Materials Science and Engineering C 57 (2015) 272–278

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Methotrexate intercalated layered double hydroxides with the mediation of surfactants: Mechanism exploration and bioassay study

Chao-Fan Dai, De-Ying Tian, Shu-Ping Li *, Xiao-Dong Li

Jiangsu Key Laboratory of Biofunctional Material, College of Chemistry and Material Science, Nanjing Normal University, Nanjing 210023, China

ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form 11 June 2015
Accepted 22 July 2015
Available online 26 July 2015

Keywords:
Methotrexatum
Layered double hydroxides
Nonionic surfactants
Bioassay experiment

ABSTRACT

Methotrexatum intercalated layered double hydroxides (MTX/LDHs) hybrids were synthesized by the co-precipitation method and three kinds of nonionic surfactants with different hydrocarbon chain lengths were used. The resulting hybrids were then characterized by X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy and transmission electron microscopy (TEM). XRD and FTIR investigations manifest the successful intercalation of MTX anions into the interlayer of LDHs. TEM graphs indicate that the morphology of the hybrids changes with the variation of the chain length of the surfactants, i.e., the particles synthesized using polyethylene glycol (PEG-7) present regular disc morphology with good monodispersity, while samples with the mediation of alkyl polyglycoside (APG-14) are heavily aggregated and samples with the addition of polyvinylpyrrolidone (PVP-10) exhibit irregular branches. Furthermore, the release and bioassay experiments show that monodisperse MTX/LDHs present good controlled-release and are more efficient in the suppression of the tumor cells.

1. Introduction

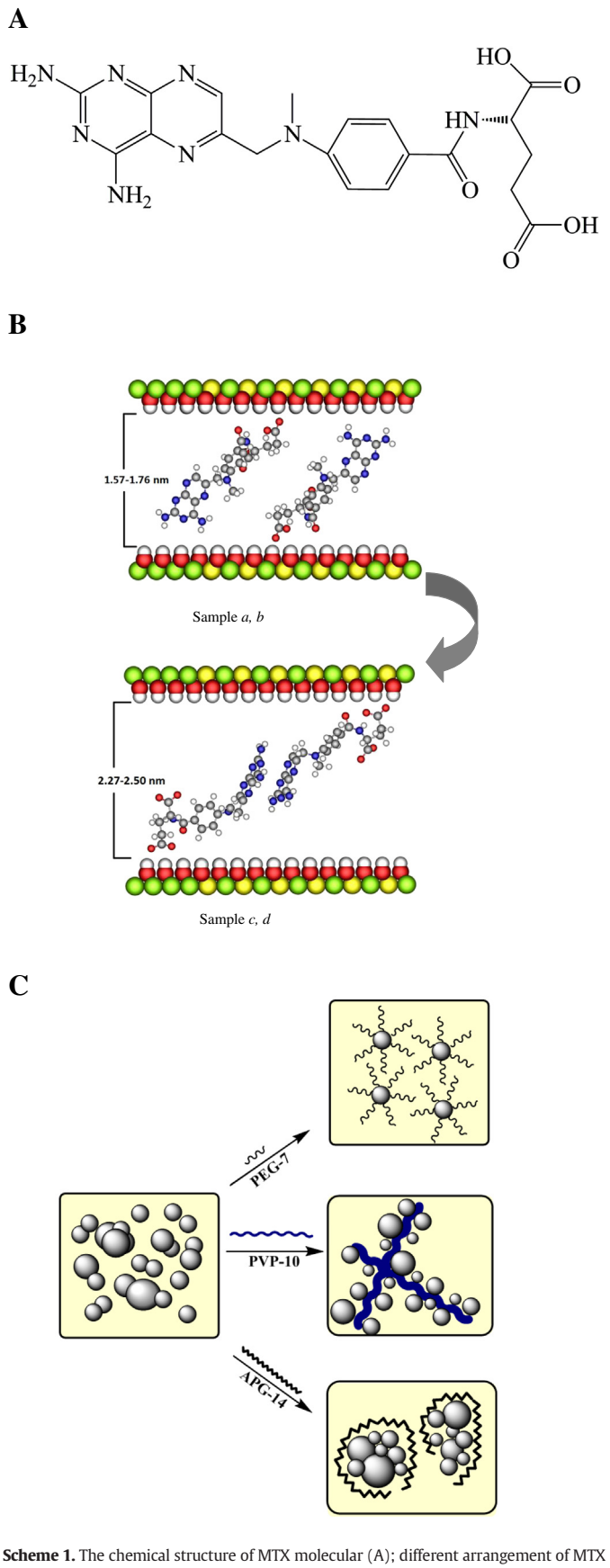
Recently nanocarrier mediated drug delivery system, particularly to treat cancers, has been a highly attractive research area for the last two decades. Especially, inorganic nanoparticles are obtaining more attention nowadays because of their high drug loading, pronounced stability and biocompatibility. Thus, various inorganic nanocarriers including magnetite, calcium phosphate, carbon, gold, silica oxide and layered double hydroxide (LDHs) have been evaluated for delivering cytotoxic drugs [1–6]. Layered double hydroxides (LDHs) are a class of anionic clays which can be represented by the general formula of $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}A^{n-}_{x/n} \cdot mH_2O$, where M^{2+} and M^{3+} are designated as the di- and tri-cations, A^{n-} refers to the interlayer anions often in hydrated form and they are usually associated with the hydroxide layer through electrostatic attraction, hydrogen bond and van der Waals forces [7,8]. Moreover, $x = M^{3+}/(M^{2+} + M^{3+})$ stands for the layer charge density [9]. Various amounts of water (mH_2O) are hydrogen bonded to the hydroxide layers or to the interlayer anions, thus forming the 3-D layered structure. Considered from the structural point, the cationic layered framework leads to safe accommodation of many biologically important molecules including genes and drugs. Furthermore, the liability to acid dissolution offers desirable discharge of loaded drugs as well as compatibility with diverse biosystems [10]. Therefore, LDHs have attracted extensive interest in the field of cellular delivery and anticancer carrier [11–14]. Compared with other inorganic nanocarriers, their outstanding superiority is that the intercalation of drugs into LDHs can not only increase their solubility or improve their therapeutic profile, but also help to decrease its pristine harmful capacity [15–17].

