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Coating of hydrophilic chalcogenide quantum dots with carboxymethyl chitosan for lateral flow immunoassay applications

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Abstract. Quantum dots (QDs) is a class of fluorescent label widely using for biological and biomedical applications. The unique optical properties of QDs make them promising tool as fluorescent markers and analytical labels of proteins. To apply them in biological fluids it is essential to coat QDs with biocompatible polymers. In this research CdTe/CdS/ZnS QDs with mercaptopropionic acid as stabilizer was coated with carboxymethyl chitosan (CMC) by electrostatic interactions. The physicochemical properties of resulting QDs-CMC were studied by absorption and fluorescence spectroscopy, dynamic light scattering and capillary zone electrophoresis.

Keywords: quantum dots, chitosan, immunoassay

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Материалы конференции
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Покрытие гидрофильных халькогенидных квантовых точек карбоксиметилхитозаном для применения в иммунохроматографическом анализе

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Аннотация. Одним из бурно развивающихся направлений в мире науке является использование квантовых точек (КТ) в различных приложениях биоанализа в качестве флуоресцентных маркеров и аналитических меток белковых молекул. Для применения КТ в биоанализе, их поверхность покрывают биосовместимыми полимерами. В данной работе покрытие КТ состава CdTe/CdS/ZnS-МПК карбоксиметилхитозаном (КМХ) осуществляли электростатической адсорбцией. Физико-химические свойства полученных КТ-КМХ исследованы методами капиллярного зонного электрофореза, динамического рассеяния света, методами спектроскопии поглощения и флуоресцентной спектроскопии.

Ключевые слова: квантовые точки, хитозан, иммуноанализ

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Introduction

Currently, colloidal quantum dots (QDs) have drawn tremendous attention as promising tool for in vivo imaging and biosensoric applications. The unique optical properties make them exciting alternative for conventional analytical labels in lateral flow immunoassay (LFIA). To apply QDs in bioanalysis their surface usually coating with biocompatible polymers with different functional groups for attachment of biological molecules. The most commonly used polymeric coating is chitosan. Chitosan is a natural, glucosamine polysaccharide which display good biocompatibility. However, chitosan is insoluble in neutral and alkaline media. As a result, the coating is conduct in an acetic acid medium. Low pH values lead to protonation of anionic stabilizers of QDs (for example: thioglycolic acid, mercaptopropionic acid (MPA), 2-mercaptoethanol, etc) and decrease of quantum yield. In addition, chitosan does not have carboxyl groups required for protein conjugation by the carbodiimide-succinimide method.

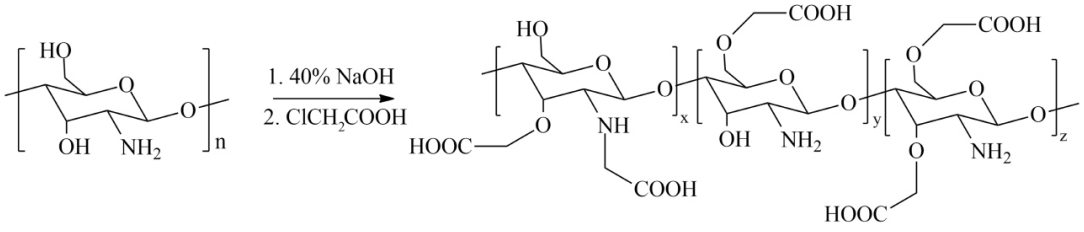
Materials and Methods

CdTe/CdS/ZnS-MPA QDs, chitosan (deacetylation degree: ≥ 80%, molecular weight ≤ 50 kDa, National Research Centre, Egypt), monochloroacetic acid (for synthesis, VEKTON, Russia), sodium hydroxide (98%, VEKTON, Russia), isopropyl alcohol (99.9%, Sigma-Aldrich), deionized water.

