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Article *in* Progress on Chemistry and Application of Chitin and its Derivatives · September 2021

DOI: 10.15259/PCACD.26.011

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PREPARATION AND CHARACTERISATION OF NEW BIOMATERIALS BASED ON CHITOSAN IODIDE WITH BIOLOGICALLY ACTIVE DYES

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Abstract

New composite materials were obtained based on chitosan iodide and organic dyes – methylene blue and fuchsine in fucorcin (Castellani liquid) – by using a simple synthetic procedure. The materials were characterised by scanning electron microscopy, X-ray diffraction, temperature-programmed desorption mass spectrometry, infrared spectroscopy and visible and ultraviolet light spectroscopy. The dyes in the composites were distributed uniformly and did not form separate phases. These composites could form structured porous sponges and films and therefore be used in various fields of application. The materials displayed antibacterial activity against antibiotic resistant gram-positive and gram-negative bacteria.

Keywords: *fuchsine, methylene blue, iodine, chitosan*

Received: 28.02.2021
Accepted: 19.05.2021

1. Introduction

Dyes and polymers are two important classes of organic compounds with wide industrial applications. These substances play an important role in the pharmaceutical, food and textile industries. They influence many biological processes. The study of the interaction between dyes and polymers is important both for their practical application and for the fundamental fields of biochemistry, analytical chemistry and pharmaceuticals. Polymers are of great importance for use in the purification of water contaminated with dyes [1]. The affinity of some polymers for dyes is used for wastewater treatment after the dyeing procedure [2]. On the other hand, coloured polymers are important as materials for various applications. Dyes in polymer matrices are used to create organic light-emitting diodes [3], to make functional optical materials, to generate photovoltaics and to create hybrid solar cells and sensors [4, 5].

Chitin is the second most common natural biopolymer after cellulose, and chitosan is a cationic polysaccharide that is produced from chitin by its deacetylation; it consists mainly of the repeating unit (1,4)-2-amino-2-deoxy-D-glucose (or D-glucosamine). Chitosan is biodegradable, biocompatible and non-toxic. It is widely used as a sorbent of metals and organic compounds (including dyes), in biomedicine, in tissue engineering, as a drug and gene carrier, as an antibacterial agent, in the textile industry, for processing fabrics and paper and in the food industry as an additive to food and food films, among other applications [6-9]. Chitosan is a good film-former. Chitosan can also be used in green electronics, for creating laboratories on a chip and in microfluidics. New compounds for colouring are obtained on the basis of chitosan and its derivatives in combination with various dyes. In the work [10], a new dye for colouring wood was obtained on the basis of carboxymethylchitosan and Acid Red GR. Dye-containing chitosan films can be used as a solder for laser tissue restoration and stitching. Chitosan with dyes is promising for the creation of flexible, biocompatible and easy-to-handle laser-activated glue, various forms of which could be used for specific surgical needs [11-14]. pH-sensitive dyes (natural and synthetic) are used to create 'smart' food films [15-17].

Natural and synthetic dyes are the subject of intense research. Chemical modification of dyes is an effective and inexpensive way to improve their properties, namely increase biocompatibility and antibacterial activity. In this work, we modified two dyes (fuchsine and methylene blue [MB]) with chitosan iodide. We have previously obtained materials based on chitosan in various solvents and brilliant green. From the results obtained, we concluded that chitosan iodide is more promising for the preparation of antibacterial dyes [18].

Fuchsine is a triphenylmethane dye. Basic fuchsine (magenta) is an antifungal agent and is active against staphylococci. Traditional Castellani solution contains boric acid, phenol, fuchsine, resorcinol, acetone, alcohol and water. The antimicrobial properties of fuchsine and Castellani paint have been known for a long time [19, 20]. However, there are interesting new applications of fuchsine. In particular, fuchsine can be used in the development of sensors and in photonic devices. Researchers presented the development of a colorimetric probe based on basic fuchsine for the detection of NO_2^- in water supply systems and wastewaters [21]. In another study, polymer films of polymethyl methacrylate doped with basic fuchsine were obtained, their characteristics were investigated and a conclusion was drawn about the possibility of using them in photonic devices [22]. New fuchsine dye can be used in solar cells [23]. Chitosan is generally considered a sorbent for binding fuchsine [24, 25]. The combination of Castellani dye as an antiseptic with chitosan has not been previously considered.

MB is a basic thiazine dye with interesting properties and points of application in various fields. MB has been used as an antiseptic since its discovery in 1876, and it continues to find

new applications as an antimicrobial agent [26], including in association with the surface of medical polymers [27]. Effective antimalarial compositions are being developed on the basis of MB [28, 29]. Of note, it has potential in slowing down the neurodegenerative Alzheimer's disease; the mechanism of the inhibitory action of MB is being actively studied [30-32]. The photophysical properties of MB are used in photodynamic cancer therapy [33-37]. The use of MB in nanotechnology is based on its fluorescence, redox potential and aggregation properties. MB has been used to create biodegradable micro- and nanospheres as a contrast agent for optical coherence tomography, while the binding of MB in a polymer (poly lactic-co-glycolic acid [PLGA]) reduces toxicity and improves signal strength [38]. MB and gold nanoparticles have been incorporated into commercial polyvinyl chloride (PVC) catheters [39]. Exposure to red laser light on modified catheters caused fatal photosensitisation of *Staphylococcus epidermidis* and *Escherichia coli*. MB is an object of molecular biophysics. Researcher have investigated interactions of MB with serum albumin [40]. Mass spectrometric methods for studying the interaction of MB with various compounds are being developed [41, 42]. The ability of MB to transfer electrons has led to its use as an intermediary component in biosensors. In the new generation of electrochemical amperometric biosensors, MB and DNA hybrids are actively used [43]. MB immobilised on cellulose acetate modified with titanium dioxide has been used as a sensor for the determination of ascorbic acid [44]. The possibilities of using MB in solar cells are also interesting [45-48].

Hybrid materials based on MB and chitosan could have a number of useful applications. However, the data on the creation of such materials are extremely scarce. Chitosan and chitin, as well as their derivatives, are mainly considered MB sorbents and means of cleaning the environment from dyes. Thus, in this work, a sorbent for sorption of MB was obtained on the basis of chitosan and potassium iodate [49]. In addition, chitosan with MB was used for electrochemical detection of DNA (biosensor) [50]. Moreover, hydrogels of chitosan and MB were used in photodynamic therapy [51]. Our study should breathe new life into well-known organic dyes with biological activity.

