Exploiting Fatty Acid-Polymer-Based Lauric Acid and Chitosan as Coating Material for Drug Encapsulation

[1]Sabiqah Tuan Anuar, [1]Patricia Nellane Ithurayasamy and [2]Laili Che Rose

[1]School of Marine and Environmental Sciences,
Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia
[3]School of Fundamental Sciences, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia

Abstract: A hybrid of saturated fatty acid and natural polymer was exploited as a coating material to encapsulated drug. Thus in this research, both lauric acid (C12 fatty acid) and chitosan were used as coating formulation for drug release at two different pH (1.2 and 6.8). The combination of fatty acid and chitosan, a linear polysaccharide, provides a better control of the drug release by selectively releasing the encapsulated drug in the selected pH. Results from FTIR and SEM showed that lauric acid-chitosan capsule coating is proven to have a film forming capability and stability with a controlled drug release at the mimicking human colon area pH. Moreover, initial study using in-vitro dissolution method indicated their capability to form a membrane and protect the drug from degradation.

Key words: C12 fatty acid · Chitosan · Drug encapsulated · Cross-linking · *in-vitro* method · FTIR

INTRODUCTION

Recently, there were lots of researches done on many fatty acids and polymers as a drug-encapsulated ingredient due to its variety of application. However, using them alone has provided several disadvantages, in terms of stability, solubility and biodegradability. For instance, a number of pharmaceutics have chosen liposomes in drug encapsulation due to their composition of amphiphilic phospholipids and cholesterol [1]. Their small structures of 80-100 nm in size have enabled them to encapsulate hydrophilic drugs in the aqueous interior or hydrophobic drugs within the bilayer [1]. However, there some drawbacks in using liposomes for targeted drug delivery, which is leakage of drug in the blood, their capability of coupling with low encapsulation efficiency and unstable during their storage [2].

On the other hand, polymer are more favorable as they do not easily cross through some biological barriers, offer stability of various forms of active drugs and have achieved an effective controlled drug release property [2, 3, 4, 5]. Nevertheless, biodegradable polymers have difficulty in sustaining the drug release time owing to its slow degradation time. Using both polymer and fatty acids based coating can solve this problem, which can provide a better-controlled release of encapsulated drug. Lauric acid, C12 fatty acid is a medium chain saturated fatty acid that is a relatively stable and degrades naturally as well as environment friendly. Meanwhile, chitosan, a polysaccharide comprising copolymers of glucosamine and N-acetyl glucosamineare, is a good biocompatible and biodegradable natural polymer with excellent film-forming ability and is considering as non-toxic material [6]. Due to the primary hydroxyl and amine groups located at the backbone, any chemical modification is allowed to take place in order to control its properties [4, 6].

Thereby, this article describes the initial findings of a combination of C12 fatty acid and chitosan that will provides a better control and stability of the drug release.

Experimental Procedures:
Formulation of Fatty-Acid And Polymer Based Coating Material: The microspheres of fatty acid and polymer blends was prepared by water-in-oil (w/o) cross-linking method. In general, 2 % (w/v) of lauric acid (C12 fatty acid) and polymer were dissolved in acetic acid and were

continuously stirred until a homogeneous solution is achieved. Then, 5% of drug was dissolved in the blend solution and was emulsified with liquid paraffin oil (100 g, w/w), containing 1 % (w/w) Span-85. The solution was stirred at 600 rpm speed using a stirrer for 15 minutes. 5 ml of glutaraldehyde with 0.5 ml of 0.1 N HCl was added and stirred for 3 hours. The final product was then filtered, washed with petroleum ether and distilled water. Finally, the final product obtained was dried at 40°C for 24 hours.

Characterization of Synthesized Coating Material by FTIR: The microspheres was crushed and mixed with potassium bromide (KBr) in the ratio 1:7 into an agate mortar. The samples was mixed and ground until homogenous and fine powder is obtained before it was made into a pallet. The identification was perbormed by FTIR spectroscopy (Perkin Elmer; Spectrum 100) in the mid-IR range of $4000 - 400$ cm^{-1} and 16 scans per analysis.

