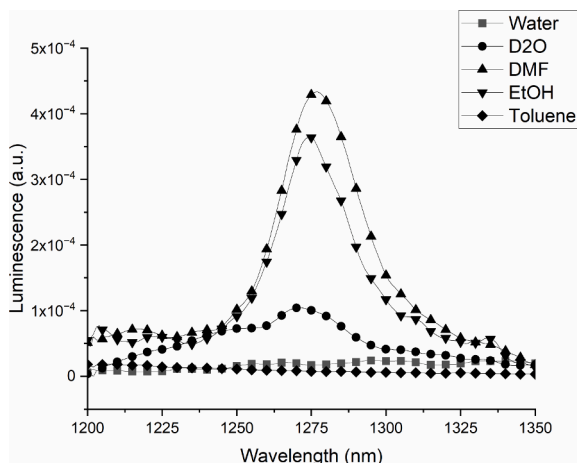


Fig. 9. Singlet oxygen emission spectra after excitation of LASU at 590 nm in water, D<sub>2</sub>O, DMF, EtOH and toluene.

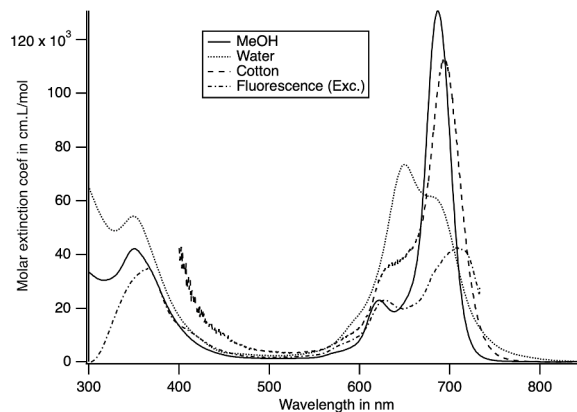


Fig. 10. Absorption and fluorescence of LASU on cotton.

seen in the excitation spectrum. The main Q band in the excitation spectrum of LASU was broadened because of the saturation of the absorption that reached 100 % of the incoming light at the Q band maximum.

All the textile samples were showing a fluorescence emission upon light excitation, an example of the fluorescence spectra is given in Fig. 11.

We also detected the formation of singlet oxygen after excitation at 650 nm or 700 nm of the textile (Fig. 12).

LASU Bleaching.

The resistance to bleaching was measured by submitting the samples to the light exposure at 36,000 Lux (a 1/3 of the intensity of a bright sunlight, or  $a \times 60$  times intensity of a typical indoor light) for up to 220 h and washing in a laundry machine (Fig. 13). The concentration of the remaining dye in the fabric was calculated from an exponential regression according to:

$$\frac{[Dye]}{[Dye_0]} = \exp(-k_{Bleach}Iot - k_{Leach}n)$$

From the slope of the curve the estimated bleaching time  $1/Iok_{Bleach}$  in sun light (108 000 Lux) was therefore found to be 36 h, or 260 days under indoor light. Such a big difference reflected the light flux difference between the natural sun light and a typical indoor light conditions. The leaching rate  $k_{Leach}$  of LASU was 0.2 per wash.

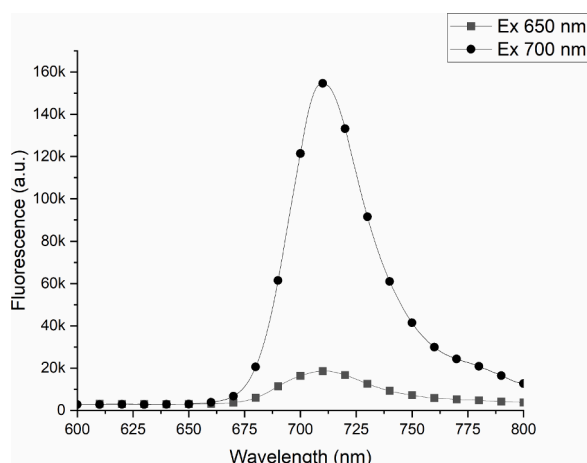


Fig. 11. Fluorescence emission spectra of LASU in the textile upon excitation at 650 nm (squares) and 700 nm (circles).

