Eco. Env. & Cons. 30 (October Suppl. Issue) : 2024; pp. (S14-S20)
Copyright@ EM International
ISSN 0971–765X

DOI No. http://doi.org/10.53550/EEC.2024.v30i06s.002

A Review on Trending Applications of Pullulan in Pharmaceutical Industry

K.M. Gaikwad, K.R. Jagtap and Y.R. Mulay*

Department of Microbiology, Anekant Education Society's (Autonomous), Tuljaram Chaturchand College of Arts, Science & Commerce, Baramati 413 102, Dist. Pune, M.S., India

(Received 1 March, 2024; Accepted 3 May, 2024)

ABSTRACT

Nowadays pullulan is an emerging as a biopolymer for various industrial applications. According to the global pullulan market research report 2024, the major market size is occupied by the pharmaceutical industry. Pullulan is a versatile polymer used worldwide due to its properties like biocompatibility, thin film formation, solubility, and flexibility. This review traced the trending applications of pullulan in pharmaceutical industry. Based on the study's findings, pullulan and its derivatives are often utilised in many applications such as targeted drug administration, molecular chaperon, tissue engineering, immunization, plasma expanders, and gene transfer. It involved in almost all industries and is trending as a sustainable material. In conclusion, pullulan is being increasingly recognized for its sustainability and eco-friendly nature.

Keywords : *Pullulan, Biopolymer, Sustainable*

Introduction

Biopolymers are the products obtained from natural sources like plants, animals and microbes. Recently biopolymers are getting more attention for industrial applications. Many biopolymers such as cellulose, chitosan, starch, collagen, gelatin, albumin, silk fibroin and alginate are used in pharmaceutical and biomedical applications. Easy availability, affordability, biocompatibility, biodegradability and renewability are the most attraction seeking properties of biopolymers. Generally used synthetic polymers like acrylic polymers, poly methyl methacrylate and poly hydroxyethyl methacrylate found non biodegradable. This environmental concern urges for the use of biodegradable and biocompatible biopolymers.

One exopolymeric substance that is mostly produced by the fungus *Aureobasidium* species- which looks like black yeast- is pullulan. The chemical formula of this substance is [α-D-Glucopyranosyl-(1→4)-α-D-Glucopyranosyl-(1→4)-α-D-Glucopyranosyl-(1→6)]n. The lack of branches and the linear structure are both caused by maltotriose repeating units (Singh *et al*., 2017). The outcomes include both α-(1→6) and α-(1→4) glycosidic linkages, enhanced solubility, and structural flexibility. The end product is a polymer with excellent shaping and spinning properties. The powdered form of amorphous pullulan is white and dry. There is no aroma, no flavour, and no taste to it. The US Food and Drug Administration have determined that Pullulan is safe (Singh *et al*., 2021). This natural polymer has been shown in several studies to be safe for consumption, non-toxic, and free of carcinogens; only a few examples include Raychaudhuri *et al.* (2020) and Trinetta *et al*. (2016). This polymer can degrade between 250 and 280 °C and has remarkable thermal stability, as reported by Farris *et al*. (2012). Farris *et al.* (2014) found that Pullulan dis-

(*Vice-Principal & Professor)

solves in water, formamide, and diluted alkali dimethyl sulfoxide, but it does not dissolve in organic solvents generated from alcohols. It makes a thick, colourless liquid with great adherence when combined with other binder components. Falsafi *et al.* (2023) states that it might form a thin layer that prevents oxygen from reaching the surface and can keep moisture in. One great alternative to the non-biodegradable polymers is Pullulan. This review aimed to highlight the potential therapeutic applications of Pullulan.

Applications of Pullulan in pharmaceutical industry

Biopolymers have wide use in the pharmaceutical industry. They are generally used for packaging, encapsulation, coating and in formulation of drug delivery systems. Cellulose, chitosan and Pullulan are largely used biopolymers for the pharmaceutical and biomedical applications. Pullulan works as the best vehicle for the delivery of targeted drugs, genes, and proteins because of its encapsulation capacity, retention ability, and flexibility in nature. Formulations of Pullulan such as nanoparticles, nanogels, nanomicelles, co-polymers, conjugates, micro particles, microspheres, hydrogels, electrospun fibers, and films are mostly used. The biocompatibility of pullulan makes it a promising polymer for biomedical and pharmaceutical applications.

