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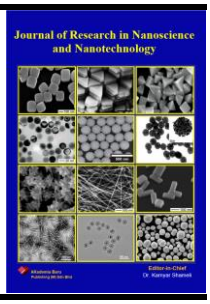

Preparation and Application of Cross-linked Alginate Nanoparticles as Drug Carrier: A Review

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ABSTRACT

Nanotechnology also called as nanotech is referring to the science, technology and engineering to synthesis, manipulation and investigation of materials and devices at nanoscales. Nanoparticles (NPs) owns a special place in nanotechnology due to their extreme-small size, multifunctionality, and modifiable surface for wide range of applications especially in new drug delivery application as the stability, solubility, biocompatibility and drugs releasing of NPs are highly controllable. The variation of nanomaterials with different physiochemical and morphological properties will affect the materials-living cells interaction and determination of the route of clearance and potential toxic effects. Thus, it requires more cross-disciplinary research on the designing and developing of drugs delivery NPs based on the diagnosis and treatment of devastating diseases. Biopolymers-based NPs have gained great attention for drug delivery system over the past few decades compared to other materials-based NPs such as liposomes, ceramics and lipids as well as magnetic NPs. There are extensive studies and developments of biopolymers-based NPs for drug delivery and tissue engineering as it capable of controlling the release of drugs, stabilizing labile molecules from degradation, and site-specific drug targeting. Alginate is an anionic copolymer that extracted from algae. The NPs composed alginate have become one of the most extensively researched materials for biomedical applications such as tissue engineering, wound dressing, protein/enzyme carrier and drug delivery due to its biodegradability, biocompatibility, non-toxicity and mucoadhesive properties. Furthermore, by physiochemical crosslinking and cooperating with other polymers, the mechanical strength, cell affinity and drug release profile can be modified and improved. In this brief review paper, several aspects about using alginate or crosslinked alginate for drug delivery application will be discussed, mainly focus on the structure and properties of alginate such as biocompatibility, immunogenicity and biodegradability, as well as the effect of mucoadhesion and pH sensitivity of using alginate in drug delivery nanoparticles developments.

Keywords:
Alginate nanoparticle, Gelation technique,
Emulsification technique, Drug carrier,
Cross-linked biopolymer.

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1. Introduction

At December 1959 meeting of the American Physical Society, the concept of nanotechnology was introduced in a lecture titled 'There's plenty room at the bottom' by a physics Nobel laureate Richard P Feynman [1]. Ever since that, nanotechnology has changed the scientific landscape in all technology developments as well as in medical technology developments in terms of disease diagnosis, treatment, and prevention [2, 3].

Nanoparticles (NPs) owns a special place in nanotechnology, gaining an increased interest and attention in biomedical application especially in drug carrier application. The introduction of NPs in drug carrier application enables the drugs to be absorbed, encapsulated and distributed at nanoscale level with minimum adverse effect and improves the therapeutic efficiency [4, 5]. However, it is a crucial concern on the consumer safety because of the potential translocation of NPs into tissues due to their nano-size and the difference between the concentration of biologically active substance delivered in the tissue and the physiological normal concentration [6]. There are increasing demand of formulated dosage forms with controlled drug release and long-lasting therapeutic effect at low dosing frequency over the past decade. There are many substances showed pharmacological effects in vitro, but most of them required high enough concentration on the site to trigger the pharmacological response where the concentration of drugs delivered to the site of action is depending on the administration dose, rate and extend of absorption as well as their distribution throughout the body. The clinical effects will maintain until the concentration of drug dropped below certain level caused by the excretion and metabolism. A dosage form including one or more drugs together with various number of substances (excipients) have been added to the formulation for facilitating the preparation and administration, promoting the continuous drug release, bioavailability and degradation protection of drugs. Besides, it is important to consider the influences of excipients on the physiochemical characteristics of final products which related to the rate or extent of absorption of one or multiple drugs. Therefore, the cautious selection of excipients is crucial for the formulation of a stable and effective dosage form [7].

