



SPIN COATED CARBOXYMETHYL DEXTRAN LAYERS ON TiO₂-SiO₂ OPTICAL WAVEGUIDE SURFACES

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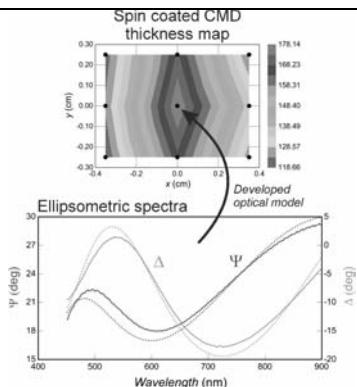
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Received October 13, 2016

Carboxymethyl dextran (CMD) layers were fabricated on optical waveguide sensor chips by spin coating technique. The dependence of the layer thickness on the rotational speed as a technical parameter of the fabrication process, was investigated by spectroscopic ellipsometry. Optical model for the evaluation of the ellipsometric data was developed and the refractive index dispersion (Cauchy B parameter) of the CMD layer was determined based on our high sensitivity measurements on gold substrate. The mapping measurements provided details about the extent of the inhomogeneity in the thickness and refractive index of the CMD layers. The chemical composition of the CMD layer, as well as the completeness of the coverage was examined by X-ray photoelectron spectroscopy (XPS).



INTRODUCTION

Hydrogels for biosensor applications are of high importance due to the advantages provided by the highly hydrated, extended, 3D structured polymer chains.¹ The benefits are the high capacity for analyte binding, the hydrophilic characteristic for reducing the non-specific binding, as well as the chemical variability. The dextran is a glucose polysaccharide bearing α -(1,3) glycosidic bonds in its backbone chains and α -(1,6) glycosidic bonds at

the branching points, providing c.a. 5 % branching degree for the polymer. The dextran is able to absorb a huge amount of water even in its native form and its chemical composition makes the polymer a potential candidate for further crosslinking reactions. The dextran is mainly used in its carboxymethylated form especially for the conjugation of bioreceptor molecules and for achieving the covalent grafting of the dextran chains to the transducer's sensing surface. Covalent bonds between an aminated surface and

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the carboxyl groups of the carboxymethyl dextran (CMD) can be formed using the carbodiimide reagent based linking chemistry.²⁻⁴ Due to the ability to suppress the non-specific binding, the dextran is particularly attractive for the widely used label-free optical biosensor, the surface plasmon resonance (SPR) technique, and the feasibility of the dextran has been proven by several applications.⁵⁻⁶ The waveguide based optical biosensors have great perspective owing to its ultrahigh sensitivity and the ability to realize the high throughput analysis. However, in spite of the attractive properties and the need for applications of dextran layers, methodologies for the fabrication of dextran coatings on waveguide surfaces are narrowly available and they are generally suffering from the lack of details.

Spin coating is a common technique for the deposition of polymer layers on various substrates.⁷ This procedure is suitable for the fabrication of layers in wide range of thickness controlled by adjustable parameters, such as the rotational speed or the concentration of the coating solution. The ability of fine-tuning the thickness of the coated layers could open wide area of applications for label-free biosensors, *e.g.* in multidepth waveguide sensors.⁸⁻⁹ The effect of the rotational speed and the concentration of the dextran solution on the layer thicknesses in spin coating fabrication processes has been investigated.¹⁰ In this work dextran and poly(acrylic acid) have been used as sacrificial layers for microstructure fabrication. The thickness of the film was determined by profilometry, and values were obtained in the range of 40-1100 nm. In a different study, Piehler *et al.* prepared amino-functionalized dextran layers by spin coating on silica substrates,¹¹ however the results are not detailed for the different parameters. For parameters selected the thickness of the dextran layers was measured using ellipsometry and a 2-parameter Cauchy model was used for the data evaluation. The thickness of the dry dextran layers was 500 - 5000 nm, and the corresponding surface concentration was 700-7000 ng/mm². Ellipsometry is an attractive optical measurement technique in our laboratory for the determination of the properties of thin surface layers, such as the thickness and the refractive index of the layers on top of even transparent glass substrates.^{12,13} The ellipsometry has been verified as a suitable technique in the characterization of thin biofilms (*e.g.* proteins)^{14,15} or synthetic polymers.¹⁶

