Synthesis and Physico-chemical Characterization of Nanohybrid Materials Based on Isonicotinic Acid Hydrazide

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The usual treatment for tuberculosis is difficult by its long-term therapy, raised dosing frequency and adverse effects of anti-tuberculosis drugs. Anionic clays, also called layered double hydroxides are a class of hydrotalcite like structures having high compositional variety and being able to self-assembly in the presence of drug molecules. The work refers to the development of nanohybrid materials like delivery systems that intercalates isonicotinic acid hydrazide, known as isoniazid anti-tuberculosis drug, into layered structure of hydrotalcite compounds. Loaded drug molecules of isoniazid were proved to be released in a controlled way from the as synthesized nanostructure in a simulated buffer solution. The obtained sample was characterized by advanced techniques thus demonstrating the inclusion of isoniazid antibiotic between hydrotalcite sheets. These results revealed important expectations for further researches of these organic-inorganic nanocomposites for tuberculosis treatment.

Keywords: nanohybrids, tuberculosis, Koch bacillus, infection, newborn, fetus, mother, infant, epidemiology.

One of the most interesting areas of research with importance in medicine is the delivery of therapeutic and active molecules. Conventional therapy has many disadvantages such as rapid release of drug, lack of control over release rate and oscillations in drug levels in the blood stream or other organs of interest, poor water solubility, enzymatic degradation, the use of high drug doses and many adverse effects.

Nowadays, a priority of medical research is the development of efficient drug delivery nanosystems. Therefore, a fascinating progress in science is the designing of nanomaterials possessing various physical and chemical properties at nanolevel, high reactivity and raised specific surface area. Nanohybrids are composite nanomaterials with different features in which one of phases has at least one dimension at nanosize order.

Layered nanohybrids are formed by intercalation of a guest anion into the interlayer space of the inorganic sheets with no change in the pristine layered structure. These organic-inorganic nanohybrid compounds are very ingenious structures that ensure a wide variety of nanocomposites due to the different combination of the inorganic and organic components.

Layered double hydroxides, also known as hydrotalcites, are inorganic materials used as hosts to create diverse organic-inorganic nano-biomaterials which consist of two-dimensional thin layers offering extensive uses in various fields [1-5].

In last few years, hydrotalcites appeared as biocompatible drug delivery systems with great features such as cost effective materials, easiness of preparation, possibility of loading various active molecules, release of the drugs in a controlled manner and biodegradability [6-16].

Layered double hydroxides (LDHs) are derived from a naturally occurring magnesium hydroxide type brucite being composed of a centering Mg$^{2+}$ ion between octahedral arranged HO$^-$ ions. Each hydroxide ion is bonded to three magnesium atoms.

Their general formula can be expressed $\left[M^{2+}, M^{3+}(OH)_2\right]^{x-} (A^{n-})^{x/n} \cdot mH_2O$, where $M^{2+}$ represent a divalent cation, such as Mg$^{2+}$, Zn$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, Co$^{2+}$, etc., and $M^{3+}$ represent a trivalent cation, such as Al$^{3+}$, Cr$^{3+}$, Fe$^{3+}$, etc., and $A^{n-}$ are exchangeable organic or inorganic anions with charge (n), such as NO$_3^-$, CO$_3^{2-}$, Cl$^-$, SO$_4^{2-}$, H$_2$O$, \text{compensating the positive charge of layers due to the isomorphical divalent/trivalent substitution in the interlayer region, while m represents water molecules that are located in the interlayer region (fig. 1)} [17].

Hence, LDHs used as drug delivery systems can protect drugs from physic-chemical and enzymatic degradation releasing the drug at the target site in a controlled manner. These features result in a decrease in therapeutic concentration, frequency of doses, toxicity as well as side effects [18, 19].

The synthesis of nanodelivery systems for the treatment of tuberculosis refers to intercalation of isonicotinic acid hydrazide (INH), also called isoniazid, into interlayer space of hydrotalcites.

Isoniazid belongs to the drugs group called antibacterial agents being a prescription medication to present and to treat tuberculosis. INH is one of the strongest anti-

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humans population from one continent to another reflecting that this pathogen was able to adapt to migration of died because this illness [20-22]. There are studies revealing global tuberculosis report claims that and about 1.3 million 8.6 million people infected with this disease and the latest human beings. In last few years, there were approximately tuberculosis drugs, multidrug prescriptions, and frequent due to long treatment period, side effects of anti-in the treatment of tuberculosis is patient non-compliance in the intestine, bones and brain. The most common challenge other organs such as the tonsils, liver, spleen, kidneys, extrapulmonary tuberculosis where the microb infects in 1882) when the bacterium infects the lungs, and M.Tuberculosis kinds and social interactions formed by rifampin, etambutol and pirazinamide. tuberculosis drug along with other three drugs namely activating the catalase-peroxidase enzyme being important components of the mycobacterial cell wall. Among other radicals, nitric oxide is produced by activation of catalase-peroxidase enzyme being important in the activity of pretomanid, an experimental antituberculosis prodrug [31-33]. The evolution of drug resistance mycobacteria was relatively slow compared to other bacteria pathogens. Multidrug-resistant tuberculosis implies prolonged treatment with toxic drugs although treatment failure is common [34].

There is why numerous studies had focused on obtaining drug delivery systems in order to enhance the efficacy of anti-tuberculosis therapy. This study reports the synthesis and characterization of a nanodelivery system based on layered double hydroxides for the treatment of tuberculosis by the intercalation of isoniazid into the ZnAlLDHs structure.