Biodegradable polymers and natural polysaccharides are the most studied materials for fabricating nanoparticles for drug carrier application due to their excellent biocompatibility, biodegradability, good mechanical stability, and site-specific drug targeting. Furthermore, Biodegradable polymers and natural polysaccharides are the most versatile materials used for drug delivery nanoparticles development in order to avoid the potential toxicity which limiting their diverse clinical applications [8]. The diverse properties of different biopolymers such as chitosan, sodium alginate and cellulose are some of the commonly used in controlled drug delivery that act as inert excipients mainly in the areas of cancer therapy and controlled delivery of vaccines [9-12].

These low-cost biopolymers are able to degrade in human body which considered innocuous. By incorporating therapeutic agent into a polymeric matrix or capsule, the degradation protection of the biologically active compound, absorption and release profile can be controlled and improved resulting increased therapeutic effect but reduced the dosage frequency. Moreover, the crosslinking or association of multiple polymeric materials can significantly advance the development of modified release drug delivery systems. Other than that, different polymer blends also a promising approach to obtain nanoparticles with desired biopharmaceutical properties for drug delivery and targeting while preventing the high costs.

In this short review, different aspects related to the use of alginate to fabricate nanoparticles for drug delivery and targeting will be discussed. The structure of alginate and its applications in the

forms of hydrogels and nanoparticles will be briefly introduce, followed by the biocompatibility, immunogenicity and biodegradability of alginate. Besides, the influence and important of mucoadhesivity and pH-sensitivity of alginate in the drug delivery applications will be explained. Some comparisons between different studies, and the overview of the publications and citations of alginate and its drug delivery application will also be carried out in this short review.

2. Alginate as a Biopolymer from Marine Sources

Alginate is one of the most utilized natural polysaccharide in biomedical application such as tissue engineering, wound dressing, protein/enzyme carrier and drug delivery for last decades and it also acclaimed permission from Food and Drug Admission (FDA) for human use [13]. Alginates as anion copolymers consist large amount of (1, 4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers (**Figure 1.**). The presence of these 2 monomers will affect the drug release properties while the physical and chemical properties are influenced by the molecular weight, composition and sequence of M and G monomers [6, 14, 15]. Commonly, alginate has the characteristics of pH sensitivity, non-toxicity, biocompatibility, biodegradability, low cost, mucoadhesive, and non-immunogenic, being attractive for modified delivery of drugs. The mucoadhesion mechanism of alginate provides bio-adhesive property to the drug delivery system to become adhesive to mucous membrane on hydration which allows drugs to deliver to particular mucus tissue for a period of time comparable to the drug releasing time [16, 17].

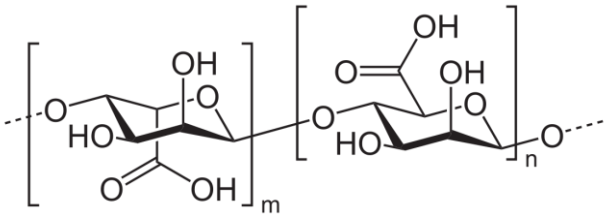

Figure 1. Chemical structure of alginate.

Alginate hydrogels prepared by various crosslinking methods play an important role in wound healing, drug delivery and tissue engineering applications. These hydrogels contain similar structure like extracellular matrices in tissues, and can create a physiologically moist microenvironment which able to minimize bacterial infection and promote wound healing. Besides, it able to encapsulate and release small chemical drugs and macromolecular proteins under controlled manner which depending on the crosslinking methods and type of crosslinker. The gelation of alginate occurs by an exchange of sodium ions from the guluronic acid (G) blocks with multivalent cations such as calcium ions, the exchanged calcium ions will stack layers of G blocks and forming a “egg box” structure (**Figure 2.**). The ratio of M and G blocks is one of factors that affecting the properties of alginate hydrogels, the higher G content tend to form stronger, stiffer, more brittle, high porosity and greater restriction to solute transport; while higher M content resulting more elastic and weaker hydrogels, this is crucial to understand the optimization of using alginate as a useful delivery system for therapeutic applications as well as other factors such as pH effect, and molecular weight of encapsulated drugs [18].