We have been working on immobilization chemistries for biosensing.^{17,18} As a continuation of our work in the field of dextran coating, the current work deals with dextran layers prepared by spin-coating. The CMD layers were fabricated on the surface by spin coating technique, using different rotational speed aiming at thicker layers than our previous results obtained in a flow-cell by *in situ* depositions.¹⁹ Our findings were that only a few nanometer thick CMD layer can be deposited on the biosensor chip surface mainly in a flat conformation measured under aqueous conditions (*i.e.* laying polymer chains), which is not appropriate for most biosensing experiments. In this work, we aimed at about 100 nm thickness and investigated the technical possibility of tuning the thickness value. For the fabrication of CMD layers, an optical sensor chip was used as substrate, which are generally applied in our biosensor measurements performed by optical waveguide lightmode spectroscopy (OWLS). The structure, as well as the optical parameters of the layers were measured by spectroscopic ellipsometry in atmospheric environment, focusing on the effect of the variable parameter, the rotational speed on the layer properties. As the best of our knowledge, spin coated CMD layers have not been analyzed by ellipsometry and optical model for the evaluation of such dextran based coatings has not been revealed in details. X-ray photoelectron spectroscopic (XPS) measurements were conducted to determine the chemical composition of the CMD layer and to gain data about the extent of the coverage on the investigated area.

EXPERIMENTAL

Chemicals

Dextran T-500 (with 500 kDa molecular weight) was obtained from Pharmacosmos A/S, Holbaek, Denmark. 3-aminopropyltriethoxysilane (hereafter aminosilane), Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich. The water used in the experiments was ultrapure grade Milli-Q water with the resistivity of 18 MΩ·cm and produced by Millipore system. All chemicals and reagents were of analytical grade.

Sample substrates

1.5 cm x 1.5 cm sized BK7 glass covered by 160 - 180 nm thick TiO₂-SiO₂ sol-gel layer were used as substrates for the spin coating of the CMD. The samples were sliced from the genuine, 1.6 cm x 4.8 cm OWLS sensor chips obtained from Microvacuum Ltd., Hungary. In addition, gold surfaces were used for determining the Cauchy *B* parameter of the bulk CMD (BK7 glass covered with 30 - 40 nm thick gold layer).

Sample preparation

Sensor chip cleaning

The surfaces were cleaned using chromic acid followed by neutralization with 1 M potassium hydroxide solution and intensive rinse with ultrapure water. The samples were dried under nitrogen stream and kept in vacuum desiccator. The gold surface was exposed to UV ozone in an UV ozone cleaner apparatus (Microvacuum Ltd., Hungary).

Aminosilylation of sensor chips

The cleaned surfaces were silylated by 3-aminopropyltriethoxysilane in a temperature controlled vacuum chamber (Büchi B-585). The aminosilane reagent was evaporated at 80 °C and 6 mbar vacuum and kept in this conditions for 10 min. After closing the chamber and turning off the heating power the reaction was completed in 20 min. The vacuum was subsequently restored and the samples were baked at 120 °C for 2 hours.

Fabrication of CMD layers

The CMD was synthesized from 500 kDa molecular weight native dextran. The synthesis procedure was based on the recipe of Huynh et al.²⁰ and our detailed method used in this work can be found in our former publication¹⁹. The carboxymethylation degree (DS as degree of substitution) of

$$\tan \Psi \cdot e^{i\Delta} = \bar{\rho} = \bar{\rho}(\bar{n}_0, \bar{n}_1, \dots, \bar{n}_{m+1}, d_1, d_2, \dots, d_m, \phi_0, \lambda) \quad (1)$$

Here $\bar{\rho}$ is the complex reflection coefficient, the Ψ and Δ independent parameters reflect to the change in the amplitude and in the phase of the polarized light, respectively (called ellipsometric angles), m as a subscript is the number of the layers over the substrate ($m + 1$ refers to the substrate and the subscript 0 to the ambient air, respectively), \bar{n} is the complex refractive index, d is the thickness of the layer, ϕ_0 is the angle of incidence and λ is the wavelength of the reflected light. Using an appropriate optical model with estimated initial values, the acquired Ψ and Δ spectra can be fitted by minimizing the value of the calculated error function, the mean squared error (MSE) of the fit. The iteration was conducted until finding the lowest MSE value for the fit. As a result, the properties of the sample can be determined. The refractive index n was approximated by the 3-parameter Cauchy's equation, according to Eq (2):

$$n = A + B/\lambda^2 + C/\lambda^4 \quad (2)$$

where A , B , C are the Cauchy parameters that can be varied in the fitting procedure. By the de Feijter's formula²¹, mass of the examined layer can be also determined from the measured thickness and refractive index data.