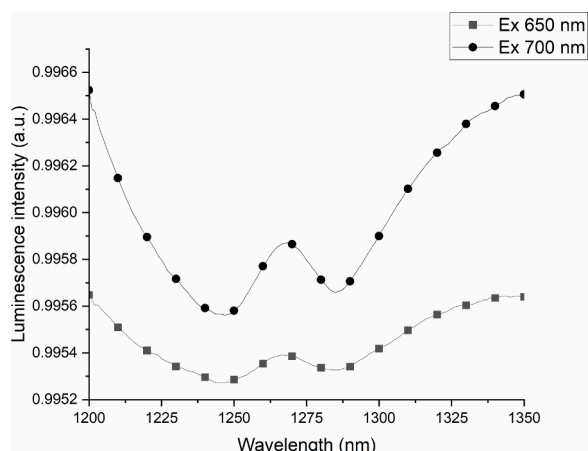


Fig. 12. Singlet oxygen luminescence spectra after illumination of LASU-2 after excitation at 650 nm (squares) and 700 nm (circles).

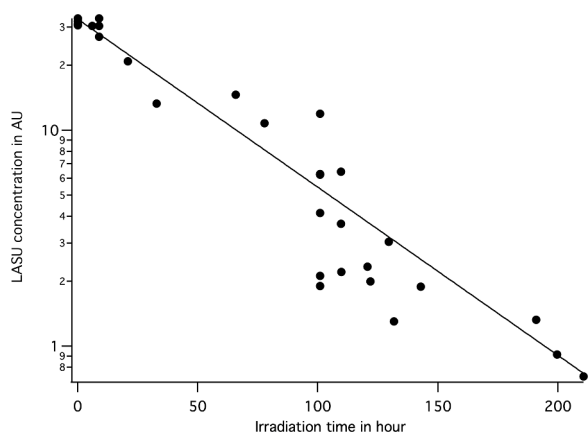


Fig. 13. The resistance of a cotton textile dyed with LASU against bleaching at 36,000 Lux and leaching.

### 3.4. Hazard tests

#### 3.4.1. Cytotoxicity in BALB / c3T3 NRU assay

LASU phthalocyanine caused a dose-dependent reduction of BALB/c 3T3 fibroblast viability as presented in Fig. 14.

The  $IC_{50}$  value was 6.4  $\mu\text{g/ml}$  ( $R^2$  0.96) (GraphPad Prism® v.6.05

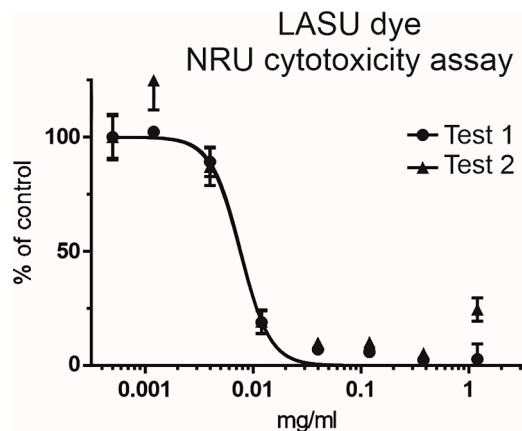


Fig. 14. The dose-response effect of LASU phthalocyanine on BALB/c 3T3 fibroblast viability. The assay was performed twice (Test 1 and Test 2) with six replicates of each concentration in both assays.

software).

