FABAD J. Pharm. Sci., 50, 1, 169-186, 2025
Doi: 10.55262/fabadeczacilik.1511505
REVIEW ARTICLE

A Review on Calcium-alginate microspheres for Drug Delivery System: Characteristics, Drug Release, Activity, Stability and *In Vivo* Studies

Amiruddin AMIRUDDIN[*,**], Mahardian RAHMADI[***], Dewi Melani HARIYADI[****,*****°]

A Review on Calcium-alginate microspheres for Drug Delivery System: Characteristics, Drug Release, Activity, Stability and In Vivo Studies

SUMMARY

Microspheres are one of the drug delivery systems that allow therapeutic agents to penetrate the body to increase their efficacy and safety by regulating their rate of release, timing, and site of action in the body. Alginate is a natural polymer of polysaccharides that has the advantages of being biocompatible, low toxicity, inexpensive, easy to obtain, and capable of chemical modifications. Calcium chloride is the most suitable crosslinker for sodium alginate. Research on calcium-alginate microspheres has been widely developed to deliver various drugs. Therefore, this review provides a comprehensive summary of research focusing on the characteristics, drug release, activity, stability, and in vivo studies of calcium-alginate microspheres for drug delivery.

Key Words: *Microspheres, alginate, calcium chloride, characteristics, in vitro studies, stability, in vivo studies*

İlaç Taşıma Sistemi için Kalsiyum-aljinat mikroküreleri Üzerine Bir Derleme: Özellikler, İlaç Salımı, Aktivite, Stabilite ve In Vivo Çalışmaları

ÖZ

Mikroküreler, terapötik ajanların vücutta salım hızını, zamanlamasını ve etki alanını düzenleyerek etkinliğini ve güvenliğini artırmak için vücuda nüfuz etmesini sağlayan ilaç taşıma sistemlerinden biridir. Aljinat, biyouyumlu, düşük toksisiteli, ucuz, kolay elde edilebilir, kimyasal modifikasyonlara uygun olma gibi avantajlara sahip, doğal bir polisakkarit polimeridir. Kalsiyum klorür, sodyum aljinat için en uygun çapraz bağlayıcıdır. Çeşitli ilaçların taşınmasında kalsiyum-aljinat mikroküreleri üzerine araştırmalar yaygınlaşmıştır. Bu nedenle, bu derleme kalsiyum-aljinat mikrokürelerinin ilaç salımı için karakteristikleri, ilaç salımı, aktivitesi, stabilitesi ve in vivo çalışmaları üzerine odaklanan araştırmaların kapsamlı bir özetini sunmaktadır.

Anahtar Kelimeler: *Mikroküreler, aljinat, kalsiyum klorür, özellikler, in vitro çalışmalar, stabilite, in vivo çalışmalar.*

Received: 06.07.2024
Revised: 22.10.2024
Accepted: 27.11.2024

[*] ORCID: 0009-0001-2376-4527, Doctoral Programme of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115, Indonesia.
[**] ORCID: 0009-0001-2376-4527, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Campus C Mulyorejo, Surabaya 60115, Indonesia.
[***] ORCID: 0000-0002-7256-2446, Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Campus C Mulyorejo, Surabaya 60115, Indonesia.
[****] ORCID: 0000-0001-9357-3913, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Campus C Mulyorejo, Surabaya 60115, Indonesia.
[*****] ORCID: 0000-0001-9357-3913, Pharmaceutics and Delivery Systems for Drugs, Cosmetics and Nanomedicine (Pharm-DCN) Research Group, Faculty of Pharmacy, Universitas Airlangga, Campus C Mulyorejo, Surabaya 60115, Indonesia.

[°] Corresponding Author;Dewi Melani HARIYADI
E-mail: dewi-m-h@ff.unair.ac.id

INTRODUCTION

Drug delivery systems are defined as formulations or devices that allow the entry of therapeutic agents into the body to increase their efficacy and safety by regulating their release rate, timing, and site of action in the body (Jain, 2020). One of the carriers for drug delivery is the microspheres. Microspheres may be used for controlled release of drugs, antibiotics, vaccines, and hormones (Prasad et al., 2014). Microspheres consist of a drug or core of active agents and polymers (Kadam et al., 2015). The type of polymer used can be derived from natural or synthetic. Still, natural polymers have the advantages of being cheap, readily to use, biocompatible, biodegradable, and able to do a multitude of chemical modifications (Rajeswari et al., 2017). Alginate is a naturally occurring polysaccharide polymer that is generally found in the cell walls of brown algae (Phaeophyceae) species (Lee & Mooney, 2012). The structure consists of β-D-Mannuronic acid (M) and α-L-Guluronic acid (G) residues linked through 1,4 bound in various proportions and arrangements. Alginic acid containing a sodium salt is sodium alginate (Patil et al., 2010).