Methotrexate (MTX) is a kind of cytostatic folate antagonist drug usually used in the treatment of cancer and inflammatory diseases (the chemical structure is shown in Scheme 1A). It is known to be eliminated from an organism in a short time and presents side effects together with toxicity when high therapeutic doses are administered [18]. Therefore, considerable attention has been paid to improve the efficacy of MTX recently. The use of matrices, either organic or inorganic, for the sustained release of MTX mainly aims to decrease the taken frequency to keep a steady level of the drug in the bloodstream [19]. This fact is in some cases of paramount importance since it would help to avoid unnecessary doses that can produce side effects, and LDHs have been used for this purpose as it has been reported that the intercalation of drugs into LDHs not only can increase their solubility or improve their therapeutic profile, but also can help to decrease its pristine harmful capacity [20–25].

The morphology of intercalation hybrids has paid attention from both fundamental and practical viewpoints. The morphology of hybrids may affect their physicochemical properties and concerns about their practical applications. Hitherto, disc-like and hexagonal MTX/LDH hybrids had been synthesized and characterized, and the results indicated that disc-like samples present good anticancer effect [26–28]. Surfactants are a good soft template to modify the morphology of the nanoparticles, and then CdS nanotubes, CdSe nanowires and ZnSe nanorods

* Corresponding author at: College of Chemistry and Material Science, Nanjing Normal University, Nanjing, China.
E-mail address: lishuping@njnu.edu.cn (S.-P. Li).

http://dx.doi.org/10.1016/j.msec.2015.07.040
0928-4931/



Scheme 1. The chemical structure of MTX molecular (A); different arrangement of MTX anions in LDHs layers for the MTX/LDHs hybrids (B); the formation mechanism of the nanostructures with the help of three surfactants (C).

etc. had been prepared with their help [29,30]. Considered from the structure viewpoint, surfactants are perhaps good candidates to change the morphology of MTX intercalated LDH hybrids. It's well known that anionic surfactants are easy to intercalate into LDH interlayers and then forbid the carriage of anticancer drug [31]. As a rule, LDH layers are positively charged and the negatively charged anionic surfactants are liable to be attracted on the surface of LDHs, even be intercalated into the interlayer of LDHs. The electrostatic interaction between the anionic surfactants and interlayer is so strong that the anticancer drugs can hardly be carried again. Meanwhile, the high toxicity of cationic surfactants also impedes their application and then nonionic surfactants were chosen here due to their good biocompatibility and feasibility. Such study is of novelty and, to the best of our knowledge, the relevant report has never been reported before.

2. Materials and methods

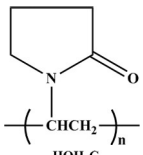
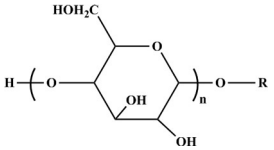
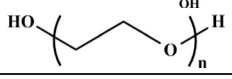
2.1. Materials

Here, three kinds of nonionic surfactants are selected and their structures are described in Table 1. Polyethylene glycol (PEG-7) was purchased from Shanghai Lingfeng Chemical Reagent Co. and the number of 7 represents the hydrocarbon chain length, Polyvinylpyrrolidone (PVP-10) and Alkyl polyglycoside (APG-14) were purchased from Sigma Chemical Co, and MTX was from Zhejiang province Huzhou prospect pharmaceutical Co. Magnesium nitrate ($Mg(NO_3)_2 \cdot 6H_2O$), aluminum nitrate ($Al(NO_3)_3 \cdot 9H_2O$), ammonia solution ($NH_3 \cdot H_2O$), methotrexate (MTX), Triton X-100, hexamethylene, n-butyl alcohol and ethanol (EtOH) were all of analytical purity. All chemicals used were of analytical grade or of the highest purity available.

2.2. Synthesis of pristine LDHs and MTX/LDHs hybrids

Pristine $Mg_2Al–NO_3$–LDHs were prepared by the typical coprecipitation method and used as reference materials [27]. While during the synthesis of MTX/LDHs hybrids, three nonionic surfactants (i.e., PEG-7, PVP-10 and APG-14) were used, and the procedure are described as follows: the mixed salt solution containing 0.032 mol/L Mg^{2+} and 0.016 mol/L Al^{3+} and a certain amount of nonionic surfactant was first mixed and stirred rigorously to form a good suspension. MTX was dissolved into 15 mL of 10% $NH_3 \cdot H_2O$ to get a 0.05 mol/L solution. Then the mixed salt solution was dropped into MTX solution at a constant rate of 3 mL/min, and the final solution was adjusted to pH of 9.5 by adding a certain amount of 10% $NH_3 \cdot H_2O$. Followed by vigorously stirring for 1 h at 60 °C, the final products were washed with deionized

Table 1
The properties of surfactants.

Name	Structure	LD50 (rats through mouth)
Alkyl polyglycoside (APG)		>10,000 mg/kg
Polyvinylpyrrolidone (PVP)		>13,000 mg/kg
polyethylene glycol (PEG)		49,050 mg/kg

LD50 means Lethal Dose 50%, which is an index commonly used in the description of toxicity of the substance.