#### 3.4.2. Phototoxicity in mouse BALB/c 3T3 cells

In the first experiment LASU was tested at concentrations of 1000, 316, 100, 31.6, 10.0, 3.16, 1.0 and 0.316  $\mu\text{g/mL}$ . In the absence of UVA irradiation cytotoxicity was obtained at 10  $\mu\text{g/mL}$  and in the presence of light cytotoxicity was obtained from 0.032  $\mu\text{g/mL}$  onwards. The calculated PIF of 9177.184 exceeded the threshold value of 5 showing that LASU had phototoxic potential.

Because the color of LASU interfered the interpretation of the test results, an additional washing step was included into the second experiment. The concentrations for the second experiment without UVA irradiation were the same as in the first experiment but the concentrations with UVA-irradiation were 1.0, 0.316, 0.1, 0.0316, 0.01, 0.00316, 0.001 and 0.000316  $\mu\text{g/mL}$ . In the absence of UVA-irradiation cytotoxicity was observed from 3.16  $\mu\text{g/mL}$  onwards. In the presence of UVA-irradiation, the respective concentration was 0.1  $\mu\text{g/mL}$ . The respective PIF value of 17.297, exceeded the threshold value of 5 showing the clear phototoxic potential. The PIF values are presented in the Table 2 of Supporting information. To conclude, the clear phototoxic potential of LASU was attributed to its mode of action.

#### 3.4.3. Skin irritation in human EpiDerm™ skin irritation test

Living reconstructed human epidermis (EpiDerm™) tissues were used to evaluate the possible skin irritation potential of LASU phthalocyanine dye. Because of the intense color of the LASU phthalocyanine, additional color interference and MTT reduction tests were carried out. According to the EU and GHS classification, a test material is classified as an irritant if the mean relative tissue viability of triplicate tissues exposed to a test material is reduced below 50 % of the mean viability of the negative controls (R38 / Category 2 or no label, [14]). In our tests, the calculated viability of EpiDerm™ tissues was 84 % after exposure to the solid LASU dye and 95 % upon exposure to the  $IC_{20}$  concentration compared to the negative controls (see Table 1).

Based on the results, we classified LASU phthalocyanine as a non-irritant substance.

#### 3.4.4. Eye irritation in human epicular test

Three experiments were performed. In the first experiment LASU showed relative viability of 61 % and classified as non-irritant. In the second experiment LASU showed relative viability of 48.0 % and classified as irritant. In the third experiment LASU showed relative viability of 38.2 % and classified as irritant. The challenge was high standard deviations in results most likely due to difficulties with washing steps. LASU tended to form clumps on the tissue construct.

Due to the uncertainties of clumping of LASU in the test system, no conclusive results were obtained. To confirm whether LASU is ocular irritant or non-irritant additional testing is needed.

#### 3.4.5. Micronucleus in vitro in human lymphocytes with/without metabolic activation

In experiment I at the end of exposure period of 4 h no precipitation of LASU at any concentration was observed without metabolic activation. However precipitation was apparent with metabolic activation at the concentrations 250  $\mu\text{g/mL}$  and higher. In experiment II without metabolic activation, precipitation was observed at the concentrations of 350  $\mu\text{g/mL}$  and higher in the end of the respective exposure of 44 h treatment.

The limit value of the relative cell growth was 70 % compared to the negative/solvent control which corresponds to 30 % of cytostasis. In experiment I without metabolic activation, no increase of the cytostasis above 30 % was noted up to the concentration of 200  $\mu\text{g/mL}$  but at concentration of 400  $\mu\text{g/mL}$  a cytostasis of 55 % was observed. In experiment I with metabolic activation, no increase of the cytostasis above 30% was noted up to a concentration of 125  $\mu\text{g/mL}$ . At a concentration of 250  $\mu\text{g/mL}$  a cytostasis of 35 % was observed. In