2. Materials and Methods

We used two forms of chitosan – one with a molecular weight of 200 kDa and a deacetylation degree (DD) of 89% and one with a molecular weight of 500 kDa and a DD of 80.5% (Bioprogress, Moscow) – and fucorcin (Phytopharm, Artemovsk Ukraine). One millilitre of active fucorcin solution contains fuchsin basic, 4 mg; boric acid, 8 mg; phenol, 39 mg; acetone, 0.049 ml; resorcinol, 78 mg; ethanol 96%, 0.095 ml; excipient fucorcin; and purified water. Other chemical used were MB (Sigma), HI (55%), polyvinyl alcohol (Sigma) and NaOH (Merck).

2.1. Preparation of Solution and Biomaterials Based on Chitosan Iodide With the Addition of Castellani Paint and Methylene Blue

The samples indications in the text is as follows: ChI chitosan iodide; ChF0.25, ChF0.5, chitosan iodide with different concentration of fucorcin; ChMB1, ChMB2, chitosan iodide with different concentrations of MB.

To obtain chitosan iodide with fucorcin (fuchsin), the following steps were performed. Two grams of chitosan (200 kDa, D 89%) was added to 50 ml of distilled water and left to activate for 1 h. Then HI (55%) was added dropwise with vigorous stirring for 20 min until chitosan (ChI) was dissolved. Next, a fucorcin solution was introduced – either 0.25 ml (ChF0.25) or 0.5 ml (ChF0.5) – and the absorbed solution was stirred actively for 10 min. As a result, the concentration of fucorcin was 0.5% in ChF0.25 and 1% in

ChF0.5%.

To obtain chitosan iodide with MB, the following steps were performed. One gram of chitosan (500 kDa, DD 80.5%) was added to 30 ml of distilled water and left for 1 h to activate the polymer. Then HI (55%) was added dropwise until chitosan was dissolved and the pH was 4.0, and the mixture was actively mixed. Next, 0.04 g of MB was dissolved separately in 20 ml of warm water (50-60°C). The two solutions were combined (total volume 50 ml) and stirred for an additional 10 min. This solution contained 0.08% MB (ChMB1). In the second case (ChMB2), the procedure was the same, but 3 times more MB was dissolved (0.12 g). Hence, this solution contained 0.24% MB.

F0.25, F0.5, MB1 and MB2 – aqueous solutions of fucorcin or MB in similar concentrations – were used as reference solutions in antibacterial tests.

From the initial solutions, films, sponges and beads were obtained. They were used for dyeing medical gauze and white coarse calico.

Thin films were obtained by drying the obtained solutions in a mixture with polyvinyl alcohol (1:1) on Teflon, at room temperature.

Nanostructured sponges were obtained by vacuum drying the resulting solutions after preliminary freezing (-5°C). Some of the sponges were neutralised in ammonia vapour and freeze-dried.

To obtain beads, the resulting solutions were added dropwise to an alkaline solution and washed with water until a neutral pH was reached. Some of the beads were lyophilised.

To stain gauze and fabric (pieces of gauze folded six times and pieces of coarse calico), 3 ml of these solutions diluted in 30 ml of distilled water were used. The solution and fabric was incubated in an ultrasonic bath for 30 min. The gauze and fabric were air dried and washed to remove excess dye.

2.2. Characterisation of Materials Using the Example of Freeze-Dried Sponges

The crystal structure of sponges was studied with X-ray diffraction (XRD), the molecular structure was determined by Fourier-transform infrared (FTIR) spectroscopy and the morphology was analysed with scanning electron microscopy (SEM). Temperature-programmable desorption mass spectrometry (TPDMS) was used to evaluate the presence and yield of iodine.

In this work, XRD was performed on an automated DRON-4-07 diffractometer (IC "Bourevestnik", St.-Petersburg, Russia). CuKα radiation (wavelength 0.154 nm) and Bragg-Brentano focusing θ-2θ (where 2θ is the Bragg angle) were used. The experimental results were processed with the software package DifWin-1 (Etalon PTC, Russia).

SEM was performed on a REMMA-102 scanning electron microscope (JSC "SELMI", Sumy, Ukraine) To prevent the accumulation of surface charge in the electron-probe experiment, the dielectric samples were covered with a thin layer (30-50 nm) of silver in a vacuum unit a VUP-5M (JSC "SELMI", Sumy, Ukraine).

The IR spectra in the range of 400-4000 cm^{-1} were recorded on a Spectrum-One spectrometer (Perkin Elmer). Before the study, the samples in powder form were mixed with KBr powder (2.5-3.0 mg of sample per 300 mg of KBr) and compressed into dense tablets of the required size. Spectra were processed using the Spectrum v5.0.2 standard software, a process that includes smoothing, baseline correction, changing the slope of the spectrum and simulating an increase in sample concentration or thickening of the absorbing layer.

TPDMS was used to evaluate the presence and yield of iodine. The absorption spectra of aqueous solutions of 2% ascorbic acid, HI, 0.01% brilliant green (for comparison) and complex preparations in liquid form based on chitosan and brilliant green were taken. The optical density of the solutions was measured on a photometer with a diffraction grating

KFK-3-01 in glass cuvettes (1-cm layer thickness). The wavelength was 305-800 nm.

2.3. Antibacterial Activity of the Resulting Solutions

To assess the antibacterial activity of the chitosan-dye compositions, we used the reference strains of *Staphylococcus aureus* and *Escherichia coli*. We determined the minimum inhibitory concentration (MIC) for test solutions against each bacterium by use of a broth macrodilution method. First, the 10 concentrations of each sample were prepared from stock solution using Mueller-Hinton broth in ratios from 1:2 to 1:1024. Then, overnight cultures of bacteria were diluted in Mueller-Hinton broth and added into each tube with chitosan-dye composites to reach a final bacterial concentration of 1×10^5 colony-forming units (CFU)/ml. The dilutions of the pure dyes were used as controls. All tubes were incubated at 37°C for 24 h. Subsequently, the tube with the lowest concentration that completely inhibited visual growth of bacteria (no turbidity) was considered the MIC.

3. Results and Discussion

3.1 Morphology and Visualisation

The formation of chitosan iodide proceeds according to the following scheme (Figure 1):

The chemical and physical nature of the interaction of chitosan iodide with fuchsine and MB (Figure 2) is not clear and may include hydrophobic interactions, hydrogen bond formation, formation of dye nanoparticles between polymer fibres and inclusion complexes.