The use of alginate nanoparticles was reported since 1989 until recent years. Alginate nanoparticles including nanoaggregates, nanocapsules and nanospheres are able to attach or encapsulate the drugs, enzymes, and other compounds in or on the particle matrix based on preparation methods. Nanoaggregates are nanosized colloidal systems that have different

morphologies and the drugs are physically dispersed into the system; nanocapsules are vesicular systems that consists of drugs confined to an aqueous or oily liquid core which surrounded by polymeric membrane; nanospheres are a spherical particle with gelled interior that entrapped drugs or other components.

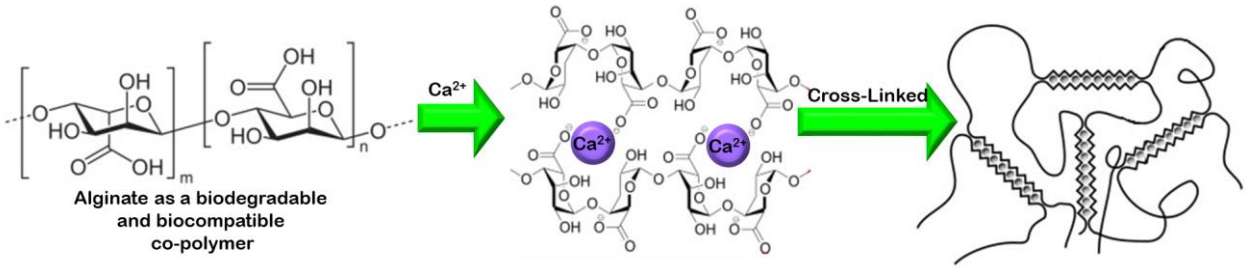

Figure 2. Structure of alginate and it's cross-linked by calcium cations.

Furthermore, similar to other alginate-based products, nanoparticles formed by alginate extracted from different sources will have varied percentage of monomers, and its purity will highly affect the properties of alginate nanoparticles such as mucoadhesive properties and immunogenicity. Therefore, there are a lot of methods such as spray drying, covalent cross-linking, ionotropic gelation and emulsification technique were used to prepare alginate nanoparticles for obtaining desired properties for different drugs delivery systems, ionotropic gelation are the most commonly used method among others [15].

2.1. Biocompatibility, Immunogenicity and Biodegradability of Alginate

Materials' biocompatibility and immunogenicity are critical criteria in their suitability as drug delivery carriers. Immunogenicity influences the chemical composition of alginates as well as the mitogenic contaminants contained in them. When alginate contact with blood, it has minimal cytotoxic effects and minimises hemolysis. The content and purity of alginic acid determine its biocompatibility and strength. Even though the biocompatibility of alginate has been thoroughly investigated in vitro and in vivo, there is still a controversy about the impact of alginate composition, which could be connected to the varied levels of purity in the alginate studied in various papers [19]. Due to alginate is derived from natural sources, it may contain impurities such as heavy metals, endotoxins, proteins, and polyphenolic chemicals, all of which can provoke an immunogenic reaction at injection or implantation sites. When alginates are implanted into animals, they do not cause any significant foreign body reaction if they are purified through a multi-step extraction procedure until they reach a very high purity. Gels fabricated from commercially available high purified alginate were subcutaneously injected into mice, a significant inflammatory response was observed [20].

Alginate is lacking an important enzyme named alginase that has the function of cleaving the polymer chains lacks which resulted alginate undegradable in mammals. However, ionic crosslinked alginate hydrogels are able to dissolve due to the releasing of divalent ions from crosslinked hydrogels into the surrounding media by the exchange reactions with monovalent cations such as sodium ions. Commercial available alginate usually has higher molecular weight than the renal clearance threshold of the kidneys, and will not be completely removed from the body [21]. Slight oxidation of alginate chains using sodium periodate ($NaIO_4$) is a promising approach for alginate to degrade in physiological conditions, presenting wide potential as delivery vehicle for various applications [19]. **Figure 3.** showed the mechanism of periodate oxidation, alginate can be oxidized with sodium periodate, and this process cleaves the carbon-carbon bond of the cis-diol group in the uronate residue and changes the chiral conformation to an open-chain adduct which enable the

degradation of the alginate backbone. The oxidation of alginate will not affect its gel-forming capability in the presence of divalent cations, although the molecular weight of alginate will be slightly reduced.