The measurements were performed using a microfocusing equipment (diameter of the spot area: 300 µm). The spectra were recorded in the wavelength range of 193 - 1690 nm, at 70° incident angle (gold samples were measured at 45 - 70°). By measuring the samples in mapping mode, Ψ and Δ spectra were recorded at multiple points of the surface and a map of the layer parameters could be taken for all the samples. The distance between the measurement positions was 0.35 cm in the x direction and 0.25 cm in the y direction. Data acquisition time of 5 s was used at each measurement point. First the aminosilylated OWLS chips (without the CMD layer) were measured and then the sensor chips spin coated by the CMD.

the CMD was 0.27 throughout the experiments. Aqueous solution of the CMD at the concentration of 50 mg/ml was prepared from lyophilized sample. The solutions were kept at 60 °C until the complete dissolution. Followed by a pH adjustment to the range of 7.0-7.5 with sodium hydroxide, the CMD solution was filtered using 0.2 µm pore size membrane. EDC and NHS reagents were added to the CMD solution to activate the carboxylic groups for covalent grafting to the aminated surface. The concentration of the EDC and NHS was set for providing the [COOH] : [EDC] : [NHS] = 3 : 1 : 1 molar concentration ratio, where [COOH] refers to the carboxylic group concentration in the CMD solution. The prepared CMD-EDC-NHS solution was incubated for 20 min at room temperature, then 200 µl droplet from the solution was pipetted and spin coated on the sensor surface. The sample was spun for 60 s at the chosen rotational speed from the values of 1000, 2000, 3000 and 4000 rpm.

Measurement techniques

Spectroscopic ellipsometry

Ellipsometric measurements were performed by a Woollam M-2000DI rotating compensator spectroscopic ellipsometer (J.A. Woollam Co., Inc.). The measured raw parameters, Ψ and Δ are a function of the structure and optical properties of the sample, and can be described as follows¹²:

The ellipsometric data were collected and analyzed by the CompleteEASE software.

X-ray photoelectron spectroscopy

The chemical composition and the coverage of the CMD layers were measured by X-ray photoelectron spectroscopy. The samples were radiated by X-ray emitted from the Al-anode source. Our ESA 105 type cylindrical mirror analyzer (Staib Instruments Ltd.) with retarding field was used to gain the photoelectron spectra in a constant energy resolution of 1.5 eV. The data were collected from 8 mm diameter area. The samples were kept in 2 x 10⁻⁹ mbar ultrahigh vacuum during the whole measurements. The evaluation method of the XPS data can be found in our previous work.¹⁹

RESULTS AND DISCUSSION

The XPS spectra of the C, O, N, Ti, as well as Si peaks recorded on spin coated CMD samples can be seen in Fig. 1. The analyzed C peak clearly proves the presence of the carbohydrate molecules on the OWLS chip, owing to the high signal attributed to the C–O and O–C–O bond states of the C atoms. The averaged elemental ratios of the samples were the followings: C: 0.538, O: 0.435, Si: 0.001, Ti: 0.002, N: 0.024. The signal of the TiO₂-SiO₂ waveguide component elements, the Ti and Si could be detected demonstrating that the chip surface was not perfectly covered by the CMD layer, however, the coverage was near to be

complete (99.9 %). Since the CMD layer was much thicker (see the ellipsometric section of the results below) than the detection depth of the XPS technique (8 - 10 nm), the detected Ti and Si signals suggest that the CMD layer could be formed with small amount of cavities. The main part of the N is supposed to have originated from the N content of the EDC and NHS reagents. As only a very small amount of Ti and Si could be detected, similarly just a small amount of N must have originated from the aminosilane coverage. The aminosilane layer was determined to be 0.65 nm from the XPS measurements on separate chips covered only by the aminosilane layer.