The major applications of pullulan in pharmaceutical industry are as follows:

Pullulan in drug delivery systems

When it comes to medicines, one of the most crucial duties is drug distribution. Modern medicine favors focused medication distribution. The reason behind this is that, rather than aiming at the entire body or an organ, it focuses on transferring the medicine to a specific location. The pharmacodynamics, pharmacokinetics, and immunogenicity of medicinal substances are the primary areas of concentration in targeted medication administration. Conventional systems have drawbacks that this approach can eliminate such a short half-life, poor specificity, a poor therapeutic index, poor solubility, unstable, uncooperative patients, and negative side effects (Tewabe *et al.*, 2021). Similar to the food business, the pharmaceutical sector first uses biopolymers primarily as thickeners, binder, emulsifiers, texurizers, and moisture retaining agents. In vivo applications are a result of its usage in subsequent studies and advancements. Chitosan, starch, cellulose, and alginate are among the most common biopolymers used in the pharmaceutical industry. New studies show that pullulan might have uses in the pharmaceutical sector as well. As a polymer, pullulan has several useful characteristics, such as being flexible, elastic, strong, and stable. Entrapment, permeation, charge-based transportation, etc. are only a few of the various uses for pullulan derivatives (Kumar *et al.*, 2012).

Orodispersible films

The primary route of drug administration is oral preferred. Oral drug administration is moving towards the new technology called orally dispersible or disintegrating films. Orodispersible films are better than the hard capsules, tablets, and liquids to consume. Orodispersible films are taste masked to avoid the bitterness of the drug. Polyvinyl alcohol, maltotriose, methylcellulose and pullulan are widely used in preparations of these films. Pullulan found safe, edible, more soluble polymer and have acceptable mouthfeel. As pullulan is tasteless, it can be easily masked by other tastes and flavors. Taste masked iron loaded ordispersible films of pullulan are prepared for the treatment of iron deficiency mainly in anemic children and pregnant ladies (Gupta *et al*., 2023). A patients of high blood pressure that is hypertension takes antihypersensitive drug amlodipine besylate on daily basis. For patients compliance it is entrapped in pullulan and formed orally disintegrating films (Pezik *et al*., 2021).

Capsule formulation

The good film forming capacity, tastelessness and non toxicity, edible etc properties makes pullulan a good candidate for the formation of capsules. Recently pullulan was used for formulation of hard capsule for target usage (Kalmer *et al*., 2024). Zhou *et al.*, 2023 used plasticizers glycerol and sorbitol mixture to form soft capsule of pullulan. A carboxyl-modified pullulan nanocomposite used for hard capsule formation (Ding *et al*., 2023). Many researches also reported that pullulan can also used as microcapsules and capsule shell preparation.

Bio responsive and controlled drug release

Over an extended length of time, controlled drug release maintains a steady pace. Dissolution, diffusion, and osmosis are the primary processes that al-

low for control of drug release. Liu *et al.* (2024) developed spray-dried microparticles based on pullulan for regulated drug administration, while Shengan Hu *et al.* (2024) made enteric capsules utilising pullulan. Pullulan has the potential to be a bioresponsive delivery system for a range of therapies. For regulated insulin release, a glucose-sensitive carboxymethyl pullulan improves insulin's biological activity and bloodstream stability over the long term.

Tumor and cancer cell targeting

Kaneo *et al*., 2001 ; Research conducted by Constantin *et al*. (2007), Lu *et al*. (2002), and Lin *et al.* (2019) found that pullulan nanoparticles modified with cholesterol, pullulan with folate particles, and carboxymethyl pullulan nanoparticles enhanced drug entrapment and cancer cell targeting capabilities in various organs and tissues. In order to deliver hydrophobic medications to tumour cells specifically, Jung *et al*., 2003 and Kumar *et al*., 2012 used pullulan acetate formulations to encapsulate the pharmaceuticals.