Characterization of Synthesized Coating Material by SEM: The SEM instrument was used to study the morphology of the formulated coating material. These coating material was taken on copper stub, sputtered with 10 nm thickness of gold coating for 2 minutes and mounted on SEM Microscope (JEOL 6301F; ?86 JEOL Ltd., Akishima-Tokyo, Japan).

In-vitro Drug Release: The *in-vitro* release method was conducted based on [7][8] with some modifications. Briefly, the drug release from the coating material were studied in two different medium which are the mimic of stomach fluid (pH 1.2) for 2 hours and mimic of colonic fluid (pH 6.8) for 2 hours in 37°C of water bath filled in a beaker and was stirred at 100 rpm speed. Aliquot sample was withdrawn from the medium at intervals of time to study the composition of drug release using UV–Vis spectrophotometer (Shimadzu, Kyoto Japan) at a fixed λ_{max} of 243 nm.

Validation Method: The method applied in this research was validated for different parameters such as linearity, accuracy, specificity and precision, limit of detection (LOD) as well as limit of quantitation (LOQ). In addition, the characteristics of regressions such as slope, intercept, correlation coefficient (r), standard deviation, limit of detection (LOD) and limit of quantitation (LOQ) were determined for both pH 1.2 (mimic of stomach fluid) and pH 6.8 (mimic of colonic fluid).

RESULT AND DISCUSSION

Synthesis of Lauric Acid-Chitosan Coating Material: In this article, we describe the preparation for the formulation of lauric acid and chitosan as coating material. The synthesized coating matrial was tested for drug encapsulating and release in two different pH of 1.2 and 6.8. Initially, a C12 fatty acid (lauric acid) and natural polymer, chitosan based coating material was formulated and a drug was encapsulated within the material through the water-in-oil (W/O) cross-linking method by means of glutaraldehyde.

The use of lauric acid offers high solubility to the drug as well minimizes the volume of the formulation to transport the drug in an encapsulated form [9]. Glutaraldehyde provides the covalent attachment in the cross-linking method that should prevent the coating material from leaking, as describe elsewhere [10]. Figure 1 shows the formulated coating material. The resulted coating material demonstrated to have a good film forming capabilities, owing that advantages of using chitosan in the formulation [11]. It should be noted that the combination of chitosan and lauric acid in the formulation does not alter the film-forming capabilities of the material.

Characterization of The Formulated Coating Material: FTIR spectroscopy was used to observe the presence of the functional groups involved in the synthesis method. Figure 2 shows the IR spectra recorded for pieces of coating material at various stages throughout the synthesis processes. The pure lauric acid shows strong absorption for C=O stretching vibrations at around 1696 cm^{-1} together with C-H stretching at 2848 cm^{-1}, similar to the previously reported study by El-Leithy *et al.* [12].

FTIR spectra for chitosan shows the presence of hydroxyl and C=N imines stretchings at 3445 and 1652 cm^{-1}respectively. The C-O bond recorded at 1151 cm^{-1} while 869 cm^{-1} showed the C-H wagging for chitosan structure [13, 14, 15 16]. The introduction of glutaraldehyde crosslinker in the water-in-oil cross-linking method results in the shift in the C=N vibration [17, 18] to 1653 cm^{-1} of amide I band. Additionally, the presence of additional functional groups in each stage proposes the chemical interaction in between the coating material and the drug [19]. The presence of O-H, C=O, N-H and C=C were all identified around 3447 cm^{-1}, 1701 cm^{-1}, 1637 cm^{-1} and 1562 cm^{-1}, respectively. In summary, these characteristic absorptions indicate the formation of the bonds between the new developed coating material and