The resulting solutions were coloured pink when fucorcin was added, and blue in case of MB, with different intensities depending on the concentration of dyes. The optical characteristics of solutions are presented in Figure 3.

Various materials were obtained from the solutions (Figure 4).

When staining fabrics, the final colour did not differ visually depending on the concentration of solutions, especially when staining gauze (Figure 5).

The morphology of the sponges was studied with optical microscopy and SEM (Figures 6–8). The sponges prepared from all three solutions had a porous, fairly regular structure with a pore size of about 50-150 μm. There was no remarkable difference in morphology.

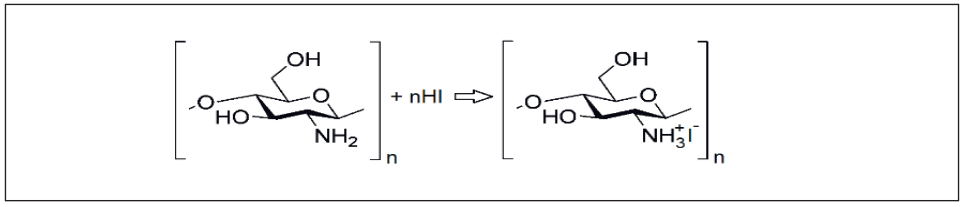

Figure 1. Chitosan iodide synthesis

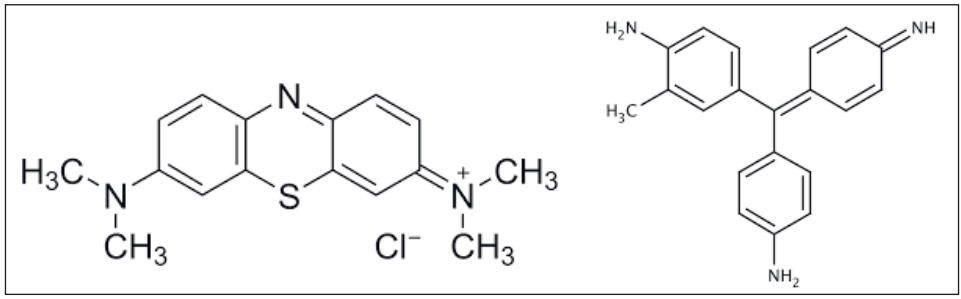

Figure 2. Chemical formulae of methylene blue (left) and fuchsine (right)

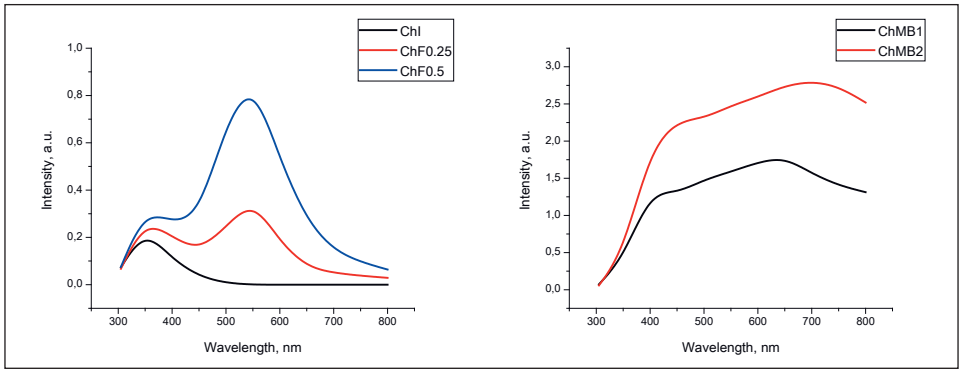

Figure 3. Ultraviolet-visible light spectra of ChI, ChF0.25, ChF0.5 (left), ChMB1, ChMB2 (right). The peak at 350-400 nm corresponds to iodine and its compounds, 550 nm to fuchsine and 650 nm to methylene blue

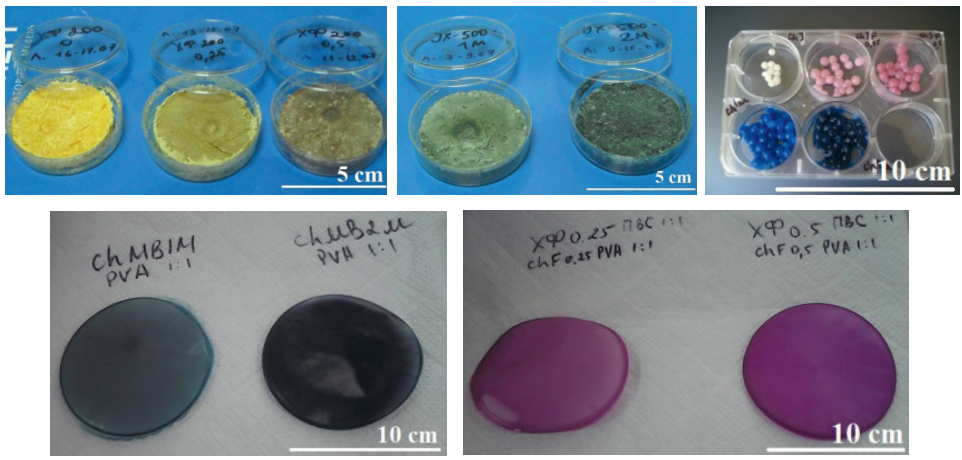

Figure 4. Some materials prepared from chitosan iodide and organic dyes: sponges (above left), beads (above right) and films (below)

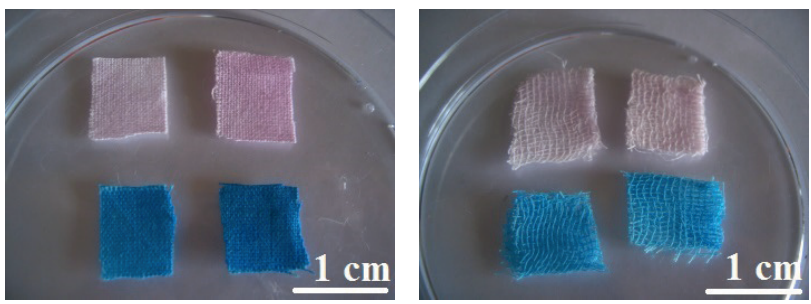

Figure 5. Fabrics, stained with resulting solutions: calico in the left dish and gauze in the right dish. Pink coloured fabrics are stained with fuchsine containing solution, blue coloured fabrics are stained with methylene blue containing solutions.

