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Effects of extraction solvents on photoluminescent properties of eysenhardtia polystachia and their potential usage as biomarker

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ABSTRACT

Currently, nanomaterials had been used for several applications; one of them is as bio-markers. These nanomaterials contain fluorescent compounds as effective indicators for imaging and other applications in Biotechnology. In previous studies, we proposed a functionalized nanomaterial-based biomarker from silica and *Eysenhardtia Polystachia*, a medicinal tree known in Mexico as "palo azul" (Kidneywood). Our previous results showed the feasibility of the nanomaterial obtained as bio-marker. In this article, our purpose is to evaluate the effects of extraction solvents on fluorescence of that biomarker. The photoluminescence (PL) effect was evaluated at different pH (4, 7.4 and 8); four extraction solvents, ethanol, methanol, methanol-ethanol and methanol-ethanol-water were evaluated. A molecular dynamics simulation was performed to recognize molecular interaction between the compounds of the extracts with solvent molecules and to investigate the solvent molecules effect on photoluminescence spectra. The results were also compared with rhodamine 6G and we found that, at physiological pH (7.4), the fluorescent-coated silica nanoparticles obtained were also stable. We found that extraction solvents could be used for obtaining different nanomaterials for specific applications, and also found the best extraction solvent for obtaining *EP* nanomaterials for health care applications, specifically for imaging techniques.

1. Introduction

Nowadays, nanomaterials had been used for several applications; one of them is as bio-markers. These nanomaterials contain fluorescent compounds as effective indicators for imaging and other applications in Biotechnology. Modern bio-imaging technologies are used to measure physical and biochemical parameters such as surface area, tissue properties, and chemical concentration; or even to achieve temporal views of biological functions.

The data obtained by these techniques is the basis for mathematical modeling of protein kinetics, for studying biochemical networks, and testing computational models already developed [1]. Also, the bio-images obtained with the fluorescent nanomaterials are helping to develop sophisticated experiments in genomics, proteomics and metabolomics; and providing a basis for new types of research in cancer pathologies [2].

Fluorescent compounds had been studied for over twenty years, improving quality and applicability for nanomaterials modification. For example, fluorescent and pH-sensitive dyes along with the recombinant fluorescent protein technology could allow labeling almost every cellular structure for its study [1], [3–6]. Other fluorescent compounds could be: organic dyes with fluorophores; genetically encoded fluorescent proteins; and Quantum dots (QDs). The last ones (QDs) were used for Shah, et al. for labeling stem cells, but due to their complex surface chemistry, organic dyes are preferred by some researchers for coupling different nanomaterials [7–13].

Organic dyes such as rhodamine, fluorescein, 4′,6-Diamidino-2-Phenylindole (DAPI), and Red Fluorescent Protein (DsRed) are easy to use and relatively inexpensive for labeling cells in culture. Nevertheless, they usually lose fluorescence quickly, therefore they are useful only in short-term tests. It is well known that changes in pH causes chemical degradation of these organic dyes [9], [10], [14].

Also, some of these dyes could have a level of toxicity. For example, Rhodamine 6G has high toxicity and teratogenic effects. As a consequence, it was not approved by FDA, and yet it is an important chromophore used in biomedical applications. Thaler, et al., 2008, reported toxicity of Rh6G in the rat retine up to 2 μL at 5.0 mg/mL concentration; the Rh6G accumulation cause severe damage to rat retinal ganglion cells. The effect of Rhodamine in mice is, mainly, inhibition of enzymes, such as ATPasa, and at cellular level in mitochondria. In some studies, it has been suggested that the use of rhodamine, at concentrations of about 10 to 12 mg/kg in mice, could even cause cancer, due to the accumulation of the substance in some tissues and/or by inhibiting enzymes important in cellular processes.

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This toxicity was the reason why we proposed the use of an organic dye, which could be less toxic than rhodamine, obtained by *Eysenhardtia Polystachia* (*EP*), a medicinal tree known in Mexico as "palo azul" (Kidneywood) [15]. There is a discussion about which is/are the fluorescent component/s from this tree. Previous studies have reported the main components of *EP* extracted with methanol, ethanol and water, as solvents, in combination or separately [16–20]. Among these components, aurones, flavonoids, isoflavonoids and matlaline had been found.

It is known that all flavonoids contain a 15-carbon skeleton, their core structure is a 2-phenylbenzopyranone, in which the three-carbon bridge between the phenyl groups is commonly cyclised with oxygen, therefore they could efficiently absorb UV light [21]. Other components of the plant extract are methylated flavonoids; for example, Burns, et al. reported 7-hydroxy-2′,4′,5′ trihydroxyisoflavones; Alvarez, et al. reported (3*S*)-3′,7-Dihydroxy-2′,4′,5′,8-tetramethoxyisoflavan and (3*S*)-7-Hydroxy-2′,3′,4′,5′,8-pentamethoxyisoflavan. Also, according to Acuña et al., coatline B could be in the plant; and is an C-glycosyl-α-hydroxydihydrochalcone [17,20,22]. As seen in Fig. 1, coatline B is a precursor of matlaline.

Matlaline could be the compound related with fluorescence from *EP* extracts showed at certain pH. Its absorption spectrum is from 283 to 430 nm at pH = 9. Matlaline is a four-ring tetrahydromethanobenzofuro[2.3-*d*]oxacine which is not present in the tree but is the end product of spontaneous oxidation, as we have mentioned. The first report of the natural fluorescence of this compound was in 1565 by Nicolas Monadares, who observed a peculiar blue in the plant infusion.