Gene delivery

Foreign DNA is transferred to host cells using gene delivery in order to carry out gene therapy. Genome transfer agents often include polyamide dendrimers, cationic polymers, polyethylene imide, and calcium phosphate. The major problem with chemical methods is their low transfection effectiveness and toxicity to cells when dealing with big DNA. The biodegradability and biocompatibility of collagen, gelatin, and pullulan make them ideal for use in oral medication administration. One potential use for pullulan is as a gene carrier, as it is an effective agent for encapsulating genes. It prevents enzymes that break down DNA from damaging genes. Gene delivery makes extensive use of pullulan formulations including conjugates, micelles, nano carriers, cationic systems, and magnetic nanoparticles. Pullulan hydrogels were discovered by Gupta *et al*., 2004 to have sustained DNA release to cancer cells and high loading efficiency for plasmid DNA. Juan *et al*. (2007) used diethylaminoethylamine pullulan to transfer genes to cells in arteries or muscles. Using Pullulan spermine, researchers in Nagane *et al*. (2009) and Thakor *et al*. (2009) were able to transfer genes associated to neurons. Wang *et al*. (2014) replaced some of the folate in pullulan to make gene transfection more effective and to mute genes. Derivatives of pullulan, such as pullulan-g-poly(L-lysine), pullulan-protamine, and succinylated pullulan, have demonstrated efficacy in targeted gene delivery with reduced cytotoxicity (Kim *et al.,* 2010; Park *et al*., 2012; Liu *et al*., 2014).

Tissue engineering

Aspects of tissue engineering include organ regeneration, tissue healing, and transplantation. Cellulose and agarose are two examples of the biopolymers used extensively in tissue engineering due to their self-healing capabilities. Tissue engineering is a better fit for pullulan polymer because of its self-healing and polymerization capabilities. It could be useful in tissue engineering, wound healing, and preventing infections. Based on its biocompatibility, high water holding capacity, and strong strength, the material is very appropriate for tissue engineering (Chaouat *et al*., 2006; Hashimoto *et al*., 2015; Lack *et al.,* 2004 and Yang *et al*., 2011). For tissue engineering purposes, pullulan derivatives such as phosphorylated pullulan (Shiozaki *et al*., 2011), carboxylated pullulan (Li *et al.,* 2015), and pullulan-cellulose acetate (Atila *et al*., 2015) have been used. The principal usage of 3D scaffold pullulans was found to be bone, endothelial cell, and skin cell regeneration (Arora *et al.,* 2015; Bang *et al*., 2016). Aydogdu *et al.* (2016) demonstrated the successful healing of small to large bone lesions utilizing pullulan microspheres. Malekzadeh *et al*. (2024) reported the use of oxidized carboxymethylated pullulan for bone tissue regeneration. Yeasmin *et al.,* 2024 created and used wound dressings, self-healing materials, and pullulan and polyvinyl alcohol films. Wound dressings with chitosan-oxidized pullulan hydrogels infused with essential clove oil were utilised by Suflet *et al*., 2024 to avoid infections and speed up the healing process. The antimicrobial and antifungal capabilities of these hydrogels were demonstrated.

Vaccination

The vaccine needs protective carrier for delivery. Polymer pullulan can form micelle, microspheres with hydrophobic core helps in protective transfer of vaccine. Kageyama, *et al.,* 2013; I.G. Kong *et al.,* 2013 and Yulki *et al.,* 2012 reported that cholesteryl pullulan based NY-ESO-1 vaccine and cationized form of cholesteryl pullulan are used for immunization in esophagous cancer treatment and in mucosal respiratory infection. Hasegawa *et al.,* 2005 found

that cationized form of cholesteryl pullulan could conjugate tetanus toxoid and boosts the immune response.

Plasma expanders

In order to increase the plasma volume, a substance known as a plasma expander raises the osmotic pressure. Plasma expansion makes use of crystalloids and colloids. Plasma expanders are often made with albumin, gelatin, and dextran. Pullulan has excellent bio compatibility and is entirely safe for human use. It aids in blood circulation by keeping the intravascular osmotic pressure constant. In order to regulate the microcirculation of the blood, the index of blood circulation, the pace of cardiac contractions, and the cardiac output, Igarashi *et al.* (1983) proposed that Pullulan can be employed as a substitute for is ovolumetric blood. Shingel *et al.* documented gãmma-irradiated pullulan, which had a low viscosity, in 2002 for the purpose of expanding blood plasma. One possible alternative to blood plasma is pullulan and its derivatives, according to research published by Kulicke *et al*. (2006).

