Al-Azhar Bulletin of Science

Volume 29 | Issue 2 Article 10

12-1-2018
Section: Botany, Microbiology and Zoology

ELUSIVE PHARMACEUTICAL FUNCTION OF MULTI-ADVANTAGES DRUG DELIVERY SYSTEM FROM NATURAL MUSHROOM

Mahmoud Mohamed
Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt

Nehal El-Deeb
Biopharmaceutical Product Research Department, City of Scientific Research and Technological Applications(SRTA-City), New Borg El-Arab City 21934, Alexandria, Egypt

Mohamed El-Assar
Polymer Materials Research Department Advanced Technology and New Material Research Institute, City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab City 21934, Alexandria, Egypt

Mohamed Farag
Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt

Ayman Ahmed
Professor of Microbiology and Director of Al-Azhar Center for Fermentation Biotechnology and Applied Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt

How to Cite This Article

Mohamed, Mahmoud; El-Deeb, Nehal; El-Assar, Mohamed; Farag, Mohamed; and Ahmed, Ayman (2018) "ELUSIVE PHARMACEUTICAL FUNCTION OF MULTI-ADVANTAGES DRUG DELIVERY SYSTEM FROM NATURAL MUSHROOM," *Al-Azhar Bulletin of Science*: Vol. 29: Iss. 2, Article 10.
DOI: https://doi.org/10.21608/absb.2018.33808

Al Azhar Bulletin of Science Vol. 29, No. 2 (December), 2018, pp. 19-25.

ELUSIVE PHARMACEUTICAL FUNCTION OF MULTI-ADVANTAGES DRUG DELIVERY SYSTEM FROM NATURAL MUSHROOM

Mahmoud A. Mohamed[1], Nehal M. El-Deeb[2], Mohamed R. El-Assar[3], Mohamed M. S. Farag[1], Ayman F. Ahmed[4]

[1]Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt
[2]Biopharmaceutical Product Research Department, City of Scientific Research and Technological Applications(SRTA-City), New Borg El-Arab City 21934, Alexandria, Egypt.
[3]Polymer Materials Research Department Advanced Technology and New Material Research Institute, City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab City 21934, Alexandria, Egypt.
[4] Professor of Microbiology and Director of Al-Azhar Center for Fermentation Biotechnology and Applied Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt

ABSTRACT

The κ-carrageenan/sodium alginate beads were prepared for drug delivery system. The presented study; describes the preparation of κ-carrageenan/sodium alginate beads and their feasibility as potential carriers for delivery of polysaccharide drugs was evaluated. Moreover, the pH sensitive behavior of beads was measured in simulated gastrointestinal tract condition. Furthermore, the hydrogel beads were loaded with polysaccharides extracted from naturalmushroom, and their release was monitored. This results showed that; the swelling ratio of beads indicated pH-dependent properties with maximum water absorbing at pH 7.4. Moreover, the highest amount of released polysaccharide was 27.2% at pH value 7.4 within 6 h. It was observed that the encapsulation of κ-carrageenan with sodium alginate reduced the premature release of drug in the stomach mimicked condition acidic pH and released the drug more specifically to the colon mimicked condition alkaline pH. Therefore, these results suggest that microcapsules could be further developed as effective drug delivery system with pH sensitive drug release ability.

Keywords: Drugdelivery; Mushroom; Polysaccharide; κ-carrageenan/sodium alginate; Beads.

