Materials Letters 212 (2018) 78–81

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/mlblue

Green synthesis of lignin based fluorescent nanocolorants for live cell imaging

Mamatha M. Pillai [a,1], K.R. Karpagam [b,1], Rosina Begam [b], R. Selvakumar [a], Amitava Bhattacharyya [c,*]

[a] Tissue Engineering Lab, PSG Institute of Advanced Studies, Coimbatore 641004, India
[b] Functional, Innovative and Smart Textiles Lab, PSG Institute of Advanced Studies, Coimbatore 641004, India
[c] Department of Electronics and Communication Engineering, PSG College of Technology, Coimbatore 641004, India

ARTICLE INFO

Article history:
Received 4 August 2017
Received in revised form 15 September 2017
Accepted 15 October 2017
Available online 16 October 2017

Keywords:
Lignin
Nanocolorants
Electrolysis
Fluorescence staining
Antibacterial

ABSTRACT

In this study, lignin has been used as stabilizing agent during electrochemical synthesis of copper based nanocolorants. Brown, green and blue colored colloidal suspensions of nano particles have been produced under different electrolysis process conditions using copper electrodes. Average particle sizes are found to be 11 nm, 50 nm and 151 nm for green, blue and brown, respectively. Nanocolorants exhibit fluorescent properties under blue, green and UV filters and possess good antibacterial efficiency (9–12 mm zone of inhibition). They are nontoxic and efficient for live cell fluorescent imaging with L929 cells. Cells stained with green nanocolorant show highest fluorescent intensity among all synthesized colorants and pure lignin.

1. Introduction

Lignin, an amorphous, three-dimensionally cross-linked gum like polymeric material, contains complex methoxylated phenylpropane substructures [1]. Lignin extracted from plant sources like coir has many applications in water treatment, natural dyes, dust suppression, oil fields additives and agriculture [2]. As lignin showed auto fluorescence property during plant cell imaging [3], it would be interesting to study its fluorescent staining potential for mammalian live cells. Fluorescent imaging of live cells, an important tool to analyze cellular processes, has challenges like minimization of photo quenching and cell division without any physical damage. Lignin, being a biopolymer, may be useful in this aspect as commonly used fluorescent dyes for mammalian cell imaging are both cyto and phototoxic [4].

Further, complex structure of lignin enables it to be used as a stabilizing or capping agent during preparation of copper nanoparticles [5]. Researchers successfully produced copper oxide (Cu_2O) nanoparticles from a mixture of pure lignin and $CuSO_4$ solution by centrifuge and precipitate method [6]. Green approach to synthesize copper nanoparticles using a plant extract was also reported [7]. Among the synthesis methods, electrochemical route is easy, cost effective and eco-friendly [8]. Researcher synthesized copper nanoparticles using citrate buffer, DNA, etc as capping agent in this process [9]. However, there is no report on a method to synthesize lignin capped copper based nanocolorants through electrochemical route.

In this study, lignin was used as capping agent for synthesizing colloidal suspensions of copper nanoparticles using electrochemical technique at various electrolysis bath conditions. L929 mouse fibroblast cells were stained to evaluate the nanocolorants' potential as fluorescent cell staining agent. They were also studied for cyto-toxicity, antibacterial effect and fabric dyeing ability.

2. Experimental

Lignin was isolated from coir by sulphite pulping [10]. Sodium bisulphate (2%) solution was added to coir (material to liquor, 1:20), autoclaved for 2 h at 121 °C, pH 4.5 (0.01 g citric acid) and extracted lignin was filtered. Electrolysis bath (40 ml) was prepared with different lignin concentrations, 0.1 g NaCl and 0.01 g citric acid. Copper electrodes (8 × 2 cm, LxB; thickness 0.06 mm) were used for electrolysis. Once the electrolytic setup was powered using DC power source, copper ions were released from anode (+), lignin present in the solution stabilized the ions to form nanoparticles expressing different colors (Supplementary Fig. 1). The synthesized nanocolorants were characterized for their morphology,

* Corresponding author.
E-mail address: abh@psgias.ac.in (A. Bhattacharyya).
[1] Equal contribution.

https://doi.org/10.1016/j.matlet.2017.10.060
0167-577X/

Table 1
Electrochemical conditions and lignin concentration for color formation.

S. No	Lignin (gpl)	Current (mA)	Voltage (V)	Time taken (min)	Colour obtained
1.	17.5	0.05	3.5	30	Brown
2.	4.3	0.05	2.4	30	Green
3.	2.1	0.02	2	20	Blue

particle size and fluorescence properties. Nanocolorants (10 mg) were studied with L929 cells for cytotoxicity assay using MTT (3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) [11]. For imaging, cells were treated with 200 µl of pure lignin and synthesized nanocolorant dispersions (brown, blue and green) and incubated for 10 min. Fluorescence intensity was assessed using surface intensity plot. Cotton fabrics (7.5 g) were dyed with these solutions. CIE L a* b* color values and physical properties of the dyed fabrics were measured.

