pH-sensitive polymer-based carriers as a useful approach for oral delivery of therapeutic protein: A review

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Abstract

There are a lot of proteins and enzymes in the human body and their dysfunction can lead to disease. The use of proteins as a drug is common in various diseases such as diabetes. Proteins are hydrophilic molecules whose spatial structure is critical to their correct function. There are different ways to administration of proteins. Protein structures are degraded by gastric acid and enzymes in the gastrointestinal tract and also have a small ability to permeation from the gastrointestinal epithelium due to their large hydrophilic nature. Therefore, their oral use has limitations. Since the oral use of drugs is one of the best and easiest routes for patients, many studies have been done to increase the stability, penetration and ultimately increase the bioavailability of proteins through oral administration. One of the studied strategies for oral delivery of protein is the use of pH-sensitive polymer-based carriers. These carriers use different pH-sensitive polymers such as eudragit, chitosan, dextran, and alginate. The use of pH-sensitive polymer-based carriers by protecting the protein from stomach acid (low pH) and degrading enzymes, increasing permeability, and maintaining the spatial structure of the protein leads to increased bioavailability. In this review, we focus on the various polymers used as pH-sensitive polymer-based carriers for the oral delivery of proteins.

Keywords: pH-sensitive polymer-based carriers; Protein delivery; Oral administration; Bioavailability

1 Introduction

Oral administration of the drug is the most choice of administration route; due to its simplicity, suitability, less associated pain, and better patient compliance especially when used for the treatment of chronic therapy (1, 2). This route also has some advantages, such as flexible dosing, fewer person-hours and training requirement for staff, reduced costs associated with clinic or hospital visits or direct contact requirements and related expenses, which is attractive to the pharmaceutical industry (1). An important type of drug that helps patients most in new therapies is therapeutic protein and used for the treatment of several diseases such as diabetes, cancers, etc. (3). Protein as large and hydrophilic molecules and sensitive to acid and enzyme degradation have low bioavailability when administered orally through the GI tract; therefore, other delivery routes such as subcutaneously or intravenously, to achieve higher bioavailability are usually adopted (4-6). pH-sensitive polymer-based carriers (pH-SCs) are typically used in the oral route to overcome the low bioavailability. Such pH-SCs for oral drug delivery are used to enhance the stability of the drug in the low-pH of the stomach and allowing their controlled release in intestines at higher pH (7). pH-SCs have various benefits such as controlled drug release, enhanced solubility and stability, protection from acid and enzyme, increase efficacy and reduced toxicity (8). For example, Abdallah Makhlof et al., (9) designed novel pH-sensitive chitosan (CS) nanoparticles and Han et al., (10) developed hyaluronic acid (HA) pH-sensitive polymer-based carriers for oral insulin delivery. The main goal of the present review was to summarize various polymers used as pH-sensitive polymer-based carriers reported used for oral delivery of protein.

2 pH-sensitive polymer-based carriers

pH-SCs are chemical molecules/compounds that use as oral drug delivery to enhance the stability and control release of the drug delivered through the stomach (1). According to the

structure, pH-sensitive carriers are categorized mainly as polymeric compounds that may someone is also known as pH-sensitive nanoparticles carriers or pH-sensitive hydrogel carriers (11, 12).

2.1 Nanoparticles (NPs) carriers

NPs are solid particles with a size in the range of 10-1000nm. The main purpose of designing such a delivery system is to control particle size and surface properties to permit the suitable rate of release of the drug and be used for oral delivery of proteins (13, 14). They include the following:

2.1.1 Polyanions based pH-sensitive polymer-based nanoparticle

Eudragits are poly (methacrylic acid-co-methyl acrylate) copolymers that are widely used for pH-sensitive NPs formulation. Several types of Eudragits exist, such as Eudragit L100 and Eudragit S100, that separately dissolve at pH>5.5 and pH>7.0, respectively (7, 15). This range of pH illustrates that they are suitable for drug delivery to the duodenal and ileal parts of the small intestine. Dai et al., worked on polyanions pH-sensitive NPs to improve the bioavailability of cyclosporine A (CyA). They showed that approximately 99% of the drug was encapsulated with S100 NPs (16).

2.1.2 Polycations based pH-sensitive polymer-based nanoparticle

Chitosan, a cationic polymer used for preparing pH-sensitive NPs and enhances the absorption of these particles by the intestinal epithelium. There are several modified CS derivatives such as thiolated CS, PEGylated CS, and carboxylate CS that used to improve CS properties for oral delivery (17). Modified CS derivatives developed and the properties of CS significantly improved by various methods such as copolymerization, free radical graft, N-substitution and O-substitution (18). Intestinal penetration and improved antigen stability upon using CS NPs and antigen-cyclodextrin enhanced. These NPs could be promising antigen-delivery systems

for oral vaccination (19). Mumuni et al., research also showed that the formation of insulin CS NPs can be a potentially safe approach to protect insulin from the acidic environment of the stomach (20).