Molecular chaperon

Molecular chaperons are the agents used to control protein conformational changes within cells which encompass essential cellular processes such as proper folding of newly synthesized proteins, protein transportation and translocation, signal transduction, and maintaining protein quality control. Dimethyl sulphoxide, glycerol and phenylbutyric acid are used as molecular chaperons. Due to the inefficiency and other side effects there is need of biopolymer usage. As a molecular chaperone, the cholesterol-modified pullulan may self-aggregate into a hydrophobic core. It aids in the restoration of the natural form of denatured proteins by encasing them. Pullulan chaperons improve the heat stability of protein structures and the enzymatic activity of proteins. Proteins may be released from CHP-hyaluronan hydrogels in a denaturation-free manner, according to research by Hirakura *et al*., 2010. Enzyme aggregation can be prevented by CHP nanogels, according to Nomura *et al*., 2003; Sawada *et al*., 2010 and Sawada *et al*., 2011. Using heat-and light-sensitive CHP nanogels as molecular chaperones to improve citrate in syntheses activity was described by Hirakura *et al*., 2004.

Conclusion

In conclusion, this study found that pullulan is sustainable and biocompatible polymer for various pharmaceutical applications. It is majorly used in formation of orodispersible films and for targeted drug delivery. Protein and gene can be efficiently delivered by using pullulan based carriers. Pullulan is effectively used in tissue engineering due to its polymerizing capacity and self healing nature. The stability, safe encapsulation based transportability of pullulan is efficient character for its use in vaccination, plasma expander and molecular chaperons. Thus pullulan is the versatile biolpolymer for phamaceutical and biomedical applications.

Acknowledgement

The special thanks to Principal, Tuljaram Chaturchand College of Arts, Science & Commerce (Autonomous) Baramati, for funding this study under the Seed Money for Teachers Scheme 2023-24.

Conflict of Interest -None

References

Amrita, A., Arora, A. and Sharma, P. 2015. Pullulan-based composite scaffolds for bone tissue engineering: Improved osteo conductivity by pore wall mineralization. *Carbohydrate Polymers.* 123*:* 180-189.

Atila, D., Keskin, D. andTezcaner, A. 2015. Cellulose acetate based 3 dimensional electrospun scaffolds for skin tissue engineering applications. *Carbohydrate Polymers.* 133: 251-261.

Aydogdu, H., Keskina, D., Baran, E.T. andTezcaner, A. 2016. Pullulan microcarriers for bone tissue regeneration. *Materials Science and Engineering: C. 63:* 439-449.

Bang, S., Lee, E., Ko, Y.G., Kim, W.I. and Kwon, O.H. 2016. Injectable pullulan hydrogel for the prevention of postoperative tissue adhesion. *International Journal of Biological Macromolecules.* 87: 155-162.

Carullo, D., Rovera, C., Bellesia, T., Buyukta, D., Ghaani, M., Santo, N., Romano, D., & Farris, S. 2023. Acid-derived bacterial cellulose nanocrystals as organic filler for the generation of high-oxygen barrier bionanocomposite coatings. Sustainable Food Technology, 1(1), 941-950. https://doi.org/10.1039/D3FB00147D

Chaouat, M., Le Visage, C., Autissier, A., Chaubet, F. and Letourneur, D. 2006. The evaluation of a small-diameter polysaccharide-based arterial graft in rats.

Biomaterials. 27(25): 5546-5553.

Constantin, M., Fundueanu, G., Bortolotti, F., Cortesi, R., Ascenzi, P. and Menegatti, E. 2003. A novel multicompartimental system based on aminated poly(vinyl alcohol). *Journal of Controlled Release.* 91(1-2): 173-187.

Ding, Y., Zhong, B., Yang, T., Zhang, F., Liu, C., & Chi, Z. 2023. Carboxyl-modified nanocellulose (cNC) enhances the stability of cNC/pullulan bionanocomposite hard capsule against moisture variation. SSRN Electronic Journal. https://doi.org/10.2139/ssrn.4606197

Erceg, T., Šovljanski, O., Tomi´c, A., A´cimovi´c, M., Stupar, A. and Baloš, S. 2024. Comparison of the Properties of Pullulan-Based Active Edible Coatings Implemented for Improving Sliced Cheese Shelf Life. *Polymers.* 16(2): 178.

