Carbon Quantum Dots (CQDs) Prepared from Waste Biomass as a New Class of Biomaterials with Luminescent Properties

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Abstract

Carbon Quantum Dots (CQDs) are objects with a size less than 10 nm that have the ability to emit radiation in the visible range from blue to red depending on the excitation radiation used. Quantum dots are used in in vitro bioimaging of cell structures or creation of biosensors. In contrast to classic nanodots, which are obtained from simple sulphides, selenides or metal tellurides, carbon quantum dots are constructed from a non-toxic, biocompatible carbon core, thanks to which it is possible to apply quantum carbon dots in bio-imaging in-vitro or in-vivo biological structures with minimal cytotoxic effect on cells.

The aim of the research was to obtain carbon nanodots capable of emitting fluorescence using lignin from waste biomass. The CQDs were functionalized with amino-acids. The result of the work was to obtain a series of CQDs with advanced luminescence properties using hydrothermal and microwave assisted methods. Ready products were investigated over their cytotoxicity.

Keywords: *carbon quantum dots, nanomaterials, waste biomass, biomaterials, luminescence*

Introduction

In the past few years, the scientists have focused on the nanomaterials development since the objects of the size below 100 nm tend to exhibit extraordinary optical, electrical and biological properties. Among nanorods, nanofibers or nanowires a new type of nanoparticles have been discovered – quantum dots. The nanomaterials can be described as objects of the size below 10 nm. Their most unique property is the ability to emit radiation in the visible region from blue to red. The emission depends on the excitation radiation which is applied. Therefore, such nanomaterials have a great potential in the industry and may successfully replace traditional fluorescence dyes [WANG, Youfu et al. 2014, LIANG, Zicheng et al, 2016, ZUO, Jun et al. 2015]. A special attention is paid to the so-called carbon quantum dots (CQDs) which are superior to other types of quantum dots with metallic or semi-metallic cores due to their lack of cytotoxicity [CAYUELA, Angelina et al 2016]. CQDs can be prepared from both synthetic and natural raw materials. The most interesting carbon quantum dots preparation strategy involves the application of biomass. As a feedstock, any organic component may be used. Carbon quantum dots can be obtained by various methods, including laser ablation, microwave radiation, conventional heating or ultrasounds. Carbon nanomaterials have a great potential in medicine and pharmacy, since they are water-soluble [ZUO, Jun et al. 2015, WANG, Ru et al. 2017]. Moreover, CQDs can undergo surface modification due to the presence of certain functional groups, which may differ depending on the raw material applied.

The surface functionalization may result in the enhancement of water-solubility [ZHANG, Miaomiao et al. 2016]. Also, it can positively affect luminescence properties such as fluorescence quantum yield or photostability as well as resistance to photobleaching and photoblinking. The functionalization can be carried out using natural substances containing sulphur or nitrogen atoms [ROY, Prathik et al 2015, PIRES, Natalia et al 2015]. Currently, it is believed, that carbon quantum dots can replace traditional dyes, drug carriers and labelling agents in medicine and pharmacy due to their nanosize and ability to permeate cell membranes. CQDs can be applied in cells labelling and diagnostics since they may help to visualise various cell components. Carbon quantum dots can be modified by various biomolecules including proteins which help them to detect cancer cells [DAS, Rahul et al. 2017]. Functionalization of the nanomaterials with compounds containing phenol rings may give them antioxidant properties.

The functionalization process can be performed simultaneously with the nanodots formation during carbonization process, or after. The right choice of the modifying agent results in the preparation of the nanomaterials with desired characteristics [GAO, Xiaohui et al, 2016, PANDA, Snigdharani et al. 2018].

Lignin which is one of the most abundant polymers in the environment rich in carbon atoms [CHEN, Jao et al, 2016]. Thus, it is a cheap and easily accessible raw material for the quantum dots synthesis.

In this article, a strategy for the novel type of nanodots synthesis is proposed. The obtained nanomaterials were investigated over their physicochemical, especially spectroscopic and luminescence properties. Also, their cytotoxicity was investigated to verify their potential in medicine and pharmacy.

Materials and methods

Materials

Tab. 1. CQDs synthesis parameters
Tab. 1. Parametry syntezy CQD

Sample	Lignin, g	HCl, ml		water, ml	Modifying agent, g	Modifying agent	Time, h	Temperature, °C
1	0.50	0.50		5.00	0.10	*L*-lysine	12	180
2						*L*-glutamic acid		
3						*L*-aspartic acid		
4						*L*-cystein		

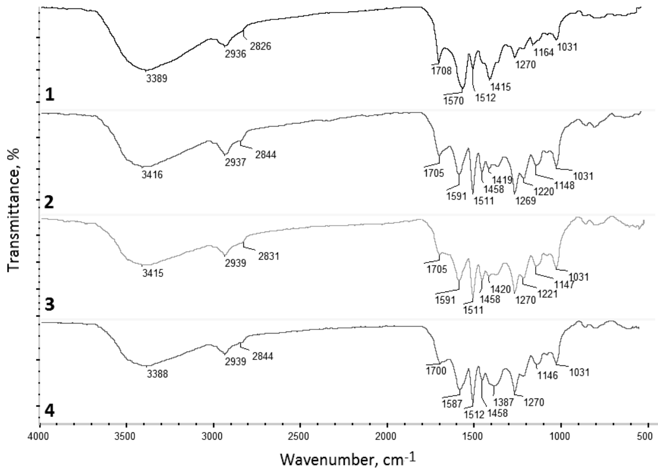

Fig. 1. Presents FTIR spectra of the obtained samples
Rys. 1. Przedstawia widma FTIR uzyskanych próbek