1. INTRODUCTION

Biopolymers and their derivatives have drawn increasing attention in medical field as carriers for controlled drug delivery due to their safety, and biocompatibility **(Zare-Akbari *et al.* 2016, Noisri, Nongkhai, and Trakulsujaritchok 2017 and Dai *et al.* 2008).** The pH-sensitivity of hydrogel is an important factor in designing polymers for controlled drug release in the gastrointestinal tract, which has a variation of pH from the stomach to the intestine.Hydrogels from natural polymers; especially polysaccharides have been widely used because of their advantageous properties such as nontoxicity, biocompatibility, and biodegradability. Among these polymers, carrageenans which normally ionic polymers consisted of a linear of polysaccharides and extracted from red seaweed**(Khan et al. 2017).** Kappa-carrageenan (K-CG) is one of the carrageenan family which broadly used as gelling-stabilizing and thickening agent in the food industry **(Xu et al. 2014).** It has one sulfate group per a disaccharide repeating unit and can interface with oppositely charged polymers by ionic interaction**(Navikaite et al. 2016).** An advantage of K-CG is capacity to form hydrogel with green condition be that as it may, its high swelling leads to burst initial drug release. This deficiency can be solved by blending or coating the K-CG with other polymers with lower swelling characteristic for example, sodium alginate. Sodium alginate (SA) is an anionic linear polysaccharide extracted from brown seaweed, and consists of 1→4 linked D-mannuronic and L-glucuronic acids. It gel is pH-sensitive because of the presence of carboxylic group along the backbone. Sodium alginate based structured polymers are especially attractive in pharmaceutical and encapsulation application

(Noisri et al. 2017). Sodium alginate was chosen as the biopolymer for this research because alginates are polysaccharides produced naturally that are biodegradable, non-toxic, and biocompatible and have the capacity to incorporate acid labile drugs into the matrix formed after cross-linking(Almeida and Almeida 2004, Tong et al. 2017). Alginates are hydrocolloids, water soluble biopolymer, extracted from brown seaweed and composed of α-L-glucuronic acid β-D-mannuornic residues **(Almeida and Almeida 2004, Prabu and Tnk 2017 andK. Y. Lee 2012 and Andrew D Clarke., et al.2017).** Polysaccharides are the major category of bioactive compounds found in mushroom. The overall therapeutic effects of polysaccharides are antioxidant, antidiabetic, anti-bacterial, anti-inflammatory, anticancer and immunomodulators**(Puri 2017, Elsayed et al. 2014).**Polysaccharides from Mushrooms have isolated and characterized such as pleuran from *Pleurotus* species, lentinan and erothionine in *Lentinula. edodes*, ganoderan from *Ganodermalucidium* and agaritine from *Agaricuss*p and calocyban from *Calocybeindica* **(Rathore, Prasad, and Sharma 2017).** Many polysaccharides have been accounted for that show immuno-stimulatory and antitumor activities(Zhang et al. 2017). In this study, microcapsules frombio polymeric hydrogels as a drug delivery device in simulated gastrointestinal system (SGI) were investigated. Moreover, K-CG/SA beads was prepared and investigated as microcapsule drug carrier for the controlled release of polysaccharide extracted from mushroom.

2. MATERIALS AND METHODS

2.1. Materials

Alginic acid sodium salt (High viscous), Kappa Carrageenan, potassium chloride and calcium chloride anhydrous were obtained from Sigma–Aldrich. Distilled water was used throughout this research. All other chemicals were of analytical grade and were used without any further purification.

2.2. Methods

2.2.1 Mushroom fruiting bodies and culture conditions

This study consisted of nine wild mushrooms growing species. Mushroom fruiting bodies were cultured on Potato Dextrose Agar (PDA) medium and incubated at 25^{o}C for 5-7 days then purified on PDA media.Purified mushrooms were stored in 50% glycerol at -80C^{0}.

2.2.2 Isolation of polysaccharides extracts

Mushroom polysaccharide extracted according to Jian-Yong Wu method with some modifications **(Wu 2017).** The filamentous

