SYNTHESIS AND CHARACTERIZATION OF CALCIUM HYDROXIDE ENCAPSULATE ALGINATE MICROSPHERES AS DRUG DELIVERY

Mazlisya Nadhirah Mazelan[1] and Nurazreena Ahmad[1*]

[1]School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia, 14300 Nibong Tebal, Malaysia

*nurazreena@usm.my

Abstract. Calcium hydroxide ($Ca(OH)_2$) has a pH range of 12.5-12.8 and as a result of its high pH, it exerts antibacterial properties. Therefore, $Ca(OH)_2$ has been widely used in dentistry as a root filling material and as a wound treatment dressing for deep cavities and pulpal wounds respectively. However, due to its short residence time in the root canal, $Ca(OH)_2$ must be renewed on a regular basis hence, increasing the number of appointments required and resulting in patient non-compliance. To encounter this shortcoming, the encapsulation of $Ca(OH)_2$ within alginate microspheres (AMs) was proposed to prolong the slow release of $Ca(OH)_2$ onto the targeted site with sufficient concentration. To enable this $Ca(OH)_2$ are encapsulated in AMs via emulsification/gelation method. The effect of $Ca(OH)_2$ to alginate ratio on the formation of the microspheres was evaluated. FTIR analysis showed a peak shift from 1632 and 1425 cm^{-1} to 1632 and 1430 cm^{-1} respectively which is due to an ionic bonding between Ca^{2+} and COO- groups of alginate during crosslinking process of alginate with Ca^{2+} ions and the presence of calcium as verified microspheres prepared by external gelation which indicated the conjugation between alginate and $Ca(OH)_2$. The morphology of the microspheres was observed via scanning electron microscope (SEM) while the particle size measurement was evaluated using Particle Size Analysis (PSA). SEM observation showed that the AMs and $Ca(OH)_2$ encapsulated AMs (CH-AMs) obtained were spherical in shape, however, the microspheres were entangled between the alginate film. The particle size of the microspheres obtained were less than 200 μm which were within the targeted value regardless of the $Ca(OH)_2$ to alginate ratio.

Keywords: Calcium hydroxide, Alginate microspheres, Emulsification/gelation method

Article Info

Received 6th October 2021
Accepted 22nd November 2021
Published 20th December 2021

ISSN: 1823-7010, eISSN: 2600-7444

Introduction

Teeth, periodontal tissues, oral mucosa, and other soft and hard tissues are the anatomical structures of oral cavity which is a part of digestive system. 700 microorganism species tend to colonize the complex ecological niche in oral cavity as it involves food consumption on daily basis which could affect someone's oral health [1]. Rather than specific types of bacteria, microbial dysbiosis are thought to be the cause of oral infectious diseases such as dental caries, periodontitis, peri-implantitis, and oral candidiasis [2].

The selection of an appropriate drug and drug administration route will contribute to the success of restoration treatment. Local therapy is preferable to execute a localized disease such as periodontitis over the systemic therapy in order to avoid the complications associated with systemic drug administration. Hence, local drug delivery (LDD) is a method of delivery drugs by effectively employing local antimicrobials into oral infectious diseases or any individual areas which have failed the effectiveness under the traditional mechanical therapy [3].

Consequently, drug delivery systems (DDS) have received a lot of attention in the last few decades in the field of oral infectious diseases. The transportation and release of therapeutic agents or bioactive substances to the target areas at specific rates could be done by DDS [4], typically, carriers and associated therapeutics are included [5]. The utilization of local drug administration and controlled drug release in DDS, higher curative efficiency and fewer side effects can be achieved [6].

The deficiency of traditional drug administration in terms of therapeutic efficiency and patient compliance [7] can be overcome by the use of microspheres which are known as one of the controlled-release devices of DDS that is being widely utilized in biomedical applications for drug delivery due their impactful therapeutic effect over traditional dosage [8]. The development of biodegradable polymeric microspheres has been advanced in recent years, with the inclusion of controlled drug release behaviors and drug protection from the effects of the *in vivo* environment [9] having the critical advantage of leading to the formation of non-toxic degradation products that are eventually absorbed or excreted by the body [10]. Sodium alginate, for example, has been extensively researched as a DDS candidate.

