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Synthesis of protocatechuic acid–zinc/aluminium–layered double hydroxide nanocomposite as an anticancer nanodelivery system

Farahnaz Barahuie [a], Mohd Zobir Hussein [a,*], Shafinaz Abd Gani [b,c], Sharida Fakurazi [c,d], Zulkarnain Zainal [a]

[a] Materials Synthesis and Characterisation Laboratory, Institute of Advanced Technology (ITMA), Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[b] Department of Biochemistry, Faculty of Biotechnology & Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[c] Laboratory of Vaccines and Immunotherapeutics, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[d] Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

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ABSTRACT

Protocatechuic acid, an active anticancer agent, has been intercalated into Zn/Al–layered double hydroxide at Zn/Al=2) using two different preparation methods, co-precipitation and ion-exchange, which are labelled as PZAE and PZAC, respectively. The release of protocatechuate from the nanocomposites occurred in a controlled manner and was fitted satisfactorily to pseudo-second order kinetics. The basal spacing of the resulting nanocomposites PZAE and PZAC was 10.2 and 11.0 Å, respectively, indicating successful intercalation of protocatechuate anions into the interlayer galleries of Zn/Al–NO_3–LDH in a monolayer arrangement with angles of 24 and 33° from the *z*-axis in PZAE and PZAC, respectively. The formation of nanocomposites was further confirmed by a Fourier transform infrared study. Thermogravimetric and differential thermogravimetric analyses indicated that the thermal stability of the intercalated protocatechuic acid was significantly enhanced compared to its free protocatechuic acid, and the drug content in the nanocomposites was estimated to be approximately 32.6% in PZAE and 29.2% in PZAC. Both PZAE and PZAC nanocomposites inhibit the growth of human cervical, liver and colorectal cancer cell lines and exhibit no toxic effects towards normal fibroblast 3T3 cell after 72 h of treatment.

1. Introduction

Cancer nanotechnology is a field of science that provides new avenues that are not accessible by conventional technology, especially in the prevention, diagnosis and therapy of cancer diseases. Cancer nanotechnology offers the design of nanoscale materials and devices with unique therapeutic properties that increase the solubility, half-life and bioavailability of attached drugs to deeply infiltrate tumours with a high level of specificity and to administer novel therapies to curb the problems of drug delivery in cancer.

Cancer is the major cause of death in this century; therefore, there is an ongoing need for the development of new anti-cancer drugs, drug combinations and chemotherapy strategies through scientific exploration. Natural products have provided a fertile source of cures for various diseases; more recently, the effectiveness of herbal drugs in cancer therapy has provided a new way to overcome the cancer cell resistance to chemotherapy [1,2].

Protocatechuic acid is one of the most outstanding natural products, having been found to possess significant antioxidant [3] and anticancer features. Protocatechuic acid is beneficial in the treatment of breast, lung, liver, cervix and prostate cancers [4].

Layered double hydroxides (LDHs), a type of inorganic nanolayered material, are promising candidates for anticancer drug delivery. LDHs have fascinated researchers due to their properties, and these inorganic nanolamellar solids have demonstrated their suitability for different applications in the pharmaceutical industry and continue to attract considerable attention for encapsulation and stabilisation of anticancer drugs due to their amazing properties for drug delivery. LDHs are able to host a large numbers of active anticancer drugs, including protocatechuic acid [5], camptothecin [6], etoposide [7] and methotrexate [8,9], due to their controllable ion-exchange mechanism, pH dependence [10], ease of penetration into the cells because of the positively charged outer layer [11,12], the ability to participate in sustained-release and selective release of the guest in a precise site where its action is expected [13–15] and reduced toxicity compared to other drug carriers [16] are other properties of LDHs that make them unique nanocarriers for drug delivery systems.

* Corresponding author. Tel.: +60 3 89468092; fax: +60 3 89467006.
E-mail address: mzobir@upm.edu.my (M.Z. Hussein).

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LDHs can be found in nature or can be synthesised through a direct route in the laboratory [17].

LDHs, or hydrotalcite-like compounds, are a family of natural and synthetic compounds, having a general formulation of $\left[M^{2+}_{1-x}M^{3+}_{x}(OH)_2\right]^{X+}(A^{m-})_{x/m}\cdot nH_2O$, where M^{2+} and M^{3+} are, respectively, divalent and trivalent metal cations that are octahedrally coordinated to hydroxyl ions, A^{m-} represents the anion between the layers, n refers to the number of interlayer water molecules, and $x=M^{3+}/(M^{2+}+M^{3+})$ is the molar ratio, which generally ranges between 0.2 and 0.4 and determines the positive charge density and hence the anion exchange capacity of the layered double hydroxide [18–20].

Previous work has shown that protocatechuic acid incorporated into Mg/Al–LDH exhibited great anticancer activities [5], but no report on the use of Zn/Al–LDH as the carrier could be found in the literature. Therefore, this communication reports the intercalation of protocatechuic acid into the Zn/Al–NO_3–layered double hydroxide through ion-exchange and direct methods. In addition, we focused on the thermal properties, structure and slow-release properties of the synthesised drug–LDH nanocomposites. The synthesised nanocomposites were later subjected to cytotoxicity studies using in vitro 3T3 normal fibroblast cells and HeLa human cervical, HT29 colorectal and HepG2 human liver cancer cells.