Fig. 2. Luminescence properties of the prepared samples
Rys. 2. Właściwości luminescencyjne przygotowanych próbek

Fibroblast growth medium (FGM) and human dermal fibroblasts (HDF), aminoacids (L-lysine, L-glutamic acid, L-aspartic acid, L-cystein), XTT assay were purchased from Sigma Aldrich, Poland. Ethanol, methanol, NaOH were purchased from Avantor, Poland. Lignin was obtained from the wood during extraction process.

Methods

Carbon quantum dots synthesis

Carbon quantum dots were obtained by hydrothermal method using lignin as a raw material.

As modifying agents aminoacids were used (L-lysine, L-glutamic acid, L-aspartic acid, L-cystein).

The nanomaterials synthesis was carried out in autoclave (parameters given in Table 1). After reaction, the products were neutralized with NaOH solution and purified on membranes for 96h and dialysed on 500–1000 MWCO to remove contaminants.

Antioxidant properties study

To perform antioxidant properties study a solution of DPPH in methanol was prepared with absorbance = 1. The measurements were performed using Aligent 8453 spectrophotometer at 517 nm.

To determine the capability of free radicals removal, 0.10 g of each sample was placed in 5 ml of DPPH solution and left in darkness for 1 hour. Then, the absorbance of each solution was measured at 517 nm. The percentage of the free radicals removed was calculated using following Equation:

$$\%S = \frac{A_s - A_c}{A_c}$$

where:
%S – the % of the free radicals which were neutralized
A_c – the absorbance of the DPPH solution without the sample
A_s – the absorbance of the DPPH solution containing sample

Luminescence properties analysis

The quantum yield of the obtained products was determined according to following Equation:

$$Q_s = Q_r \left(\frac{A_r}{A_s}\right)\left(\frac{E_s}{E_r}\right)\left(\frac{\eta_s}{\eta_r}\right)^2$$

Q = Fluorescence quantum yield
η = Refractive index of the solvent
A = Absorbance of the solution
E = Integrated fluorescence intensity of emitted light

Tab. 2. CQDs fluorescence quantum yield
Tab. 2. Wydajność kwantowa fluorescencji CQD

Sample	Fluorescence Quantum Yield, %	Fluorescence Quantum Yield after 30 days, %
1	9	9
2	6	5
3	7	7
4	11	11

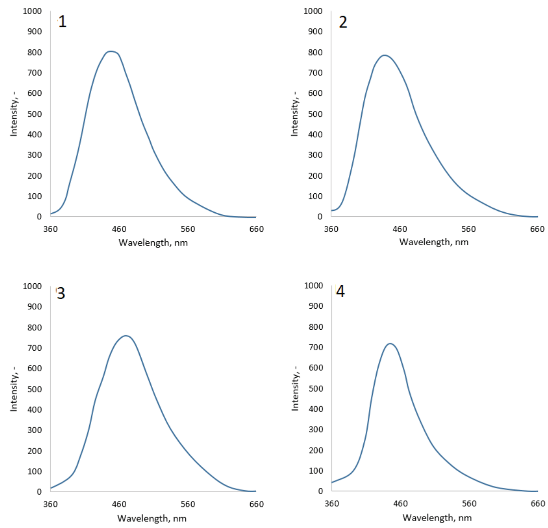

Fig. 3. Fluorescence spectra of the prepared samples
Rys. 3. Widma fluorescencji przygotowanych próbek

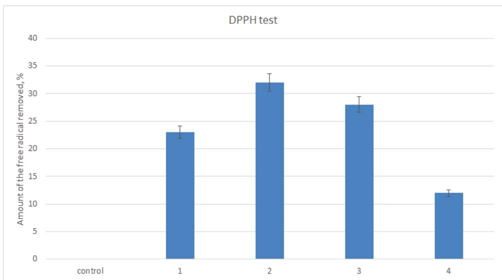

Fig. 4. Antioxidant properties of the prepared CQDs
Rys. 4. Właściwości przeciwutleniające przygotowanych CQD

Subscripts 'r' and 's' refer to the reference and unknown fluorophore respectively

The fluorescence quantum yield was determined by comparison to quinine sulphate standard solutions, which exhibited a fluorescence quantum yield of 0.54 at 365 nm.

Cytotoxicity study

Cytotoxicity of the CQDs was investigated using HDF (primary cells). The culture was performed for 48h at 37°C (95% CO_2). As a culture medium complete FGM was applied. The cells were monitored under Delta Optics inverted microscope. The cytotoxicity was measured by XTT assay.