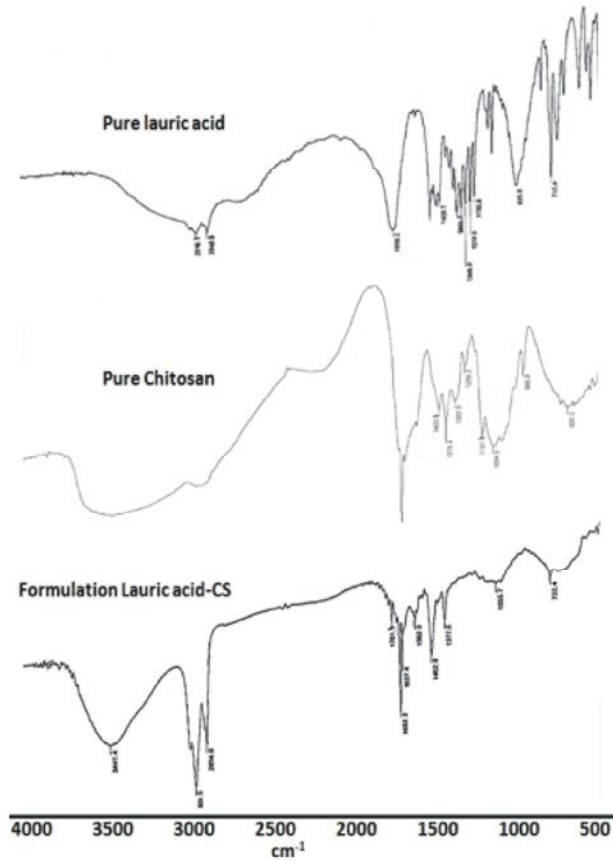Fig. 1: Lauric acid-chitosan based coating material

Fig. 2: Comparison of IR spectrum of pure lauric acid, pure chitosan and Coating material
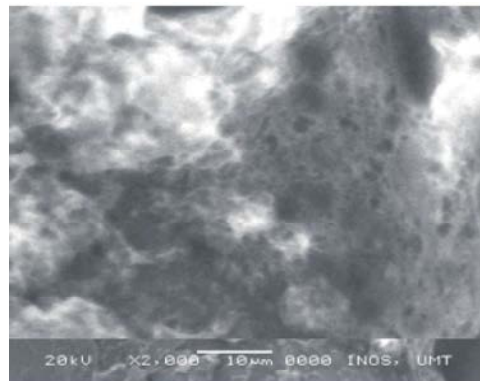