Microcentrifuge Eppendorf 5425 (Eppendorf, Germany) , magnetic stirrer IKA C-MAG HS 7 (IKA-Werke, Germany), analytical balance CAUX-120 (CAS Corporation, Republic of Korea), polyethersulfone syringe filters with 0.22 μm pores, Amicon ultra-0.5 mL centrifugal filters (Merck, Germany), spectrophotometer UNICO-2100 (United Products & Instruments, USA), spectrofluorometer FluoroLog 3 model FL3–21 (Horiba Jobin Yvon SAS, France), Zetasizer Nano S Size Analyzer (Malvern, Germany), Agilent Capillary Electrophoresis System 7100 (Agilent Technologies, USA), Elix Advantage 5 Water Purification System (Millipore, USA).

Preparation of carboxymethyl chitosan

Analyzing a number of articles [1–6], usually chitosan is dissolved in acetic acid and mix with solution of negative charged nanoparticles (NPs). Since chitosan does not have carboxylic groups which required to protein conjugation by carbodiimide-succinimide method and negatively charged QDs degrade in acid solutions it is necessary to modify chitosan.

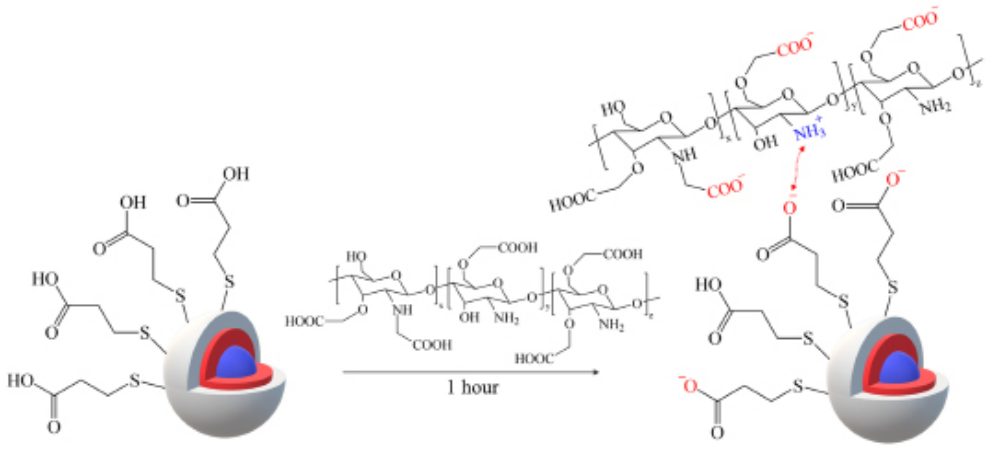


Scheme 1. Carboxymethylation of chitosan

In this study, to ensure solubility in water and the introduction of carboxyl groups into the structure, chitosan was modified with monochloroacetic acid in a sodium hydroxide medium. Chitosan is dispersed in isopropyl alcohol for 20 minutes. 40% sodium hydroxide solution and monochloroacetic acid are added to the reaction mixture with constant stirring at a temperature of 45 °C. The resulting CMC was washed with anhydrous isopropyl alcohol and dried.

Carboxymethylation of chitosan is conducted by alkylation of chitosan with monochloroacetic acid in sodium hydroxide medium according to [7] (Scheme 1).

Further, the resulting CMC was used for coating CdTe/CdS/ZnS-MPA QDs by electrostatic interaction according to Scheme 2.



Scheme 2. Coating of CdTe/CdS/ZnS-MPA QDs with CMC

The obtained QDs-CMC were studied by absorption and fluorescence spectroscopy, dynamic light scattering and capillary zone electrophoresis.

Results and Discussion

To determine the presence of carboxyl groups, the obtained CMC was studied by IR spectroscopy with a frustrated total internal reflection attachment (Fig. 1).

Analyzing the obtained CMC IR spectrum, it can be noted that after carboxymethylation, chitosan retains its native structure, as evidenced by the presence of vibrations in the region of 1024–877 cm^{-1}. Vibrations in the region of 1668 and 1409 cm^{-1} points the successful introduction of carboxyl groups into the structure of chitosan.