3. Results and discussion

3.1. Optimization of electrochemical process

Table 1 shows the optimized electrochemical bath parameters for the synthesis of nanocolorants (brown, green and blue). It can be observed that, brown shade requires more lignin concentration than green. Lighter shade like blue requires very less concentration of lignin and time required for synthesis is also low. Alcoholic group present in lignin can react with citric acid to form ester linkage while the copper may react with phenolic group of lignin to form phenoxide. Electrolysis bath contains 0.25 gpl citric acid while the lignin concentration is much higher in all electrochemical baths. Hence, the presence of lignin is more expected for stabilizing the nanoparticle structure than citric acid. As biopolymer lignin has complex, amorphous structure and it interacts with copper as well as citric acid in the electrolytic bath, a combination of different complex structures is expected in each synthesized dyes.

3.2. Morphology and composition of nanocolorants

Nano sized particles ranges from 11-151 nm were observed for different nanocolorants in particle size analyzer (Zetasizer version 7.11). Average sizes of the particles were approximately 11 nm and 50 nm for green and blue colorants, respectively. The brown has higher particle size (151 nm) with a broad size distribution compared to blue and green. This may be due to higher lignin content where the biggest and complex structure formation is expected. Structural stabilization by the large, complex lignin molecules [12] at high concentrations may lead to higher size of brown dye. Lignin nanoparticles synthesized using other processes have also shown similar bigger sizes [13,14].

Under transmission electron microscopy (HRTEM, Jeol JEM 2100, Japan), all particles were found to be spherical in shape and sizes were in line with particle size analyzer results (Fig. 1a–c). Different sizes of particles were observed in brown dyes with majority having more than 100 nm; few smaller particles have 2

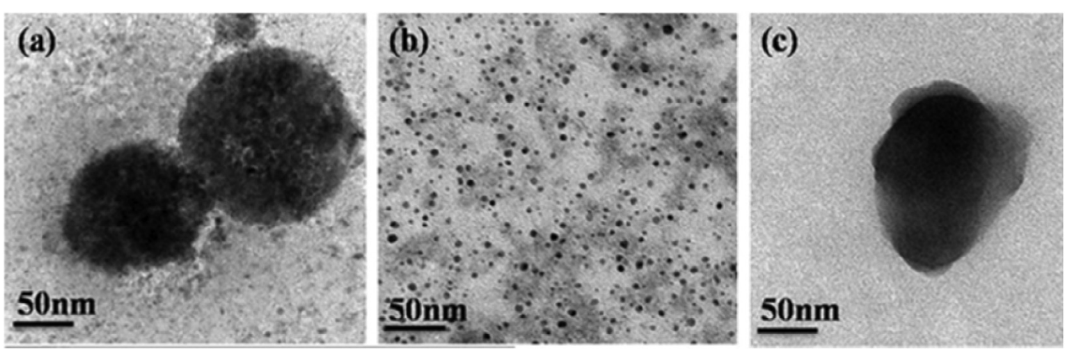

Fig. 1. Transmission electron microscopic images of (a) brown dye, (b) green dye, (c) blue dye. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

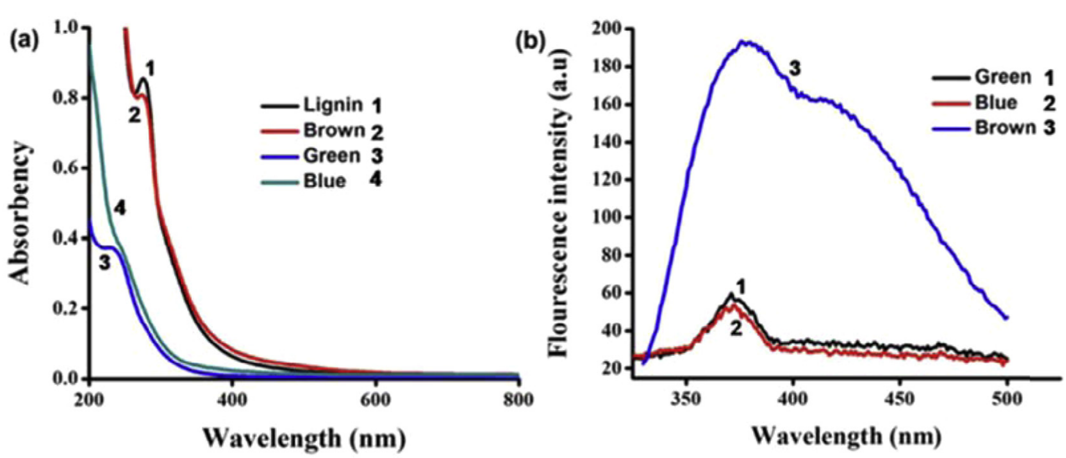

Fig. 2. (a) UV visible spectra (b) Photoluminescence spectrum of nanocolorants.