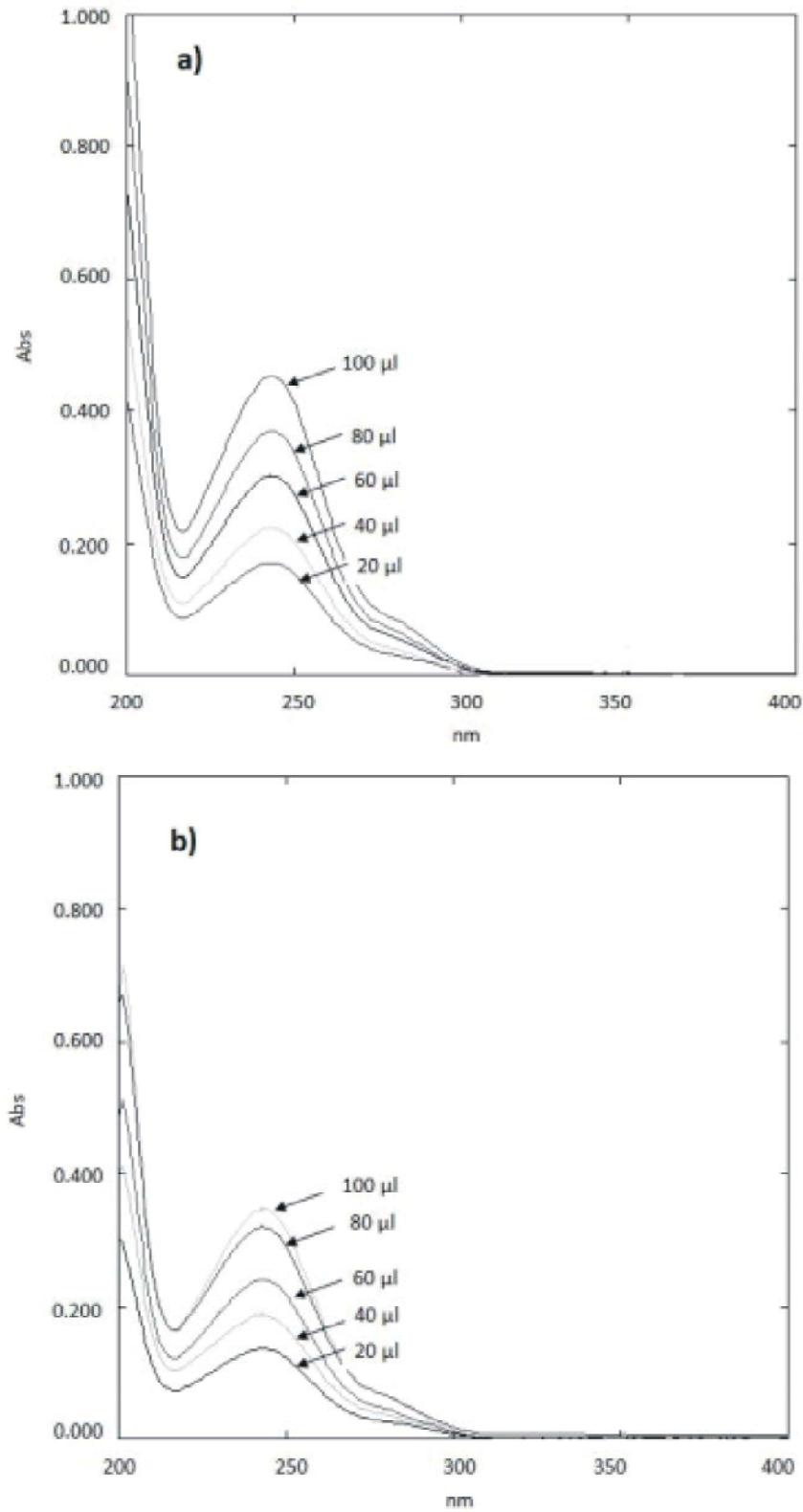Fig. 3: SEM image of lauric acid-chitosan formulated coating material (×2000 magnifications).

Fig. 4: UV spectrum at wavelength 243 nm for the coating material with presence of drug in (a) pH 6.8 and (b) pH 1.2 at 5 different concentrations.
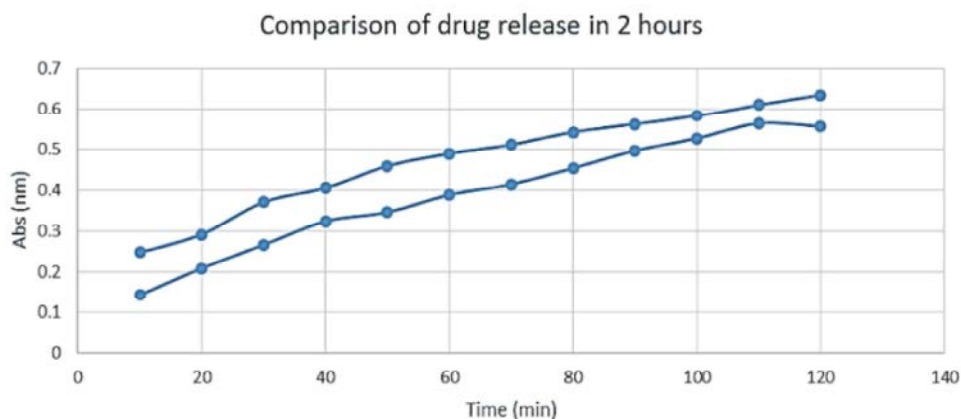
Comparison of drug release in 2 hours

Fig. 5: Comparison of drug release from lauric acid-chitosan based coating material in mimic of stomach fluid (pH 1.2) and mimic of colonic fluid (pH 6.8) for 2 hours reaction.

drug. This also indicates that the drug was chemically stable within the lauric acid-chitosan□coating material [19]. SEM was used to investigate the surface morphology of the formulated lauric acid-chitosan based coating material. The SEM study were taken on × 500 and × 2000 magnifications have revealed that the lauric acid-chitosan formulated coating material have a rough and porous surface (Figure 3) and this is due to the presence of a dense of wrinkles of the coating material. This morphology has also been reported in other studies [20]. While, the porosity of the material might be due to the addition of emulsifiers and surfactant during the sample preparation. ?

