Research Article

TO PREPARE AND EVALUATE THE NANOPARTICLES OF ANTITUBERCULAR DRUG WITH MONTMORILLONITE NANOCLAY IN CHEMOTHERAPY OF TUBERCULOSIS

Raghvendra[*1], **DR. R. Saraswathi**[2]
[1]Research Scholar, Institute of Pharmaceutical Sciences and Research Center, Bhagwant University, Ajmer (dt), Rajasthan, India.
[2]AL Shifa College of Pharmacy, Perinthalmanna, Malappuram (dt), Kerala, India.
*Corresponding author's E-mail: raghvendra_mpharm@rediffmail.com

ABSTRACT

Isoniazid is a potent antitubercular agent. Montmorillonite, one of the smectite type nanoclay, consists of hydrated aluminium silicate with large space between layers. It has a high cation exchange capacity. As our drug has shorter half life, a controlled release formulation of Isoniazid is a good choice for the better therapeutic action. Objective of our study is to intercalate Isoniazid into the interlayers of montmorillonite. Isoniazid/montmorillonite nanocomposites will be suitable as a controlled drug delivery system of Isoniazid. Isoniazid/ montmorillonite nanocomposites were produced by solution method in which solutions of Isoniazid in various concentrations were used. In this, we try to intercalate Isoniazid into interlayers of montmorillonite to find out optimum condition such as sonication time, pH value, initial drug concentration, clay concentration etc. Thermo gravimetric analysis, Fourier transform infrared spectroscopy, elemental analysis, differential scanning calorimetry, X-ray diffraction analysis are the techniques to characterize the Isoniazid/ montmorillonite nanocomposites. Intercalation efficiency and yield of Isoniazid/ montmorillonite nanocomposites shall be observed and calculated. From the different analytical techniques, we can find that Isoniazid was successfully intercalated into the interlayers of montmorillonite. From the observation we can finalise that the optimum conditions for effective intercalation of Isoniazid into montmorillonite. X-ray diffraction analysis verifies the intercalation by the increase in the interlayer distance of montmorillonite system. The in vitro release study will also prove the release profile of Isoniazid/ montmorillonite nanocomposites.

Keywords: Isoniazid, montmorillonite, antitubercular agent, nanoclay.

INTRODUCTION

Montmorillonite is an actual bendable phyllosilicate accumulation of minerals that about anatomy in diminutive crystals, basic a clay. It is called afterwards Montmorillon in France. Montmorillonite, an affiliate of the smectite family, is 2:1 clay, acceptation that it has 2 tetrahedral bedding sandwiching an axial octahedral sheet. The particles are plate-shaped with a boilerplate bore of about one micrometre. Members of this accumulation cover saponite. It is the capital basic of the volcanic ash weathering product, bentonite[1]. The water agreeable of montmorillonite is capricious and it increases abundantly in aggregate if it absorbs water. Chemically it is hydrated sodium calcium aluminium magnesium silicate hydroxide (Na, Ca) 0.33 (Al, Mg) 2 (Si4O10) (OH) 2 ·nH$_2$O.

Potassium, iron, and added cations are accepted substitutes; the exact arrangement of cations varies with source. Clay minerals possess excellent properties such as low or null toxicity, good biocompatibility, and promise for controlled release, thus give rise to the incessant interest to their development for biological purposes, for example, pharmaceutical, cosmetic, and even medical ones[2]. The properties which make clay minerals useful in pharmaceutical applications are the high adsorption ability, high internal surface area, high cation exchange capacity, interlayer reaction, chemical inertness, and low or null toxicity. Clay minerals are used as excipients in pharmacological applications to improve the organoleptic properties, for example, taste, smell and colour or the physical and chemical ones, and to facilitate and promote the pharmacological formulations[3, 4].

Their colloidal properties make them useful as emulsifying, gelling and thickening agents to avoid the segregation of the components of the pharmaceutical formulations. Contrary to these traditional applications of clay minerals, novel attempts have recently been explored to develop new potentials as drug carrier, protecting matrix, release controlling agents, and chemical modifiers[5,6].

MATERIALS AND METHODS

Preparation of MMT colloid

For effective intercalation of drug into the MMT, a colloidal dispersion of MMT in Millipore water with a suitable pH was essential. So for getting optimized conditions for clay swelling, different parameters analyzed such as

Swelling time: Three samples of 1% clay was prepared in Millipore water and allowed to swell for 12 hrs, 24 hrs & 48 hrs separately.

pH: Four samples of 5.0% clay suspension were prepared in Millipore water and adjusted the pH to 3, 5, 7 and 13.Sonicated samples for 1.5 hrs. Kept for 24 hours and colloidal stability measured with sedimentation ratio.