Esra, P., Tugba, G., Selma and Vural, Ä. 2021. Development and characterization of pullulan-based orally disintegrating films containing amlodipine besylate, *European Journal of Pharmaceutical Sciences.* 156: 105597.

Farris, S., Introzzi, L., Fuentes-Alventosa, J.M., Santo, N., Rocca, R. and Piergiovanni, L. 2012. Self-assembled pullulan-silica oxygen barrier hybrid coatings for food packaging applications. *Journal of Agricultural Food Chemistry.* 60(3): 782-790.

Farris, S., Unalan, I.U., Introzzi, L., Fuentes-Alventosa, J.M. and Cozzolino, C.A. 2014. Pullulan-based films and coatings for food packaging: present, applications, emerging opportunities and future challenges. *Journal of Applied Polymers.* 131: 40539.

Gupta, M.S., Kumar, T.P., Reddy, D., Pathak, K., Gowda, D.V., Babu, A.V.N., Aodah, A.H., Khafagy, E.S., Alotaibi, H.F. and Abu Lila, A.S. 2023. Development and Characterization of Pullulan-Based Orodispersible Films of Iron. *Pharmaceutics*. 15: 1027.

Gupta, M., & Gupta, A. 2004. Hydrogel pullulan nanoparticles encapsulating pBUDLacZ plasmid as an efficient gene delivery carrier. Journal of Controlled Release, 99(1), 157-166.

Hasegawa, U., Nomura, S.I.M., Kaul, S.C., Hirano, T. and Akiyoshi, K. 2005. Nanogel-quantum dot hybrid nanoparticles for live cell imaging. *Biochemical and Biophysical Research Communications.* 331(4): 917-921.

Hashimoto, Y., Mukai, S.A., Sawada, S.I., Sasaki, Y. and Akiyoshi, K. 2015. Nanogel tectonic porous gel loading biologics, nano carriers, and cells for advanced scaffold. *Biomaterials.* 37: 107-115

Hirakura, T., Nomura, Y., Aoyama, Y. and Akiyoshi, K. 2004. Photoresponsivenanogels formed by the self-assembly of spiropyrane-bearing pullulan that act as artificial molecular chaperones. *Biomacromolecules.* 5(5): 1804-1809

Hirakura, T., Yasugi, K., Nemoto, T., Sato, M., Shimoboji, T., Aso, Y. and Akiyoshi, K. 2010. Hybrid hyaluronan hydrogel encapsulating nanogel as a protein nanocarrier: New system for sustained delivery of protein with a chaperone-like function. *Journal of Controlled Release.* 142(3): 483-489.

Igarashi, T., Nomura, K., Naito, K. and Yoshida, M. 1983. Plasma extenders. US Patent 4: 370,472.

Juan, A.S., Ducrocq, G., Hlawaty, H., Bataille, I., Guenin, E., Letourneur, D. and Feldman, L. J. 2007. Tubular cationized pullulan hydrogels as local reservoirs for plasmid DNA. *Journal ofBiomedical Materials Research Part A.* 83(A): 819-827

Jung, S.W., Jeong, Y.I. and Kim, S.H. 2003. Characterization of hydrophobized pullulan with various hydrophobicities. *International Journal of Pharmaceutics.* 254(1): 109-121.

Kageyama, S., Wada, H., Muro, K., Niwa, Y., Ueda, S., Miyata, H. and Shiku, H. 2013. Dose-dependent effects of NY-ESO-1 protein vaccine complexed with cholesteryl pullulan (CHP-NY-ESO-1) on immune responses and survival benefits of esophageal cancer patients. *Journal of Translational Medicine.* 11(1): 246.

Kaneo, Y., Tanaka, T., Nakano, T. and Yamaguchi, Y. 2001. Evidence for receptor-mediated hepatic uptake of pullulan in rats. *Journal of Control Release.* 70: 365-373.

Kim, H. and Na, K. 2010. Evaluation of succinylated pullulan for long-term protein delivery in poly(lactide-co-glycolide) microspheres. *Macromolecular Research.* 18: 812-819.

Kong, I. G., Sato, A., Yuki, Y., Nochi, T., Takahashi, H., Sawada, S. and Yamamoto, M. 2013. Efficient targeted pDNA/siRNA delivery with folate-low molecular-weight polyethyleneimine-modified pullulan as non-viral carrier. *Materials Science and Engineering: C.* 34: 98-109.