2.1.3 Mixed polyanions and polycations pH-sensitive polymer-based Nanoparticles

The combination of polyanions and polycations NPs can be more useful because they do not require a homogenizer or a cross-linker. Therefore protein-containing NPs drugs can be prepared using two oppositely charged polymers, which prevent the denaturation of the protein drug and improve oral absorption and uptake in the certain region of GI such as the colon (21). NPs systems composed of a positive-charged and a negative-charged polymer have been developed, such as CS mixing with Eudragit (22-24). Recently, layer by layer (LBL) coated NPs have attracted considerable attention (15, 25). Zhou et al. used LBL chitosan/alginate coatings on poly(lactide-co-glycolide) NPs for antifouling protection and folic acid-binding (26). Sarmento et al. worked on alginate and CS nanoparticles for oral delivery of insulin and reported improved oral absorption and bioactivity (27). As a way to pass interferon-alpha (INFα), the tight junctions of the GI epithelium, pH-SCs nanoparticles improved oral delivery of protein as chemotherapeutic agent (28).

Some studies of the different pH-SCs for improved oral bioavailability of proteins are summarized in table1.

Table1. Some Examples of different studies that use of pH-SCs for oral administration of proteins

Protein	A main polymer of pH-SCs	Research object	Main result	Ref.
Calcitonin	Eudragit P-4135F	Rat model	After 8-12 h, the relative pharmacological effect became most extreme based on the oral delivery by pH-SCs	(29)
Insulin	Chitosan/alginate coated polyelectrolyte complexes	Diabetic rat	The insulin-loaded NPs can effectively reduce blood sugar levels and prolong the release of insulin after the oral route.	(30)
Bovine serum albumin (BSA)	Eudragit L100–55 (EGAC-BSA)	Rat model	Oral use of BSA loaded pH-SCs significantly enhanced the intestinal permeation of BSA compared to free BSA	(31)
Low-molecular-weight heparin (LMWH)	Eudragit P4135F	*In Vitro*	Observed an efficient LMWH encapsulation, and a pH-controlled drug release	(32)
Interferon-α	Poly(methacrylic acid-grafted-ethylene glycol)	*In Vitro*	Increase the permeation of IFN-α through the tight junctions of the GI epithelium model	(33)
Insulin	Alginate/Gum Tragacanth	*In Vitro*	Retention of nearly all of the trapped insulin in a simulated gastric system and controlled release of insulin in a simulated intestinal buffer	(34)

Cp1-11 peptide/insulin	CS/Alginate	Diabetic rat	The oral delivery of insulin appeared to show excellent hypoglycemic effect and higher bioavailability of insulin	(35)
Insulin/heparin sodium	CS and Methacrylic acid	Diabetic rat	Oral delivery of NPs showing the relief of diabetic symptoms	(36)
Cyclosporine A	Eudragit S100 and Sylysia 350	Rat model	The findings of a comparative pharmacokinetic analysis showed a relative bioavailability of 90.8 percent for the optimized carrier	(37)
Insulin	Trimethyl CS and fucoidan	*In Vitro*	The NPs were able to modify the Caco-2 intestinal epithelial cell monolayer barrier and enhance paracellular insulin transport across the intestinal barrier	(38)
Recombinant protein as the subunit of the vaccine	Thiolated Cellulose Acetate Phthalate	BALB/c mice	Oral immunization of a recombinant protein effectively delivered to Peyer's patches eliciting mucosal IgA response	(39)
Insulin	PGLA, alginate and the positively charged CS	Diabetic rats	A major hypoglycemic effect only 1 h after gavage and continuous release of insulin for at least 6 h was observed	(40)
BSA	Carboxymethyl-β-cyclodextrin grafted CS	*In Vitro*	The drug-loaded carriers showed controlled release profiles and the amount of BSA released from the carriers was much higher in simulated intestinal fluid	(41)
Protein as vaccine	Alginate-CS	*In Vitro*	Significantly increase the internalization of proteins at the Caco2 and macrophage cells	(42)

Insulin	Carboxymethyl Starch/Poly(2-isobutyl-acrylic acid)	Diabetic rats	The oral bioavailability of the insulin-loaded microgels enhanced 23–38 times compared to insulin solution	(43)