The developed optical model used for the evaluation of the ellipsometric data is presented in Fig. 2. The model will be explained in details below. The spectra of the Ψ and Δ were fitted in the wavelength range of 450-900 nm out of the full measured range for each measurement on the chips.

The measurements were separated into two parts, beginning with the measurements on the chips without the CMD layer and then with the CMD. The evaluation followed the same method: first, the measured map data on the aminosilylated sensor without the CMD layer were analyzed, which was followed by the evaluation of the CMD layers using the separate measurements. In the optical model, the refractive index of the different layers were fitted by the (2) three-parameter Cauchy's equation. The BK7 glass was handled as substrate and its parameters were kept fixed for all the samples (optical parameters of BK7 glass were referenced from the database of the CompleteEASE program²²). Our model hypothesized linear inhomogeneity of the

refractive index in the depth of the $\text{TiO}_2\text{-SiO}_2$ waveguide layer (gradient layer). The layer was divided into 5 slices in which the Cauchy A parameter was a linear function of the depth and the fitted inhomogeneity number (IH) presented the percentage of the difference of the A between at the bottom and the top of the $\text{TiO}_2\text{-SiO}_2$ layer. Additionally, the parameter B was also fitted while keeping C at zero. A fixed aminosilane layer was also implemented in the model. The thickness of the aminosilane layer was set to 0.61 nm based on our XPS measurements. The refractive index of the aminosilane was derived from the literature and found to be 1.427 at the wavelength of 632.8 nm.²³ This value was used to gain the Cauchy function of the aminosilane for implementing in the optical model, where the B was chosen to be 1.0×10^{-2} . The evaluation of the samples with the CMD layer was performed using constant parameters for the $\text{TiO}_2\text{-SiO}_2$ layer. These values were derived from the preceding evaluation by averaging the map data to get one value for each parameter for representing the whole waveguide of the individual chips. We had these specific parameter values for all the samples which could be applied for calculating the CMD layer parameters over its specific $\text{TiO}_2\text{-SiO}_2$ layer. The Cauchy parameters of the waveguides were measured to be rather uniform, represented by the $A_{\text{TiO}_2\text{-SiO}_2} = 1.682 \pm 0.015$ and $B_{\text{TiO}_2\text{-SiO}_2} = 6.9 \times 10^{-3} \pm 2.1 \times 10^{-3}$ (average value \pm standard deviation, considering all examined chips). The IH values were found to be -1.8 ± 0.6 %, which taking into account could significantly enhance the quality of the fit.

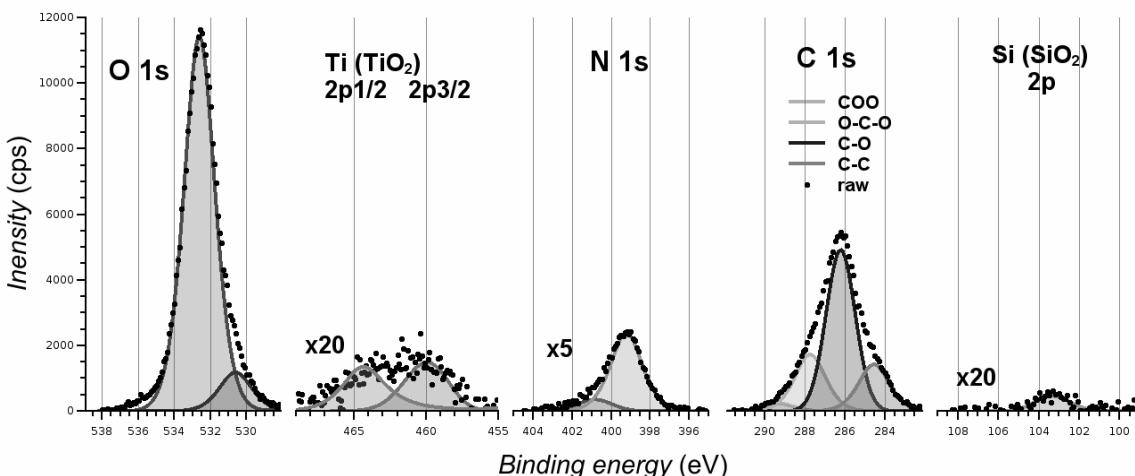


Fig. 1 – C, O, N, Ti, as well as Si peaks of the XPS spectrum measured on spin coated CMD layer (4000 rpm). The $\text{Ti}(\text{TiO}_2)$ and $\text{Si}(\text{SiO}_2)$ labels refer to the oxide type bond state of the Ti and Si atoms, respectively. The N, Ti and Si peaks were enlarged according to the x5, x20, and x20 labels.