2. Materials and methods

2.1. Materials

PA ($C_7H_6O_4$, molecular weight 154.12) was purchased from Acros Organics (Geel, Belgium) at 97% purity and used as received. Phosphate-buffered saline was obtained from Sigma-Aldrich. Chemicals including NaOH, $Zn(NO_3)_2\cdot 6H_2O$ and $Al(NO_3)_3\cdot 9H_2O$ were purchased from Friendemann Schmidt chemical USA and were used without further purification. Deionised water was used in all of the experiments.

2.2. Synthesis

2.2.1. Synthesis of Zn/Al–NO_3 LDH

Zn/Al–NO_3 precursor was synthesised by the direct co-precipitation method using a previously established protocol [21]. Briefly, to prepare Zn/Al-NO_3, a 2 M solution of NaOH was added by dropwise addition to a mixed salt solution (250 mL) containing $Zn(NO_3)_2$ and $Al(NO_3)_3$ (molar ratio of Zn^{2+} to Al^{3+} is 2:1) until a final pH of 7 was reached under vigorous stirring and a nitrogen atmosphere; this was followed by aging at 70 °C for 18 h. Pure LDH was obtained via centrifuge separation and washing and then drying in an oven at 60 °C overnight.

2.2.2. Synthesis of protocatechuic acid–Zn/Al nanocomposite via the ion-exchange method

The protocatechuate–Zn/Al–LDH nanocomposite was prepared using the ion-exchange method.

Next, 50 mL of an aqueous solution of 1.6 g (0.2 M) of PA was added slowly to the suspension of 0.2 g of Zn/Al–NO_3–LDH in 50 mL of deionised water under vigorous stirring at room temperature, and the pH was maintained at 7 by the addition of a 1 M NaOH solution. The slurry was kept under magnetic stirring for 3 h at room temperature, and then aged at 70 °C for 18 h. The precipitate was collected by centrifugation, washed with deionised water and dried in an oven at 60 °C. The resulting product was labelled PZAE.

2.2.3. Synthesis of protocatechuic acid–Zn/Al nanocomposite via the co-precipitation method

PA was intercalated into Zn/Al–NO_3 ($R=2$) via the co-precipitation route. A mixed solution containing $Zn(NO_3)_2$ and $Al(NO_3)_3$ (the molar ratio of Zn^{2+} to Al^{3+} was 2:1) was added to 50 mL of an aqueous suspension containing 3.2 g (0.4 M) of protocatechuic acid under vigorous stirring and a nitrogen atmosphere. The pH of the mixture was raised to 7 by dropwise addition of a 2 M NaOH solution. The reaction mixture was aged at 70 °C for 18 h and then centrifuged, washed and dried in an oven at 60 °C. The resultant material was labelled PZAC.

2.3. Characterisation

Powder X-ray diffraction patterns were recorded in the range of 2–70° with a dwell time of 0.5°/min on a Shimadzu diffractometer, XRD-6000 using a Cu-$K\alpha$ radiation source ($\lambda=1.5418$ Å) driven at 30 kV and 30 mA. Fourier transform infrared (FTIR) spectra of the materials were recorded over the range of 400–4000 cm^{-1} on a Thermo Nicolet Nexus FTIR (Smart Orbit model) with 4 cm^{-1} resolution, using the KBr disc method with 1% of the sample in 200 mg of spectroscopic-grade potassium bromide. The pellet was prepared at 10 t of pressure. For carbon, hydrogen, nitrogen and sulphur (CHNS) analyses, a CHNS-932 LECO instrument (St. Joseph, MI) was used. The chemical compositions of the samples were analysed for magnesium and aluminium ions via inductively coupled plasma atomic emission spectrometry using a Perkin-Elmer spectrophotometer, model Optima 2000 DV (Perkin-Elmer, Boston, MA), under standard conditions. Thermogravimetric and differential thermogravimetric analyses were performed using a Mettler Toledo instrument with a heating rate of 10 °C/min in the range of 20–1000 °C under a nitrogen atmosphere (N_2 flow rate, 50 mL/min). Surface characterisation of the material was performed via the nitrogen gas adsorption–desorption technique at 77 K using a Micromeritics ASAP 2000 instrument, and all samples were degassed at 105 °C for 6 h. A field emission scanning electron microscope (Nova Nanosem 230 model) was used to determine the surface morphology of the samples. Ultraviolet–visible spectra were obtained to determine the optical properties. A controlled release study was performed using a Perkin Elmer ultraviolet–visible spectrophotometer (Lambda 35).

2.4. Controlled release study

The release of PA from PZAE and PZAC nanocomposites into the medium of phosphate-buffered solution at pH 4.8 and 7.4 [22,23] was performed. Approximately 72.4 mg of each nanocomposite was added to 500 mL of the phosphate buffer solutions. The accumulated amount of PA released into the solutions was measured at regular time intervals at a wavelength of 255.8 nm using a Perkin Elmer ultraviolet–visible spectrophotometer. To compare the release rate of PA from the PZAE and PZAC nanocomposites with that from the physical mixture of PA with pristine Zn/Al–layered double hydroxide (prepared for this purpose), 0.50 mg of the physical mixture of PA (0.16 mg) and the pristine layered double hydroxide (0.34 mg) was used. Release of the protocatechuic acid was determined as described above.

2.5. Cell culture

Healthy HeLa, HepG2, and HT29 cancer cells and 3T3 normal fibroblast cells were obtained from ATCC; these cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% foetal bovine albumin (FBS), penicillin, and streptomycin in an atmosphere maintained with 5% of CO_2 at 37 °C. Furthermore, the