Over several decades, alginates have been extensively utilized in the design and improvement of various biopolymeric systems for a multitude of scientific and biomedical purposes, one of which is drug delivery due to its capability of carrying out many chemical modifications with the simple addition of crosslinkers such as divalent calcium ions from $CaCl_2$ (Dhamecha et al., 2019; Hasnain et al., 2020). Calcium chloride with sodium alginate can be cross-linked because sodium alginate has a carboxyl group that bonds with Ca^{2+} ions (Fadhilah et al., 2019). Research on microspheres using sodium alginate polymers and $CaCl_2$ crosslinkers has been widely developed to deliver various drugs. This review aims to comprehensively analyze characteristics, drug release, activity, stability, and *in vivo* studies of calcium-alginate microspheres for drug delivery.

Alginate

Alginate is a naturally occurring polysaccharide polymer that is generally found in the cell walls of brown algae (Phaeophyceae) species (Lee & Mooney, 2012). The structure consists of β-D-Mannuronic acid (M) and α-L-Guluronic acid (G) residues linked through 1,4 bound in various proportions and arrangements. Alginate when compared to gelatin, agar, and other polysaccharides, has a more remarkable ability to form gels that are not affected by temperature (Ching et al., 2017). Among the many alginates, that have received the most attention in the pharmaceutical and biomedical industries is sodium alginate (Szekalska et al., 2016). Sodium alginate is an alginic acid containing sodium salt with a chemical structure $(C_6H_7NaO_6)n$ and a mean molecular weight of 216.121g/mol (Frent et al., 2022). Sodium alginate has the advantages of being cheaper, biocompatible, biodegradable, low toxicity, readily to be had, having excellent thickening and gelling properties, and provides the highest mucoadhesive ability compared to other polymers, so it is widely used in drug delivery systems (Batista et al., 2019; Adrian et al., 2019; Fernando et al., 2020). Sodium alginate was exploited within the design and improvement of various biopolymeric systems for a multitude of scientific and biomedical purposes, one of which is drug delivery because sodium alginate is capable of carrying out many chemical modifications with the simple addition of crosslinkers such as divalent calcium ions from $CaCl_2$ (Dhamecha et al., 2019; Uyen et al., 2019; Hasnain et al., 2020).

Calcium Chloride

Calcium chloride is an inorganic salt substance with the chemical formula $CaCl_2$. Organoleptic calcium chloride is a white crystalline powder, granules, or crystalline mass and is hygroscopic (Rowe et al., 2009). Ca^{2+} ions are most preferred for developing microparticles compared to Rb^+, Cs^+, K^+, Na^+, Li^+, Ba^{2+}, Sr^{2+}, and Mg^{2+} because Ca^{2+} is the safest for the body (Tecante et al., 2012; Thi et al., 2019). Calcium

chloride is often used as a crosslinker to make alginate beads (Skrzypczak et al., 2019). Calcium chloride with sodium alginate can crosslink because sodium alginate has a carboxyl group at the guluronic acid (G) position, which binds to Ca^{2+} ions (Fadhilah et al., 2019). Based on competitive inhibition studies, the mechanism involved in the complexation of divalent cations and the G-block region of the polymer is the dimerization of G residues, is two G chains are bonded to opposite sides of the alginate polymer by the addition of Ca ions. This alignment creates hydrophilic cavities with diamond-shaped holes that bind calcium ions through the multi-coordination of the oxygen atoms of the carboxyl groups. This tightly bonded polymer configuration causes the formation of a junction zone with an "egg box" shape (Ching et al., 2017). Figure 1. shows the egg-box structure of calcium-alginate.