With this information and having in mind the applications mentioned in the first part of this section, we proposed a functionalized nanomaterial in a previous study, using silica and *Eysenhardtia Polystachia* [15]. Our previous results showed the feasibility of this nanomaterial as bio-marker, because we obtained a low-decay of fluorescence and proofs of cell internalization of the dye. Those results were obtained with a nanomaterial synthesized with *EP* extracted with ethanol. Therefore, it is necessary to investigate more possibilities of functionalized nanomaterials obtained with different solvents.

We are interested on investigating photoluminescense from different extracts from the same tree. Therefore our purpose in this article is to evaluate the effects of extraction solvents on fluorescence of silica-*Eysenhardtia Polystachia* nanomaterials. Also, we performed a molecular dynamics simulation of molecules derivated from EP extract, to discuss experimental results. And we compared extracts with rhodamine 6G (Rh6G).

We believe that the approach presented here, will help to understand which is the best nanomaterial and the path to obtain it with the specific purpose to be applied as bio-marker in healthcare industry.

2. Materials and methods

2.1. Materials

Heartwood from *Eysenhardtia polystachya* (*EP*) was collected from Uruapan, Michoacán, México. Samples were macroscopically and microscopically identified as *Eysenhardtia polystachya* (Ortego) Sarg. by MSc. Josefina Barajas, at the "Xiloteca" (National Herbarium), Biology Institute, Universidad Nacional Autónoma de México (UNAM). Samples were kept at the National Herbarium under registration code MEXU 1850.

Tetraethylorthosilicate (TEOS) (98%), 3-aminopropyltriethoxysilane (APTES), methanol (CH_3OH), ethanol (C_2H_5OH) (90%), diethyl ether $(C_2H_5)_2O$ (anhydrous), toluene (C_7H_8) and rhodamine 6G (Rh6G) were purchased from Sigma Aldrich Co. Hydrochloric acid (HCl), sodium hydroxide (NaOH), ammonium hydroxide (NH_4OH), phthalate buffer solution (pH = 4), sodium phosphate monobasic (NaH_2PO_4) and sodium phosphate dibasic (Na_2HPO_4) were purchased from J.T. Baker. Phosphate buffer solution of pH = 7.4 and pH = 8 were prepared using adequate quantities of mono and di basic sodium phosphate. All chemicals were used without further treatment and deionized water was used in all experiments.

2.2. Eysenhardtia Polystachia extraction

Four powdered *EP* organic dyes were obtained with different solvents, using an extraction procedure previous reported [15,16]. *EP* powder was subjected to soxhlet extraction using four different solvents; methanol, ethanol, 50:50% v/v methanol-ethanol and 1/3:11/3:1/3%v/v methanol-ethanol-deionized water.

The extraction systems obtained were transferred into a separator funnel for obtaining different phases with $(C_2H_5)_2O$ and 5 M NaOH solution. Then pH was changed by adding small quantities of HCl, assuring that only poliphenolic compounds were precipitating [21–25]. Finally, the precipitate obtained was dissolved with deionized water and dried at 60 °C for 24 h to obtain four *EP* organic dyes; EPD-M, EPD-E, EPD-ME and EPD-W corresponding to the four solvents already mention. Dyes samples were stored in amber glass vials, sealed, labeled and kept under controlled temperature for further use.

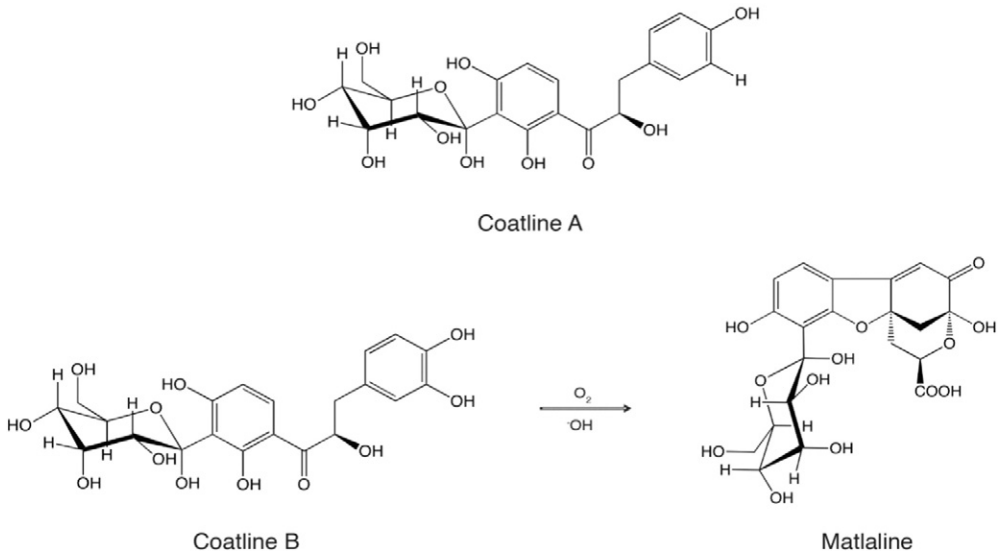

Fig. 1. Chemical structure of coatline A and the heterocycle matlaline from the coatline B.