Clay concentration: Three samples of clay suspension were prepared at different clay concentrations such as 1%, 3% and 5% in Millipore water and adjust the pH to 7. Sonicated samples for 1.5 hrs. Kept for 24 hours and colloidal stability measured with sedimentation ratio.

Sonication time: Three samples of clay suspension were prepared at 5.0%, in Millipore water and adjusted the pH to 7.Sonicated samples for 30, 60 & 120 minutes. Kept for 24 hours and colloidal stability measured with sedimentation ratio.

Temperature: Three samples of clay suspension was prepared at 5.0%, in Millipore water and adjusted the pH to 7.Sonicated samples for 120 minutes by keeping temperatures in 293K,313K & 333K. Kept for 24 hours and colloidal stability measured with sedimentation ratio. The intercalated amount was determined by U.V. Spectrophotometric method.

Intercalation of isoniazid into MMT[7-10]

We have attempted using solution method to facilitate the intercalation of drug with the MMT matrices.

Solution intercalation method

Clay suspensions were prepared at 5.0% concentration in Millipore water and adjust the pH to 7.0. Samples sonicated for 1.5hrs by keeping solution temperature of 313K, Kept for 24 hours. After 24 hours, drug dissolved in Millipore water was added to the colloidal suspension and sonicated for 10 minutes for the intercalation. The drug was added to the clay suspension by different ratios to optimize the best ratio which yields higher intercalation. After sonication the resultant solution was centrifuged for 30 minutes at 4000rpm. The supernatant obtained was used for the analysis by U.V. method to find out the unintercalated drug. The fine powder obtained was dried and kept airtight.

Adsorption isotherm and kinetic studies

Isotherm studies were conducted by keeping the same parameters as said in solution method. Clay suspension of 1ml. containing 50.0 mg was pipetted out and transferred to five separate RBFs and added 9 ml. of water. The varying concentrations (20mg, 40mg, 60mg, 80mg & 100mg) of drug were added to RBFs. Isotherm studies were conducted for different time intervals like 2hr, 4hr, 6hr, 8hr & 10hr.Then the process was completed and as explained in the solution method. U.V. analysis was performed with the supernatant after centrifugation. Adsorption kinetic studies were conducted as explained above, but only keeping for 24hr contact time to get equilibrium concentration and avoided different intervals. The experiments were repeated also at 293K & 333K other than 313K temperatures and the readings were recorded.

Characterisation of intercalated INH/MMT[11-15]

The intercalated samples were analyzed and characterized by FTIR, XRD, TGA, and TEM. The

concentration of INH was investigated by recording the UV spectra in the range of 262 nm by using a SHIMADZU UV-240 spectrophotometer. The samples were measured in quartz cells.

X-ray diffraction patterns were recorded using SIEMENS D-500 diffractometer using CuK$_\alpha$ radiation (λ= 1.5405 Å). Diffraction angle pattern was recorded from 1° to 60°. The Fourier transform infrared (FT-IR) analysis for the samples were done using KBr pellets on a PERKIN-ELMER (SpectrumRX1, FTIR V.2.00) spectrophotometer. The thermogravimetric measurements were carried out on TGA Q50, TA instruments under nitrogen atmosphere. The analysis temperature used in this study was from room temperature to 800^0C at a rate of 10°C/ min. The transmission electron micrographs (TEM) and high resolution transmission electron micrographs (HRTEM) were taken for morphological analysis of the MMT matrices with JEOL 3010 field emission electron microscope with an accelerating voltage of 300 KV. The samples were analyzed by preparing a dilute solution made in distilled water, drop casted on a carbon coated copper grid, followed by drying the sample at ambient conditions before it was attached to the sample holder on the microscope.

Adsorption isotherm studies [16, 17]

Langmuir adsorption isotherm

The Langmuir adsorption isotherm is obtaining by plotting C_e /q_e against q_e. The slope and intercepts will give the value of Langmuir constants as explained in introduction part. Slope will give $1/Q_0$ and intercept will give $1/Q_0.K_L$. C_e /q_e and q_e are tabulated below. The isotherms at 20°C, 40°C and 60°C are plotted using these data.