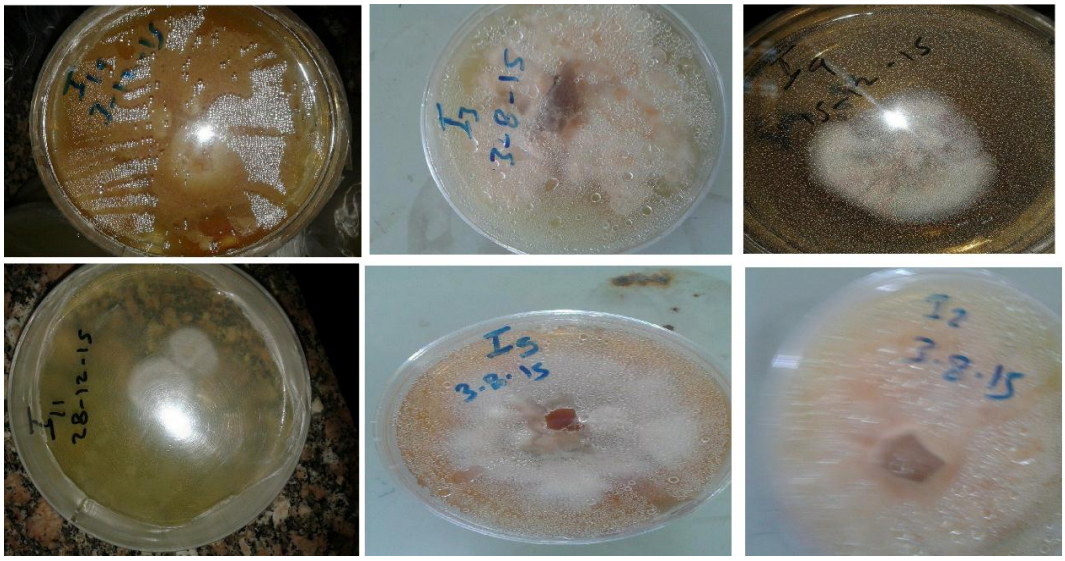


Photo1: Showing different mushroom strains culturedon PDA medium.

hyphae of mushroom were cultured into Potato Dextrose broth medium at 27 °C for eight days. The mycelium were harvested and centrifuged at 2,000 rpmfor 15min; the culture supernatants were separated carefully and filtered through filter paper. The resulting supernatantwas heated at 90°C for 1 hr. To precipitate of polysaccharide add ethanol and put the mixture in 4°C for 24 h. Then put mixture in oven at 50 °C for 72 h. Then precipitate scratched and collected in Eppendorf.

2.2.3. Preparation of polymers for microcapsules

Alginate (Alg) and kappa carrageenan (KCG) solutions were prepared separately by dissolving the biopolymer in distilled water and heating at 70°C (for Alg) and 80°C (for KCG), while stirring constantly for 30 min. a mixture containing 1 % of KCG solution, 0.5 % of Alg solution with ratio 5:10 respectively.

2.2.4. Formulation of pharmaceutical polysaccharide microcapsules

Polysaccharide (100 mg) dissolved in 2.5 ml of distilled water were mixed with 5 ml of 0.5 % sodium alginate and 2.5 ml of 1 % kappa carrageenan solution pre-sterilized at 121°C for 15 min. under continuous stirring until complete homogenization is reached. For polymer beads preparation, 10 ml of the latest solution is poured into a stirring salt solution composing of 100 ml of 3.0 % KCl and 100 ml of 3.0% $CaCl_2$ using the encapsulator and using a100 µl spray nozzle. The resulted beads are continuously stirred in$CaCl_2$ and KCl solution for at least 30 min. The obtained beads are filtered out by washing with distilled water. The process is described briefly in **Fig. 2**

2.2.5. *In vitro* release studies

In vitro polysaccharide release study was carried out in simulated gastric fluid at pH 1.2, phosphate buffer saline pH 7.2, and acetate buffer solutions at pH 3.6, 4.6 and 5.6. The release profile was studied on beads obtained with size of 100µm. The beads were suspended in 50 ml of dissolution media at different pH values. These dissolution media were stirred at 120 rpm in a horizontal laboratory shaking water bath and maintained at 37 ± 0.5°C. At different time intervals, the release medium was withdrawn and the same volume of fresh medium was replaced to maintain the constant volume. The amount of released polysaccharide was measured by a spectrophotometer at 490nm. and calibrationcurve of polysaccharide standard solutions ranging from 1 to 100 µg/ml using distilled water as blank in spectrophotometer**(Cuesta et al. 2003).** All release tests were performed in triplicate.

