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NANOCOMPOSITE (CLAY BASED) AS A SUITABLE CARRIERS FOR BIOACTIVE MOLECULES: STABILITY AND ANTIMICROBIAL ASPECT

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Abstract

Recent research on nanocomposites as potential pharmaceutical carriers, is focused on utilization of inorganic matrices with layered structure in which bioactive molecules/drugs are incorporated. One of the promising inorganic material with such layered structure is clay, which is quite common ingredient in pharmaceutical products, both as excipient or active substance. Clay minerals are not only "inert ingredients", but they can also be used to decrease or increase dissolution rate, delay and/or target drug release, to prevent possible side effects, taste masking or increase stability. Prosperity of clay-based drug delivery systems depends on the amount of a drug retained by the clay, on its release kinetics and on the total amount released during therapeutic regime. Thus, it is very essential to understand and to improve the physico–chemical aspects of drug–clay complexes. The aim of this research is to provide more information on drug-clay stability formulations.

Pyrophyllite clay, (Parsovići, Konjic, Bosnia and Herzegovina), $Al_2Si_4O_{10}(OH)_2$, is a smectite clay type (talc-pyrophyllite group) and was used here as a potential pharmaceutical carrier. Stability of pyrophillite and bioactive molecule/drug was estimated by use of thermal analysis methods (differential thermal analysis/ thermogravimetric analysis - TGA/DTA) and Fourier-transform infrared spectroscopy (FTIR). Additionally detailed kinetic study was done using contemporary kinetic software in order to evaluate kinetics of clay/ bioactive molecule (drug) complex. Antimicrobial study was performed against *Staphylococcus aureus* (Gram positive bacteria) and *Escherichia coli* (Gram negative bacteria) using batch experimental method. In epruvetes with 9.9 mL of Muller- Hinton broth and 0.1 mL of bacterial inoculum adjusted to 0.5 McFarland standard was added 1 gram of sterilized pyrophillite. Incubation with 120 rpm shaking was performed during 24h at 37 ^{0}C.

Decomposition of neat pyrophillite is one-step process with following kinetic triplet: Ea (activation energy) - 216 kJ/mol; A (pre-exponential factor) - 4.1687 x 10^{10} s^{-1} and N-th order model, while clay/drug mixture decomposition is a four step process. Third stage in mixture decomposition is of special interest, because decomposition of pyrophillite is happening there. Kinetic parameters for the third stage are as follows: Ea - 176 kJ/mol, A – 6.537 x 10^8 s^{-1} and the N-th order model also gave the best fit. After overnight incubation, we noticed highly significant removing of both bacteria from broth. *Staphylococcus aureus* was reduced from 2.3 x 10^9 cfu/mL to 5.5 x 10^6 cfu/mL, while *Escherichia coli* was reduced from 4.5 x 10^{11} cfu/mL to 3.5 x 10^8 cfu/mL.

Based on the kinetic results we can conclude that thermal stability of pyrophillite is slightly lowered comparing to neat pyrophillite data, but on the other hand pyrophillite did not make any impact on thermal stability of other components in mixture. This information is of great interest for stability assessment of clay/drug mixture.

***Key words**: Clay, Alumosilicate (pyrophillite), Drug delivery, Antimicrobial actitivity.*

1. Introduction

Last decade abounds with numerous scientific papers and research on development of new and improved drug delivery systems (DDS). Perfect DDS would be the one who increase stability and efficiency of drugs, decrease doses and side-effects and enlarge biocompatibility and reduce costs, as well. The main issue during oral drug delivery is weak adsorption. There are 40% of new generation drugs and about 75% of compounds in pre-formulation studies that show very low or almost none solubility in water [1]. So low water solubility of drugs contributes to low bioavailability and that is one of the major problems for lypophillic drugs. Finding a proper solution for solubility issue is one of the major task, but also a challenge for pharmacy field. As one of the promising ideas for stability enhancement of drugs, is incorporation of bioactive molecules of active compounds into some inorganic or inorganic/organic matrices [1, 2]. These systems are known as nanocomposites. Composite material, in general, is a material that consists of two or more phases mostly with different properties, and nanocomposite presume that at least one of the phases is less than 100 nm. Novelty research on nanocomposites are based on synthesis of so called hybrid materials which use inorganic matrix with layered structure. Such hybrid materials enhance binding between active compound and matrix due to two mechanism: adsorption and incorporation into layered structure of matrix. It is well known that better adsorption of bioactive molecules contributes to better solubility, stability and activity of drugs [3].