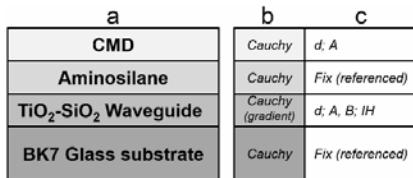


Fig. 2 – Optical model developed for the characterization of spin coated CMD layers. Schematic structure of the sample (a), the applied model for determining the refractive index (b) and the fitted parameters (c) are also presented.

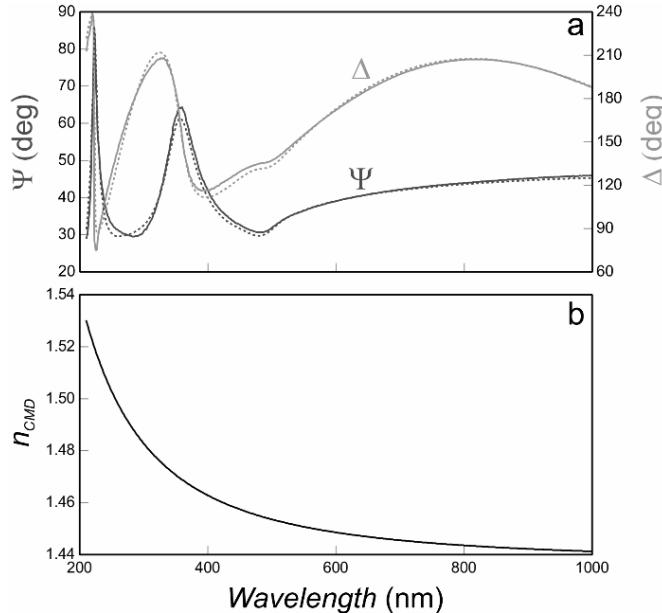


Fig. 3 – Ψ (—) and Δ (—) spectra (a) measured on spin coated CMD layer (3000 rpm) on top of a gold substrate for the determination of the wavelength dependence of the CMD refractive index (b). On the (a) part of the figure, the solid lines refer to the measured spectra, the dashed lines (--- and - - -) to the fitted curves. The measurement was performed at the incident angle range of 45 - 70° (with 5° angle shift) and in the wavelength range of 210 - 1000 nm. The plotted spectra here were recorded at 55°. The applied optical model was based on considering the following structure: BK7 glass / Cr₂O₃ / Au / CMD. The varied parameters were the CMD thickness, the A and B parameters, as well as the extinction coefficient of the CMD layer. The fixed parameters for the underneath layers were derived from the database of the CompleteEASE program.²² MSE of the presented fit: 39.