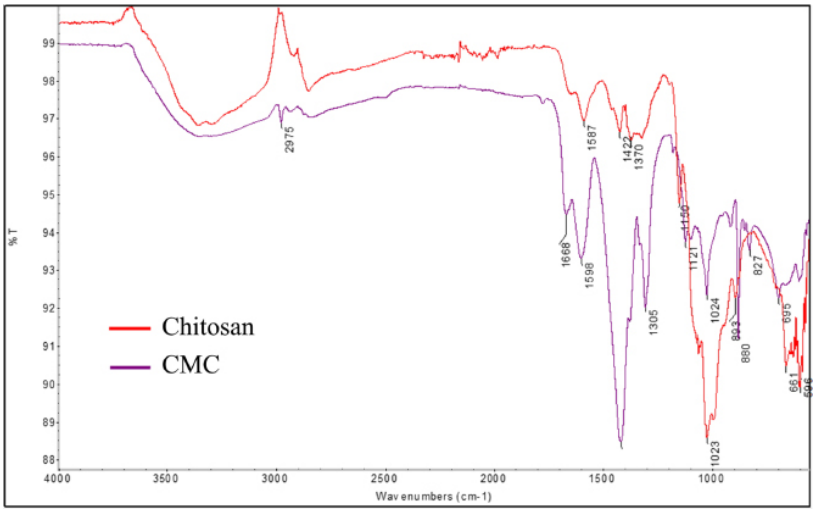


Fig. 1. IR spectra of chitosan (blue) and CMC (violet)

Table 1

Characteristic frequencies in the IR spectra of chitosan and CMC

Frequency, cm^{-1}	Characteristic frequencies
3455–3445	O-H, N-H
2860–2850	C-H
1668	C=O
1409	$-CH_2COONa$
1024–877	C-O, C-O-C

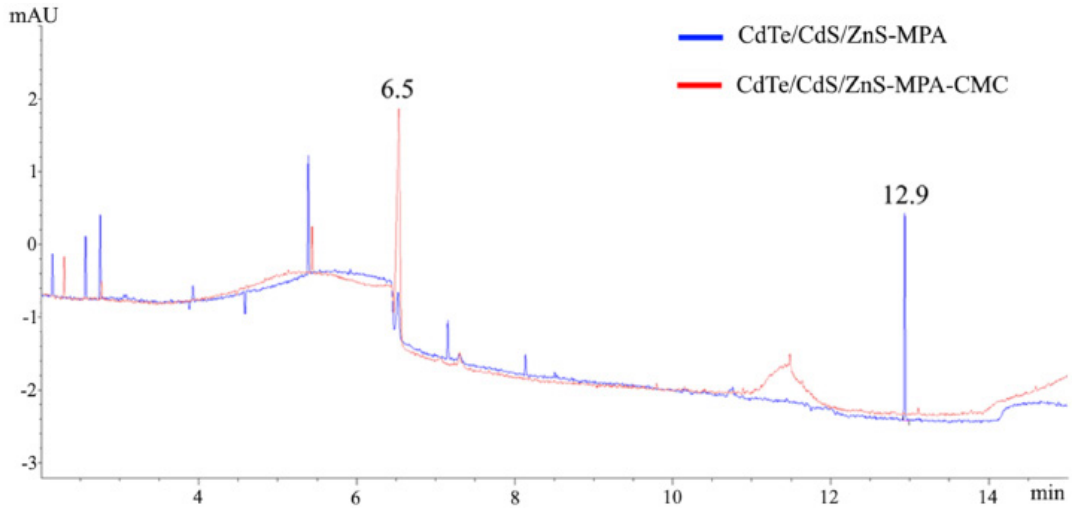


Fig. 2. Overlay of electrophoregrams of CdTe/CdS/ZnS-MPA (blue) and CdTe/CdS/ZnS-MPA-CMC (red) QDs. Analysis conditions: detection wavelength 220 nm, voltage +30 kV, supporting electrolyte 25 mM borate buffer solution pH = 9.2

To define the physicochemical properties and the possibility of application in bioanalysis, the obtained QDs-CMC were studied by absorption and fluorescence spectroscopy, dynamic light scattering and capillary zone electrophoresis.