Sodium alginates are retrieved from the cell walls of brown algae such as *Macrocystic pyrifera* which classifies it as a naturally occurring anionic polysaccharides. Sodium alginate structures are composed of two monomers including β-d-mannuronic acid (M) and α-l-guluronic acid (G) which are linked by 1→4 glycosidic bond [11]. Sodium alginate has biodegradability, biocompatibility, and non-toxicity properties, which have led to the applications of alginate-based materials in the food and biomedical industries [9]. Therefore, sodium alginate is the most natural polymer candidate used in biomedical applications, including DDS.

Calcium hydroxide ($Ca(OH)_2$) with pH of 12.5-12.8 has been suggested as a root filling material for deep cavities and as a therapeutic dressing for pulp wounds due to its inherent high pH, which exerts antibacterial effects and induces periapical tissue healing. $Ca(OH)_2$ has been used in treating a variety of endodontic diseases such as periapical lesions or traumatically injured immature teeth by the means of pulpal dressing and apical closure.

Temporary root canal filling with $Ca(OH)_2$ has been shown in clinical studies to be very effective in halting root resorption [12]. Apical periodontitis prevention is the ultimate biological goal of this treatment. However, short residence time in the root canal due to degradation of $Ca(OH)_2$ into CaO requires multiple treatment appointments and prolong the treatment duration. Typically, the treatment lasts for about nine months [13].

Therefore, to allow prolong release of $Ca(OH)_2$ it is suggested that the encapsulation of the $Ca(OH)_2$ within the alginate microspheres. The drug release mechanism of $Ca(OH)_2$ encapsulate AMs is contributed by the combination of drug release via the degradation of alginate network and the diffusion of drug through alginate network [14]. The diffusion of the drug is caused by the swelling behavior of alginate matrix through an ionic exchange of Ca^{2+} ions with Na^+ ions during cross-linking process of sodium alginate with $CaCl_2$ after absorbing a huge volume of water or aqueous solutions [15] which later results in dissolution/erosion at the edge of the microspheres [16].

Hence, this study aims to encapsulate $Ca(OH)_2$ in alginate microspheres (AMs) via emulsification/gelation and to investigate the effect of $Ca(OH)_2$ to alginate ratio on the formation of $Ca(OH)_2$ encapsulate AMs (CH-AMs).

Materials and Methods

Materials. Sodium alginate ($C_6H_7O_6Na$) was acquired from Sigma (Saint Louis, MO, USA). Systerm, Malaysia provided liquid paraffin oil ($C_{15}H_{11}ClO_7$). Petroleum ether (C_6H_{14}) and ethanol (C_2H_5OH) were supplied by QRec, Malaysia. Calcium hydroxide ($Ca(OH)_2$), sorbitan monooleate (Span 80, $C_{24}H_{44}O_6$), and calcium chloride dihydrate ($CaCl_2.2H_2O$) were purchased from Sigma (Saint Louis, MO, USA).

Preparation of $Ca(OH)_2$ encapsulated alginate microspheres. $Ca(OH)_2$ encapsulated alginate microspheres (CH-AMs) were prepared via emulsification/gelation method. $Ca(OH)_2$ to alginate ratio were varied at 1/50, 1/175, 1/100, 1/125, and 1/150 where they will be dissolved in 1 ml ethanol solution with the use of magnetic stirrer for 3-5 minutes. Later, 9 ml of alginate solution at 4% w/v was poured into the beaker for 30-60 min by magnetic stirring in order to obtain homogeneous $Ca(OH)_2$-alginate mixture. The mixture was then emulsified in liquid paraffin oil at ratio 1:10 containing 2% v/v Span 80 (surfactant). The emulsification process was maintained under mechanical stirring at 300rpm to form the water-in-oil emulsion. The addition of dropwise 10% w/v of $CaCl_2.2H_2O$ into the emulsion was for gelation process with constant stirring speed for 90 min. Petroleum ether was used to wash and filter the CH-AMs. Lastly, the microspheres were air-dried at room temperature.

