Iron Oxide Magnetite-Polyethylene Glycol-Layered Double Hydroxide Core Shell Nanoparticles for Drug Delivery

Mona Ebadi¹, Saifullah Bullo¹,², Mohd Zobir Hussein¹∗

¹Materials Synthesis and Characterization Laboratory, Institute of Advanced Technology (ITMA), University Putra Malaysia, Selangor, Malaysia.
²Laboratory for vaccine and immunotherapeutic, institute of Biosciences, University Putra Malaysia, Selangor, Malaysia

E-mail: mzobir@upm.edu.my

Abstract. Recently, micro- and nanoparticles with magnetic core and functional shell structures have received much attention because of their potential applications in drug delivery, biosensors catalysis, selective separation and chromatography etc. Layered double hydroxides (LDHs) nanoparticles (NPs) are an efficient cellular delivery vehicle for many negatively charged chemical species. Recently, Mg and Zn/Al-layered double hydroxide (Mg/Al-LDH) and (Zn/Al-LDH) has emerged as a promising nanocarrier for drug delivery system due to its unique properties such as low cytotoxicity, pH-controlled release, good biocompatibility, tunable particle size, and protection of drugs in the interlayer. Various anionic biofunctional molecules, including DNA, SiRNA, drugs and vitamins have been successfully intercalated into LDH interlayers, protected from enzyme degradation and delivered into the cytoplasm in mammalian cells through the clathrin-mediated endocytosis pathway. In this study, a magnetite core shell nanodrug delivery formulation was designed based on Fe₃O₄-PEG-Zn/Al-LHDs or Mg/Al-LDHs loaded with anticancer drug 5-Fluoro-2,4(1H,3H)-pyrimidinedione commonly called 5-fluorouracil. Designed core shell magnetite nanoparticles formulation was characterized using XRD, zeta sizer and high performance liquid chromatography (HPLC).

1. Introduction
Nanomedicine is an advanced field of medical science where nanomaterials are designed and applied for various biomedical applications. Lately, nanomedicine is widely getting attention for theranostics applications i.e. simultaneous disease diagnosis and treatment. In addition, the use of magnetic nanomaterials is highly sought-after for nanomedicine applications particularly for targeted delivery, hyperthermia and biosensors. As important biomedical nanomaterials, magnetic nanomaterials need to have (1) superparamagnetic property which prevents residual magnetism induced aggregation;(2) sufficient magnetic responsiveness, which ensures the feasibility for external magnetic field induced separation and delivery;(3) monodispersing in size, which is critical for the electrical and magnetic properties of any nanoparticles due to their fundamental size dependent properties; (4) easily
functionalizable surfaces, which are important for further functionalization, such as the linkage of recognition molecules. Core–shell structural superparamagnetic iron oxide hybrid nanoparticles are exceptionally promising materials. Polymer coatings could stabilize magnetic nanoparticles leading to better dispersion and biocompatibility and could also be further modified with functional molecules of various descriptions for diverse applications. In this study we describe the development of theranostic core shell nanoparticles based on superparamagnetic of Fe₃O₄ core coated with PEG fabricated with Mg/Al-LDHs and Zn/Al-LDHs. The designed core shell nanocarriers were loaded with anti-cancer drug 5FU.

2. Experimental study
2.1. Materials and Procedures

Distilled deionized water (18.2 MΩcm⁻¹) was used in all experiments. Iron (II) chloride tetra hydrate (FeCl₂·4H₂O ≥ 99%), iron (III) chloride hexahydrate (FeCl₃·6H₂O, 99%) were purchased from Merck, Germany. polyethylene glycol, average M.W. 6000 was purchased as a raw material from Acros Organics. Ammonia solution (25%) was obtained from Scharlau. Aluminum nitrate (Al(NO₃)₃·9H₂O) with 98.5% purity, Zinc nitrate hexahydrate Zn(NO₃)₂·6H₂O with 98% purity and magnesium nitrate (Mg(NO₃)₂·6H₂O) with 99% purity were supplied by ChemAR. The drug used in this study is 5-fluorouracil (C₄H₃FN₂O₂ ≥ 98%) was obtained from AKSci. Dimethyl sulfoxide ((CH₃)₂SO ≥ 99%) was purchased from Sigma Aldrich, Merck.

2.2 Experimental

In order to prepare magnetite iron oxide coated with polyethylene glycol (FPEG), the mixture of 2.43 gr ferrous chloride tetra hydrate (FeCl₂·4H₂O), 0.99 gr ferric chloride hexahydrate (FeCl₃·6H₂O) and 80 mL deionized water in the presence of 6 mL ammonia hydroxide (25 % by mass) was exposed to ultrasonic irradiation for 1 h. The precipitates were centrifuged and washed 3 times and then the washed precipitates were dispersed in 100 mL deionized water and mixed with 2 % PEG. Then the nanoparticles were placed in an autoclave at 200 °C for 24 h. The black precipitates were then collected by a permanent magnet and washed three times to remove the excess PEG which does not participate in the coating process. The washed precipitates were re-dispersed in 50 mL ethanol and added dropwise into a layered double hydroxides solution (Mg/Al) by stirring the mixture until the pH decreased to 9. Then the core-shell nanocomposite was centrifuged at 5000 rpm. The 3% of drug, 5-FU [4, 21] which was dissolved in dimethyl sulfoxide ((CH₃)₂SO ≥ 99%) was added into the nanocomposite (iron oxide-PEG-LDH (Mg/Al) and the mixture was stirred for 24h. Finally, the coated iron oxide was washed and dried in an oven. The same procedure was done to prepare iron oxide coated with LDH (Zn/Al). The structure and the composition of the iron oxide with poly ethylene glycol coating and LDH(Mg/Al) and LDH(Zn/Al) nanocarrier were characterized on an X-ray diffraction (XRD) in the 2θ range of 2°–80°, High performance liquid chromatography (HPLC) and particle size analysis (PSA).