Evaluation of the Formulated Coating Material: In order to evaluate the coating material performance, it is necessary to study the dissolution of the coating material by in-vitro realase method [2]. Hence, the in-vitro study was carried out in two different pH conditions: pH 1.2 and 6.8, mimicking pH for both stomach and colon fluid, respectively.

From the dissolution method in mimic of colonic (pH 6.8) and stomach fluid (pH 1.2), it was showed that the drug have been released gradually in both pH (Figure 4a and 4b) at five concentrations (20 – 100 ìL). For both pH, increasing the concentration from 20 to 100 ìL will gradually increase the UV absorbance peak at 243 nm, showing the stable release of the drugs. Interestingly, when comparing the trends for two hours reaction as shown in Figure 5, it can be seen that the absorbance for pH 6.8 was recorded higher than those recorded at acidic medium of pH 2.1 when detected at 243 nm (Fig.5). Thus this indicates that the lauric acid-chitosan based coating material degrades more rapidly when in contact to almost

neutral pH (pH 6.8), presumably as in colon area pH, without much degraded in the acidic medium, similar to the previously reported findings of using guar-gum based coating material [21].

Validation Method: The method used in this research was validated for its linearity, accuracy, precision, as well as the value of limit of detection (LOD) and limit of quantitation (LOQ) were calculated. Under the optimum conditions, the in-vitro dissolution method showed the excellent linearity over the concentration ranges of 20 to 100 µl for both release at pH 1.2 and 6.8 with correlation coefficient r > 0.9955 and r > 9874, respectively. The method also provided detection limits of 18 µl/ml but with slightly higher LOQ of 31 µl/ml. Nevertheless, the linearity range of 20-100 µl for pH 1.2 and pH 6.8 were satisfying with a maximum absorbance of 243 nm. In addition, the linearity of the calibration curve for both pH 1.2 and pH 6.8 shows linear response over the concentration used [11, 22]. Thus, with further analysis and verification conducted in future, these values can be brought down in order for the proposed method to be adopted for routine testing.

CONCLUSION

A coating material with the combination of lauric acid (C12 fatty acid) and a natural polymer, chitosan were successfully synthesized for drug encapsulation application using water-in-oil cross-linking method. The production of the coating material subsequently demonstrated by FTIR spectroscopy and SEM microscopy. The performance of lauric acid-chitosan coating material was verified by *in-vitro* dissolution

method of drug at pH 1.2 and pH 6.8. This article is the first demonstration in exploiting the combination of lauric acid and chitosan as a coating material for drug application that shown stability and possible release in targeting pH.

ACKNOWLEDGEMENT

REFERENCES

1. Cadgas, M., A.D. Sezer and S. Bucak, 2014. Liposomes as Potential Drug Carrier Systems for Drug Delivery. Intech.

2. Nicholas, M.E., S. Panaganti, L. Prabakaran and K.N. Jayveera, 2011. Novel colon specific drug delivery system: A review. International Journal of Pharmaceutical Sciences and Res., 2(10): 2545-2561.

3. Makadia, H.K. and S.J. Siegel, 2011. Poly lactic- co- glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers, 3(3): 1377-1397.

4. Mitra, A. and B. Dey, 2011. Chitosan microspheres in novel drug delivery systems. Indian Journal Pharmaceutical of Sciences, 73(4): 355-366.

5. Dangi, A.A., L.G. Ashok and J. Divya, 2013. Formulation and Evaluation of Colon Targeted Drug Delivery System of Levetiracetam using Pectin as Polymeric Carrier. Journal of Applied Pharmaceutical Science, 3(1): 78-87.

6. Azeredo, H.M.C., D. Britto and O.B.G. Assis, 2010. Chitosan edible films and coatings. Nova Science Publishers Inc, pp: 179-194.