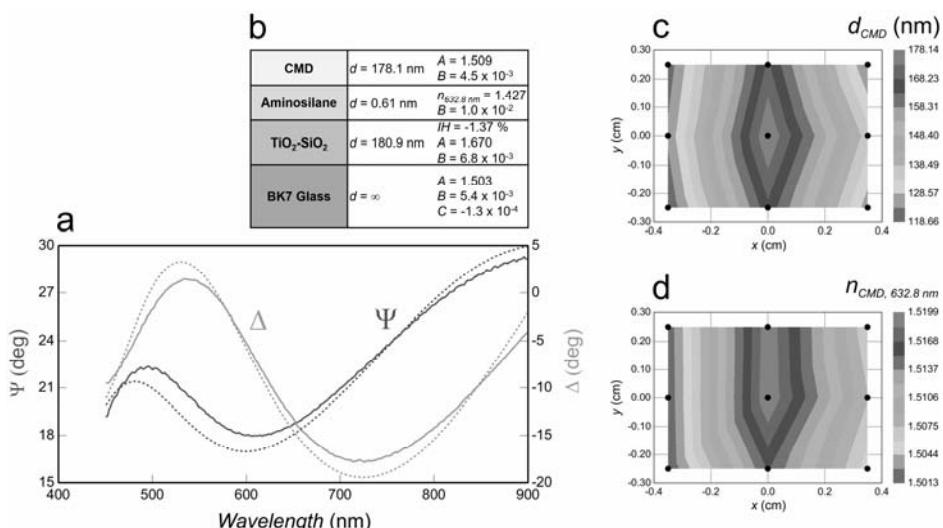


Fig. 4 – Ψ (—) and Δ (—) spectra measured for the determination of the thickness and refractive index of CMD on the TiO₂-SiO₂ waveguide (a). The solid lines refer to the measured spectra, the dashed lines (--- and - - -) to the fitted curves (MSE = 20). The spectra were recorded at the center point ($x = 0$ cm, $y = 0$ cm) of the sample fabricated by spin coating of the CMD at 3000 rpm rotational speed. Details about the optical model were also put on the figure (b). Additional figures: thickness (d_{CMD}) (c) and refractive index ($n_{CMD, 632.8\text{ nm}}$) (d) map of the CMD. The refractive index was calculated from the Cauchy equation at the wavelength of 632.8 nm. Each map contains 9 measured points (distance between the measured points: 0.35 cm in the x, 0.25 cm in the y direction).

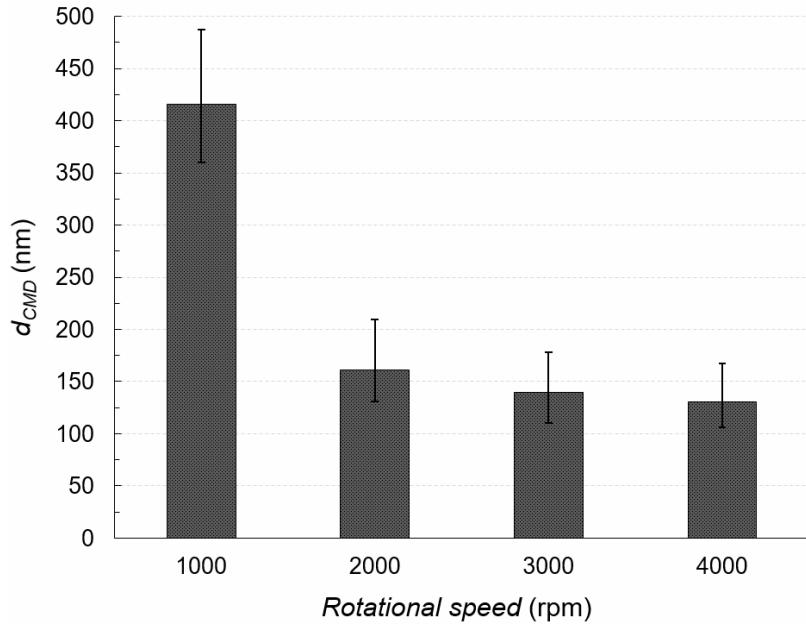


Fig. 5 – The effect of the rotational speed on the thickness of the spin coated CMD layer (d_{CMD}). The data were collected from the thickness maps of multiple parallel samples. The map values for the individual samples, as well as these data for each parallel sample was averaged. The bars on the columns represent the minimum and maximum values of the measured thicknesses at the corresponding rotational speeds.

The Cauchy B parameter of the bulk CMD was determined by the evaluation of the spin coated CMD layer on gold substrate, since measurements on gold were able to provide high optical contrast and sensitive measurement for low refractive index layers (for the fitted spectra and details see Fig. 3).

The wavelength dependence of the CMD, represented by its Cauchy B parameter was derived from this measurement, and it was used as invariable parameter for the analysis of the layers on the aminosilylated TiO_2-SiO_2 surfaces. This value was found to be $B_{CMD} = 4.5 \times 10^{-3} \pm 7.4 \times 10^{-4}$ and the average was subsequently used (C_{CMD} was set zero as its effect on the fit was negligible). Accordingly, only the thickness (d_{CMD}) and the A parameter of the CMD layer were varied in the calculations. Fig. 4a exhibits one of the fitted Ψ and Δ spectra of the CMD coated samples. The value of the MSE was in the range of 6-12 for the evaluation of the TiO_2-SiO_2 layers and in 13-47 for the CMD layers, which suggests that the quality of the fits were satisfactory.