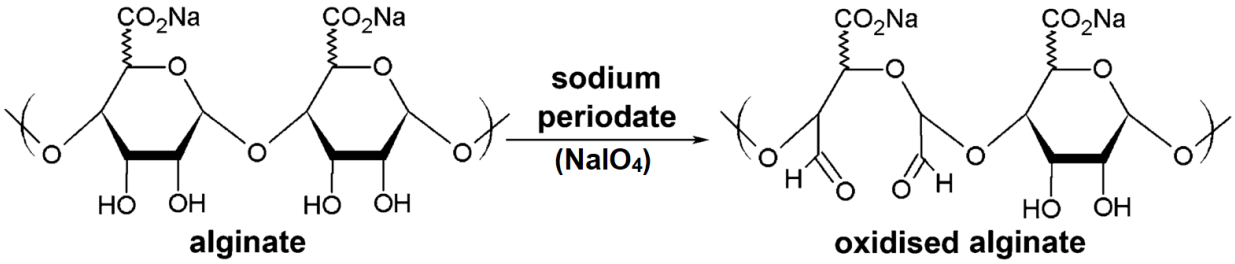

Figure 3. Mechanism of oxidation of alginate using sodium periodate ($NaIO_4$).

2.2. Mucoadhesion of alginate

"Mucoadhesion" refers to the adhesion between two surfaces, one of them is mucosal layer. As an anionic polymer, alginate has the highest mucoadhesive strength compared to other polymers such as polystyrene, chitosan, carboxymethyl cellulose and poly(lactic acid), which in fact cationic and non-ionic polymers shows less bio-adhesiveness than anionic polymers [22]. Alginate's bio-adhesive property has shown advantages in mucosal drug delivery to the gastrointestinal tract and nasopharynx. Mucoadhesive drug delivery systems can increase drug residence time at the site of activity or resorption, enabling it to be used as a potential delivery vehicle for drugs to mucosal tissues while also increasing overall drug effectiveness and bioavailability [22-24]. In the recent study from Pamlényi K. et al. [25], they optimized the formulation of sodium alginate film as mucoadhesive drug delivery system containing cetirizine dihydrochloride, and concluded that glycerol will reduce the mucoadhesivity of films while cetirizine dihydrochloride will improve the tensile strength of films.

2.3. pH-sensitivity of alginate

The release of macromolecules from alginate is significantly reduced in low pH solutions, which could be useful in developing a system for the oral administration of certain compounds. **Figure 4.** shows the mechanisms of pH-dependent swelling/shrinking behaviors. At low pH such as gastric environment, alginate appears to shrink, and the encapsulated prebiotic, probiotic microorganisms, or different drugs will not release; while alginate expand and release encapsulated compounds in alkaline environment such as intestine [26]. Besides, the hydrated sodium alginate in the stomach converted into a porous, insoluble layer of alginic acid and the alginic acid layer converted again into a soluble viscous layer once it enters the higher pH of the intestinal tract. This alginate pH condition can be used to tailor release profiles. Nonetheless, rapid dissolution of alginate matrices in higher pH ranges can result in explosive release, which is undesirable for protein drugs because it causes protein drugs to be denatured by proteolytic enzymes [27]. However, by combining or utilizing other polymers, the drawback of pH sensitivity on the physicochemical and mechanical properties can be overcome. In a novel approach from Apoorva A. et al. [28], pH-sensitive alginate hydrogel delivery system reinforced with gum tragacanth was fabricated with improved physiochemical properties for intestinal targeting of nutraceuticals. Another innovative approach from Rezaei F. et al. [29], vancomycin (VANCO) loaded silk fibroin-sodium alginate nanoparticles embedded in poly(N-isopropylacrylamide) (PNIPAM) hydrogel containing epidermal growth factor (EGF) was used on the treatment of chronic burn wound infections. This system showed ideal pH responsive release

behavior and release rate contributed by the combine pH sensitive effect from VANCO, sodium alginate and silk fibroin.