Surface morphology analysis. The morphology of the AMs and CH-AMs was observed by Scanning Electron Microscope (SEM) (Hitachi TM3000 Table Top, Japan). The sample preparation involved the placement of AMs and CH-AMs on top of carbon tape attached to the sample holder stage. Sputter coating technique was used in coating all samples that were coated with gold-platinum. The microspheres were measured by using a feature called “data entry measurement“ feature in TM3000 software. Acceleration voltage of 20.0 kV was used during the measurement.

Particle size analysis. The particle size measurement and homogeneity distribution were observed by Particle Size Analyzer (Mastersizer 3000, Malvern Instruments Ltd., United Kingdom). The wet dispersion method involved the use of Hydro dispersion unit. Hydro dispersion unit used water as the dispersant when it conducted the samples for analysis. d_{10}, d_{50}, d_{90} and Span value are used to represent the particle sizes and size distribution respectively. The d_{10}, d_{50}, d_{90} indicated the diameter for 10%, 50% and 90% of sample mass which had lesser size with respect to the Span value [8]. The Span value was calculated by using equation 3.1 [17]. The homogeneity in particle size was shown by the size distribution width which was expressed by Span value and equation (1). The Span value that lower than 1 indicates a homogeneous distribution [18].

$$Span = \frac{d_{90-}\, d_{10}}{d_{50}} \quad (1)$$

Fourier Transform Infrared (FTIR) spectroscopy. Carboxyl, hydroxyl, and amine are the examples of the functional groups of the CH-AMs, $Ca(OH)_2$ and sodium alginate that were analyzed by Fourier Transform Infrared (PerkinElmer Spectrum One, USA). The sample preparation was conducted by mixing the samples with potassium bromide powder (KBr) with the ratio of 1:10 by using agate mortar. Next, the mixture was then poured into a pellet holder and utilized pressure under a hydraulic press for 2 minutes to produce a thin semi-transparent pellet. The pellet then was subjected to the infrared beam window of FTIR. The software used was Spekwin32 software with an additional reference by using table of IR absorption in identifying the functional groups of CH-AMs. The scanning was done by setting up the transmittance mode (%T) with 4000 cm^{-1} to 400 cm^{-1} of scan range of and 8 number of scans was used.

Results and Discussion

Morphology of CH-AMs. Figure 1 shows the effect of $Ca(OH)_2$ to alginate ratio on the morphology of unloaded AMs and CH-AMs. Unloaded AMs showed the spherical shape of microspheres; however, the microspheres were entangled in the matrix of alginate with a visible crack formation on the surface. It can be observed that at all ratios, the CH-AMs were found to be spherical in shape, however they were entangled within the alginate film which could be due to an insufficient cross-linking time for the microspheres to split from the alginate matrix individually. Entanglement is the formation of a reticular or spherical structure by cross-linking points in the polymer chain or between polymer chains, preventing the polymer chains from moving normally and thus affecting the nature of the polymer. The measurement of entanglement is generally determined by the viscosity of the polymer fluid or the modulus of complete non-crystalline polymer [19]. Uyen et al. (2019) reported that at low alginate concentration of 2% and 3% w/v, the microspheres observed were clumpy and entangled, while it became discrete with the increasing alginate concentration at 4% w/v. The size of the particles was observed as directly proportional to the alginate concentration which could be influenced by the increase in viscosity of alginate solution. This entanglement behavior can be caused by an ionic exchange reaction between Na^+ and Ca^{2+} which are linked to carboxylic groups of alginate. Theoretically, the cross-linking bonds and physical entanglements are caused by the replacement of bivalent ions with monovalent ions eventually causing the breakup of the “egg-box” structure and indirectly increasing the distance between the polymeric chains [20]. At 1/150 ratio, crack formation was found on the CH-AMs which could be influenced by the insufficient amount of liquid paraffin oil and

Span 80 incorporated during the emulsification process. The rough surface and crack on the CH-AMs at 1/150 ratio could be due to the cross-linked calcium-alginate-network formed during the process is not strong enough [21]. Hence, it can be concluded that the optimum $Ca(OH)_2$ to alginate ratio is at 1/100, as the shape is in good sphericity with lesser entanglement.