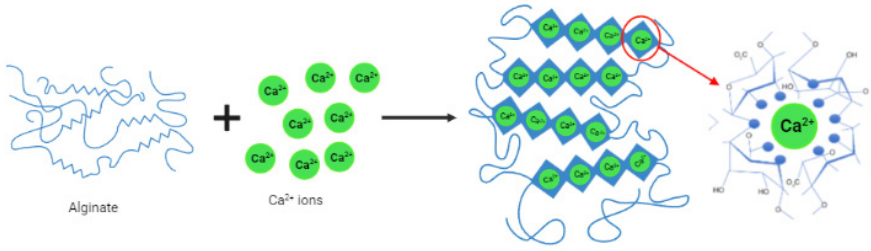

Figure 1. Schematic structure of "egg box" calcium-alginate (Ching et al., 2017).

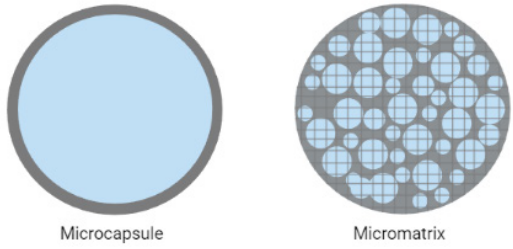

Figure 2. Schematic structure of microcapsule and micromatrix (Uyen et al., 2019).

Microspheres

Microspheres serve as carriers for drug delivery, enabling the controlled release of various substances, including drugs, antibiotics, vaccines, and hormones (Prasad et al., 2014). Microspheres have a diameter of 1 µm to 1000 µm with spherical particles, consisting of drugs and polymers (Kadam et al., 2015). Microspheres are divided into microcapsules and micromatrices depending on how they are encapsulated, as shown in Figure 2. Microcapsules contain substances entrapped and ringed by distinct capsule walls whereas in micromatrices, entrapped materials are scattered all over the matrix (Solanki, 2018; Uyen et al., 2019). Microspheres have several benefits when compared to conventional drug delivery systems, including:

a. The therapeutic effect of microspheres is prolonged and sustained.
b. Microspheres increase bioavailability and reduce the frequency or severity of side effects.
c. It improves patient adherence by reducing the frequency of dosing.
d. Microspheres are small, spherical particles that are easy to inject into the body.
e. The first-pass metabolism and toxicity of drugs are decreased by microspheres.
f. Protection of drugs against the environment (Ramteke et al., 2012; Prasad et al., 2014; Verma, 2019; Tandale et al., 2020).

On the other hand, the microspheres have limitations:

a. Release rates can vary due to diet and transit rates through the gut.
b. Variations in the rate of release between doses.
c. Materials and processing for the preparation of controlled release are significantly more expensive than for the conventional formulations (Prasad et al., 2014; Verma, 2019).

Calcium-alginate microspheres for drug delivery systems can be prepared using several methods. Three standard methods for preparing calcium-alginate microspheres are spray drying, extrusion, and emulsification/gelation techniques. The spray drying method for producing dry powder microspheres is fast, sustainable, economical, repeatable, and scalable. This method involves atomizing the alginate solution with hot air after mixing it with organic reagents (Sosnik & Seremeta, 2015). The extrusion method is the most straightforward and popular method for preparing drug-filled microspheres by alginate ionic gelation involving simple diffusion and crosslinking reactions by Ca^{2+} ions. The alginate solution is added dropwise to the crosslinking solution in the extrusion method (Uyen et al., 2019). The emulsification/gelation method can produce microspheres with a simple and inexpensive experimental setup. The emulsification/gelation process generally involves two key steps: first, the formation of stable polymer droplets, and second, the hardening of these droplets within the emulsion system. In this procedure, the gelation happens when the cross-linker ions and the alginate solution collide (Mishra, 2015). Calcium-alginate microspheres for drug delivery systems have been widely developed to date. Several evaluations of calcium-alginate microspheres have been conducted in vitro or in vivo. This evaluation aims to verify the effectiveness of the calcium alginate microspheres used in the drug delivery system, ensuring maximum and stable drug delivery.