2.2 Hydrogel

Hydrogels have various properties of their structure that can be suitable for a specific therapeutic. Cross-linked hydrogel networks protect drugs from unsuitable environments, especially from low pH and can respond to pH and released drugs at higher pH (12, 44, 45). There is two main polymer type that used for preparing pH-sensitive hydrogel carriers: 1) synthetic polymer 2) natural materials. synthetic polymer for example carboxylic acid-containing polymers like polyacrylic acid (PAA) and polymethacrylic acid (PMAA) have some advantageous features, including pH-sensitivity, enzyme inhibition, mucoadhesion, and open epithelial tight junctions that can increase the bioavailability of encapsulated drug (46). Mundargi et al. methacrylic acid and polyvinyl alcohol (PVA) carrier investigated for oral controlled drug delivery (47). a thermal and pH-sensitive hydrogel prepared by Yue et al., for the controlled and targeted delivery of the 5-Fluorouracil as an anti-cancer drug by PAA, the obtained hydrogels showed an excellent thermal and pH-sensitivities. According to the results of release profiles and cytotoxicity of hydrogels, they may have the potential to be used in some formulations for colon cancer delivery (48). Natural polymers such as alginate, HA, and CS are usually used for oral drug delivery due to their biocompatibility and physicochemical properties (49). Modification HA with carboxylic groups was developed to optimize the system for drug delivery to the colon with the pH-sensitive release (50). Abbasi et al. worked on a novel biodegradable pH-sensitive hydrogel for specific delivery of sulfasalazine in ulcerative colitis. They showed that pH-sensitive hydrogels are capable of effective drug delivery to the colon (51). In the following, some natural polymers used in the preparation of pH-sensitive hydrogel carriers are presented.

2.2.1 Chitosan

This cationic polymer is extensively used for drug delivery and pH-sensitive carriers (52). CS is safe to increase the absorption of the drug from the intestine, due to its pH-sensitivity,

biocompatibility, and mucoadhesion properties (53-55). Chemical modifications, such as trimethylated, thiolate, and carboxymethyl CS, have been studied for oral delivery of BSA (56, 57). The intestinal absorption of insulin with CS nanoparticles evaluated by Panet al. that result showed that insulin aggregation occurred at up to 80%, and its release *in vitro* showed a tremendous initial burst with a pH-sensitivity property (58).

2.2.2 Alginate

Alginate is a biodegradable, non-toxic, mucosal immunogenic and immune-deficient polymers (53). In low pH or divalent cations environments, alginate is capable to form a gel complex (59, 60). Alginate blended with water-soluble CS (N, O-carboxymethyl chitosan, NOCC) to obtain microencapsulated beads for the delivery of BSA. The result showed excellent pH-sensitive carriers for bioactive drug delivery in the intestine (61).

2.2.3 Dextran

Dextran is an extracellular polysaccharide produced by some bacteria. It is biodegradable and biocompatible and contains hydroxyl groups that allow a wide range of chemical manipulations (62). This polymer in combination with insulin has been investigated to improve pharmacokinetic and pharmacodynamics properties. In a study performed by Lopez et al., insulin was encapsulated in the CS-coated alginate core to make insulin-loaded alginate/insulin dextran nanoparticles (63). Polymersome synthesized based on amphiphilic copolymers of dextran and poly(lactic-co-glycolic acid) (PLGA) for the encapsulation of insulins showed a significant increase in the encapsulation efficiency of insulin (64).

2.2.4 Gelatin

Gelatin is a natural protein-polymer used for both oral and pulmonary insulin delivery and controlled release of protein (65, 66). It has several functional groups and has some hydrophobic properties. The release of growth factors from gelatin matrices was evaluated and

showed that the gelatin hydrogel system facilitates the release of growth factor protects and the protein from denaturing which seems to be applicable for any charged biomacromolecules (67).

2.2.5 Hyaluronic acid

HA is attractive for use in the drug delivery system due to its pH-sensitivity and protecting properties during oral administration of proteins (68, 69). Copolymer combined with HA was prepared for delivery of α-chymotrypsin that observed increases the percentage of chymotrypsin activity and this combined copolymer can preserve its activity under simulated gastric condition (70).

2.2.6 Polylactic co glycolic acid

PLGA is one of the most important drug carriers used for controlled release. PLGA breaks down to produce glycolic acid and lactic acid, which are metabolized by the body (71). This biodegradable system can be utilized as an oral drug delivery system for insulin (72). The various method of encapsulation of insulin in PLGA was investigated and its release over prolongs duration compare with the formulation that prepared by the anhydrous encapsulation method and shows the effectivity treatment of diabetic in rats significantly increased (73).