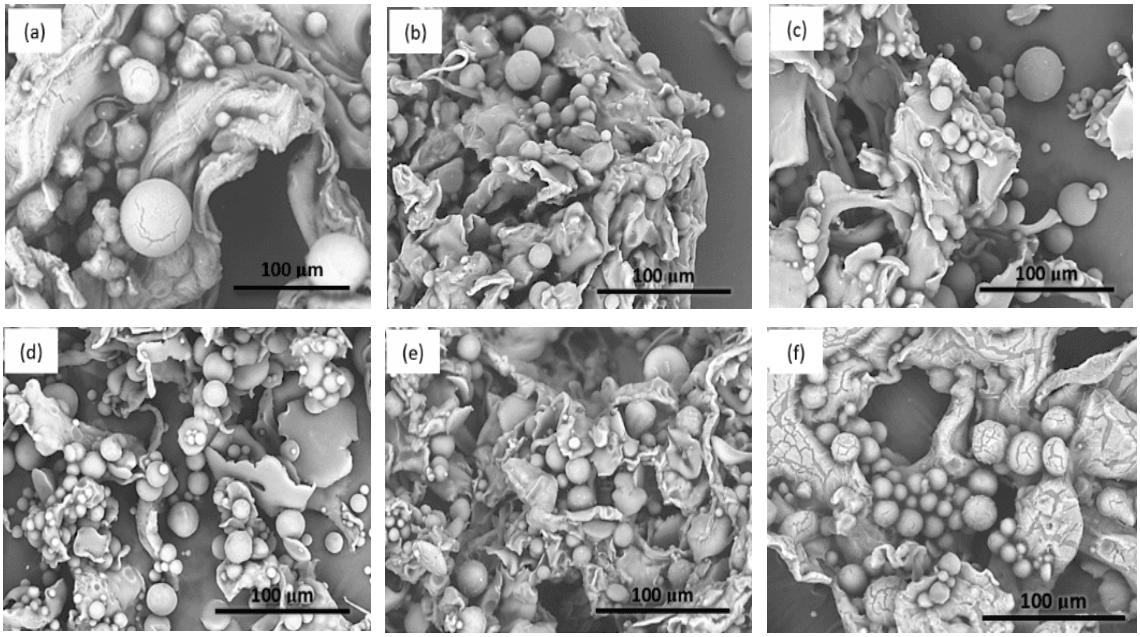

Figure 1. SEM images of CH-AMs at various $Ca(OH)_2$ to alginate ratio (a) unloaded AMs, (b) 1/50, (c) 1/75, (d) 1/100, (e) 1/125 and (f) 1/150.