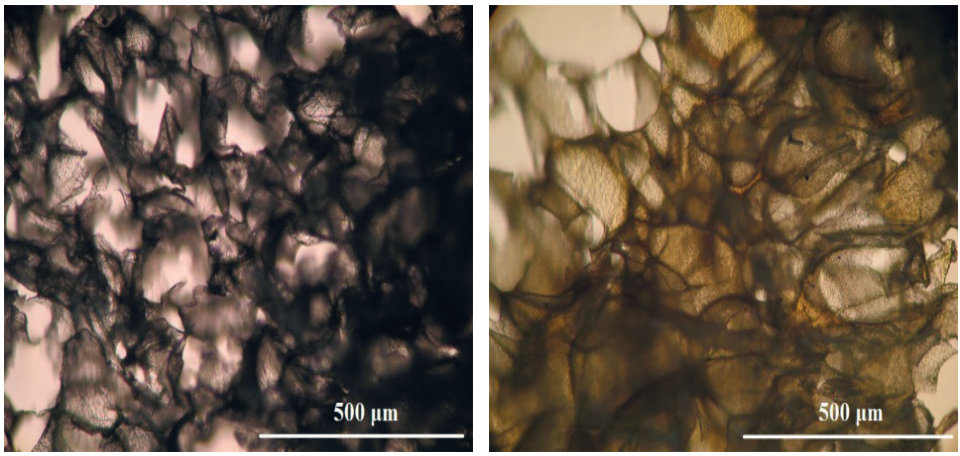

Figure 6. ChMB1 (left) and ChF0.25 (right), optical microscopy image, magnification at 100×

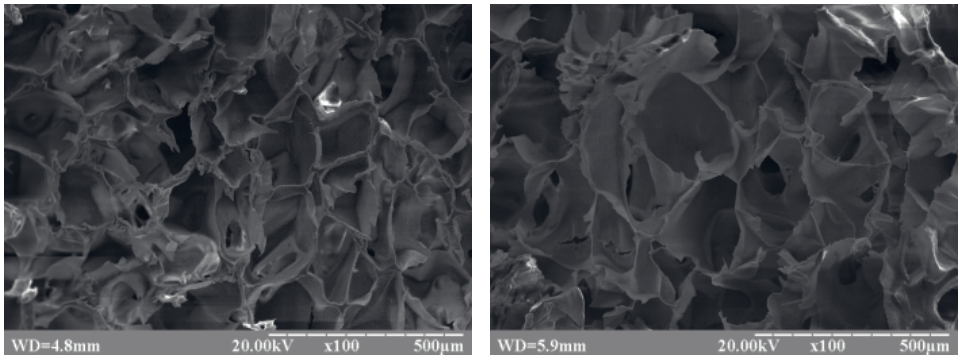

Figure 7. Scanning electron microscopy of the samples ChI, ChF0.25 and ChF0.5

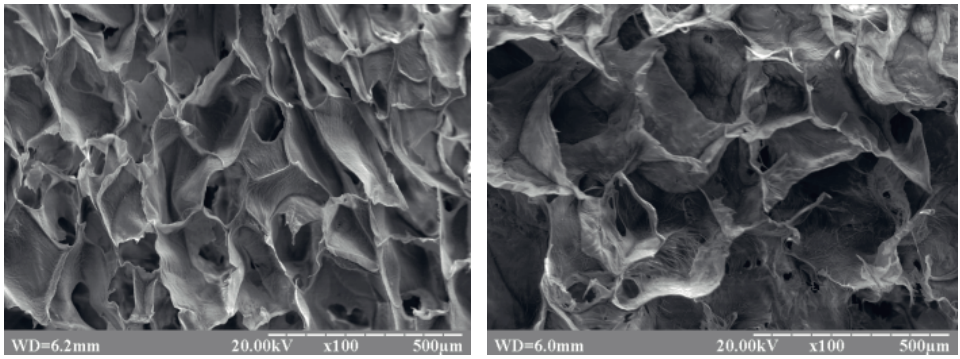

Figure 8. Scanning electron microscopy of the samples ChMB1 and ChMB2

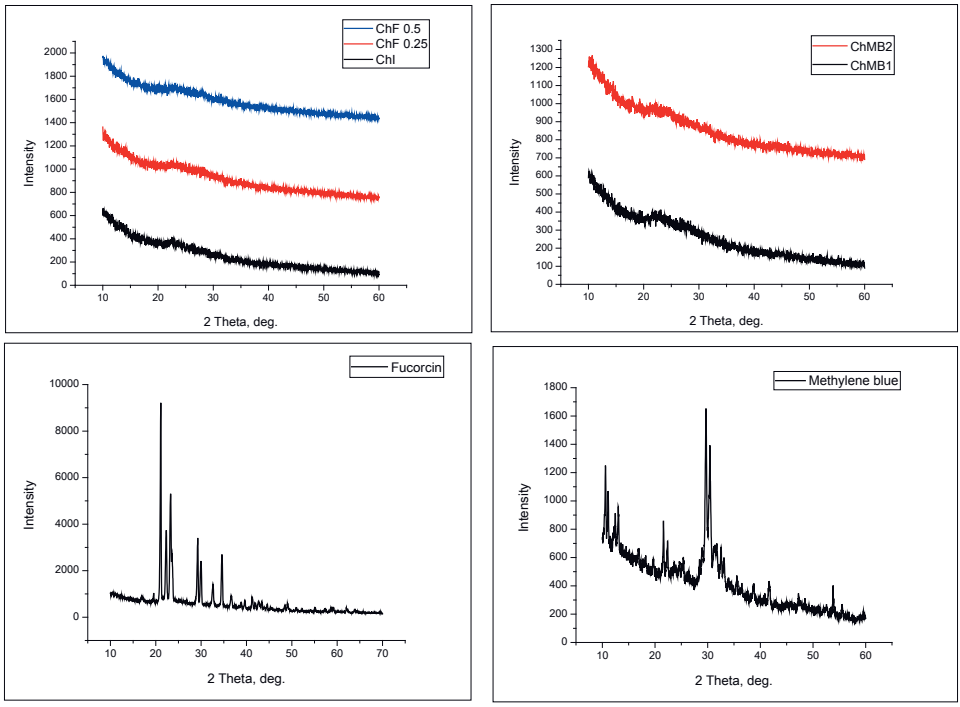

Figure 9. X-ray diffraction patterns of the composite samples (dry sponges, above) and pure fucorcin and methylene blue (below)

3.2 XRD

Figure 9 shows the diffraction patterns of the samples. Sponges based on chitosan had an amorphous structure, without distinct crystalline phases. All samples had a wide weak halo in the region of $2\theta = 20°$, which is characteristic of amorphous materials (glass and polymers). On the diffraction pattern of fucorcin, there are reflexes characteristic of the crystalline phases. On the MD diffraction pattern, there are reflexes characteristic of the crystalline phase of MB. These findings coincide with the data of the standard library of diffractograms by the Joint Committee on Powder Diffraction Standards. If the crystallisation of MB and fucorcin occurred in the samples, we would observe the same reflexes on the diffractograms of sponges as on the diffractogram of MB. The absence of crystalline phases indicates that additional components of the samples are in a state associated with the matrix.