The migration time of CdTe/CdS/ZnS-MPA QDs is 12.9 minutes, and that of the CdTe/CdS/ZnS-MPA-CMC one is 6.5 minutes, which is associated with a change in the surface charge of QDs as a result of the addition of CMCs to the surface of QDs. It can be noted that the peak has a narrow symmetrical shape, which indicates the monodispersity of QDs-CMC.

Fig. 3 shows the normalized absorption spectra of CdTe/CdS/ZnS-MPA and CdTe/CdS/ZnS-MPA-CMC QDs, and their fluorescence spectra (excitation wavelength: 350 nm).

The peak in the absorption spectrum of QDs of the composition CdTe/CdS/ZnS-MPA in the region of 569 nm corresponds to the formation energy of an electron-hole pair (exciton). After QDs coating, the position of the exciton peak slightly shifts to the red region of the spectrum due to CMC coating absorption. The maximum fluorescence peaks are at a wavelength of 621 nm. The luminescence wavelength does not change after coating since CMC does not participate in emission processes. The QDs quantum yield decreases. The relative quantum yield of CdTe/CdS/ZnS-MPA and CdTe/CdS/ZnS-MPA-CMC are 27% and 25%, respectively. The decrease in the quantum yield is due to the fact that the CMC immobilized on the QD surface shields the secondary emission.

The hydrodynamic diameters of QDs and QDs-CMC determined by the dynamic light scattering method are presented in Table 2.

Thus, after CMC coating of QDs, the hydrodynamic diameter of QDs increased by 96 nm, which is associated with the capture of a single CMC fragment of several QDs.

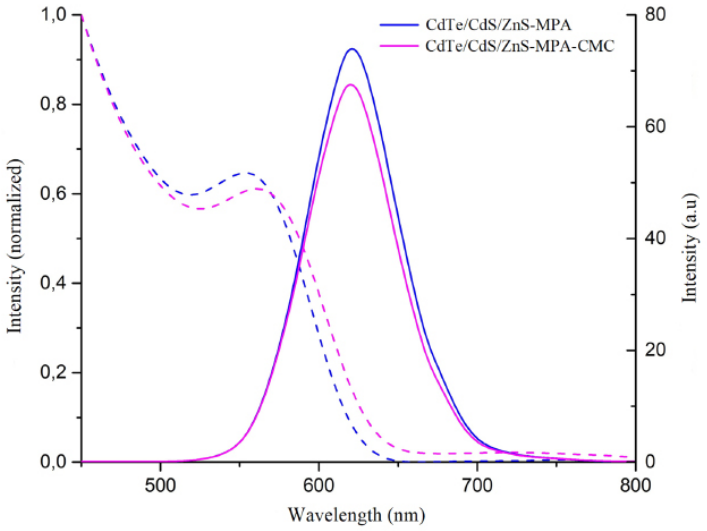


Fig. 3. Overlay of the absorption (dotted lines, left axis) and fluorescence (solid lines, right axis) spectra of the CdTe/CdS/ZnS-MPA and CdTe/CdS/ZnS-MPA-CMC QDs

Table 2

Hydrodynamic diameters of QDs and QDs-CMC

Sample	Hydrodynamic diameter, nm
CdTe/CdS/ZnS-MPA	46 ± 2
CdTe/CdS/ZnS-MPA-CMC	137 ± 5

Conclusion

Modification of QDs with CMC makes it possible to introduce functional groups required for conjugation with proteins. The functionalization of QDs based on electrostatic interactions leads to increase in the hydrodynamic size of QDs as a result of the capture of several QDs by a single polymer fragment. As a result, the CMC coating preserve the optical properties of QDs. The immobilization of CMC leads to a slight decrease in the quantum yield.

The biocompatibility of chitosan makes it a promising polymer coating for the conjugation of QDs with proteins. In further studies, it is planned to immobilize antibodies on the surface of QDs-CMC for lateral flow immunoassay applications.

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