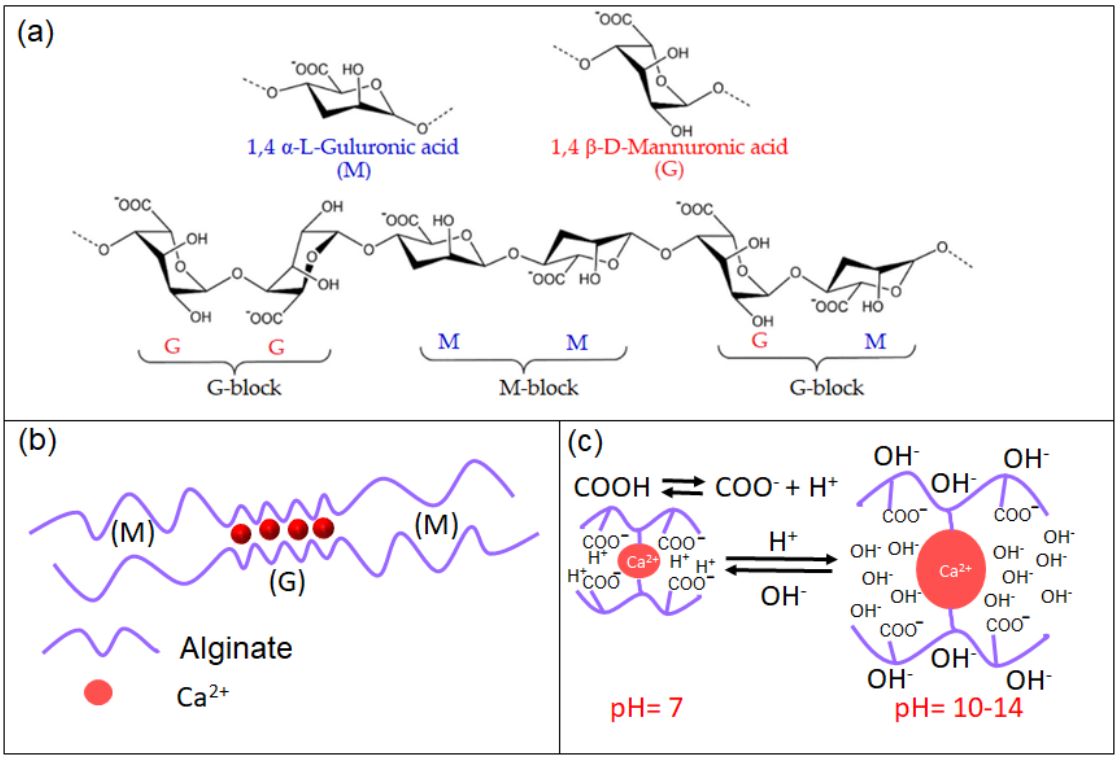

Figure 4. (a) The conformation of monomers and blocks distribution of alginate salt, (b) chemical structure of cross-linked Ca-alginate, and (c) The mechanisms of pH-dependent swelling/shrinking behaviors between the pH value of 7 to 14.

3. Preparation and drug loading of alginate nanoparticles

Ionotropic gelation method was first described by Calvo et al. [30] in 1997 for fabricating chitosan (CS) nanoparticles in the presence of sodium tripolyphosphate (TTP) as nano-encapsulation of proteins. CS is a cationic polymer with positively charged amino groups that can cross-link with the negatively charged phosphate groups at specific concentration range under constant stirring to obtain the nanoparticles for capturing protein.

Based on the preparation procedure from Calvo et al., many researchers altered and modified it and applied to different counter pairs of materials. According to Pedroso-Santana and Fleitas-Salazar [31], CS and alginate are most used cationic and anionic polymer using ionotropic gelation method to prepare nanoparticles for drug carrier applications. Therefore, CS and alginate will be taken as examples for cationic and anionic polymer in this review for procedure explanation.

Basically, there are two ways of using ionotropic gelation method to produce alginate nanoparticles. First way also called as Rajaonarivony's method [32], Li et al. [33] used this method to prepare nifedipine loaded chitosan-alginate nanoparticles. After pH adjustment of sodium alginate solution and chitosan solution, the solution of calcium chloride ($CaCl_2$) incorporated with nifedipine, and chitosan solution were added and stirred accordingly. Then, suspension was equilibrated overnight to produce nifedipine loaded nanoparticles with uniform particle size. Furthermore, another way of using ionotropic gelation method is similar to previous way. In Maan et al. [34], both chitosan and sodium alginate solution were mixed into cisplatin drug under vigorous shaking and

added $CaCl_2$ and TTP dropwise into the mixture. The cisplatin loaded nanoparticles were obtained after centrifugation and resuspended in toluene and acetone.