2.2.7 Polyvinyl alcohol

PVA is a biodegradable polymer with low toxicity and thermal stability (74). Like most synthetic polymers, it has a high level of mechanical strength. It can be mixed with natural polymers, resulting in a novel drug delivery system for enhanced features (75). BSA effectively loaded in PVA carrier (76).

2.2.8 Pluronic

Pluronics or poloxamers are polymers that are insoluble in water that are tasteless, odourless and waxy white granules that have the specific feature of being a thermal gel (77). Because of its thermal sensitivity, pluronic acid can be used in the production of injectable gels (78). The pluronic matrix also allows for the control of insulin release, and its nanoparticle may,

therefore, be suitable for use as a sustained insulin delivery system. Xiang et al., (79) investigated vesicles from pluronic/poly (lactic acid) block copolymer as new carriers for oral delivery of insulin and proved that polylactic acid -[polyethylene oxide-propylene oxide-polyethylene oxide]-polylactic acid (PLA-F127-PLA) could be effective carriers for oral delivery of insulin and used for prolonged hypoglycaemic effect.

3 Conclusion

Peptides and proteins play different roles in the human body and have great importance. Defects and disorders in some proteins cause various diseases, so the use of peptides and proteins as drugs is one way to treat those diseases. There are different routes for the administration of proteins such as intravascular, intramuscular, oral, etc. The oral route is preferred and has better compliance in most patients due to its unique characteristics and ease of use. However, due to the structure of proteins and the environment of the gastrointestinal tract, the possibility of using this route for the administration of protein is limited. The large hydrophilic structure of proteins, digestive enzymes, and stomach acidic environment are the main barriers to the oral use of proteins. The use of pH-sensitive carriers while protecting the protein from degradation by acid and digestive enzymes in the stomach, and increased protein permeability, thus lead to increases in its bioavailability. The use of pH-sensitive systems as a strategy in the oral delivery of some proteins for example insulin, LMWH, calcitonin, etc. shows improved their bioavailability. Therefore, in the future oral delivery of peptides and proteins by pH-sensitive carriers should be considered by researchers, and the development of this carrier should lead to the production of various oral pharmaceutical dosage forms of protein.

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5 Abbreviations

BSA	Bovine serum albumin
CS	Chitosan
CyA	Cyclosporine A
GI	Gastrointestinal
HA	Hyaluronic acid
INFα	Interferon-alpha
LBL	Layer by layer
LMWH	Low-molecular-weight heparin
NOCC	N, O-carboxymethyl chitosan
NPs	Nanoparticles
PAA	Polyacrylic acid
pH-SCs	pH-sensitive polymer-based carriers
PLA	Polylactic acid
PLGA	Poly(lactic-co-glycolic acid)
PMAA	Polymethacrylic acid
PVA	Polyvinyl alcohol

Compliance with Ethical Standards

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References

1. Liu L, Yao W, Rao Y, Lu X, Gao J. pH-Responsive carriers for oral drug delivery: challenges and opportunities of current platforms. Drug Delivery. 2017;24(1):569-81.

2. Homayun B, Lin X, Choi H-J. Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. Pharmaceutics. 2019;11(3):129.

3. Lagassé HAD, Alexaki A, Simhadri VL, Katagiri NH, Jankowski W, Sauna ZE, et al. Recent advances in (therapeutic protein) drug development. F1000Res. 2017;6:113-.

4. Indermun S, Luttge R, Choonara YE, Kumar P, Du Toit LC, Modi G, et al. Current advances in the fabrication of microneedles for transdermal delivery. Journal of controlled release. 2014;185:130-8.

5. Branden CI, Tooze J. Introduction to protein structure: Garland Science; 2012.

6. Chen Y, Li P, Modica JA, Drout RJ, Farha OK. Acid-Resistant Mesoporous Metal-Organic Framework toward Oral Insulin Delivery: Protein Encapsulation, Protection, and Release. Journal of the American Chemical Society. 2018;140(17):5678-81.

7. Ghaffar A, Yameen B, Latif M, Malik MI. pH-sensitive drug delivery systems. Metal Nanoparticles for Drug Delivery and Diagnostic Applications: Elsevier; 2020. p. 259-78.

8. Wang JJ, Zeng ZW, Xiao RZ, Xie T, Zhou GL, Zhan XR, et al. Recent advances of chitosan nanoparticles as drug carriers. International journal of nanomedicine. 2011;6:765.

9. Makhlof A, Tozuka Y, Takeuchi H. Design and evaluation of novel pH-sensitive chitosan nanoparticles for oral insulin delivery. European Journal of Pharmaceutical Sciences. 2011;42(5):445-51.

10. Han L, Zhao Y, Yin L, Li R, Liang Y, Huang H, et al. Insulin-loaded pH-sensitive hyaluronic acid nanoparticles enhance transcellular delivery. Aaps Pharmscitech. 2012;13(3):836-45.