Particle size distributions of CH-AMs. Figure 2 shows the size distributions of CH-AMs at various $Ca(OH)_2$ to alginate ratio. It can be observed that the particle sizes and size distribution of CH-AMs are significantly affected by $Ca(OH)_2$ to alginate ratio. PSA showed that the mean particle size of CH-AM was 12.80, 72.90, 0.72, 2.73 and 14.60 µm and the Span value of 1.842, 3.710, 3.303, 3.125 and 1.747 with a decreasing number of $Ca(OH)_2$ to alginate ratio respectively. It was found that 1/100 ratio had the smallest particle size at 0.72 µm; meanwhile, 1/75 ratio had the largest particle size at 72.90 µm. Based on the analysis, there was no specific trends in determining the particle size of CH-AMs. According to the particle size distribution showed in Table 1, all samples had a size of less than 200 µm [22], which was within the targeted value for DDS. Since the particle size distribution of CH-AMs was non-homogeneous, it could be due to the non-regular distribution of CH-AMs particle size. There was a presence of both small and large particles which contributed to non-homogenous distribution. The particle size distributions of CH-AMs were not following the trend with increasing $Ca(OH)_2$ to alginate ratio as there were other factors that influenced the formation of the microspheres such as insufficient of $CaCl_2$ concentration, crosslinking time and stirring speed that might cause the interfacial tension between alginate droplets and the oil phase increased. This results in the entanglement and the non-homogeneous distribution since the dispersion of the alginate solution is ineffective. Besides, emulsification/gelation method used in this study produces micromatrixes. Micromatrixes consist of a drug that is uniformly dispersed in the polymer matrix [23]. The release rate of $Ca(OH)_2$ is dependent on various factors such as $CaCl_2$ concentration, alginate concentration, particle size and size distribution of microspheres and drug-polymer ratio [14]. It is found that the release rate of

$Ca(OH)_2$ is inversely proportional to the particle size of CH-AMs under the influence of the surface area between the particles. Dewi Melani et al. (2020) described that with the increase in the concentration of polymer in the media can cause increased viscosity, with the result that the larger the dimension of the droplets formed, the larger the particle size of microspheres produced. The inconsistency in the particle size might also be due to the entanglement of the microsphere within the alginate film.

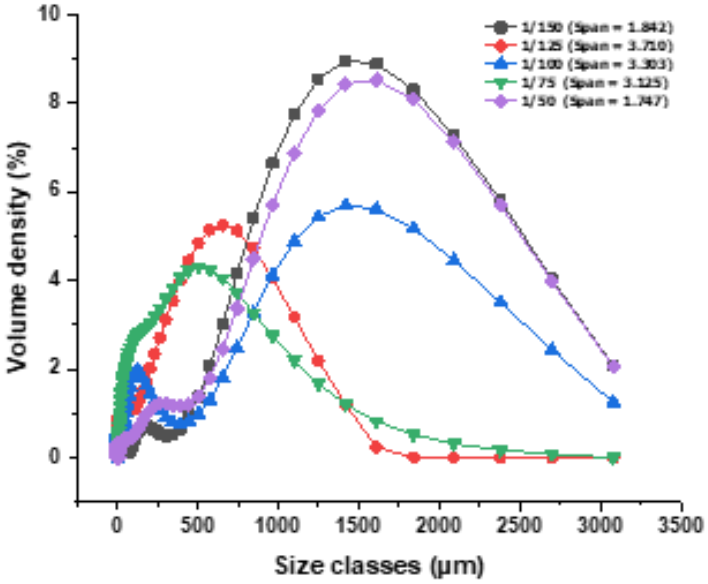

Figure 2. Particle size distribution of CH-AMs at different $Ca(OH)_2$ to alginate ratio of 1/50, 1/75, 1/100, 1/125 and 1/150

Table 1. Particle sizes of CH-AMs at different $Ca(OH)_2$ to alginate ratio of 1/50, 1/75, 1/100, 1/125 and 1/150

$Ca(OH)_2$ to alginate ratio	Size of 10% particle of CH-AMs, d_{10} (µm)	Size of 90% particle of CH-AMs, d_{90} (µm)	Mean particle size of CH-AMs (µm)
1/150	102	2470	12.80
1/125	28.7	954	72.90
1/100	0.448	2190	0.72
1/75	0.990	989	2.73
1/50	165	2480	14.60