Results and discussion

FTIR analysis

It can be notices that all samples spectra show bands typical for lignin such as 3416–3388 cm^{-1} that confirm the presence of hydroxyl functional groups coming from phenolic and alcoholic groups. Wide and broad bands in the range between 3600–2600 cm^{-1} 1708–1700 cm^{-1} indicate presence of carboxylic functional groups in the structure of polymeric nanomaterials. Moreover aromatic groups vibrations can be observed at 1512–1511 cm^{-1} and 1458–1415 cm^{-1}. What is important some additional bands can be noticed coming from the incorporated modifying agents (amino acids). Amino groups presence can be confirmed by bands located at 1591–1570 cm^{-1}.

Luminescence properties analysis

It can be noticed that all samples had luminescence properties under UV radiation and it depends on their concentration. One may also observe, that the best properties exhibit sample 1 and 4. The obtained spectra are typical for carbon quantum dot with luminescent properties.

Fluorescence quantum yield

Table 2 presents results of fluorescence quantum yield of the prepared nanomaterials. All of the CQDs have fluores-

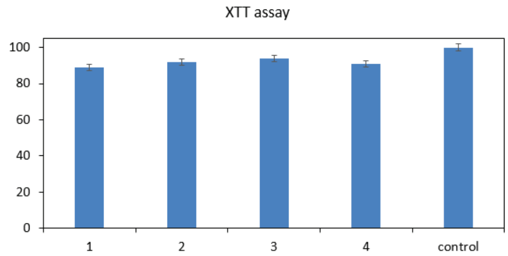

Fig. 5. Cytotoxicity of the prepared biomaterials
Rys. 5. Cytotoksyczność przygotowanych biomateriałów

cence properties. It can be noticed that this property depends on the modifying agent was applied. The highest FQY can be observed in the case of nanodots obtained from liginin and doped with cysteine. Also, very good results were obtained for the sample functionalized with lysine. Moreover, all samples had very good photostability since no decrease of luminescence properties had been noticed after 30 days.

Antioxidant properties study

Figure 4 presents results of the antioxidant properties study performed by DPPH method. It can be observed that all of the obtained samples exhibited an ability to remove free radical. The highest antioxidant activity was observed for sample 2 and 3 which can be assigned to the presence of free amino groups coming from aspartic and glutamic acids incorporated into carbon dot. The lowest ability of free radicals scavenging was noticed for sample 4 prepared using cysteine as functionalizing agent.

Cytotoxicity study

Figure 5 presents results of cytotoxicity study carried out on human dermal fibroblasts. It can be noticed, that prepared nanomaterials are non-toxic to the prepared carbon dots since no any significant decrease in the amount of living cells when comparing to the control culture can be noticed. Lack of cytotoxicity is correlated with the application of natural raw materials as well as carefully proceeded purification.

Conclusion

The aim of the following research was to obtain biocompatible carbon-based nanomaterials using waste biomass (lignin) as a raw material. Performed studies showed that proposed carbon quantum dost preparation strategy enabled obtainment of the advanced nanomaterials functionalized with aminoacids which enhance their biological activity. The modification of the carbon core was confirmed by FT-IR method. Luminescence properties study showed that CQDs had good fluorescence quantum yield and were photostable for 30 days. Moreover, it was shown that the samples had antioxidant activity. Finally, it was confirmed that prepared nanomaterials are not cytotoxic so they can be safely used in medicine and pharmacy, especially for bioimaging and cells labelling applications.

Acknowledgements

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Kropki kwantowe węgla (CQD) przygotowane z biomasy odpadowej jako nowa klasa biomateriałów o właściwościach luminescencyjnych

Kropki kwantowe węgla (Carbon Quantum Dots – CQD) to obiekty o rozmiarze mniejszym niż 10 nm, które mają zdolność emitowania promieniowania w zakresie widzialnym od od niebieskiego do czerwonego w zależności od zastosowanego promieniowania wzbudzenia. Kropki kwantowe stosuje się w bioobrazowaniu in vitro struktur komórkowych lub tworzeniu bioczujników. W przeciwieństwie do klasycznych nanodotów, które są otrzymywane z prostych siarczków, selenków lub tellurków metali, kropki kwantowe węgla są zbudowane z nietoksycznego, biokompatybilnego rdzenia węglowego, dzięki czemu możliwe jest zastosowanie kwantowych kropek węgla w bioobrazowanie struktur biologicznych in vitro lub in vivo przy minimalnym działaniu cytotoksycznym na komórki. Celem badań było uzyskanie nanodotów węglowych zdolnych do emitowania fluorescencji przy użyciu ligniny z biomasy odpadowej. CQD sfunkcjonalizowano aminokwasami. Rezultatem prac było uzyskanie serii CQD o zaawansowanych właściwościach luminescencyjnych z zastosowaniem metod hydrotermalnych i mikrofalowych. Gotowe produkty badano pod kątem ich cytotoksyczności.

Słowa kluczowe: *kropki kwantowe węgla, Carbon Quantum Dots, nanomateriały, biomasa odpadowa, biomateriały, luminescencja*