11. Karimi M, Eslami M, Sahandi-Zangabad P, Mirab F, Farajisafiloo N, Shafaei Z, et al. pH-Sensitive stimulus-responsive nanocarriers for targeted delivery of therapeutic agents. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology. 2016;8(5):696-716.

12. Lima DS, Tenório-Neto ET, Lima-Tenório MK, Guilherme MR, Scariot DB, Nakamura CV, et al. pH-responsive alginate-based hydrogels for protein delivery. Journal of Molecular Liquids. 2018;262:29-36.

13. Yi G, Hong SH, Son J, Yoo J, Park C, Choi Y, et al. Recent advances in nanoparticle carriers for photodynamic therapy. Quantitative imaging in medicine and surgery. 2018;8(4):433.

14. Xiong K, Zhou L, Wang J, Ma A, Fang D, Xiong L, et al. Construction of food-grade pH-sensitive nanoparticles for delivering functional food ingredients. Trends in Food Science & Technology. 2020;96:102-13.

15. Cazorla-Luna R, Martín-Illana A, Notario-Pérez F, Bedoya LM, Tamayo A, Ruiz-Caro R, et al. Vaginal polyelectrolyte layer-by-layer films based on chitosan derivatives and Eudragit® S100 for pH responsive release of tenofovir. Marine drugs. 2020;18(1):44.

16. Dai J, Nagai T, Wang X, Zhang T, Meng M, Zhang Q. pH-sensitive nanoparticles for improving the oral bioavailability of cyclosporine A. International journal of pharmaceutics. 2004;280(1-2):229-40.

17. Nogueira-Librelotto DR, Scheeren LE, Macedo LB, Vinardell MP, Rolim CM. pH-Sensitive chitosan-tripolyphosphate nanoparticles increase doxorubicin-induced growth inhibition of cervical HeLa tumor cells by apoptosis and cell cycle modulation. Colloids and Surfaces B: Biointerfaces. 2020;190:110897.

18. Wang J, Wang L, Yu H, Zain ul A, Chen Y, Chen Q, et al. Recent progress on synthesis, property and application of modified chitosan: An overview. International Journal of Biological Macromolecules. 2016;88:333-44.

19. He M, Zhong C, Hu H, Jin Y, Chen Y, Lou K, et al. Cyclodextrin/chitosan nanoparticles for oral ovalbumin delivery: Preparation, characterization and intestinal mucosal immunity in mice. Asian Journal of Pharmaceutical Sciences. 2019;14(2):193-203.

20. Mumuni MA, Kenechukwu FC, Ofokansi KC, Attama AA, Díaz DD. Insulin-loaded mucoadhesive nanoparticles based on mucin-chitosan complexes for oral delivery and diabetes treatment. Carbohydrate Polymers. 2020;229:115506.

21. Jelvehgari M, Zakeri-Milani P, Siahi-Shadbad MR, Loveymi BD, Nokhodchi A, Azari Z, et al. Development of pH-sensitive insulin nanoparticles using Eudragit L100-55 and chitosan with different molecular weights. Aaps Pharmscitech. 2010;11(3):1237-42.

22. Sonaje K, Chen Y-J, Chen H-L, Wey S-P, Juang J-H, Nguyen H-N, et al. Enteric-coated capsules filled with freeze-dried chitosan/poly (γ-glutamic acid) nanoparticles for oral insulin delivery. Biomaterials. 2010;31(12):3384-94.

23. Li M-G, Lu W-L, Wang J-C, Zhang X, Wang X-Q, Zheng A-P, et al. Distribution, transition, adhesion and release of insulin loaded nanoparticles in the gut of rats. International journal of pharmaceutics. 2007;329(1-2):182-91.

24. Chang C-H, Lin Y-H, Yeh C-L, Chen Y-C, Chiou S-F, Hsu Y-M, et al. Nanoparticles incorporated in pH-sensitive hydrogels as amoxicillin delivery for eradication of Helicobacter pylori. Biomacromolecules. 2010;11(1):133-42.

25. Verma A, Sharma S, Gupta PK, Singh A, Teja BV, Dwivedi P, et al. Vitamin B12 functionalized layer by layer calcium phosphate nanoparticles: A mucoadhesive and pH-responsive carrier for improved oral delivery of insulin. Acta biomaterialia. 2016;31:288-300.

26. Zhou J, Romero G, Rojas E, Ma L, Moya S, Gao C. Layer by layer chitosan/alginate coatings on poly(lactide-co-glycolide) nanoparticles for antifouling protection and Folic acid binding to achieve selective cell targeting. Journal of Colloid and Interface Science. 2010;345(2):241-7.