3.3. TPDMS

We evaluated the samples using TPDMS; of the apparatus comprised a vacuum programmable furnace and a MX7304mass spectrometer (monopoly mass analyser, ionisation of gaseous samples by electron impact). The sample was heated linearly from room temperature to 900°C with mass spectrometric analysis of gas-phase products (mass spectra were recorded in the mass range from 1 to 200 Daltons).

From the thermograms (Figures 10 and 11), the nature of the yield of water and ions containing iodine differed significantly in samples with fucorcin (ChF 0.25, ChF) and

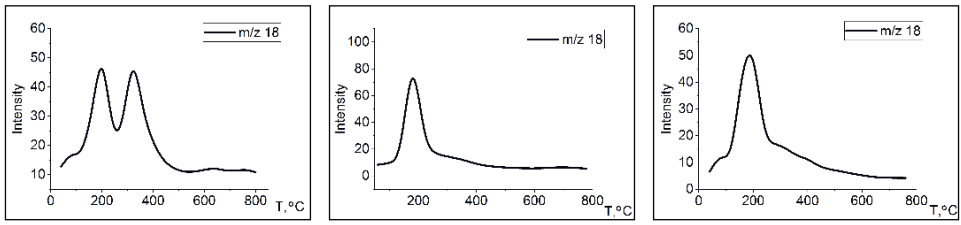

Figure 10. The temperature profile of water release (ions with m/z 18) from the samples ChI, ChF 0.25, ChF 0.5 (left to right)

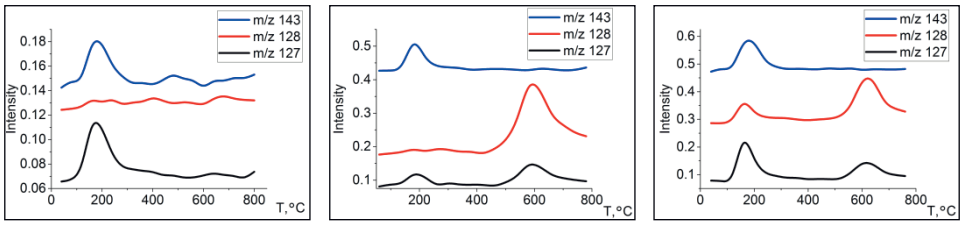

Figure 11. Temperature profile of iodine compound release (m/z 127 ($[I]^+$), 128 ($[IH]^+$) and 143 ($[IO]^+$)) from samples ChI, ChF 0.25 and ChF 0.5 (left to right)

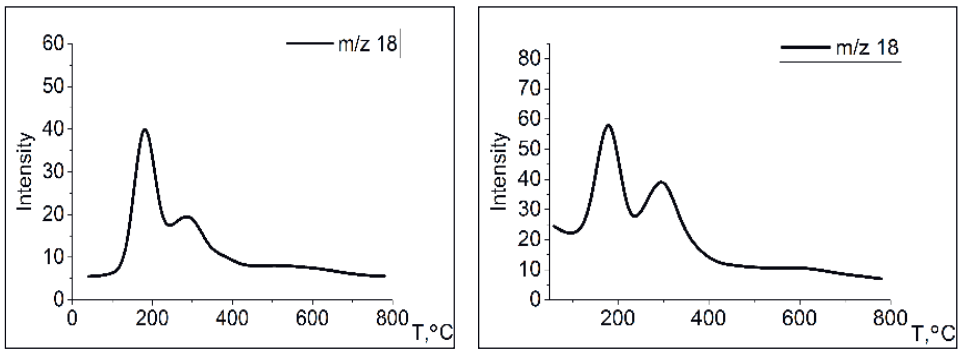

Figure 12. The temperature profile of water release (ions with m/z 18) from the samples ChMB1 and ChMB2 (left to right)

without fucorcin (ChI) These data indicate a significant difference in the chemical structure of the samples, an observation that requires further research. In samples containing fucorcin, there was one peak of water yield in the region of 200°C, while in the sample without fucorcin water was released at 200 and 350°C. In other words, the sample contains bound water.

At the same time, in the sample without fucorcin, iodine was obtained at 200°C, and in the mass spectra there were ions with mass 127 ($[I]^+$) and 143 ($[IO]^+$). In samples containing fucorcin, there was a second, high-temperature maximum of iodine yield at 600°C, in which case the mass spectra showed ions 128 ($[IH]^+$) and 143 ($[IO]^+$), which indicates that iodine in samples containing fucorcin was bound differently to the molecules of the

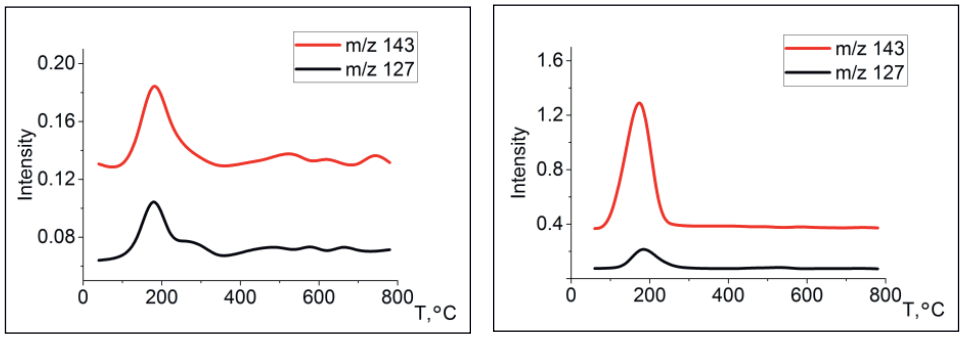

Figure 13. The temperature profile of iodine compound release (m/z 127 ($[I]^+$) and 143 ($[IO]^+$)) from samples ChMB1 and ChMB2 (left to right). Fluctuations in the high-temperature region in the left thermogram are not significant peaks, because the noise of the background signal, and the intensity of the peaks of iodine containing ions at 200°C was small

sample. This phenomenon may be due to fucorcin components or synthesis procedures.