One of the suitable inorganic matrices are clays. Clays are quite common ingredients in pharmaceutical formulation, regardless to whether they are an active components or excipients. Clays with layered structure are called phyllosilicate. Clays are divided based on number and arrangements of tetrahedral (T) and octohedral (O) planes, so we have T-O, T-O-T and T-O-T-O clay structures [3].

There are numerous research papers on clays for drug solubility improvment [4, 5, and 6], enahcement of photo/diseprsion/thermal properties of drugs [7, 8, and 9], as well as, upgrading of muco adhesive properties of drugs [10, 11, and 12]. Furthermore, alternative mineral-based therapeutics such as medicinal clay have gained attention. Clays, although used for medicinal purposes throughout millennia, have remained largely unstudied for their antibacterial activity mechanisms and medical benefits. Iliescu *et al.*, [13], synthesized hybrid montmorillonite (MMT) materials for improvement of cytostatic drugs delivery based on maintaining proper concentration of drug in blood circulation and postponed influence on cancer cells. Ghanshyam *et al.*, [14], studeied intecalation of timol maleate in MMT and proved that process of intercalation is very much dependent on pH values, where the highest concentration of timolol maleate (217 mg/g) is achieved at pH - 5.7 and t = 30 ^{0}C in 1 hour.

Clay of interest in this research is pyrophyllite. Pyrophyllite, along with talc belongs to the simplest forms of 2 : 1 phyllosilicates. Difference between talc and pyrophillite is due to talc being magnesium silicate and pyrophillite is aluminum silicate. Pyrophyllite in particular, is poorly studied, comparing to talc (even they belong to the same clay group), which is widely used as dermatological protector in creams and powders [15]. Talc is quite common in oral, topical, sublingual and rectal pharmaceutical formulations. Talc is *senso lato* pyrophyllite ore deposits are known in USA, Japan, Brasil, India, Swiss, and Sweden and there are numerous papers on potential use of pyrophillite in paper industry, textile, ceramics, dentristy, and ecology [16 - 19]. Properties of each clay differs with geological location and clay chemistry is very much dependent on it [20]. Physico-chemical properties, content, morphology of clays contribute to different commercial use [21].

With this research we wanted to explore and to pay attention to pyrophyllite clay properties and its potential use in pharmaceutical field. Firstly, since talc and pyrophyllite are structure like, the idea is to replace talc component with pyrophyllite in pharmaceutical powder mixture and, to investigate stability of pyrophyllite and possible interaction between pyrophyllite and other components in mixture. Secondly, we are all well aware of the antimicrobial properties of talc, therefor need for testing such properties in pyrophyllite is also essential. So, stability and antimicrobial study of this relatively unknown clay were in focus. Another important fact about study, is that pyrophyllite belongs to mineralogy treasure of Bosnia and Herzegovina (location Parsovici, Konjic) and its potential wide application would be of great economic interest.

2. Materials and Methods

2.1 Materials

Zinc oxide, ZnO ≥ 99% Sigma Aldrich and corn starch, $(C_6H_{10}O_5)n$ Merck were used. Pyrophyllite clay, $Al_2Si_4O_{10}(OH)_2$, pale pink colour with particle size < 5 µm was obtained by AD Harbi d.o.o, Sarajevo. Talc was purchased from local pharmacy store. Pharmaceutical powder formulation was made according to manual recipe for the preparation of galenic products as: Rp/ Zinci oxydi 25 g, amyli tritici 25 g, talci sterilisati 50 g.

2.2 Methods

2.2.1 Thermal analysis

The thermal decomposition of pure pyrophyllite, pure talc and pyrophillite/talc powder formulation was estimated by TA SDT 2060 device for simultaneous thermogravimetry and differential thermal analysis. The working atmosphere was neutral (N_2) provided from high-pressure tanks. The flowing rate was 100 cm^3 min^{-1}. The purity of gases was 99.995 vol. %. Usually, 10 mg of sample was poured into alumina sample pans of 90 µL volume. TG/DTA measurements for kinetic analysis were conducted in non-isothermal using following heating rates: 5, 15 and 30 ^0C min^{-1}. Single heating rate, 15 ^0C/min was used for pure clays. TGA/DTA measurements were carried out in triplicate and found to be repeatable.