Freundlich isotherm

Freundlich isotherm is plotting as Log q_e Vs Log C_e. Slope of the isotherm will give $1/n$ and intercept will give log K_F values. K_F is Freundlich constant. Log q_e and Log C_e values are calculated and tabulated for all the three temperatures and used to plot Freundlich isotherm.

<div style="text-align:center">RESULTS</div>

Characterization of intercalated INH/MMT [18-21]

FT-IR Studies

The FT-IR spectra of the MMT, INH drug and INH intercalated samples from solution methods are shown in Fig. In the case of MMT, a broad peak around 3449cm^{-1} observed due to the interlayer and H bonded OH stretching.

The peak at 3627.17cm^{-1} region was attributed to the valence vibration of structural OH group. The intense band at 1052cm^{-1} represents the Si-O-Si stretching and the band at 1641.82cm^{-1} corresponds to the H-O-H bending vibration of molecular water. The bending vibrations of Si-O-Si are appeared at 520 and 467cm^{-1}. The Al-OH vibration modes are present at 916 and 626cm^{-1}. In the case of INH, the peak around 1670 cm^{-1}

represents the ketone group of INH, which is due to C=O stretching and bending vibration at 1413cm^{-1}. A symmetrical stretching vibration of the primary amino group is the reason for the appearance of peak at 1600 cm^{-1} region and the NH bending vibration at 1600-1640 cm^{-1}. The characteristic aromatic ring shows peak at 1559cm^{-1}.

XRD study

The spectrum of INH/MMT system obtained almost resembled the spectrum of the MMT, but showed change in the shape and intensity of the characteristic peaks. The appearance of the peak at 1670, 1556.19, 1416.65cm^{-1} region in INH/MMT systems and the absence of the same vibration in spectrum of the MMT confirm the intercalation. Thus, the presence of characteristic functional groups evidently indicates the effective intercalation of the drug into the interlayer of the MMT. The interlayer space between crude MMT was found to be 0.968nm. This has been increased to 2.412nm in case of INH/MMT. The increase in the interlayer spacing (d) indicates the intercalation of isoniazid into MMT. According to the Bragg Law: $\Lambda = 2d \sin \theta$; the peak shifting from higher diffraction angle to lower diffraction angle is due to d-spacing increase. Compared to pure montmorillonite, the INH/MMT nanocomposite had a more open structure. It is also one of the evidence that INH has been successfully intercalated into the interlayer of montmonrillonite.

Thermo gravimetric analysis

The TGA curve for both the pure isoniazid and INH/MMT nanocomposites were performed using Universal TGA Q5 V20.6 Build 31 instruments. TGA of pure isoniazid shows almost 0.0% residues at 800°C. The initial weight taken for analysis of the pure isoniazid was 16.0170mg. Residue obtained at 800°C was 0.0694mg. The initial weight taken for analysis of the INH/MMT particles was 12.8430m.g.

Residue obtained at 800°C was 8.518m.g. The pure drug should be completely ignited at 800°C as per the TGA of pure isoniazid. So the drug present in 12.8430m.g of INH/MMT particles should be 4.3250m.g which agreed with the result obtained by U.V Spectrophotometric method and confirms the intercalation of the isoniazid.

Drug Release Kinetics of Isoniazid Nanoparticles[21-23]

Study the release kinetics, data obtained from in vitro drug release studies were plotted in various kinetic models:

Zero order (Equation 1) as cumulative amount of drug released vs. time, First order (Equation 2) as log cumulative percentage of drug remaining vs. time, and Higuchi's model (Equation 3) as cumulative percentage of drug released vs. square root of time.

$$C = K_0 t \quad .... (1)$$

Where K_0 is the zero-order rate constant expressed in units of concentration/time and t is the time in hours. A graph of concentration vs. time would yield a straight line with a slope equal to K_0 and intercept the origin of the axes.

$$\text{Log } C = \text{Log } Co - k t / 2:303 \quad .... (2)$$

where C_0 is the initial concentration of drug, k is the first order constant, and t is the time.

$$Q = K t^{1/2} \quad .... (3)$$

where K is the constant reflecting the design variables of the system and t is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time.

The zero-order rate (Equation 1) describes the systems where the drug release rate is independent of its concentration. Figure 5 shows the cumulative amount of drug release vs. time for zero-order kinetics. The first order (Equation 2), which describes the release from systems where the release rate is concentration dependent, it shows the log cumulative percent drug remaining vs. time. Higuchi's model (Equation 3) describes the release of drugs from an insoluble matrix as a square root of a time-dependent process based on Fickian diffusion. Higuchi square root kinetics, showing the cumulative percent drug release vs. the square root of time. The release constant was calculated from the slope of the appropriate plots, and the regression coefficient (r^2) was determined.