2.2.6. Swelling behavior

Swelling behavior of the beads was evaluated at pH 7.4. The sample was accurately weighed and placed in 10 ml of buffer solution.

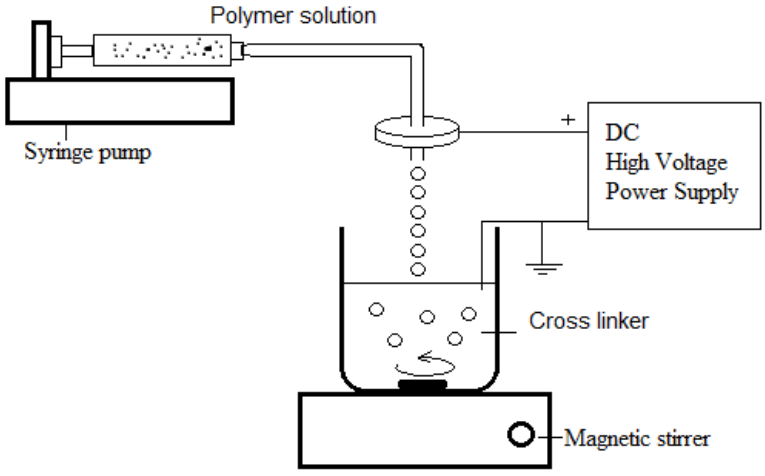


Figure (2): Schematic diagram represents the formation of beads using encapsulator.

After the specific time interval, the swollen beads were taken out, removed the excess solution and weighed. The swelling ratio was calculated by the following equation:

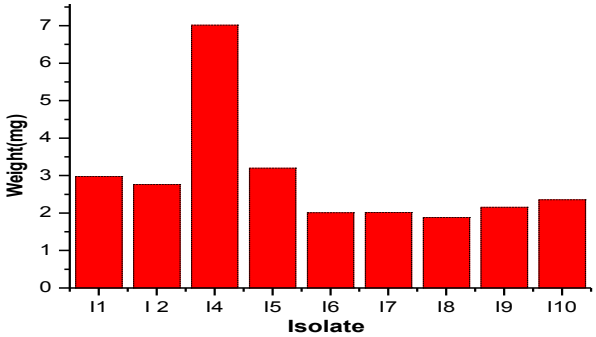
Swelling ratio (%) = (Ws - Wd)/ Wd×100

Where Ws and Wd are the weights of swollen and dry beads, respectively.

3. RESULTS AND DISCUSSION

3.1. Yield of polysaccharides from different strains

The yield of polysaccharide extracted from different strains of mushrooms cultured in 250 ml of potato dextrose broth medium was highest in isolate 4 (I4) (7.02 mg/250 ml), followed by I5 (3.2 mg/250 ml), I1 (2.98 mg/250 ml), I2 (2.77 mg/250 ml) and yield of I10, I9, I7, I6 and I8 respectively2.36, 2.16, 2.02, 2.01 and 1.88 mg/250 ml **Fig. 3.**



Figure(3): yield of polysaccharides from different strains.

3.2. In vitro *release studies*

The oral route of administration is the most convenient, favored, and desirable method of administering drugs for systemic effects. Traditional oral formulation methods employed in a majority of currently commercially available pharmaceutical products provide clinically effective therapy and safety to the patient. To overcome the limitations of conventional immediate-release dosage forms, a wide variety of oral drug delivery systems have been developed in recent days that provide sustained-release dosing and are capable of maintain steady plasma drug levels for extended periods of time with reduced side effects**(Patel et al. 2016).** The *in vitro* drug release profile of polysaccharide in different pH from the kappa-carrageenan alginate beads is shown in **Fig. 4**. The drug release from alginate beads is dependent on the penetration of the dissolution medium into the beads, swelling and dissolution of the alginate matrix, and the dissolution of the drug subsequent to leaching through the swollen matrix **(García-González et al. 2015).**