2.2.2 Antimicrobial testing

Antimicrobial study was performed against clinical isolates of *Staphylococcus aureus* (Gram positive bacteria) and *Escherichia coli* (Gram negative bacteria) using batch experimental method [22]. Overnight incubated bacterial colonies on blood agar was diluted in 2 mL of 0.9% NaCl to achieve 0.5 McFarland standard. In epruvetes with 9.9 mL of Muller- Hinton broth and 0.1 mL of bacterial inoculum was added 1 gram of sterilized pyrophyllite. Incubation with 120 rpm shaking was performed during 24h at 37 ^0C. As negative control, we used epruvetes with 9.9 mL of Muller- Hinton broth and 0.1 mL of bacterial inoculum without added pyrophyllite. All experiments were performed in triplicate.

3. Results and Discussion

3.1 Thermal stability

The purpose of this investigation is to explore potential of talc replacement with pyrophyllite clay in pharmaceutical powder formulation, since they belong to the same clay group. In order to test behaviour and stability of pyrophyllite in powder mixture, two powder pharmaceutical formulation were made. Both, pharmaceutical powder formulations contained corn starch and zinc oxide, the only difference between two formulation was wheather talc or pyrophyllite was a constituent. Ingredients in pharmaceutical mixture should be inert [1], without mutual interaction in order to maintain physico-chemical characteristics of formulation and therapeutic outcome. So investigation of stability is of great importance. Figure 1 presents thermogravimetric curves of pure talc and pure pyrophyllite recorded at 15 ^0C/min. in N_2 atmosphere. Decomposition process of both clays is given as two-step process, in which first one presents an evaporation stage, in which mass loss is contributed due to loss of absorbed or bounded molecules of water. Pyrophillite contained about 2% of water, while talc had less, about 0.5%. Second mass loss corresponds to decomposition process of neat pyrophyllite (starts about 400 ^0C) and neat talc (starts about 817 ^0C). From this TG data we can conclude that talc has much greater thermal stabily then pyrophyllite itself, since it's initial decomposition temperature is moved for almost 400 ^0C to higher temeprature region. In order to see will thermal stability of talc and pyrophyllite be preserved, TG curves of pharmaceutical mixtures were recorded.

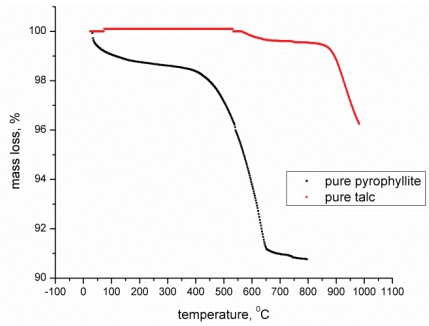
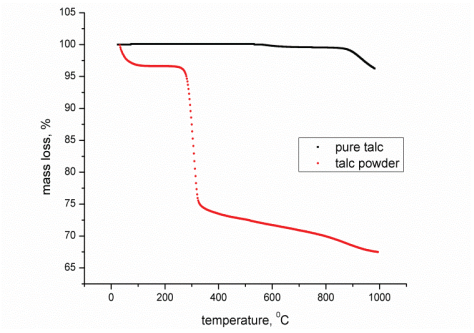
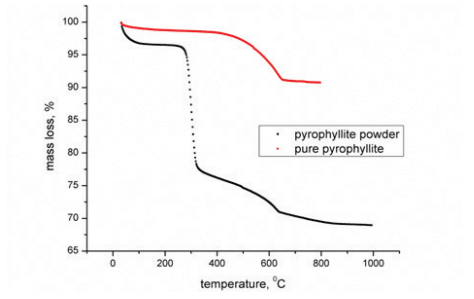


Figure 1. TG curves of pure pyrophyllite (black dots) and pure talc (red dots) in N_2 atmosphere at 15 ^0C/min heating rate