DISCUSSION AND CONCLUSION

The INH/MMT intercalation study revealed that the solution method employed to bring about the intercalation remained as a successful method. It was optimized that a good clay colloid was obtained at 5% clay concentration, at pH 7.0 and sonication time 1.5 hours by comparison of sedimentation over a period of 24 hours. The drug loading in the solution method was found to be pH dependent, wherein the rate of drug loading was favored in medium in which drug is more soluble. The intercalation was confirmed by analytical techniques such as FTIR, TGA and XRD. By peak comparison of INH/MMT with isoniazid and montmorillonite, FTIR helped to confirm intercalation. In XRD, d-spacing value of intercalated INH/MMT is 2.13 which are high when comparing to the crude MMT confirmed intercalation of drug into montmorillonite. TGA showed low residue values in case of more intercalated samples. The analytical techniques said that the mechanism in intercalation might be weak hydrogen bonding by the primary amine and the ring nitrogen also helps in adsorption. The intercalation of isoniazid into montmorillonite was optimized as better intercalation obtained in medium of pH 7.0 at clay concentration 5%, and clay to drug ratio 1:2 on sonication of 10 minutes.
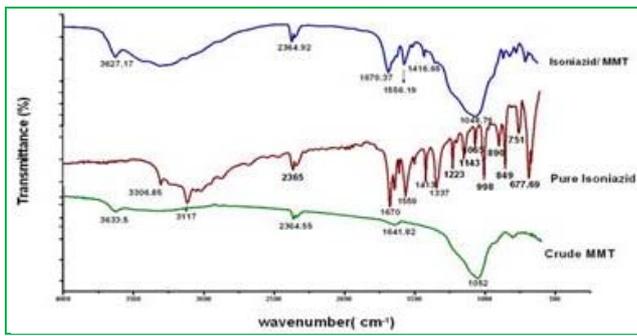
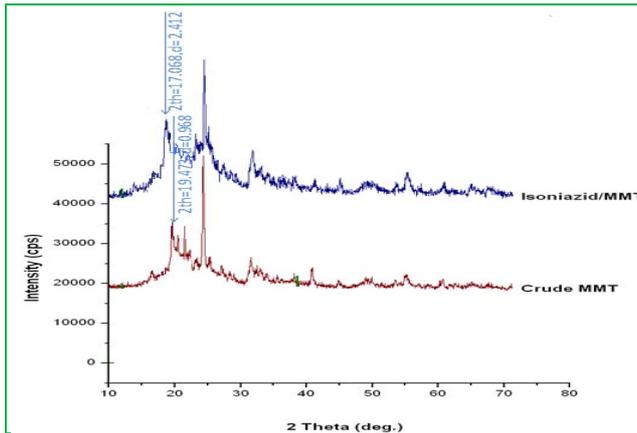**Figure 1:** Comparison of FTIR spectra of INH/MMT with crude samples

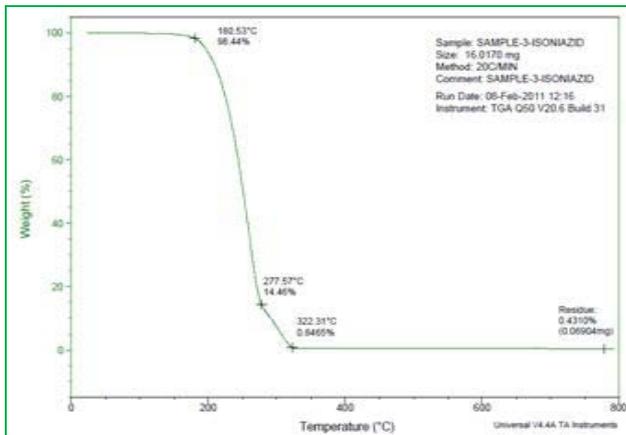**Figure 2:** XRD curves for crude MMT and INH/MMT