Interaction between $Ca(OH)_2$ and alginate polymer. The interaction between $Ca(OH)_2$ and alginate polymer were investigated by using FTIR analysis. FTIR was conducted to investigate the existence of chemical bonding of the microsphere system by evaluating the interactions between $Ca(OH)_2$ and alginate as shown in Figure 3. $Ca(OH)_2$ revealed the FTIR peaks at 3872 and 3648 cm^{-1} were attributed to -OH group, Ca is bonded to -OH group in molecular structure. The spectrum of sodium alginate showed the peaks were at 3435 cm-1 which corresponded to stretching -OH group, 2860 cm^{-1} for stretching -CH

group. The bands at 1614 and 1422 cm^{-1} disclosed the asymmetric and symmetric COO- stretching respectively. The band of 1017 cm^{-1} attributed for mixed vibration with C-O-C stretching. The FTIR spectra of AMs and CH-AMs showed there was a shift from 1632 and 1425 cm^{-1} to 1632 and 1430 cm^{-1}, respectively. This happened because of the ionic bonding between Ca^{2+} and COO- groups of alginate [25] which came from the crosslinking process of alginate with Ca^{2+} ions. According to a study reported by [26], FTIR spectra showed a strong absorption around 1594-1602 cm^{-1} that belongs to the asymmetric stretching vibration of COO-. The carboxylate carbonyl of alginate could be shifted by the presence of calcium to 1560 cm-1 as verified with microspheres prepared by external gelation which indicated the conjugation between alginate and $Ca(OH)_2$ [27]. In this study, the shifted peak occurred at 1632 and 1425 cm^{-1} to 1632 and 1430 cm^{-1}, thus confirmed the encapsulation of $Ca(OH)_2$. As for the spectrum of CH-AMs, the FTIR peaks for $Ca(OH)_2$ were not detected as the signals were too weak which was caused by the low $Ca(OH)_2$ to alginate ratio during the fabrication.

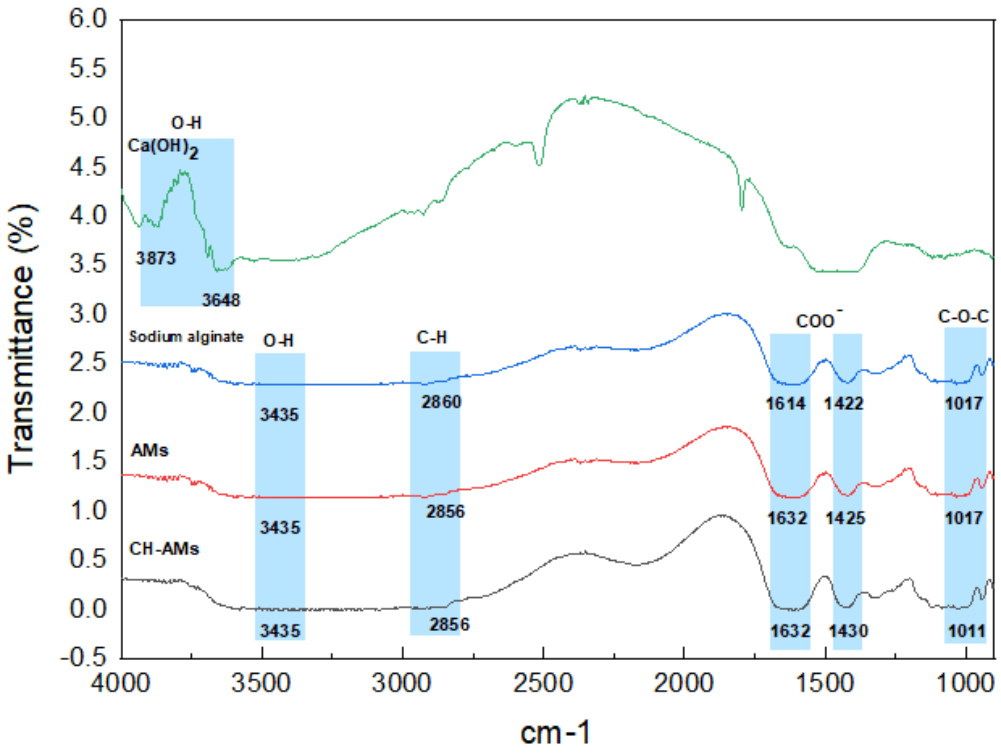

Figure 3. FTIR spectra of $Ca(OH)_2$, sodium alginate, AMs and CH-AMs.