TG curves of thermal decomposition of two pharamaceutical powder formulation are given in Figure 2. For the sake of comparasion pure talc and pure pyrophyllite were inserted in TG graphs.



a



b

Figure 2a, and 2b. TG curves of pyrophyllite powder mixture (black dots) and pure pyrophyllite (red dots); TG curves of talc powder mixture (red dots) and pure talc (black dots) recorded in N_2 atmosphere at 15 ^0C/min heating rate

Thermal decomposition of powder mixtures in Figure 2 proved to be more complex. As one can see, thermal decomposition of pyrophyllite powder formulation, as well as talc formulation is multistep process and consists of three stages: loss of water, degradation of corn starch and zinc oxide and finaly pyrophillite. Evaparation phase ends around 200 ^{0}C, decomposition of corn starch is in 280 - 350 ^{0}C range [23], zinc oxide is between 280 - 420 ^{0}C [24], and finaly pyrophillite decomposition is in temperature interval 380 - 680 ^{0}C. All this stages were confirmed with DTA curves at Figure 3.

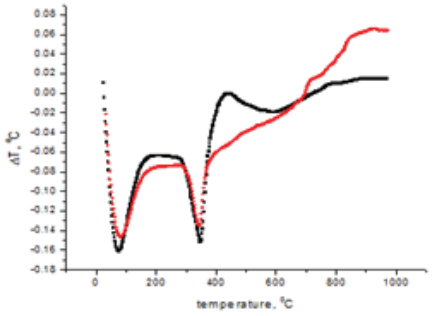


Figure 3. DTA curves of pyrophyllite mixture (red dots) and talc mixture (black dots)

Evaporation process is presented as endothermic with wide diffused peak, while melting of corn starch and zinc oxide have sharp endothermic peaks. Decomposition of talc showed broad endothermic peak, but pyrophyllite did not show any significant heat exchange in mixture.

Additionaly, Figure 4 presents comparatively DTA curves of pyrophyllite mixture and pure pyrophyllite in which endothermic nature of pyrophyllite is more pronaunced.

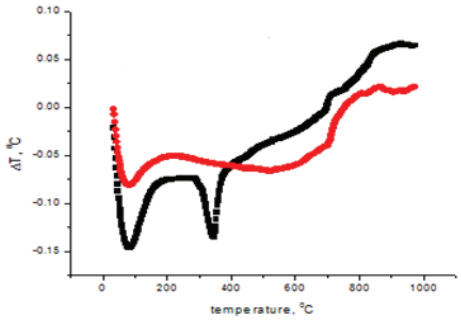


Figure 4. DTA curves of pure pyrophillite (red dots) and pyrophyllite mixture (black dots)

But what is interesting here, is that thermal decomposition of pure pyrophyllite and in powder formulation have not been changed, only unsignicificant slightly decomposition of powder mixture is moved for about 20 ^{0}C to the lower temperature region. So we can conclude that pyrophyllite maintained its stability and inertness in pharmaceutical powder formulation. Unlike pyrophillite, talc showed great thermal instability in powder mixture. This may bring us to conclusion that from the thermal stability point, neat pyrophillite have much better characteristic then pure talc in potential use as pharmaceutical carriers. Is it possbile that talc have ability to interact with other ingredinets in pharmaceutical mixture and that is why its thermal stability is decreased?

Figure 5 presents thermal decomposition of two powder clay mixture and, one can notice, that the curves are practically the same, almost parallel. There is slight deviation in the temperature range from 400 to 600 ^{0}C and if we go back to Figure 1 (pure talc) we will see that thermal stabilty of pure talc differs great deal from talc in mixture and it is quite disturbed. Based on the Figure 5, we could say that thermal decomposition of powder pharmaceutical formualtion is developing in the same temperature interval and shape of curves are quite identical, regrdless of pyrophillite or talc are present in the mixture.