**Table 2**  
Antiviral activity of neat cotton, LASU cotton and BM cotton.

Sample	Pre-bleaching time	Repetition	Virus concentration DICT <sub>50</sub> /ml	Log DICT <sub>50</sub> /ml	Average log	Average reduction	Efficiency according to ISO 18184
Cotton non treated	n.a.	1	$1.00 \times 10^5$	5.0	5.0	n.a.	n.a.
		2	$1.00 \times 10^5$	5.0			
		3	$1.00 \times 10^5$	5.0			
Cotton with LASU	0 h	1	$3.2 \times 10^1$	1.5	1.6	3.4	Excellent
		2	$3.2 \times 10^1$	1.5			
		3	$4.22 \times 10^1$	1.6			
	6 h	1	$5.62 \times 10^1$	1.8	1.7	3.3	Excellent
		2	$4.22 \times 10^1$	1.6			
		3	$5.62 \times 10^1$	1.8			
Cotton with BM	0 h	1	$1.0 \times 10^4$	4.0	4.1	0.9	n.a.
		2	$1.33 \times 10^4$	4.1			
		3	$1.78 \times 10^4$	4.3			
	6 h	1	$1.33 \times 10^5$	5.1	5.0	0.0	n.a.
		2	$7.50 \times 10^4$	4.9			
		3	$7.50 \times 10^4$	4.9			

experiment II without metabolic activation, no increase of the cytostasis above 30 % was observed up to a concentration of 350 µg/mL.

The numbers of micronucleated cells were within or below the historical control limits of the negative control and did not show a biologically relevant increase compared to the concurrent negative control. No statistically significant increases ( $p < 0.05$ ) of cells with micronuclei was noted in any dose group of LASU evaluated in experiment I and II with and without metabolic activation. The statistically significant decreases are regarded as not biologically relevant. The results are summarized in Tables 3 and 4 of Supporting information.

In conclusion, LASU did not induce structural and/or numerical chromosomal damage in human lymphocytes and therefore was considered to be non-mutagenic with respect to clastogenicity and/or aneugenicity.

### 3.5. Antiviral studies

The aim of the present study was to evaluate the ability of a dyed cotton to inactivate pathogenic viruses in real-life conditions. We have already demonstrated that the LASU-impregnated paper is efficient against bacteria and fungi [1–3], whereas in the present study we were focusing on its antiviral action. Also, in the previous study we employed a filter paper as the dye support. In the present case we employed a cotton fabric of 133 g/m<sup>2</sup> density, which could be used as a material for self-made face masks and protective cloths in case of a pandemic.

It should be noted here that in a strictly scientific study of a photodynamic activity one needs a well described light source and a quantified light dosage, preferably expressed in J/cm<sup>2</sup> at a certain wavelength value. This would be however quite irrelevant for a practical use of a self-disinfecting textile, since it should ideally work under available light sources of natural (sunlight) or artificial (incandescent, fluorescent, LED) origin. These light sources would all be that or another kind of a “white light source”, and their intensity would be easiest to evaluate in an accessible unit of SI system – Lux. The luxmeters are designed for metering of indoor and outdoor illumination, are inexpensive and easily available, and meant for use by untrained personnel. We therefore suggest that quantifying the efficiency of photoantimicrobial materials at a given Lux light intensity is justified and, most importantly, would allow to bridge the gap between the academic research and the practical use of these indeed very important materials. Considering the mentioned above, we employed the Lux units in our study. The corresponding recalculated spectral energy density for the testing conditions can be found in Supporting Information, pages 6–9.