27. Sarmento B, Ribeiro A, Veiga F, Sampaio P, Neufeld R, Ferreira D. Alginate/chitosan nanoparticles are effective for oral insulin delivery. Pharmaceutical research. 2007;24(12):2198-206.

28. Caldorera-Moore M, Vela Ramirez JE, Peppas NA. Transport and delivery of interferon-α through epithelial tight junctions via pH-responsive poly (methacrylic acid-grafted-ethylene glycol) nanoparticles. Journal of drug targeting. 2019;27(5-6):582-9.

29. Lamprecht A, Yamamoto H, Takeuchi H, Kawashima Y. pH-sensitive microsphere delivery increases oral bioavailability of calcitonin. Journal of Controlled Release. 2004;98(1):1-9.

30. Chen T, Li S, Zhu W, Liang Z, Zeng Q. Self-assembly pH-sensitive chitosan/alginate coated polyelectrolyte complexes for oral delivery of insulin. Journal of Microencapsulation. 2019;36(1):96-107.

31. Lee SH, Song JG, Han H-K. Development of pH-responsive organic-inorganic hybrid nanocomposites as an effective oral delivery system of protein drugs. Journal of Controlled Release. 2019;311-312:74-84.

32. Meissner Y, Ubrich N, Ghazouani FE, Maincent P, Lamprecht A. Low molecular weight heparin loaded pH-sensitive microparticles. International Journal of Pharmaceutics. 2007;335(1):147-53.

33. Caldorera-Moore M, Vela Ramirez JE, Peppas NA. Transport and delivery of interferon-α through epithelial tight junctions via pH-responsive poly(methacrylic acid-grafted-ethylene glycol) nanoparticles. Journal of Drug Targeting. 2019;27(5-6):582-9.

34. Cikrikci S, Mert B, Oztop MH. Development of pH Sensitive Alginate/Gum Tragacanth Based Hydrogels for Oral Insulin Delivery. Journal of Agricultural and Food Chemistry. 2018;66(44):11784-96.

35. Chen X, Ren Y, Feng Y, Xu X, Tan H, Li J. Cp1-11 peptide/insulin complex loaded pH-responsive nanoparticles with enhanced oral bioactivity. International Journal of Pharmaceutics. 2019;562:23-30.

36. Liu L, Zhang Y, Yu S, Yang Z, He C, Chen X. Dual Stimuli-Responsive Nanoparticle-Incorporated Hydrogels as an Oral Insulin Carrier for Intestine-Targeted Delivery and Enhanced Paracellular Permeation. ACS Biomaterials Science & Engineering. 2018;4(8):2889-902.

37. Dai W, Guo Y, Zhang H, Wang X, Zhang Q. Sylysia 350/Eudragit S100 solid nanomatrix as a promising system for oral delivery of cyclosporine A. International Journal of Pharmaceutics. 2015;478(2):718-25.

38. Tsai L-C, Chen C-H, Lin C-W, Ho Y-C, Mi F-L. Development of multifunctional nanoparticles self-assembled from trimethyl chitosan and fucoidan for enhanced oral delivery of insulin. International Journal of Biological Macromolecules. 2019;126:141-50.

39. Lee H-B, Yoon S-Y, Singh B, Oh S-H, Cui L, Yan C, et al. Oral Immunization of FMDV Vaccine Using pH-Sensitive and Mucoadhesive Thiolated Cellulose Acetate Phthalate Microparticles. Tissue Engineering and Regenerative Medicine. 2018;15(1):1-11.

40. Zhang L, Qin H, Li J, Qiu J-N, Huang J-M, Li M-C, et al. Preparation and characterization of layer-by-layer hypoglycemic nanoparticles with pH-sensitivity for oral insulin delivery. Journal of Materials Chemistry B. 2018;6(45):7451-61.

41. Song M, Li L, Zhang Y, Chen K, Wang H, Gong R. Carboxymethyl-β-cyclodextrin grafted chitosan nanoparticles as oral delivery carrier of protein drugs. Reactive and Functional Polymers. 2017;117:10-5.

42. Yu X, Wen T, Cao P, Shan L, Li L. Alginate-chitosan coated layered double hydroxide nanocomposites for enhanced oral vaccine delivery. Journal of Colloid and Interface Science. 2019;556:258-65.

43. Liu L, Zhang Y, Yu S, Zhang Z, He C, Chen X. pH- and Amylase-Responsive Carboxymethyl Starch/Poly(2-isobutyl-acrylic acid) Hybrid Microgels as Effective Enteric Carriers for Oral Insulin Delivery. Biomacromolecules. 2018;19(6):2123-36.