Experimental part
Materials and methods
ZnAlLDHs-isoniazid nanohybrid by coprecipitation method
Synthesis of ZnAlLDHs-isoniazid nanohybrid, denoted as ZnAlLDHs-INH1, was prepared using coprecipitation method. A solution containing zinc and aluminium nitrate salts in a ratio of 2:1 in 50 mL water was prepared and vigorously stirred for 20 min under continuous nitrogen flow. Over this solution was added 50 mL of a 1 mol/L isoniazid solution and stoutly stirred for 20 min more. The obtained sample was aged at 65°C for 48 h, centrifuged and completely washed with deionized water. Then the sample was dried in an oven at 65°C for 2 days and ground into a fine white powder being afterwards subjected to characterization.

ZnAlLDHs-isoniazid nanohybrid using ion-exchange route
Sample preparation by ion exchange, termed as ZnAlLDHs-INH2, was performed using salt solutions of Zn[^2+]/Al[^3+] at a molar ratio of 3:1 in 25 mL water, stirred for 20 min and then added dropwise to other 25 mL water maintaining the pH of a 7.5 value by simultaneous addition of 0.5M NaOH solution.

Next step consisted of dropwise adding 100 mL of a 1M isoniazid solution to the freshly prepared ZnAlLDHs under a continuous flow of nitrogen. Then, the sample was stirred for 24 h at 40°C and aged for 48 h at 70°C. The obtained product was centrifuged, washed with deionized water and dried at 65°C for 2 days. The dried sample was ground into a powder for further structural and morphological characterizations.

Results and discussions
XRD characterization
X-Ray Diffractometer was used to record XRD patterns of nanocomposites prepared by coprecipitation and ion exchange and the isoniazid sample molecule drug, respectively, presented in figure 2. Basal spacing of the ZnAlLDHs intercalated with nitrate ions between sheets was 8.8Å. For nanocomposites, XRD peaks show that the basal spacing was 12.01Å and 11.54Å being first reflection (003) for both ZnAlLDHs-INH1 and ZnAlLDHs-INH2, was prepared using coprecipitation method. A solution containing zinc and aluminium nitrate salts in a ratio of 2:1 in 50 mL water was prepared and vigorously stirred for 20 min under continuous nitrogen flow. Over this solution was added 50 mL of a 1 mol/L isoniazid solution and stoutly stirred for 20 min more. The obtained sample was aged at 65°C for 48 h, centrifuged and completely washed with deionized water. Then the sample was dried in an oven at 65°C for 2 days and ground into a fine white powder being afterwards subjected to characterization.

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FTIR spectroscopy

IR spectra of ZnAILDHs-INH1 and ZnALLDHs-INH2 nanocomposites and also of isoniazid are presented in figure 3. FTIR spectrum of pure isoniazid drug reveals several of the particularity functional group bands where the main bands are the carbonyl C=O, amino NH2, N-N single bond, C-H bond of the aromatic ring and C=C double bond.

Layered double hydroxides have characteristic IR bands from 3,000 to 3,400 cm⁻¹ due to the -OH vibration and the interlayer water molecules. Nanohybrid samples contain almost all of the bands of the isoniazid and LDHs with a minor change in the wave numbers of particular peaks due to the interaction of the drug with the inorganic matrix.

This kind of shift can be observed for carbonyl band which is shifted from 1650 cm⁻¹ to 1600 cm⁻¹ in both nanohybrid samples. FTIR peaks contain N-N stretching vibration because of the terminal amine group of the drug. It is remarkable that the presence of the characteristic bands of isoniazid in the IR spectra of nanohybrids supports the XRD pattern, thus demonstrating the successful intercalation of the drug between the interlayer space of the ZnAILDHs.

Analysis of particle size

Particle size of the simple ZnAILDHs and the two nanocomposites (presented in fig. 4) was determined using a dynamic light scattering technique by a zeta sizer. The procedure consisted of sample dispersion in deionized water followed by sonication and finally the analysis using a zeta sizer. ZNAILDHs sample had a wide distribution of sizes being ranged from 50 to 900 nm.

In compliance with the cumulative distribution frequency, almost 85% of particles were equal to or less than the 340 nm. Particle size of the two nanohybrid samples was distributed over a narrow scale of 50-300 nm and almost 70% of the particles had the size equal to or less than 165 nm.

Release characteristics of drug

Release profile of the drug from nanohybrid samples (not shown) was performed in human body simulated physiological phosphate buffer solution of pH 7.4 simulated to blood pH and pH 4.8 simulated to intracellular liposomal pH. The difference in the release features, faster at pH of 4.8 and more sustained at pH of 7.4 was due to the release mechanism since in acidic condition the drug was released by ion exchange and degradation, whereas in alkaline condition the release was accomplished by ion exchange.

Conclusions

Novel nanomaterial was designed by exploring its properties at nanoscale and further used as efficient device in pharmaceutical field. This behavior has no significant effect on their layered structure or activity in medical area as drug delivery nanosystems.

This study focused on applications of hydrotalcites in biomedicine due to their physic-chemical stability, high biocompatibility and low toxicity acting as good candidates for isoniazid delivery.

These nanohybrids are able to increase the therapeutic effect of anti-tuberculosis drug being a huge step towards developing antimicrobial agents with increased activity on pathogens.

The release of isonicotinic acid hydrazide from the nanoformulation was performed in a simulated human phosphate buffer solution.

By shortening therapy period and limiting side effects can be improved the compliance of patients to the tuberculosis treatment.

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