The studies were done in Unité de Sécurité Microbiologique à Pasteur Lille (France) as a contract work according to the ISO 18184:2019 standard [15]. Two sets of LASU dyed fabrics were employed. The first one was used as prepared after impregnation, and the second one was

photobleached for 6 h at 36 000 Lux of 400 W halogen light 2900 K (total light dose 140 J/cm<sup>2</sup> at 700 nm, see SI pages 6–9) to imitate the loss of activity upon fading out. A negligible decrease of absorption was observed upon bleaching. As another reference, the cotton samples dyed with Methylene Blue (BM) treated in a similar way were employed. Before testing, each sample was sterilized by autoclaving at 121 °C for 15 min. A 50 µL aliquots of a suspension of corona virus HCoV-229E at a concentration of  $1 \times 10^7$  TCID<sub>50</sub>/mL was deposited onto the tested fabrics. It was then left to dry under a laminar flow hood until visually dry. The dried contaminated sample was placed under a light therapy lamp Sundesk 3 (Dayvia, Lille, France) at a distance that allows exposure of 590 Lux for 30 min (total light dose 49 mJ/cm<sup>2</sup> at 700 nm). Each sample was tested in a triplicate. At the end of the exposure time each sample was placed in 10 mL of culture medium with glass beads and shaken vigorously to recover viruses remaining on the surface. The viruses were then concentrated on an Amicon Ultra-15 column and quantified by cytopathic effects titration using the dilution-limit method in 96-well microtitre plates on plated cells. The infectious titres were calculated using the Spearman and Kärber method.

The fabric treated with the LASU molecule showed antiviral activity of average 3.4 log, i.e. reduction of 99.97 % reduction in viral load when the tissue has never been previously exposed to light. The pre-bleached tissue showed 3.3 log antiviral activity, i.e. 99.95 % reduction in viral load. According to the ISO 18184:2019 standard such an activity is considered “excellent”. The results of the study are presented in the Table 1.

In comparison, a tissue treated with a common photo-disinfectant, Methylene Blue, showed antiviral activity of only 0.9 log (86.66 % reduction in viral load) when the tissue had never been previously exposed to light. When the tissue had been previously exposed to light for 7 days, it no longer showed any antiviral activity.

It should be noted here that in traditional antimicrobial studies the 3 log value is considered as borderline to claim any antimicrobial activity at all. In our work we aimed to evaluate the minimal conditions at which the LASU-dyed cotton would be effective, and the increase of efficiency can be easily achieved by increasing the light intensity or duration. We therefore conclude, also considering our previous work [1–3], that under a 600 Lux illumination in 30 min time the LASU dyed cotton with dye load 2 mg/g is efficient against viruses and bacteria, and with the increased dye load it is also efficient against fungi.

## 4. Conclusion

Photodynamic antimicrobial LASU textiles are effective against viral, bacterial, and fungal pathogens and can be activated by a moderate artificial, ambient or natural light. Considering the safety of use of the LASU dye, it offers a tolerable safety profile for a textile dye. The self-



disinfecting LASU textiles offer potential protection against combined microbial infections, and most importantly against unknown or unidentified infections.

#### Declaration of competing interest

There is no conflict of interests in this work.

#### CRediT authorship contribution statement

**Alexander Efimov:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Conceptualization. **Christophe Dagallier:** Writing – review & editing, Resources, Funding acquisition. **Céline Frochot:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Bauyrzhan Myrzakhmetov:** Writing – review & editing, Writing – original draft, Investigation. **Philippe Arnoux:** Writing – review & editing, Writing – original draft, Investigation. **Tuula Heinonen:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Marika Mannerström:** Writing – review & editing, Writing – original draft, Investigation. **Tarja Toimela:** Writing – original draft, Investigation. **Zafar Ahmed:** Writing – original draft, Investigation. **Jean Frédéric Audibert:** . **Benoît Habermeyer:** Methodology, Investigation. **Serge Mordon:** Writing – original draft, Methodology, Investigation. **Robert Bernard Pansu:** .

#### Acknowledgments

Funding from Christex Co (Lille, France), BusinessFinland (project LASU) and the Research Council of Finland (Flagship Program PREIN, decision 320165) are gratefully acknowledged.

#### Supplementary materials

Supplementary material associated with this article can be found, in

the online version, at [doi:10.1016/j.pdpdt.2024.103978](https://doi.org/10.1016/j.pdpdt.2024.103978).

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