The map of the resulted CMD thicknesses was generated considering the 9 center points. As the thickness map data reveals, the position related variation of the CMD layer thickness was relatively high, which is demonstrated by the ranges in Fig. 5 including the minimum and maximum thickness values. These results show an inhomogeneous spin coated CMD layer, of which formation may be explained by the high CMD

concentration of the applied solution. It is suggested, that for achieving layers with better homogeneity level, the concentration of the CMD should be lowered below 50 mg/ml. Fig. 4d represents the refractive index of the CMD ($n_{CMD, 632.8 \text{ nm}}$; calculated by the Cauchy equation at the wavelength of 632.8 nm) as a function of the measurement position. The $n_{CMD, 632.8 \text{ nm}}$ was found to be 1.516 ± 0.013 (average \pm standard deviation, based on map data of all the samples), without significant dependence on the rotational speed. The determined refractive index is very similar to the value of 1.52 reported in the study of Piehler and co-workers (this value was given at 589 nm).¹¹

The dependence of the thickness of the CMD layer on the rotational speed is summarized in Fig. 5. The rotational speeds of 2000, 3000 and 4000 rpm resulted in similar layers. The tendency of the observed small effect was in the expected direction, however, the minimum and maximum values suggest that in fact there was no significant difference among the thicknesses. The 1000 rpm rotational speed showed huge effect on the d_{CMD} compared to the layers at 2000, 3000 and 4000 rpm. The amplification of the rotational speed influence could be also reached by optimizing the CMD concentration in the spun solution to get more tunable thicknesses. Linder et al. presented a ~ 40 -170 nm thickness range for spin coated dextran layers, where concentration of the dextran solution was 50 mg/ml (5 % (v/w)) and the applied rotational speed range was the same as in

our work¹⁰. Our layers were thicker compared to their prepared ones, which is supposed to be mainly due to the higher molecular weight of the CMD applied here (500 kDa in contrast to their 66 kDa dextran). This molecular weight difference could lead to high difference in the viscosities, which could strongly affect the thickness of the resulted layers. The deposited mass of the CMD could be calculated from our thickness and refractive index data by the de Feijter's formula and a range of 161-511 ng/mm² was determined, regarding the varied rotational speeds.

CONCLUSIONS

We have presented our results on the characterization of CMD layers spin coated on the aminosilylated TiO₂-SiO₂ type waveguide surface of the OWLS sensor chips. Our XPS results confirmed the presence of the CMD on the chip surface, as well as the layers were found to be not perfect in the surface coverage, however, the uncovered parts were only 0.1 %. The CMD layers were examined by spectroscopic ellipsometry. This type of analysis on the CMD as the best of our knowledge has been unrevealed in the literature so far. Optical model for the ellipsometric evaluations was developed and the Cauchy *B* parameter (refractive index dependence on the wavelength) of the CMD was also determined to be 4.5 x 10⁻³ providing a useful parameter for the analysis of the data presented here and for prospective studies. The ellipsometric data showed high rotational speed dependence on the CMD layer thickness comparing the thickness values at 1000 and 2000 rpm. However, varying the rotational speeds to 3000 and 4000 rpm did not cause significant difference in the CMD layer thickness. The spin coated CMD layers were not perfectly homogeneous in thickness, and the fabrication process can be optimized to get more homogenous layers with fine-tunable thickness for further biosensor applications. The detailed ellipsometric analysis presented here is expected to provide useful support for the characterization and fabrication of spin coated dextran and other type of biopolymer layers.

Acknowledgement: The Lendület program, the ERC-HU Program of NKFIH and the János Bolyai Research Scholarship (SK) of the Hungarian Academy of Sciences are

gratefully acknowledged for funding this research. The work was also supported from the M-ERA-net "WaterSafe" OTKA NN 117847, OTKA K115852 and TÉT-12-DE-1-2013-0002. Support from the Pro Progressio Foundation and Varga József Foundation are also acknowledged.

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