Ionotropic gelation method has been widely applied for preparing alginate nanoparticles loaded with various drugs as drug carriers due to the highly modifiable procedures. **Table 1.** shows a list of alginate nanocarriers from different types of cationic polymer or surfactant, types of drug loaded, nanoparticles size, and encapsulation efficiency. The encapsulation efficiencies are calculated by Equation (1) below:

$$\text{Encapsulation efficiency (\%)} = \frac{Initial\ Drug\ Amount - \ Final\ Drug\ Amount}{Initial\ Drug\ Amount} \times 100\%$$

Table 1. List of alginate nanoparticles fabricated by ionotropic gelation method and their representative data.

Cationic polymer/ Surfactant	Type of Drug Loaded	Nanoparticle Size with Drug (nm)	Encapsulation Efficiency (%)	Ref.
Chitosan	Gatifloxacin	347	79.63	[35]
Chitosan	Curcumin diethyl disuccinate	414 ± 16	54.9 ± 1.3	[36]
Chitosan	Cisplatin	10 – 30	80	[34]
Chitosan	Lysozyme	901.8 ± 75.50	92.79 ± 0.53	[37]
Chitosan	Doxorubicin	300	99	[38]
ε-polylysine	Bovine Serum Albumin (BSA)	133.2 ± 0.5	57 ± 6.4	[39]
Green tea synthesized sliver nanoparticle	Bovine Serum Albumin (BSA)	414.2 ± 22.6	98.7 ± 0.1	[40]
Natural honey	Rifampicin	65 ± 25	38.48 ± 2.85	[41]
Corn Starch & Natural honey	Theophylline (THP) & BSA	61.8 ± 9.4	68.3 ± 5.6 (THP) & 70.5 ± 4.2 (BSA)	[42]

By comparing the encapsulation efficiency of nanoparticles from the list above, it is clearly that the alginate nanoparticles with chitosan as cationic polymer showed better encapsulation efficiency compared to other cationic polymers or surfactants. Besides, the chitosan-alginate nanoparticles have overall improvement in encapsulation efficiency indicates that the maturity of technology is improving over years. Between the studies of chitosan-alginate nanoparticles, nanoparticles fabricated by Maan et al. [34] and Wu et al. [37] have unique particle size of 10-30 nm and 901.8 ± 75.50 nm respectively. By comparing both preparation procedures with Yoncheva et al. [38], the usage of crosslinker, chitosan-alginate ratio, and type of drugs loaded are the possible factors affecting the nanoparticle size which lack of studies so far.

Moreover, in recent years, green materials are gaining attention in developing novel alginate nanoparticles as drug carriers [40-42] which showed a new path and high potential in future. Natural honey was used by Thomas et al. [41, 42] as surfactant and stabilizer, the stability of nanoparticles is improved when certain amount of honey is added and it is non-toxic with excellent biocompatibility. In addition, they also used starch for replacing other polymer to improve drug releasing performance of nanoparticles.

Figure 5. showed the number of publications and citations in Web of Science. A total of 767 publications have been listed since 1995 in Web of Science related to alginate-based drug delivery applications, the sum of citations also increased significantly over years. There is a reduced

publication and citation numbers by the end of September 2021, might due to the reduced level of physical activities caused by the global COVID-19 pandemic which also limited the research activities in industries and universities.