For ChMB1 and ChMB2 (Figures 12 and 13), the nature of the yield of water and ions containing iodine was the same for both samples. Water was obtained at 200 and 300°C, which indicates that the water in the samples is in two forms, associated with the substance in different ways. In both samples there was one maximum iodine yield at 200°C, in the mass spectra there are ions with mass 127 ($[I]^+$) and 143 ($[IO]^+$), but in sample ChMB2, the signal intensity of these ions (ionic current) was much higher.

3.4. FTIR

FTIR spectra of the composite compounds do not contain 'fingerprint' peaks in the region below 1500 cm^{-1} characteristic for pure fucorcin and MB that indicates that the dyes are in the bound state.

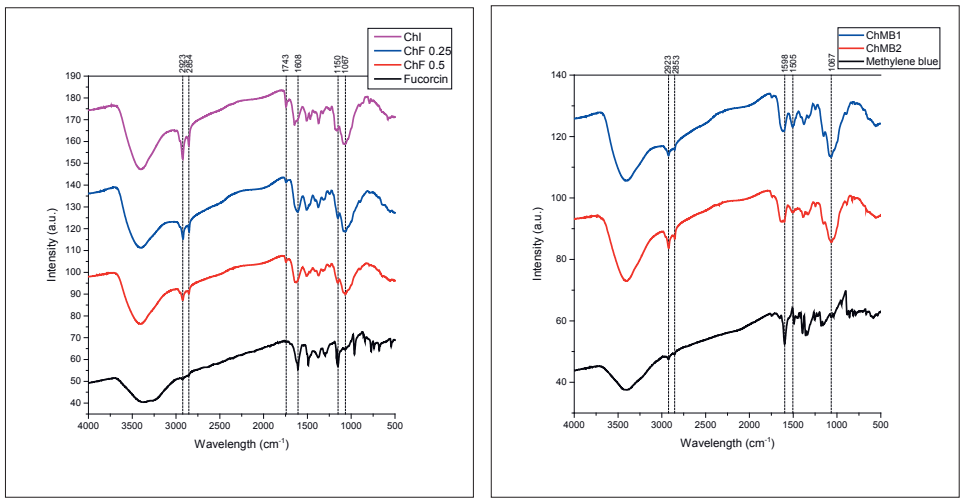

Figure 14. Fourier-transform infrared spectra of the investigated materials

3.5 Assessment of Antibacterial Activity

The pure solutions of the dyes were more effective against *S. aureus* than *E. coli*, and the activity depended on the dye concentrations. The combination of the dyes with chitosan solution increased the antibacterial effect against both microorganisms. The MIC of the solutions and composite materials are shown in the Table 1.

Table 1. Minimum inhibitory concentration (MIC) of the pure dyes and composite materials against *Staphylococcus aureus* and *Escherichia coli*

Bacteria Sample	***Staphylococcus aureus* dilution**	***Escherichia Coli* dilution**
MB1	1:32	1:2
MB2	1:128	1:2
F0.5	1:32	no effect
F0.25	1:2	no effect
ChMB1	1:512	1:512
ChMB2	1:1024	1:1024
ChF0.5	1:1024	1:128
ChF0.25	1:1024	1:128

4. Conclusions

The composite materials based on chitosan comprising organic dyes and iodine can be the basis of structured porous sponges and films and be used in various fields (e.g. 'green' electronics, biomedical applications, etc.). The materials display antibacterial activity against antibiotic resistant gram-positive and gram-negative bacteria.

5. References

[1] Chatterjee S., Chatterjee T., Lim S.R., Woo S.H.; (2011) Adsorption of a cationic dye, methylene blue, on to chitosan hydrogel beads generated by anionic surfactant gelation. Environmental Technology 32:13, 1503–1514. **DOI**:10.1080/09593330.2010.543157

[2] Kellner-Rogers J.S., Taylor J.K., Masud A.M., Aich N., H. Pinto A.H.; (2019) Kinetic and thermodynamic study of methylene blue adsorption onto chitosan: insights about metachromasy occurrence on wastewater remediation. Energ Ecol Environ 4, 85–102. **DOI**: 10.1007/s40974-019-00116-7

[3] Pschenitzka F., Sturm J.C.; (2001) Solvent-enhanced dye diffusion in polymer thin films for color tuning of organic light-emitting diodes. Appl Phys Lett. 78(17), 2584–2599.

[4] Mohd-Nasir S.N.F, Sulaiman M.Y., Ahmad-Ludin N., Ibrahim M.A., Sopian K., Mat-Teridi M.A.; (2014) Review of polymer, dye-sensitized, and hybrid solar cells. International Journal of Photoenergy 2014, Article ID 370160. **DOI**: 10.1155/2014/370160

[5] Mehedi Hasan M., Islam D., Ur Rashid T.; (2020) Biopolymer-based electrolytes for dye-sensitized solar cells: a critical review. Energy & Fuels 34 (12), 15634–15671. **DOI**:10.1021/acs.energyfuels.0c03396

[6] Goy R.C., de Britto D., Assis O.B.G.; (2009) A review of the antimicrobial activity of chitosan. Polímeros: Ciência e Tecnologia 19 (3), 241–247.

[7] Yilmaz Atay H.; (2019) Antibacterial activity of chitosan-based systems. In: Jana S. and Jana S. (eds) Functional chitosan, Springer, Singapore. **DOI**:10.1007/978-981-

15-0263-7_15

[8] Ahsan S.M., Thomas M., Reddy K.K., Sooraparaju S.G, Asthana A., Bhatnagar I.; (2018) Chitosan as biomaterial in drug delivery and tissue engineering. Int J Biol Macromol 110, 97–109. **DOI**:10.1016/j.ijbiomac.2017.08.140

[9] Croisier F., Jérôme C.; (2013) Chitosan-based biomaterials for tissue engineering. European Polymer J 49, 780–792. **DOI**: 10.1016/j.eurpolymj.2012.12.009

[10] Wang X., Tang R., Zhang Y., Yu Z., Qi C.; (2016) Preparation of a novel chitosan based biopolymer dye and application in wood dyeing. Polymers 8, 338. **DOI**:10.3390/polym8090338

[11] Matteini P., Ratto F., Rossi F., Pini R.; (2014) Laser-activated nanobiomaterials for tissue repair and dosage drug release. Kvantovaya elektronika (in Russian) 44 (7), 675–682.