44. Doostmohammadi M, Ameri A, Mohammadinejad R, Dehghannoudeh N, Banat IM, Ohadi M, et al. Hydrogels For Peptide Hormones Delivery: Therapeutic And Tissue Engineering Applications. Drug Design, Development and Therapy. 2019;13:3405.

45. Ata S, Rasool A, Islam A, Bibi I, Rizwan M, Azeem MK, et al. Loading of Cefixime to pH sensitive chitosan based hydrogel and investigation of controlled release kinetics. International Journal of Biological Macromolecules. 2020;155:1236-44.

46. Gao X, Cao Y, Song X, Zhang Z, Zhuang X, He C, et al. Biodegradable, p H-R responsive Carboxymethyl Cellulose/P poly (Acrylic Acid) Hydrogels for Oral Insulin Delivery. Macromolecular bioscience. 2014;14(4):565-75.

47. Mundargi R, Patil S, Kulkarni P, Mallikarjuna N, Aminabhavi T. Sequential interpenetrating polymer network hydrogel microspheres of poly (methacrylic acid) and poly (vinyl alcohol) for oral controlled drug delivery to intestine. Journal of microencapsulation. 2008;25(4):228-40.

48. Yue Z, Che Y, Jin Z, Wang S, Ma Q, Zhang Q, et al. A facile method to fabricate thermo-and pH-sensitive hydrogels with good mechanical performance based on poly (ethylene glycol) methyl ether methacrylate and acrylic acid as a potential drug carriers. Journal of Biomaterials Science, Polymer Edition. 2019;30(15):1375-98.

49. George M, Abraham TE. Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan—a review. Journal of controlled release. 2006;114(1):1-14.

50. Sharpe LA, Daily AM, Horava SD, Peppas NA. Therapeutic applications of hydrogels in oral drug delivery. Expert opinion on drug delivery. 2014;11(6):901-15.

51. Abbasi M, Sohail M, Minhas MU, Khan S, Hussain Z, Mahmood A, et al. Novel biodegradable pH-sensitive hydrogels: An efficient controlled release system to manage ulcerative colitis. International Journal of Biological Macromolecules. 2019;136:83-96.

52. Javanbakht S, Shaabani A. Encapsulation of graphene quantum dot-crosslinked chitosan by carboxymethylcellulose hydrogel beads as a pH-responsive bio-nano composite for the oral delivery agent. International Journal of Biological Macromolecules. 2019;123:389-97.

53. Naghshineh N, Tahvildari K, Nozari M. Preparation of Chitosan, Sodium Alginate, Gelatin and Collagen Biodegradable Sponge Composites and their Application in Wound Healing and Curcumin Delivery. Journal of Polymers and the Environment. 2019;27(12):2819-30.

54. Gull N, Khan SM, Khalid S, Zia S, Islam A, Sabir A, et al. Designing of biocompatible and biodegradable chitosan based crosslinked hydrogel for in vitro release of encapsulated povidone-iodine: A clinical translation. International Journal of Biological Macromolecules. 2020;164:4370-80.

55. Guaresti O, García–Astrain C, Palomares T, Alonso–Varona A, Eceiza A, Gabilondo N. Synthesis and characterization of a biocompatible chitosan-based hydrogel cross-linked via 'click' chemistry for controlled drug release. International Journal of Biological Macromolecules. 2017;102:1-9.

56. Xu Y, Du Y, Huang R, Gao L. Preparation and modification of N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride nanoparticle as a protein carrier. Biomaterials. 2003;24(27):5015-22.

57. Chen L, Tian Z, Du Y. Synthesis and pH sensitivity of carboxymethyl chitosan-based polyampholyte hydrogels for protein carrier matrices. Biomaterials. 2004;25(17):3725-32.

58. Pan Y, Li Y-j, Zhao H-y, Zheng J-m, Xu H, Wei G, et al. Bioadhesive polysaccharide in protein delivery system: chitosan nanoparticles improve the intestinal absorption of insulin in vivo. International Journal of Pharmaceutics. 2002;249(1):139-47.

59. Mansoor S, Kondiah PP, Choonara YE, Pillay V. Polymer-based nanoparticle strategies for insulin delivery. Polymers. 2019;11(9):1380.

60. Sun X, Liu C, Omer AM, Yang L-Y, Ouyang X-k. Dual-layered pH-sensitive alginate/chitosan/kappa-carrageenan microbeads for colon-targeted release of 5-fluorouracil. International Journal of Biological Macromolecules. 2019;132:487-94.