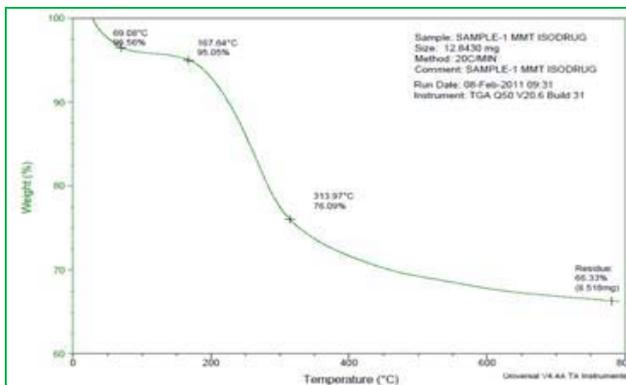

Figure 3: TG curve for pure INH

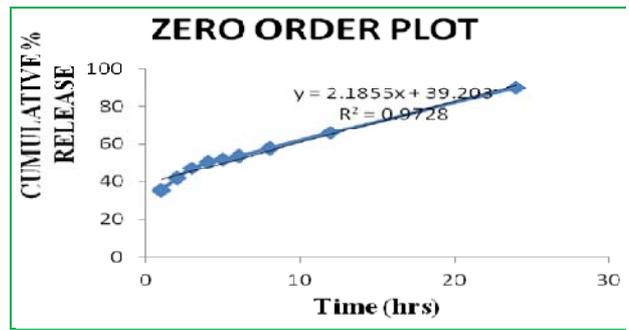**Figure 4:** TG curve for INH/MMT Nano Composites

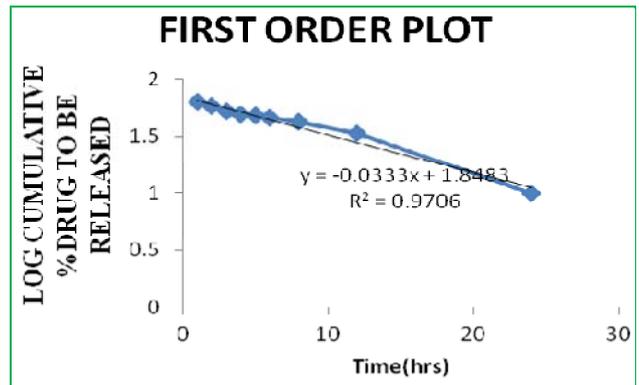**Figure 5:** Zero order plot of INH/MMT particles

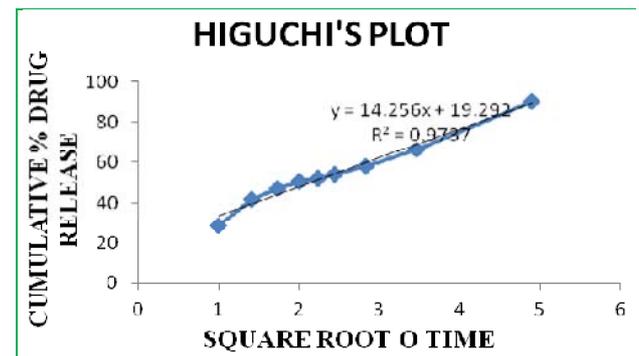**Figure 6:** First order plot of INH/MMT particles

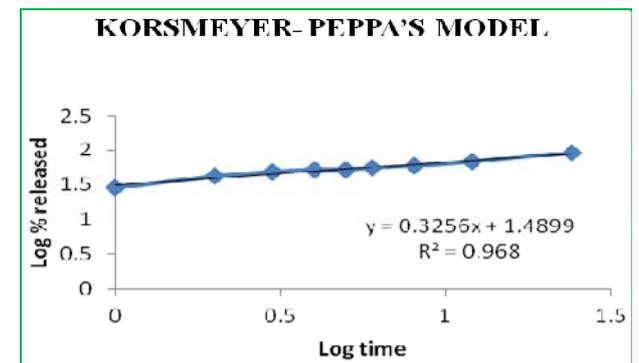**Figure 7:** Higuchi's plot of INH/MMT particles

Figure 8: Korsmeyer Peppa's Model of INH/MMT particles

The adsorption isotherm studies and adsorption kinetics were conducted. The pattern of drug loading onto clay seemed to be a kind of chemical adsorption as it follows Freundlich isotherm and second order adsorption kinetics. The drug dissolution study showed that the rates of drug release from the INH/MMT systems followed zero order release pattern. The INH/MMT particles were

formulated into capsule by hand filling method. The filled capsules were enteric coated with cellulose acetate phthalate to restrict the release of INH/MMT particles only in the intestine. The release profile of INH/MMT intercalated particles and the formulated capsules containing INH/MMT intercalated particles followed similar release characteristics. So it was clear that the prepared INH/MMT did not show any obstruction to formulate it as an oral drug delivery system.