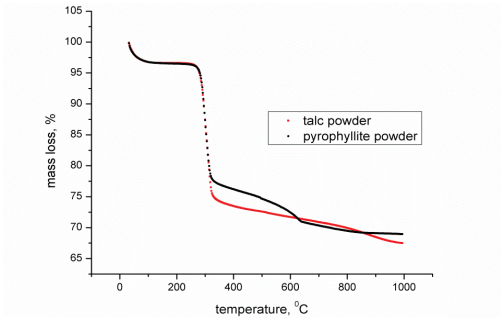


Figure 5. TG curves of talc powder (red dots) and pyrophyllite powder (black dots) in N_2 atmosphere at 15 °C/min heating rate

In order to further confirm stabilty of powder formulation, kinetic analysis was done. Kinetics2015 software which was used for thermal stability assesment, require thermal decomposition of at least three different heating rates (non-isothermal conditions). Figure 5 presents TGA curve of non-isothermal decomposition of pyrophillite powder mixture at the following heating rates: 5, 15 and 30 ^{0}C/min. TGA data served as input data for kinetic software and as an output data we have so called kinetic triplet (activation energy, factor A and mechanism) which are crucial for thermal stability assessment. Details about Kinetic2015 and kinetic models was published elsewhere [25]. Table 1 contains all the relevant kinetic parameters for decomposition process of neat pyrophyllite and pyrophillite in mixture.

Table 1. Kinetic analysis of pure pyrophyllite, pyrophyllite and talc mixture

Kinetic parameters	Materials		
	Pure pyrophyllite	Pyrophyllite powder	Talc powder
Ea, kJ/mol	216	176	111
A, 1/s	4.1687×10^{10}	6.5376×10^{8}	3.025×10^{6}
Model	N-th order	N-th order	N-th order

Since the focus of this research is thermal stability of pyrophyllite, pure and in mixture, we will put emphasis on the third stage of the decomposition process. Kinetic parameters for pure pyrophyllite and pyrophyllite in mixture for the third stage showed slight decreasment of activation energy for 40 kJ/mol. Based on the literature data for pure talc, the value of activation energy are quite high (288 kJ/mol) [26], which is in our case great decreasment of activation energy, for approximately 110 kJ/mol. Lower value of activation energy, in general, refers to instabilty. That would mean that powder mixtures are less thermaly stable then pure clays, and instability may rise from interaction between components joined in mixture. But in order to get a whole picture, pre-exponetnial factor A, which refers to molecules collisions, is of great importance. Evethought pyrophyllite showed decreasment for 40 kJ/mol, we think that stabilty of pyrophyllite is maintained and it is governed by value of pre-exponential factor, A. Thermal stability of talc is quite decreased, but we think that talc mixture stability is maintained due to decreasement of factor A. Additional confirmations on stability of these two powder mixtures could be obtained by infra red spectroskopy or DSC thermal technique which are planned as further activity.

3.2 Antimicrobial activity

After overnight incubation, using agar plate counting, we noticed highly significant removing of both bacteria from broth with pyrophyllite in relation to negative controls without clay. *Staphylococcus aureus* growth was reduced from 2.3 x 10^9 cfu/mL (negative control) to 5.5 x 10^6 cfu/mL (test tubes with clay), while *Escherichia coli* was reduced from 4.5 x 10^{11} cfu/mL (negative control) to 3.5 x 10^8 cfu/mL (test tubes with clay).

Antibacterial activity of clays is still insufficiently analysed and examined. Our experiments tested removal of bacteria from aqueous media, such as Muller Hinton broth. In batch conditions, bacteria could be removed due to their adhesion to the surfaces of pyrophyllite, respectively to aluminium as major component of this clay [22]. Besides this effect, researchers supposed that aluminium is, also, capable to attach to bacterial surface envelope, interfering with influx of nutrients or efflux of waste from bacteria [27]. Our results showed excellent antibacterial activity of pyrophyllite, suggesting further experiments and testing in order to develop it as efficient alternative antibacterial compound.

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4. Conclusions

- Based on simulataneous TG/DTA data we conclude that pyrophyllite kept good thermal stability in powder mixture.

-Thermal stability and inertness of pyrophyllite is a very good base for it's potential usage as pharmaceutical carrier. Based on the pre-exponential factor value we assume that both pyrophyllite and talc do not interacte with other consituents in mixture.

- According to good stability and excellent capacity for bacterial removal, our results sugest further experiments about concrete and practical usage of pyrophyllite in pharmacy field.

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