61. Lin Y-H, Liang H-F, Chung C-K, Chen M-C, Sung H-W. Physically crosslinked alginate/N, O-carboxymethyl chitosan hydrogels with calcium for oral delivery of protein drugs. Biomaterials. 2005;26(14):2105-13.

62. Wang B, Song Q, Zhao F, Zhang L, Han Y, Zhou Z. Isolation and characterization of dextran produced by Lactobacillus sakei L3 from Hubei sausage. Carbohydrate Polymers. 2019;223:115111.

63. Hu Q, Luo Y. Recent advances of polysaccharide-based nanoparticles for oral insulin delivery. International journal of biological macromolecules. 2018;120:775-82.

64. Alibolandi M, Alabdollah F, Sadeghi F, Mohammadi M, Abnous K, Ramezani M, et al. Dextran-b-poly(lactide-co-glycolide) polymersome for oral delivery of insulin: In vitro and in vivo evaluation. Journal of Controlled Release. 2016;227:58-70.

65. George A, Shah PA, Shrivastav PS. Natural biodegradable polymers based nano-formulations for drug delivery: A review. International journal of pharmaceutics. 2019.

66. Chen R, Cai X, Ma K, Zhou Y, Wang Y, Jiang T. The fabrication of double-layered chitosan/gelatin/genipin nanosphere coating for sequential and controlled release of therapeutic proteins. Biofabrication. 2017;9(2):025028.

67. Tabata Y, Ikada Y. Protein release from gelatin matrices. Advanced Drug Delivery Reviews. 1998;31(3):287-301.

68. Huang D, Chen Y-S, Green CR, Rupenthal ID. Hyaluronic acid coated albumin nanoparticles for targeted peptide delivery in the treatment of retinal ischaemia. Biomaterials. 2018;168:10-23.

69. Tian H, He Z, Sun C, Yang C, Zhao P, Liu L, et al. Uniform Core–Shell Nanoparticles with Thiolated Hyaluronic Acid Coating to Enhance Oral Delivery of Insulin. Advanced Healthcare Materials. 2018;7(17):1800285.

70. Fiorica C, Pitarresi G, Palumbo FS, Di Stefano M, Calascibetta F, Giammona G. A new hyaluronic acid pH sensitive derivative obtained by ATRP for potential oral administration of proteins. International journal of pharmaceutics. 2013;457(1):150-7.

71. Elmowafy EM, Tiboni M, Soliman ME. Biocompatibility, biodegradation and biomedical applications of poly(lactic acid)/poly(lactic-co-glycolic acid) micro and nanoparticles. Journal of Pharmaceutical Investigation. 2019;49(4):347-80.

72. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids and surfaces B: biointerfaces. 2010;75(1):1-18.

73. Satheesh Kumar P, Ramakrishna S, Ram Saini T, Diwan P. Influence of microencapsulation method and peptide loading on formulation of poly (lactide-co-glycolide) insulin nanoparticles. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2006;61(7):613-7.

74. Nargesi khoramabadi H, Arefian M, Hojjati M, Tajzad I, Mokhtarzade A, Mazhar M, et al. A review of Polyvinyl alcohol / Carboxymethylcellulose (PVA/CMC) composites for various applications. Journal of Composites and Compounds. 2020;2(3).

75. Gómez-Aldapa CA, Velazquez G, Gutierrez MC, Rangel-Vargas E, Castro-Rosas J, Aguirre-Loredo RY. Effect of polyvinyl alcohol on the physicochemical properties of biodegradable starch films. Materials Chemistry and Physics. 2020;239:122027.

76. Gao H, Wang Y, Fan Y, Ma J. Synthesis of a biodegradable tadpole-shaped polymer via the coupling reaction of polylactide onto mono (6-(2-aminoethyl) amino-6-deoxy)-β-cyclodextrin and its properties as the new carrier of protein delivery system. Journal of controlled release. 2005;107(1):158-73.

77. Buyana B, Aderibigbe BA, Ray SS, Ndinteh DT, Fonkui YT. Development, characterization, and in vitro evaluation of water soluble poloxamer/pluronic-mastic gum-gum acacia-based wound dressing. Journal of Applied Polymer Science. 2020;137(21):48728.

78. Chowdhury P, Nagesh PK, Kumar S, Jaggi M, Chauhan SC, Yallapu MM. Pluronic nanotechnology for overcoming drug resistance. Bioactivity of engineered nanoparticles: Springer; 2017. p. 207-37.

79. Xiong XY, Li YP, Li ZL, Zhou CL, Tam KC, Liu ZY, et al. Vesicles from Pluronic/poly(lactic acid) block copolymers as new carriers for oral insulin delivery. Journal of Controlled Release. 2007;120(1):11-7.