REFERENCES

1. Lisa Brannon- Peppas, Polymers in controlled drug delivery, Biomaterials, 1, 1997, 678-685.

2. Ritschel.W.A, Biopharmaceutic and pharmacokinetic aspects in the design of controlled release per oral drug delivery systems, Drug development and industrial pharmacy, 15 (6), 1989, 1073-1103

3. Batycky R P, Hanes J , Langer R , Edwards D A, A Theoretical model of erosion and macro molecular drug release from biodegrading microspheres, J Pharm Sci., 86 (12), 1997, 1464-1477.

4. Kinam P, Perspective nano technology: What it can do for drug delivery, J Control Release, 120 (1-2), 2007, 1-3.

5. Jin-Ho Choy, Soo -Jin Choy, Jae-Min Oh , Clay minerals and layered double hydroxides for novel biological applications, Applied clay science, 36, 2007,122-132.

6. Gamiz E, Linares J, Delgado R, Assessment of two Spanish bentonites for pharmaceutical uses, Applied clay science, 6, 1992, 359-368.

7. Lin F H, Lee Y H, Jian C H, Wong J M, A study of purified montmorillonite intercalated with 5-flourouracil as drug carrier, Biomaterials, 23, 2002, 1981-87.

8. Carretero M I, Clay minerals and their beneficial effects upon human health; A review, Appl Clay Sci., 21, 2002, 155-63.

9. Ito T, Sugafuji T, Maruyama M, Takahashi T, Skin penetration by indomethacin is enhanced by use of indomethacin/smectite complex, J.Supramol.Chem, 1, 2001, 217-219.

10. Delhugo C, Vincente M A, Rives V, Preparation of drug montmorillonite UV radiation compounds by gas solid adsorption, Clay. Miner, 36, 2001, 541-46.

11. Ray S S, Okamoto M, Polymer/layered silicates nano composites: A Review from preparation to processing, Prog.Polym.Sci, 28, 2003, 1539-1641.

12. Margarita D, Montserrat C, Edwardo R, Chosen clay nano composite films: structure characteristic and drug delivery behavior, Carbohyd.Polym, 69, 2007, 41-49.

13. Oguz O, Wilhelm O, Polyacryllamide clay nano composite hydrogels: Rheological and light scattering characterization, macro molecule, 40, 2007, 3378-3387.

14. Browne J L, Feldkamp J R, WhiteJ L , Hem S L, Acid-base equilibria of tetracycline in sodium montmorillonite suspensions, J. Pharm. Sci., 69, 1980, 811-815.

15. Bujdak J, Slosiarikova H, The reaction of montmorillonite with octa decylamine in solid and melted state, Appl. Clay. Sci., 7, 1992, 263-269.

16. Forteza M, Galan E, Cornejo J, Interaction of dexamethasone and montmorillonite. Adsorption-degradation process, Appl. Clay. Sci., 4, 1989, 437-448.

17. Gurhan G, Yoldas S, Murat K, Kadir Y, Equilibrium and kinetics for the sorption of promethazine hydrochloride on to K10 montmorillonite, J. Coll. Int.Sci., 299, 2006, 155-162.

18. Handa T, Kuroda, K, Kato C, Formation of organoammonium-mont -morillonites by solid-solid reactions, Chem. Lett, 2, 1990, 71-74.

19. Hermos M C, Perez-Rodriguez, Interaction of chlordimeform with clay minerals, Clays Miner., 24, 1981, 147–152.

20. John, Lyons G, Mark H, Preparation of monolithic matrices for oral drug delivery using a supercritical fluid assisted hot melt extrusion process, Int. J. Pharm., 319, 2007, 62-71.

21. Lin F H, Lee Y H, Jian C H, Jay-Min Wong, Ming-Jium Shieh, Cheng-Yi Wang, A study of purified montmorillonite intercalated with 5-fluorouracil as drug carrier, J. Bio. Mat., 23, 2002, 1981-1987.

22. Linda S, Porubcan, Gordon S, Born, Joe L, White, Stanley L, Hem Interaction of digoxin and montmorillonite: Mechanism of adsorption and degradation, 3, 2006, 358-361.

23. Ogawa M, Kuroda K, Kato C, Preparation of montmorillonite-organic intercalation compounds by solid-solid reactions, Chem. Lett., 1989, 1659-1662.

****