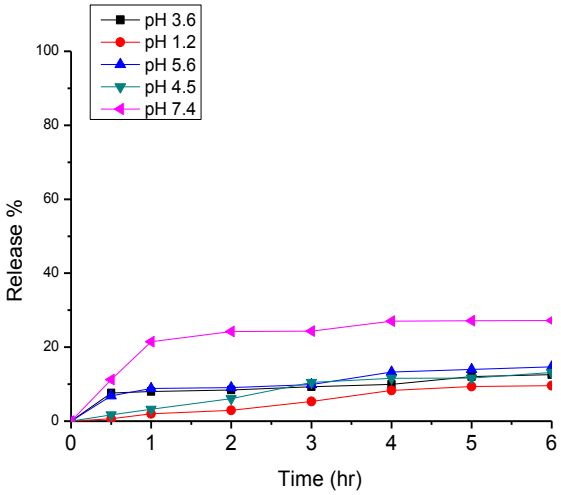


Figure (4):*In vitro* Cumulative release percentage of polysaccharide in different pH.

The effect of pH on the amount of drug release behavior at 37°C was presented in figure (4). Release behavior of polysaccharide from alginate and kappa carrageenan beads was also investigated at acidic conditions pH 1.2, gastric pH conditions, in order to determine their potential to promote fast release in the gastric environment. Polysaccharide exhibited low solubility and dissolution rate under acidic conditions. Moreover, cross-linked kappa carrageenan and alginate beads are resistant to acidic conditions and are not able to dissolve in aqueous media, likely influencing the release behavior of the drug incorporated to these carriers. In contrast to drug release profiles at pH 7.2, alginate beads accelerated polysaccharide release at simulated gastric pH conditions, which is consistent with previous reports**(Radin, Chen, and Ducheyne 2009).** The release of drug in pH 1.2 solution was slower as compared to that in pH 7.4 buffer solution **(Dai et al. 2008).** This may be due to a

higher swelling of hydrogel beads in alkaline pH condition. It was observed that, the rate of polysaccharide release was depending upon the crosslinking; as the concentration of cross-linking agent was increased in the beads, the drug release was decreased. It may be due to the fact that at higher crosslinking, free volume of the polymer matrix decreases, thereby blocking the movement of solutes through the polymer matrix **(Kulkarni et al. 2012).**

Moreover, the results showed that the calcium alginate beads exhibited lower diffusion coefficient for polysaccharide in an acidic medium. The initial burst release behavior can be described as the fast diffusion of polysaccharide molecules in the surface layer of the beads. At pH below thepKa of alginate (3.2 and 4 for guluronic acid and mannuronic acid, respectively), most of the carboxyl groups were in the form of COOH, thus reducing the electrostatic repulsion, and the intermolecular hydrogen bond would restrict the relaxation of polymer chains. A more compact network would be formed and thus reduce the release of polysaccharide. On the other hand, the amount of released polysaccharide in pH =7.4 reached to the highest (27.2 %) within 6 h. This higher release rate may be related to the higher swelling ratio of the beads and the weak H-bonding interaction between polysaccharide and polymer network in the neutral phosphate buffer and in the neutral and alkaline media. In these conditions, the carboxyl groups became ionized (COO groups) which would induce the electrostatic repulsion among negative charges in the alginate beads. This would cause the alginate hydrogel network to expand and increase the free volume spaces in the matrix, thus a higher diffusion coefficient was observed. A similar behavior and results with other drugs have also been reported by other researchers (El-aassar et al. 2014).

3.3. The swelling behavior

Swelling behavior of the prepared beads was studied in order to gain a better understanding of the pH-sensitive. The swelling behavior of the beads is mainly attributed to the hydration of the hydrophilic groups (Hoffman 2012). This factor is an important factor for drug delivery system, because it takes part in controlling the diffusion and release of drug from the beads. The equilibrium swelling study of the beads was carried out in buffer solutions at pH 7.4. The results showed that, the swelling increased with time Fig. (5). Moreover, the pH-dependent swelling behavior was due to the carboxylate and sulfate groups of sodium alginate and kappa carrageenan. However, in basic solution pH 7.4, the carboxylate and sulfate groups became ionized, and the electrostatic repulsion caused the hydrogels to significantly swell (Noisri, Nongkhai, and Trakulsujaritchok 2017,Dai et al. 2008).