Conclusion

$Ca(OH)_2$ with poor water solubility was successfully encapsulated in AMs via emulsification/gelation method to encourage prolong effectiveness of $Ca(OH)_2$ during the restoration treatment, resulting in longer residence time in the root canal and patience compliance with lesser number of appointments. The obtained results shown that the $Ca(OH)_2$ to alginate ratio had a significant effect on the particle size of CH-AMs. At all ratios, the particle size of CH-AMs obtained were less than 200 μm, however the best shape obtained was at 1/100 ratio. The morphological characteristics of CH-AMs was done by SEM where it showed the microspheres were spherical in shape, however they were entangled

within the alginate film. The interaction of $Ca(OH)_2$ and alginate polymer can be observed by FTIR analysis where there was a peak shift. This expressed an ionic bonding between Ca^{2+} ions and carboxyl (COO^-) group of alginate in the microspheres system. Hence, it is possible to conclude that CH-AMs can be synthesized with a controlled particle size via emulsification/gelation method and may have a promising future in the therapeutic delivery.

Acknowledgements

The authors gratefully acknowledge Malaysia Ministry of Education for awarding Fundamental Research Grant (FRGS/1/2018/TK05/USM/02/2) and to the School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia for providing the facilities for this research.

Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of conflict of interest

The authors have no disclosures to declare.

Compliance with ethical standards

The work is compliant with ethical standards.

References

[1] Scannapieco, F. (2013). The oral microbiome: Its role in health and in oral and systemic infections. *Clinical Microbiology Newsletter*, 35(20) 163–169.

[2] Lamont, R. J. (2018). The oral microbiota: dynamic communities and host interactions. *Nature Reviews Microbiology*, 16(12) 745-759.

[3] Sholapurkar, A., Sharma, D., Glass, B., Miller, C., Nimmo, A., & Jennings, E. (2021). Professionally delivered local antimicrobials in the treatment of patients with periodontitis-a narrative review. *Dentistry Journal*, 9(1) 1-19.

[4] Jain, K. K. (2014). Chapter 1 Current Status and Future Prospects of Drug Delivery Systems. *Drug Delivery System*, 1141.

[5] Fenton, O. S., Olafson, K. N., Pillai, P. S., Mitchell, M. J., & Langer, R. (2018). Advances in Biomaterials for Drug Delivery. *Advanced Materials*, 1705328 1–29.

[6] Kohane, D. S. (2007). Microparticles and Nanoparticles for Drug Delivery. *Biotechnology*

and Bioengineering, 96(2) 203–209.

[7] Bee, S. L., Hamid, Z. A. A., Mariatti, M., Yahaya, B. H., Lim, K., Bee, S. T., & Sin, L. T. (2018). Approaches to Improve Therapeutic Efficacy of Biodegradable PLA/PLGA Microspheres: A Review. *Polymer Reviews*, 58(3) 495–536.

[8] Uyen, N.T.T., Zainatul Ain, A. A., & Nurazreena, A. (2019). Fabrication and characterization of alginate microspheres. *Materials Today: Proceedings*, 17 792–797.

[9] Sharma, S., Sanpui, P., & Sankar, S. (2012). *RSC Advances Fabrication of antibacterial silver nanoparticle — sodium alginate – chitosan composite films*, 13 5837–5843.

[10] Singh, P. (2011). Biodegradable Polymeric Microspheres as Drug Carriers; A Review. *Indian Journal of Novel Drug Delivery*, 3(2) 70-82.

[11] Dragnet, K. L. (2000). *Handbook of Hydrocolloids*. (CRC Press) pp. 381–383.

[12] Hammarström, L., Blomlöf, L., Feiglin, B., & Lindskog, S. (1986). Effect of calcium hydroxide treatment on periodontal repair and root resorption. *Dental Traumatology*, 2(5) 184–189.