3. RESULTS AND DISCUSSION
3.1. X-Ray Diffraction of the Nanoparticles

The XRD patterns of the Zn/Al-LDH and the Mg/Al-LDH nanocarrier are shown in Figure 1. The results display for pure Zn/Al-LDH layered structure characteristic with strong peaks. Corresponding to the diffraction planes of (003) and (006) at the 2θ positions of 10.9° and 22.1°, respectively. The XRD peaks observed for pure Mg/Al-LDH with high intensity in three main characteristic diffraction planes (003), (006) and (009) at the 2θ positions of11.5° and 23.2° and 34.8°, respectively.
The XRD patterns of the Mg/Al-LDH and the Zn/Al-LDH nanocarrier coating formed on iron oxide-PEG-drug nanocomposites are shown in Figure 1(a) and (b), respectively. All iron oxide nanoparticles have six characteristic peaks at 2θ = 30.16°, 35.95°, 43.34°, 54.17°, 57.27° and 62.98°. From the XRD patterns, it has also been found that these six diffraction peaks correspond to the pure magnetite nanoparticles with a cubic inverse spinal structure (Reference JCPDS Number 82-1533). Owing to observing these characteristic peaks in two nanocomposites (Figures 1(a) and (b)) it is evident that the coating process did not result in a phase change of the iron oxide nanoparticles. In addition, the XRD also indicates that PEG was well loaded onto the iron oxide nanoparticles. The peaks belong to LDH and drug are also observed on both samples, which means that the combination of nanocomposite and drug are well integrated into the LDH.

It is noted that obvious Zn/Al and Mg/Al nanocarrier peaks appeared on the coated samples. The XRD phases of iron oxide-PEG-drug composite coating remained the same as those of the Zn/Al-LDH and Mg/Al-LDH coating, but the crystallization intensity of iron oxide, drug and PEG the phases weakened. Obviously, the thick LDH coating lessens the penetration of the X-ray effectively.

![Figure 1. XRD patterns of pure LDH(Mg/Al), LDH(Zn/Al), Poly ethylene glycol, 5-FU, core shell nanoparticles with LDH-Mg /Al(a), core shell nanoparticles with LDH-Zn/Al(b) and Iron oxide nanoparticles.](image)

### 3.2. Particle Distribution Measured by PSA with different nanocarrier

Particle size of nanocomposites with Mg/Al-LDHs and Zn/Al-LDHs as a nanocarrier (a and b) were determined with a dynamic light scattering technique by a zeta sizer. The samples were dispersed in methanol at once exposed to ultrasonic irradiation for 60 min at 30% power in 40 temperature and then they were analyzed with a particle size analysis (PSA) method. According to the cumulative distribution frequency, ≈62% of particles in sample which coating with Mg/Al-LDHs as a nanocarrier were less than the 120 nm (Figure a) and the average hydro-particle size is 120 nm following the size distribution in the range of 90 to 150 nm. in addition, The particle size of core shell nanoparticles sample that coating with Zn/Al-LDH as a nanocarrier was distributed over a narrow range of 240–260 nm and ≈63% of the particles were found to have the size less than 260 nm as shown in Figure 2.
Figure 2. Hydrodynamic size of nanocomposites (a) with Mg/Al-LDHs and nanocomposites (b) with Zn/Al-LDHs.

3.3. high-performance liquid chromatography (HPLC) analysis

Figure 3 shows the calibration curve standard for the nanocomposites. The correlation coefficient, R², for the standard solutions was 0.923. The percentage loading of drug in the nanocomposites determined by HPLC is given in figure 4. According to the results, the percentage of drug loading for iron oxide–PEG-Mg/Al-LDHs is higher than iron oxide–PEG-Zn/Al-LDHs, with 68.41% and 50.80% loading, respectively. Importantly, it was also observed that drug loading percentage in sample (a) that using Mg/Al-LDHs as the nanocarrier agent is more than when the using Zn/Al-LDHs for nanocarrier (sample b).

Figure 3. Calibration curve of 5-flourouracil drug determined using HPLC with standard concentrations of 0 mg/mL, 50 mg/mL, 150 mg/mL, and 200 mg/mL.
4. Conclusion

X-ray diffraction results showed that both nanoparticles with cubic inverse spinal structures were successfully prepared using the co-precipitation method. High performance liquid chromatography indicated that when using of Mg/Al-LDHs as nanocarrier resulted in markedly enhanced percentage drug loading with smaller particle size and narrow size distribution. Further studies will be carried out such as sustained release, cytotoxicity and anticancer assays.

Acknowledgements

The authors would like to thank University Putra Malaysia and the Ministry of Higher Education of Malaysia (UPM-MOHE) for funding this project under NANOMITE programme Vot No 5489100.

References

[1] Dorniani, D. Kura, A. U. Hussein, M. Z. B. Fakurazi S. Shaari, A. H. 2014 *Journal of Materials Science*, 49 8487
[2] Hong, K. Khwaja, A. Liapi, E. Torbenson, M. S. Georgiades, C. S. 2006 *Clin Cancer Res*, 12 2563
[3] Mirza, A. Z. & Siddiqui, F. A. 2014 *International Nano Letters* 4 1
[4] Sophie Laurent, Delphine Forge, Marc Port, Alain Roch, Caroline Robic, Luce Vander Elst and Robert N. 2008 *Chem. Rev.* 108 2064