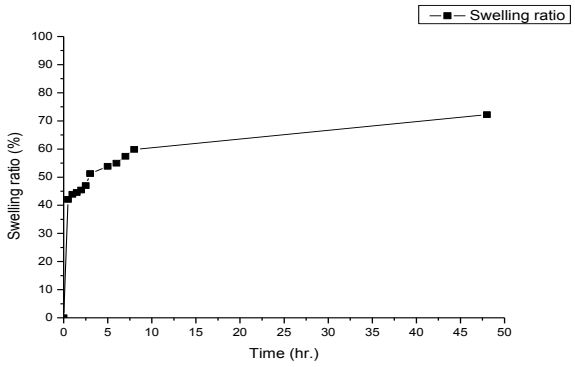


Fig. (5) Swelling profiles of the beads in buffer solution pH 7.4

CONCLUSION

In this study beads of kappa carrageenan/ sodium alginate were prepared as a pH-sensitivedrug carrier for colon-targeted drug delivery. The beads loaded with polysaccharide extracted from natural wild mushroom. The beads showed pH-dependent swelling and drug release. The*in vitro* release assay demonstrated that the alginate/carrageenan beads provided a sustained and controlled release of the polysaccharide for an extended period of time. The drug release was significantly increased when pH of the medium was changed from acidic to alkaline.

ACKNOWLEDGEMENT

Authors would like to thank Prof. Dr. AymanFarrag Ahmed, Director of Al-Azhar

Center for Fermentation Biotechnology and Applied Microbiology for his support, precious advices and for sharing in supervision. Special thanks to Prof. Mahmoud Mohamed Sakr, president of The Academy of Scientific Research & Technology (ASRT), Cairo, for his kindness, care within funding supported by Scientists for Next Generation program (SNG); grant for a new generation of graduates to a master's degree.

REFERENCES

Almeida, P. Ferreira and A. J. Almeida. 2004. "Cross-Linked Alginate-Gelatine Beads: A New Matrix for Controlled Release of Pindolol." *Journal of Controlled Release* 97(3):431–39.

Anon. 2017. "Measurement of Total Sodium Alginate in Restructured Fish Products Using Fourier Transform Infrared Spectroscopy." 1:33–45.

Cuesta, Gabriela, Norma Suarez, Maria I. Bessio, Fernando Ferreira, and Hugo Massaldi. 2003. "Quantitative Determination of Pneumococcal Capsular Polysaccharide Serotype 14 Using a Modification of Phenol-Sulfuric Acid Method." *Journal of Microbiological Methods* 52(1):69–73.

Dai, Ya Ni, Ping Li, Jun Ping Zhang, Ai Qin Wang, and Qin Wei. 2008. "Swelling Characteristics and Drug Delivery Properties of Nifedipine-Loaded PH Sensitive Alginate-Chitosan Hydrogel Beads." *Journal of Biomedical Materials Research - Part B Applied Biomaterials* 86(2):493–500.

El-aassar, M. R., Elsayed E. Hafez, Nehal M. El-deeb, and Moustafa M. G. Fouda. 2014. "International Journal of Biological Macromolecules Microencapsulation of Lectin Anti-Cancer Agent and Controlled Release by Alginate Beads , Biosafety Approach." *International Journal of Biological Macromolecules* 69:88–94.

Elsayed, Elsayed A., Hesham El Enshasy, Mohammad A. M. Wadaan, and Ramlan Aziz. 2014. "Mushrooms : A Potential Natural Source of Anti-Inflammatory Compounds for Medical Applications." 2014.