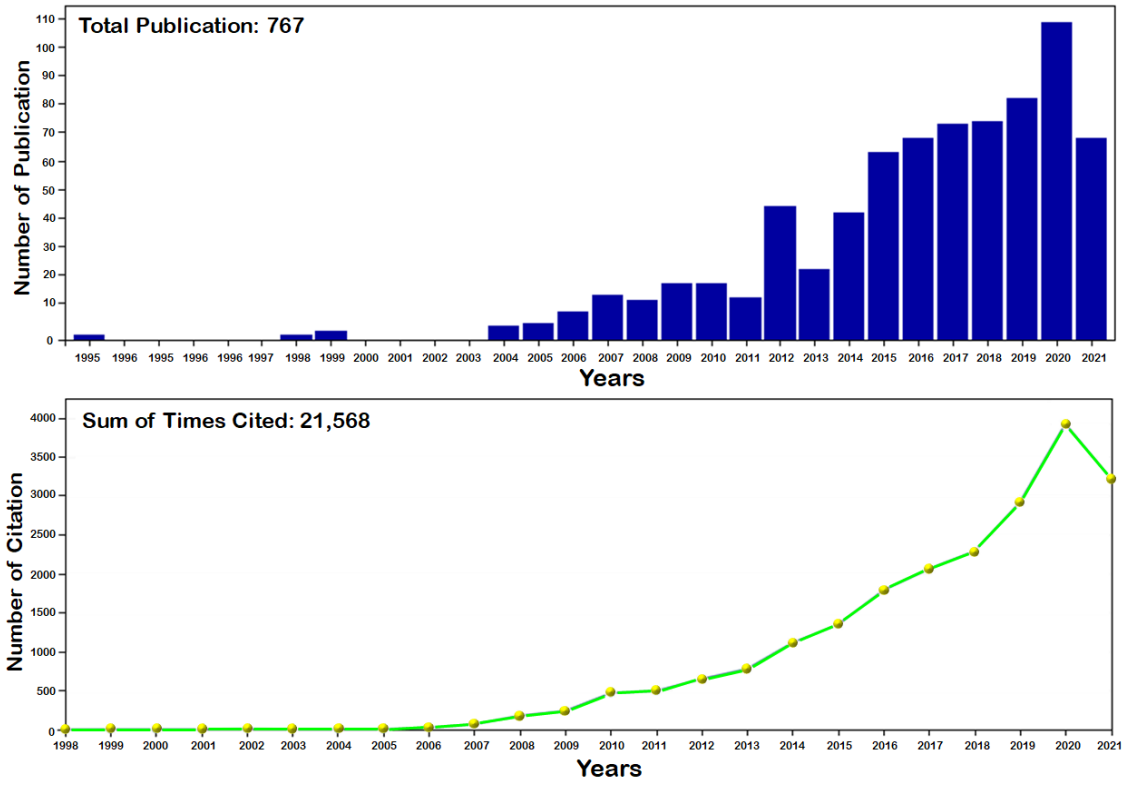

Figure 5. Number of published papers and citations on alginate and its drug delivery application in Web of Science. (1995- Sept 2021)

4. Conclusion

In conclusion, furthering the current understanding of alginate unique properties and the alginate nanoparticles prepared by ionotropic gelation method, the design of experiment and choices of polymers with better drug carrying performance can be potentially discovered and studied in the future. Natural and synthetic polymers such as poly(ethylenimine), poly(2-dimethyl(aminoethyl) methacrylate) and poly(l-lysine) which are often being used in gene and drug delivery have the potential of replacing chitosan. From literature studies in this review, modifications and adjustments on the procedures of ionotropic gelation method were done based on their approaches and types of drugs loaded, and their performance were improved every year. Furthermore, it also proved that similar ionotropic gelation procedures and conditions are suitable for different cationic polymers or surfactants which make it easy to handle. Besides, the whole process of this method does not require large amount of time or expensive equipment that reduced the costing and technical requirement for industrial production in future. Based on the studies reviewed, chitosan crosslinked alginate nanoparticles have promising drugs loading (> 80 %) and release (> 60 %) properties based on various parameters and time taken in different *in-vitro* release studies. However, the drawbacks of using chitosan cross-linked alginate nanoparticles are due to the low solubility of chitosan in neutral and alkaline conditions which might be overcome by using crosslinker, but the effect of crosslinking chitosan with alginate for clinical use are still lacking and maintain in *in-vitro* stages. There are some interesting materials were used recently but some of the studies show relatively low drug carrier performance compared to chitosan-alginate nanoparticles. Nevertheless, there are limited numbers

of studies about new pairs of cationic polymers cooperating with alginate, and so far, there are also limited studies on using multiple crosslinkers for both cationic polymer and alginate in drug delivery nanoparticles applications.

Acknowledgement

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