[12] Esposito G., Rossi F., Matteini P., Scerrati A., Puca A., Albanese A., Rossi G., Ratto F., Maira G., Pini R.; (2013) In vivo laser assisted microvascular repair and end-to-end anastomosis by means of indocyanine green-infused chitosan patches: a pilot study. Lasers Surg Med 45 (5), 318 –325. **DOI**:10.1002/lsm.v45.5

[13] Ratto F., Aluigi A., Centi S., Milanesi A., Khlebtsov B., Khlebtsov N., Delfino V., Calonico C., Lo Nostro A., Magni G., Borri C., Cavigli C., Paolo P., Pini R., Rossi F.; (2020) New materials for laser welding of connective tissue and controlled release of antimicrobial principles. Proc SPIE 11223, Photonic Diagnosis, Monitoring, Prevention, and Treatment of Infections and Inflammatory Diseases 112230S. **DOI**:10.1117/12.2545141

[14] Colasanti R., Iacoangeli M., Marini A., Aiudi D., Carrassi E., Di Rienzo A., Scerrati M., Orlando F., Provinciali M., Giannoni L., Pieri L., Fagnani F., Dallari S., Magni G., Matteini P., Ratto F., Pini R., Rossi F.; (2020) Preliminary ex vivo and in vivo evaluation of laser bonding in dura mater. Proc. SPIE 11225, Clinical and Translational Neurophotonics 112250G. **DOI**:10.1117/12.2543750

[15] Ebrahimi Tirtashi F., Moradi M., Tajik ., Ezati M.F.P., Kuswandi B; (2019) Cellulose/chitosan pH-responsive indicator incorporated with carrot anthocyanins for intelligent food packaging. International Journal of Biological Macromolecules 136 (1), 920–926. **DOI**: 10.1016/j.ijbiomac.2019.06.148

[16] Li Y., Ying Y., Zhou Y., Ge Y., Yuan C., Wu C., Hu Y.; (2019) A pH-indicating intelligent packaging composed of chitosan-purple potato extractions strength by surface-deacetylated chitin nanofibers. International Journal of Biological Macromolecules 127 (15), 376–384. **DOI**:10.1016/j.ijbiomac.2019.01.060

[17] Chalitangkoon J., Monvisade P.; (2021) Synthesis of chitosan-based polymeric dyes as colorimetric pH-sensing materials: potential for food and biomedicalapplications, Carbohydrate Polymers. **DOI**:10.1016/j.carbpol.2021.117836

[18] Sklyar A.; (2020) Biopolymer composite nanostructured material based on chitosan and brilliant green triarylmethane dye. 2020 IEEE 10th International Conference Nanomaterials: Applications & Properties (NAP), Sumy, Ukraine, 2020, pp. 02BA04-1-02BA04-5, **DOI**: 10.1109/NAP51477.2020.9309679

[19] Riley K., Flower Jr. A.H.; (1950) A comparison of the inhibitory effect of castellani's paint and of gentian violet solution on the in vitro growth of Candida albicans. Journal of Investigative Dermatology 15(5) 355–361. **DOI**: 10.1038/jid.1950.114

[20] Rogosa M.; (1934) The bacteriostatic action of gentian violet, crystal violet, basic fuchsin, and acid fuchsin on certain Gram positive bacteria. Masters Theses, University of Massachusetts Amherst. Retrieved from https://scholarworks.umass.edu/theses/1918

[21] Kowsalya V., Vaishali C., Ligy P.; (2019) Fabrication of portable colorimetric sensor based on basic fuchsin for selective sensing of nitrite ions. Journal of Environmental

Chemical Engineering 7(5). **DOI**: 10.1016/j.jece.2019.103374

[22] Mohammed M.I., Yahia I.S.; (2018) Synthesis and optical properties of basic fuchsin dyedoped PMMA polymeric films for laser applications: wide scale absorption band. Opt Quant Electron 50, 159. **DOI**:10.1007/s11082-018-1425-0

[23] Kokala R.K., Bhattachary S., Cardoso L.S., Miranda P.B., Soma V.R., Deep P.C., Sai M., Santosh Raavie K.; (2019) Low cost 'green' dye sensitized solar cells based on new fuchsin dye with aqueous electrolyte and platinum-free counter electrodes. Solar Energy 188, 913–923. **DOI**: 10.1016/j.solener.2019.06.066

[24] Ibrahim A.G., Sayed A.Z., Abd El-Wahab H., Sayah M.M.; (2020) Synthesis of a hydrogel by grafting of acrylamide-co-sodium methacrylate onto chitosan for effective adsorption of fuchsin basic dye. Int J Biol Macromol 15, 159:422–432. **DOI**:10.1016/j.ijbiomac.2020.05.039

[25] Wu X.L., Xiao P., Zhong S., Fang K., Lin H., Chen J.; (2017) Magnetic ZnFe2O4@ chitosan encapsulated in graphene oxide for adsorptive removal of organic dye. RSC Adv 7, 28145–28151. **DOI**: 10.1039/C7RA04100D

[26] Ash S.R., Steczko J., Brewer L.B.; Winger R.K.; (2006) Microbial inactivation properties of methylene blue – citrate solution. ASAIO Journal 52(2), 17A.

[27] Méndez D.A.C., Gutierrez E., Dionísio E.J., Oliveira T.M., Buzalaf M.A.R., Rios D., Machado M.A.A.M., Cruvinel T.; (2018) Effect of methylene blue-mediated antimicrobial photodynamic therapy on dentin caries microcosms. Lasers Med Sci 33(3), 479–487. **DOI**: 10.1007/s10103-017-2379-3.