7. Tomuta, I., L. Vlase, A. Popa and S.E. Leucuta, 2010. In vitro – in vivo evaluation of a novel drug delivery system for colonic targeting. Farmacia, 58(3): 368-377.

8. Behera, S., S. Ghanty, F. Ahmad, S. Santra and S. Banerjee, 2012. Uv – visible spectrophotometric method development and validation of assay of paracetamol tablet formulation. Journal of Analytical and Bioanalytical Techniques, 3(6): 1-6.

9. Jha, S.K., S. Dey and R. Karki, 2011. Microemulsions potential carrier for improved drug delivery. Asian Journal of Biomedical and Pharmaceutical Sciences, 1(1): 5-9.

10. Anuar, S.T., Y.Y. Zhao, S.M. Mugo and J.M. Curtis, 2013. The Development of a Capillary Microreactor for Transesterification Reactions using Lipase Immobilized onto a Silica Monolith. J. Mol. Catal B: Enzym., 92: 67-70.

11. Tripathi, S., G.K. Mehrotra and P.K. Dutta, 2008. Chitosan based antimicrobial films for food packaging applications.

12. El-Leithy, E.S., M. Nasr and R.A. El-Moneum, 2012. Development and characterization of solid lipid dispersion as delivery system for hydrophilic antihypertensive drug atenolol. International Journal of Drug Delivery, 4(2): 219-228.

13. Darder, M., M. Colilla and E. Ruiz-Hitzky, 2003. Biopolymer- clay nanocomposites based on chitosan intercalated in montmorillonite. Chemical Materials, 15: 3774-3780.

14. Yuan, Q., J. Shah, S. Hein and R.DK. Misra, 2010. Controlled and extended drug release behavior of chitosan-based nanoparticle carrier. Acta Biomaterialia, 6: 1140-1148.

15. Paluszkiewicz, C., E. Stodolak, M. Hasik and M. Blazewicz, 2011. FT-IR study of montmorillonite-chitosan nanocomposite materials, Spectrochimica Acta Part A., 79: 784-788.

16. Silva, S.M.L., C.R.C. Braga, M.V.L. Fook, C.M.O. Raposo, L.H. Carvalho and E.L. Canedo, 2012. Application of Infrared Spectroscopy to Analysis of Chitosan/Clay Nanocomposites, Infrared Spectroscopy - In Theophanides Theophile (Ed.) Materials Science, Engineering and Technology, InTech.

17. Hiraoui, M., M. Guendouz, N. Lorrain, A. Moadhen, L. Haji and M. Oueslati, 2011. Mat. Chem. Physic., pp: 151-156.

18. Tlili, A. Ali Jarboui, M. Abdelghani, A. Fathala, D.M. and M.A. Maaref, 2005. Mat. Sci. Eng. C, 25: 490-495.

19. Sullad, A.G., L.S. Manjeshwar and T.M. Aminabhavi, 2014. Blend microspheres of chitosan and polyurethane for controlled release of water- soluble antihypertensive drugs. Polymer Bulletin., 72(2): 265-280.

20. Gangurde, H.H., M.A. Chordiya, S. Tamizharasi and T. Sivakumar, 2013. Optimization of Budesonide pH Dependent Coated Pellets for Potential Colon Targeted Drug Delivery. Insight Pharmaceutical Sciences, 3: 1-13.

21. Gandhi, S.D., P.R. Pandyal, N.N. Upadhyay and U. Nagaich, 2010. Design, formulation and evaluation of a colon specific drug delivery system for a model anthelminthic drug- Ivermectin. Journal of Chemical and Pharmaceutical Research, 2(5): 229-243.

22. Kumar, P., A.L. Ganure, B.B. Subudhi and S. Shukla, 2015. Design and Comparative Evaluation of In-vitro Drug Release, Pharmacokinetics and Gamma Scintigraphic Analysis of Controlled Release Tablets Using Novel pH Sensitive Starch and Modified Starchacrylate Graft Copolymer Matrices. Iranian Journal of Pharmaceutical Research, 14(3): 677-691.