García-González, C. A., M. Jin, J. Gerth, C. Alvarez-Lorenzo, and I. Smirnova. 2015. "Polysaccharide-Based Aerogel Microspheres for Oral Drug Delivery." *Carbohydrate Polymers* 117:797–806.

Hoffman, Allan S. 2012. "Hydrogels for Biomedical Applications." *Advanced Drug Delivery Reviews* 64(SUPPL.):18–23.

K. Y. Lee, D. J. Mooney. 2012. "Alginate : Properties and Biomedical Applications." *Progress in Polymer Science (Oxford)* 37(1):106–26.

Khan, Abida Kalsoom et al. 2017. "Carrageenan Based Bionanocomposites as Drug Delivery Tool with Special Emphasis on the Influence of Ferromagnetic Nanoparticles." *Oxidative Medicine and Cellular Longevity* 2017.

Kulkarni, Raghavendra V., Rashmi Boppana, G. Krishna Mohan, Srinivas Mutalik, and Navanath V. Kalyane. 2012. "PH-Responsive Interpenetrating Network Hydrogel Beads of Poly(Acrylamide)-g-Carrageenan and Sodium Alginate for Intestinal Targeted Drug Delivery: Synthesis, in Vitro and in Vivo Evaluation." *Journal of Colloid and Interface Science* 367(1):509–17.

Navikaite, Vesta, Deimante Simanaviciute, Rima Klimaviciute, Valdas Jakstas, and Liudas Ivanauskas. 2016. "Interaction between κ-And ι-Carrageenan and Anthocyanins from Vaccinium Myrtillus." *Carbohydrate Polymers* 148:36–44.

Noisri, Nirynn, Piyaporn Na Nongkhai, and Thanida Trakulsujaritchok. 2017. Kappa-Carrageenan / Sodium Alginate Based Core-Shell Hydrogels for PH Sensitive Drug Delivery Systems." 22:269–78.

Patel, Nachiket et al. 2016. "Development and Evaluation of a Calcium Alginate Based Oral Ceftriaxone Sodium Formulation." *Progress in Biomaterials* 5(2):117–33.

Prabu, Lakshmana and Suriyaprakash Tnk. 2017. "Nov Appro Drug Des Dev Role of Natural Polymers in Drug Delivery Systems as Challenging Ailments." *Mini Review* 3(1):1–5.

Puri, Munish. 2017. *Food Bioactives: Extraction and Biotechnology Applications*.

Radin, Shula, Tiffany Chen, and Paul Ducheyne. 2009. "The Controlled Release of Drugs from Emulsified, Sol Gel Processed Silica Microspheres." *Biomaterials* 30(5):850–58.

Rathore, Himanshi, Shalinee Prasad, and Satyawati Sharma. 2017. "Mushroom Nutraceuticals for Improved Nutrition and Better Human Health:

A Review." *PharmaNutrition* 5(2):35–46.

Tong, Zongrui et al. 2017. "Preparation, Characterization and Properties of Alginate/Poly(γ-Glutamic Acid) Composite Microparticles." *Marine Drugs* 15(4).

Wu, Jian-Yong. 2017. "Ultrasound-Assisted Extraction of Polysaccharides from Edible and Medicinal Fungi: Major Factors and Process Kinetics." *MOJ Food Processing & Technology* 4(2).

Xu, Wei et al. 2014. "Curcumin Loaded and Protective System Based on Complex of κ-Carrageenan and Lysozyme." *Food Research International* 59:61–66.

Zare-Akbari, Zhila et al. 2016. "PH-Sensitive Bionanocomposite Hydrogel Beads Based on Caborboxymethyl Cellulose/ZnO Nanoparticle as Drug Carrier." *International Journal of Biological Macromolecules* 93(October):1317–27.

Zhang, Lin, Chun Guang Li, Henry Liang, and Narsimha Reddy. 2017. "Bioactive Mushroom Polysaccharides : Immunoceuticals to Anticancer Agents." *J Nutraceuticals Food Sci.* 2(2):6.