Kulicke, W.M. and Heinze, T. 2006. Improvements in polysaccharides for use as blood plasma expanders. *Macromolecular Symposia.* 231(1): 47-59.

Kumar, B.S., Kumar, M.G., Suguna, L., Sastry, T.P. and Mandal, A.B. 2012. Pullulan acetate nanoparticles based delivery system for hydrophobic drug. *International Journal of Pharma Bio Sciences.* 3(1): 24-32.

Kumar, D., Saini, N. and Pandit, V. 2012. An insight to pullulan: a biopolymer in pharmaceutical approaches. *International Journal of Basic & Applied Science.* 1(3): 202-219.

Lack, S., Dulong, V., Le Cerf, D., Picton, L. and Argillier, J.F. 2004. Hydrogels based on pullulan cross linked with sodium trimetaphosphate (STMP): Rheological study. *Polymer Bulletin.* 52(5) : 429-436.

Li, H., Yang, J., Hu, X., Liang, J., Fan, Y. and Zhang, X. 2011. Superabsorbent polysaccharide hydrogels based on pullulan derivative as antibacterial release wound dressing. *Journal of Biomedical Materials Research Part A.* 98A: 31-39.

Li, X., Xue, W., Zhu, C., Fan, D., Liu, Y. and Ma, X. 2015.

Novel hydrogels based on carboxyl pullulan and collagen crosslinking with 1, 4-butanediol diglycidyl ether for use as a dermal filler: Initial *in vitro* and in vivo investigations. *Materials Science and Engineering: C.* 57: 189-196.

Lin, K., Yi, J., Mao, X., Wu, H., Zhang, L.M. and Yang, L. 2019. Glucose-sensitive hydrogels from covalently modified carboxylated pullulan and concanavalin A for smart controlled release of insulin. *Reactive and Functional Polymers.* 139: 112-119.

Liu, T., Gong, X., Cai, Y., Li, H.Y. and Forbes, B. 2024. Pullulan-Based Spray-Dried Mucoadhesive Microparticles for Sustained Oromucosal Drug Delivery. *Pharmaceutics.* 16: 460.

Liu, Y., Wang, Y., Zhang, C., Zhou, P., Liu, Y., An, T., Sun, D., Zhang, N. and Wang, Y. 2014. Core-shell nanoparticles based on pullulan and poly(â-amino) ester for hepatoma-targeted codelivery of gene and chemotherapy agent. *Applied Materials and Interfaces. 6(*21): 18712-18720.

Lu, Y. and Low, P.S. 2002. Folate-mediated delivery of macromolecular anticancer therapeutic agents. *Advanced Drug Delivery Reviews.* 54(5): 675-693.

Nagane, K., Kitada, M., Wakao, S., Dezawa, M. and Tabata, Y. 2009. Practical induction system for dopamine-producing cells from bone marrow stromal cells using spermine-pullulan mediated reverse transfection method. *Tissue Engineering Part A. 15:* 1655-1665.

Nomura, Y., Ikeda, M., Nozomi, M., Yamaguchi, N., Aoyama, Y. and Akiyoshi, K. 2003. Protein refolding assisted by self-assembled nanogels as novel artificial molecular chaperone. *FEBS Letters, 553(*1-2): 271-276.

Park, J.S., Park, J.K., Nam, J.P., Kim, W.S., Choi, C., Kim, M.Y. and Nah, J.W. 2012. Preparation of pullulan-g-poly(L-lysine) and its evaluation as a gene carrier. *Macromolecular Research.* 20: 667-672.

Pezik, E., Gulsun, T., Sahin, S., & Vural, 0´. 2021. Development and characterization of pullulan-based orally disintegrating films containing amlodipine besylate. European Journal of Pharmaceutical Sciences, 156, 105597.

Ramezani Kalmer, R., Karimi, A., Ramezanalizadeh, H., Ghanbari, M., Samandarian, D., Sadjadinia, A., Gholizadeh Dogaheh, S. and Moosavi, S. 2024. Design and preparation of a novel pullulan hard capsule formulation: A promising green candidate and study of crucial capsule features. *Heliyon*. 10(7): e28969.

Raychaudhuri, S.K., Abria C., Mitra A., Raychaudhuri S.P. 2020. Functional significance of MAIT cells in psoriatic arthritis,*Cytokine*. 125: 154855.