[13] Ajram, J., Khalil, I., Gergi, R., & Zogheib, C. (2019). Management of an immature necrotic permanent molar with apical periodontitis treated by regenerative endodontic protocol using calcium hydroxide and MM-MTA: A case report with two years follow up. *Dentistry Journal*, 7(1) 1–6.

[14] Marković, D., Zarubica, A., Stojković, N., Vasić, M., Cakić, M. & Nikolić, G. (2016). Alginates and similar exopolysaccharides in biomedical application and pharmacy: Controled delivery of drugs. *Advanced Technologies,* 5(1) 39-52.

[15] Lin, N., Huang, J., Chang, P. R., Feng, L. & Yu, J. (2011). Effect of polysaccharide nanocrystals on structure, properties, and drug release kinetics of alginate-based microspheres. *Colloids and Surfaces B: Biointerfaces,* 85(2) 270-279.

[16] Tønnesen, H. H. & Karlsen, J. (2002). Alginate in drug delivery systems. *Drug development and industrial pharmacy,* 28(6) 621-630.

[17] Ismail, H., Fahmi, A., Mohamed, F., Muizzuddin, L., Rosli, M., Affendi, M., & Shafri, M. (2016). Spectrophotometric Determination of Gentamicin Loaded PLGA Microparticles and Method Validation via Ninhydrin-Gentamicin Complex as a Rapid Quantification Approach. *Journal of Applied Pharmaceutical Science*, 6(01) 007-014.

[18] Wang, E., Wang, X., Wang, K., He, J., Zhu, L., He, Y., & Lai, W. (2018). Preparation, characterization and evaluation of the immune effect of alginate/chitosan composite microspheres encapsulating recombinant protein of Streptococcus iniae designed for fish oral vaccination. *Fish and Shellfish Immunology*, 73(December 2017), 262–271.

[19] Kuo, C. J., & Lan, W. L. (2014). Gel spinning of synthetic polymer fibres. *Advances in Filament Yarn Spinning of Textiles and Polymers*. (Woodhead Publishing Limited) 2014 100-112.

[20] Segale, L., Giovannelli, L., Mannina, P., & Pattarino, F. (2016). Calcium Alginate and Calcium Alginate-Chitosan Beads Containing Celecoxib Solubilized in a Self-Emulsifying Phase. *Scientifica*, 2016.

[21] Hu, L., Kong, D., Hu, Q., Yang, X., & Xu, H. (2016). Preparation and Optimization of a Novel Microbead Formulation to Improve Solubility and Stability of Curcumin Preparation and Optimization of a Novel Microbead Formulation to Improve Solubility and Stability of Curcumin. *Particulate Science And Technology*, 35(4) 448-454.

[22] Sailaja, A. K., & Anusha, K. (2017). Review on microspheres as a drug delivery carrier, 06(05) 96–102.

[23] Solanki, N. (2018). Microspheres an innovative approach in drug delivery system. *MOJ Bioequivalence & Bioavailability*, 5(1) 56–58.

[24] Dewi Melani, H., Yashwant, P., Esti, H., Tristiana, E., Hidayah, I., & Elizabeth, S. (2020). Formulation of Metformin-Loaded Alginate Microspheres by Ionotropic Gelation-Aerosolization Technique. *Sains Malaysiana*, 49(10) 2513–2525.

[25] Kusuktham, B., & Prasertgul, J. (2015). Morphology and Property of Calcium Silicate Encapsulated with Alginate Morphology and Property of Calcium Silicate Encapsulated with Alginate Beads. *Silicon*, 6(3) 191 – 197.

[26] Ribeiro, A. J., Silva, C., Ferreira, D., & Veiga, F. (2005). Chitosan-reinforced alginate microspheres obtained through the emulsification/internal gelation technique. *European Journal of Pharmaceutical Sciences*, 25(1), 31–40.

[27] Sookkasem, A., Chatpun, S., Yuenyongsawad, S. & Wiwattanapatapee, R. (2015). Alginate beads for colon specific delivery of self-emulsifying curcumin. *Journal of Drug Delivery Science and Technology*, 29, 159-166.