[28] Heiner Schirmer R., Coulibaly B., Stich A., Scheiwein M., Merkle H., Eubel J., Becker K., Becher H., Müller O., Zich T., Schiek W., Kouyaté B.; (2003) Methylene blue as an antimalarial agent. Redox Report 8(5). **DOI** 10.1179/135100003225002899

[29] Lu G., Nagbanshi M., Goldau N., Jorge M., Meissner P., Jahn A., Mockenhaupt F.P., Müller O.; (2018) Efficacy and safety of methylene blue in the treatment of malaria: a systematic review. BMC Medicine 16:59. **DOI**:10.1186/s12916-018-1045-3

[30] Atamna H., Kumar R.; (2010) Protective role of methylene blue in Alzheimer's disease via mitochondria and cytochrome C oxidase. J Alzheimers Dis 20 Suppl 2:S439-52. **DOI**: 10.3233/JAD-2010-1004142

[31] Yoshiyuki S., Saito M., Maeda S., Ishida K., Nakamura A., Kojima S., Takashima A.; (2019) Methylene blue inhibits formation of tau fibrils but not of granular tau oligomers: a plausible key to understanding failure of a clinical trial for Alzheimer's disease. J Alzheimer Dis 68(4), 1677–1686. **DOI:** 10.3233/JAD-181001

[32] Oza M., Lorke D.E., Petroianu G.A.; (2009) Methylene blue and Alzheimer's disease. Biochemical Pharmacology 78, 927–932. **DOI**:10.1016/j.bcp.2009.04.034

[33] Wu P.T., Lin C.L., Lin C.W., Chang N.C., Tsai W.B., Yu J.; (2019) methylene-blue-encapsulated liposomes as photodynamic therapy nano agents for breast cancer cells. Nanomaterials 9, 14. **DOI**:10.3390/nano9010014

[34] dos Santos A., Terra L.F., Wailemann R.A.M.; (2017) Methylene blue photodynamic therapy induces selective and massive cell death in human breast cancer cells. BMC Cancer 17, 194. **DOI**:10.1186/s12885-017-3179-7

[35] Kofler B, Romani A., Pritz C.; (2018) Photodynamic effect of methylene blue and low leve.l laser radiation in head and neck squamous cell carcinoma cell lines. Int J Mol Sci 19(4), 1107. **DOI**:10.3390/ijms19041107

[36] Tardivoa J.P., Del Giglioa A., de Oliveira C.S., Gabrielli D.S., Junqueira H.C., Tada D.B., Severino D., Turchiello R.F., Baptista M.S.; (2005) Methylene blue in photodynamic therapy: From basic mechanisms to clinical applications. Photodiagnosis and Photodynamic Therapy 2, 175—191. **DOI**:10.1016/S1572-1000(05)00097-9

[37] Yu J., Hsu C.H., Huang C.C., Chang P.Y.; (2015) Development of therapeutic Au-methylene blue nanoparticles for targeted photodynamic therapy of cervical cancer

cells. ACS Appl Mater Interfaces. 7(1):432-41. **DOI**: 10.1021/am5064298

[38] Palma-Chavez J.A., Kim W., Serafino M., Jo J.A., Charoenphol P., Applegate B.E.; (2020) Methylene blue-filled biodegradable polymer particles as a contrast agent for optical coherence tomography. Biomed Opt Express 11, 4255–4274.

[39] Noimark S., Dunnill C.W., Kay C.W.M., Perni S., Prokopovich P., Ismail S., Wilsond M., Parkin I.P.; (2012) Incorporation of methylene blue and nanogold into polyvinyl chloride catheters; a new approach for light-activated disinfection of surfaces. J Mater Chem 22, 15388. **DOI**: 10.1039/c2jm31987j

[40] Vardevanyan P.O., Antonyan A.P., Parsadanyan M.A., Shahinyan M.A., Mikaelyan M.S.; (2019) Study of methylene blue interaction with human serum albumin. Biophysical Reviews and Letters 14(01). **DOI**: 10.1142/S1793048019500012

[41] Kosevich M.V., Boryak O.A., Chagovets V.V., Shelkovsky V.S., Pokrovskiy V.A.; (2016) Interactions of biologically active redox-sensitive dyes with nanomaterials: mass spectrometric diagnostics. Nanobiophysics: Fundamentals and Applications, 2016.

[42] Kosevich M.V., Boryak O.A., Shelkovsky V.S., Zobninaa V.G., Orlova V.V.; (2018) Paradoxical secondary emission mass spectrum of the leuco form of methylene blue 8. Journal of Analytical Chemistry 73(14), 1327–1333. **DOI**:10.1134/S1061934818140058

[43] Lin X., Ni Y., Kokot S.; (2015) An electrochemical DNA-sensor developed with the use of Methylene Blue as a redox indicator for the detection of DNA damage induced by endocrine-disrupting compounds, Analytica Chimica Acta **DOI**:10.1016/j.aca.2015.02.050

[44] Hoffmann A.A., Dias S.L.P., Rodrigues J.R., Pavan F.A., Benvenutti E.V., Eder C. Lima E.C.; (2008) Methylene blue immobilized on cellulose acetate with titanium dioxide: an application as sensor for ascorbic acid. J Braz Chem Soc 19(5), 943–949.

[45] Jana A.K.; (2000) Solar cells based on dyes. Journal of Photochemistry and Photobiology A: Chemistry 132, 1–17.

[46] Sharma S.K.; (2013) Methylene blue (MB):PYR(G) based dye sensitized solar cells: morphology and its implications. International Journal of Innovative Research in Science & Engineering ISSN (Online) 2347–3207.

[47] Nurhidayani, Muzakkar M.Z., Maulidiyah, Wibowo D., Nurdin M.; (2017) A novel of buton asphalt and methylene blue as dye-sensitized solar cell using TiO2/Ti nanotubes electrode. IOP Conf Series: Materials Science and Engineering 267 012035. **DOI**:10.1088/1757-899X/267/1/012035

[48] Reda S., El-Sherbieny S.; (2010) Dye-sensitized nanocrystalline CdS and ZnS solar cells with different organic dyes. Journal of Materials Research, 25(3), 522–528. **DOI**:10.1557/JMR.2010.0077

[49] Pama A.A., Ande S., Eneji I.S., Sha'Ato R.; (2019) Sorption of methylene blue on iodate-chitosan assembled composite from aqueous solution. Desalination and Water Treatment, 388–395, www.deswater.com. **DOI**:10.5004/dwt.2019.24464164

[50] Zhang A., Zhou C.; (2011) DNA electrochemical biosensor based on chitosan and gold nanoparticles using methylene blue as electrochemical indicator. Advanced Materials Research 298, 128–134. www.scientific.net/AMR.298.12

[51] El-Sayed A.M., Ali M.F., Mohamed E.E.M., El-Mahdy M.M., Saddik M.S.; (2015) A novel treatment of freckles by photodynamic therapy using chitosan- methylene blue hydrogel. AAMJ 13 (3), suppl 1.