Sawada, S.I. and Akiyoshi, K. 2010. Nano-encapsulation of lipase by self-assembled nanogels: Induction of high enzyme activity and thermal stabilization. *Macromolecular Bioscience.* 10(4): 353-358.

Sawada, S.I., Sasaki, Y., Nomura, Y. and Akiyoshi, K. 2011. Cyclodextrin-responsive nanogel as an artificial chaperone for horseradish peroxidase. *Colloid and Polymer Science.* 289(6): 685-691.

Shengan Hu, Chongmei Xu, Yinghui Zhang, Yue Du, Jinbao Tang, Lu Chen. 2024. Preparation of enteric capsules with pulsatile drug delivery potential using pullulan and polyacrylic acid resin III, Arabian Journal of Chemistry, Volume 17, Issue 4,2024,105691,ISSN 1878-5352, https://doi.org/10.1016/j.arabjc.2024.105691.

Shingel, K.I. and Petrov, P.T. 2001. Hydrodynamic and molecular characteristics of gamma-irradiated pullulan. *Polymer Science Series B.* 43(2): 81-84.

Shingel, K.I. and Petrov, P. T. 2002. Behavior of ã-ray-irradiated pullulan in aqueous solutions of cationic (cetyltrimethylammonium hydroxide) and anionic (sodium dodecyl sulfate) surfactants. *Colloid and Polymer Science.* 280(2): 176-182.

Shiozaki, Y., Takahata, T., Yoshida, A., Nakamura, M., Yoshida, Y., Tanaka, M., Matsukawa, A. and Ozaki, T. 2011. Novel bioadhesive material: 'Phosphorylated pullulan' bioadhesive for hard-tissue reconstruction. *Orthopaedic Research Society Annual Meeting*, Poster No. 1084.

Singh, R.S., Kaur, N., Rana,V. and Kennedy, J.F. 2017. Pullulan: A novel molecule for biomedical applications. *Carbohydrate Polymers.* 171: 102-121.

Singh, P., Mishra, B., Mandal, S. K., Agrawal, D. C., & Kruthiventi, C. 2021. An insight into pullulan and its potential applications. Polysaccharides of Microbial Origin, 1-32. https://doi.org/10.1007/978-3-030-35734-4_15-1

Suflet, D.M., Constantin, M., Pelin, I.M., Popescu, I., Rimbu, C.M., Horhogea, C.E. and Fundueanu, G. 2024. Chitosan–Oxidized Pullulan Hydrogels Loaded with Essential Clove Oil: Synthesis, Characterization, Antioxidant and Antimicrobial Properties. *Gels.* 10(4): 227.

Tewabe, A., Abate, A., Tamrie, M., Seyfu, A. and Abdela Siraj, E. 2021. Targeted Drug Delivery - From Magic Bullet to Nanomedicine: Principles, Challenges, and Future Perspectives. *J Multidiscip Healthc*. 5(14):1711-1724. Doi: 10.2147/JMDH.S313968. PMID: 34267523; PMCID: PMC8275483.

Thakor, D.K., Teng, Y.D. and Tabata, Y. 2009. Neuronal gene delivery by negatively charged pullulan-spermine/DNA anioplexes. *Biomaterials. 30:* 1815-1826.

Trinetta, V. and Cutter, C.N. 2016. Pullulan: A Suitable Biopolymer for Antimicrobial Food Packaging Applications. In: *Antimicrobial Food Packaging* (pp. 385–397). Elsevier Inc.

Yeasmin, S., Jung, J.H., Lee, J., Kim, T.Y., Yang, S. B., Kwon, D.J., Kim, M.O. and Yeum, J. H. 2024. A Novel Fabrication of Heterogeneous Saponified Poly(Vinyl

Alcohol)/Pullulan Blend Film for Improved Wound Healing Application. *International Journal of Molecular Sciences. 25:* 1026

Zhou, K., Yang, Y., Zheng, B.; Yu, Q., Huang, Y., Zhang, N., Rama, S.M., Zhang, X., Ye, J. and Xiao, M. 2023. Enhancing Pullulan Soft Capsules with a Mixture of Glycerol and Sorbitol Plasticizers: A Multi-Dimensional Study. *Polymers*. 15: 2247.