PHYSICAL CHEMISTRY 2014

12th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

The Conference is dedicated to the
25. Anniversary of the Society of Physical Chemists of Serbia

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September 22-26, 2014
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SYNTHESIS OF CHROMOGENIC SUBSTRATE FOR SCREENING OF PULLULAN-DEGRADING MICROORGANISMS

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ABSTRACT
A chromogenic substrate was obtained by coupling azo dye Congo red to microbial polysaccharide pullulan. The synthesized product was characterized by spectral techniques and elemental analysis. Results showed that dye-labelled glycan can be successfully used for the screening of microorganisms possessing enzymes that selectively catalyze the cleavage of glycosidic linkages characteristic for this polysaccharide.

INTRODUCTION
Methods based on stained polysaccharide as chromogenic substrates have been reported for the routine assay of different enzymes that specifically hydrolyses chemical linkages significant for certain polysaccharides [1,2]. These substrates are sensitive and highly specific for the target enzymes. Pullulan, one of the extracellular polysaccharides produced by yeast like fungus *Aureobasidium pullulans* is biodegradable, nontoxic and soluble in water, why has great applications in various field, from food industry to medicine and pharmacy [3].
It is a linear α-D-glucan having D-glucopyranose units connected by α-(1,4)- and α-(1,6)-glucosidic bonds. Its main structural characteristic are maltotriose repeating units mutually connected by (1,6)-α-D-glucosidic linkages (Fig.1). Congo red, the sodium salt of 3,3'-([1,1'-biphenyl]-4,4'-diyl)bis(4-aminonaphthalene-1-sulfonic acid) is a benzidine-based anionic diazo dye (Fig. 2) that is widely used for staining different substrates, some of which have commercial application in medicine and microbiology [4].

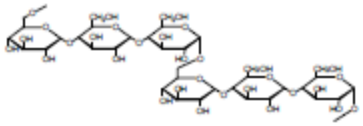

Figure 1. Pullulan

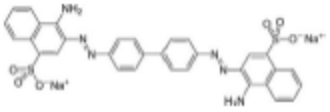

Figure 2. Congo red

In this work a synthesis of stained pullulan with dye Congo red has been reported. The resulting product was characterized by HNMR, UV-Vis and elemental analysis. The aim of this work was the obtaining a new dye labeled glucan as a potential chromogenic material for sensitive screening test of microorganisms that selectively hydrolyzes characteristic linkages of this polymer. Also, this material could potentially be used for the determination of activity of crude enzyme preparations.

EXPERIMENTAL

Pullulan used in this work is produced by *A. pullulans*, strain CH-1 (IChTM, Collection of Microorganisms) [5,6]. Other reagents and solvents were purchased from commercial sources and used without further application. Pullulan and Congo red were prepared separately, by dissolution in distilled water and then mixed together. The reaction was performed with stirring (50°C, 1 h) and, at different times, equal portions of Na_2SO_4 were added. Then aqueous solution of a Na_3PO_4 was added and reaction maintained at 50°C with extensive stirring for 1 h. Reaction mixture was centrifuged (4000 rpm, 15 min) and the precipitate was resuspended in distilled water and purified of unreacted dye by dialysis. Resulting solution was lyophilized. The ^{1}H-NMR spectra (Fig. 3) were done on a *Varian Gemini (200 MHz) NMR* spectrometer. UV-VIS spectral characteristics of free dye and product (Fig. 4 and Fig. 5) were recorded using a GBC Cintra 40 spectrophotometer. Elemental analysis (C,H,N,S) was performed using a Vario EL III, CHNS/O Elemental Analyzer, Elementar Analysen systeme GmbH.

RESULTS AND DISCUSSION

The coupling pullulan with Congo red was confirmed by ^{1}H-NMR data (Fig. 3). The ^{1}H-NMR spectrum of stained pullulan showed a characteristic signals relating to the polysaccharide and Congo red. In ^{1}H-NMR spectrum among signals at 5.0 ppm, and 5.4 ppm corresponding to the anomeric protons of α-(1,6)- and α-(1,4)-glucopyranoses, respectively, characteristic for pullulan, can be seen peaks in aromatic part of the spectrum, in the region of 7.0 ppm - 9.0 ppm, related to the signals of aromatic rings of the Congo red dye.

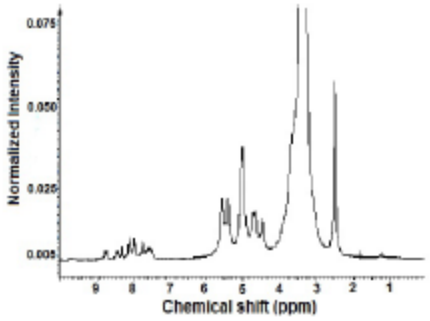

Figure 3. ^{1}H-NMR spectrum of stained pullulan with Congo red

Additional evidence that pullulan was coupled with Congo red was found in the results of elemental analysis (Table 1.). The content of dye in coupled polysaccharide is reflected by the increase of nitrogen and sulphur content.

Table 1. Elemental analysis of starting substances and reaction product

	% N	% C	% H	% S
Pullulan	/	44.4	6.2	/
Congo red	12.1	55.1	3.1	9.2
Pullulan-Congo red	1.4	35.4	5.2	0.9

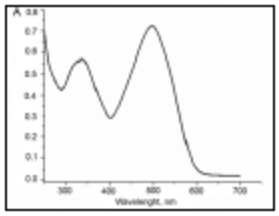

Figure 4. UV-VIS spectrum of pullulan-Congo red

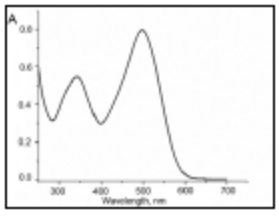

Figure 5. UV-VIS spectrum of Congo red dye

UV-VIS spectral properties of pullulan coupled with Congo red (Fig. 4) did not differ from those of free dye (Fig. 5). Free dye in distilled water (0.8

mg/ml) show two maximum absorption peaks of different intensity, the first peak at 340 nm and the second peak at 498 nm. The spectrum of pullulan-coupled Congo red (2,0 mg/ml) showed a identical shape of the curve with the maximums at the same wavelengths of the spectrum.

CONCLUSION

The dye labeled pullulan was obtained through coupling of this polysaccharide and azo dye Congo red. This novel stained material can be used as potential chromogenic substrate for rapid and sensitive screening test for microorganisms possessing enzymes that selectively hydrolyze α-(1,6)-glycosidic bonds characteristic for pullulan.

ACKNOWLEDGEMENT

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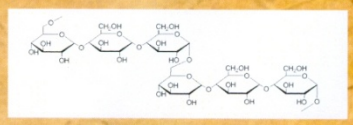

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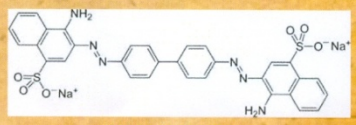

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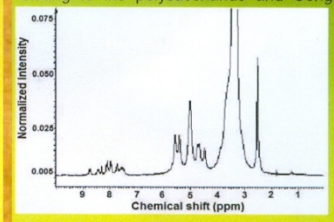

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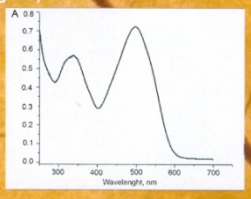

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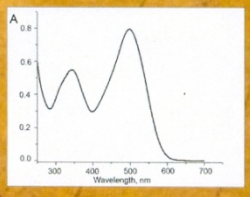

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