Characteristics of Calcium-Alginate Microspheres for Drug Delivery System

Generally, the characteristics of microspheres are influenced by several factors such as sodium alginate concentration, crosslinker concentration, crosslinking time, and preparation method (Kadam et al., 2015). Factors that affected the physical characteristics of calcium-alginate microspheres are as follows:

Alginate concentration

The concentration of alginate is a significant variable affecting the characteristics of calcium-alginate microspheres (yield, particle size, drug loading, and entrapment efficiency). The size of the droplet would increase due to the increase in the solution's viscosity brought on by the rise in alginate concentration, and the larger particle size of the large microsphere would result in an improvement in drug loading and entrapment efficiency (Hariyadi et al., 2019).

Crosslinker concentration

The concentration of $CaCl_2$ used as a crosslinker, significantly influences the characteristics of the calcium-alginate microspheres. When the $CaCl_2$ crosslinker solution comes into contact with the alginate polymer solution, it forms an egg-box structure (Ching et al., 2017). This occurs because of the bond between Ca^{2+} ions and the G-alginate structure containing carboxyl groups (Fadhilah et al., 2019). The increase in crosslinker concentration would provide more availability of Ca^{2+} ions to bind with the alginate polymer solution, causing the drug loading and entrapment efficiency to increase (Amiruddin et al., 2023). On the other hand, it was discovered that as the calcium chloride concentration increased, the mean particle size of the microspheres decreased. According to some reports, gelation happens right away when drops of alginate solution interact with calcium ions. When Ca^{2+} ions enter the droplet's interior, the water is squeezed out of the droplet, which causes the microspheres to contract and cause a smaller particle size (Manjanna et al., 2010).

Crosslinking time

The crosslinking time significantly impacts the characteristics of calcium-alginate microspheres, especially in particle size and encapsulation efficiency. It was discovered that as the crosslinking time increased, the mean particle size of the microspheres shrank (Loquercio et al., 2015; Choukaife et al., 2020). According to some reports, gelation happens right away when drops of alginate solution interact with calcium ions. Longer crosslinking time causes Ca2+ ions to enter the interior of the droplets, and water to be squeezed out of the droplets, which causes the microspheres to contract and smaller particle sizes to form (Manjanna et al., 2010). On the other hand, crosslinking time may decrease the entrapment efficiency of microspheres because longer stirring duration may cause polymer molecules to aggregate on the surface of microspheres and reduce the amount of free space in the alginate matrix, but shorter stirring time may not be enough to generate strong electrolyte interactions that condense polymer chains on the microspheres (Mali et al., 2010; Łętocha et al., 2022).

Preparation method

The method of preparation of calcium alginate microspheres affects the particle size because each method has advantages and disadvantages. In spray drying, the percent yield of the laboratory scale is not optimal due to product loss on the drying chamber walls and a low cyclone capacity to separate fines, less than <2 μm (Sosnik & Seremeta, 2015). The extrusion method has large particle sizes when compared to other methods, which can range from hundreds of micrometers to millimeters (Uyen et al., 2019). In the drop method, the crosslinker solution is added to the alginate-protein dispersion drop by drop using a burette, resulting in larger microsphere particle sizes comparing the aerosolization technique used in spray drying, so the resulting particle size is smaller (Mishra, 2015; Uyen et al., 2019). A summary of research on the characteristics of calcium-alginate microspheres for drug delivery systems is shown in Table 1.

Table 1. Characteristics of Calcium-Alginate Microspheres for Drug Delivery System

No.	Drug	Use	Alginate Conc.	$CaCl_2$ Conc.	Method	Characteristics	Ref.
1.	Ciprofloxacin HCl	Antibiotic	1%; 1.5%; 2%	3%; 5%	Ionotropic gelation method involving aerosolization	Microspheres were smooth and spherical, with a particle size of less than 5 μm, a moisture content <10%, a yield of 70.63%-82.94%, and drug loading and entrapment efficiencies between 2.58%-4.32% and 27.39%-80.74%. Particle size less than 5 μm with 89% yield, 80% drug loading, and entrapment efficiencies up to 95%.	(Hariyadi et al., 2019)
			2% - 3.5%	0.5M; 1.5M			(Hariyadi et al., 2020)
2.	Gatifloxacin	Antibiotic	1% - 2.2%	7%; 10%	Ionotropic gelation method	The various formulations of Gatifloxacin microspheres exhibited distinct spherical shapes, and it was observed that those with a higher drug concentration had a rougher surface texture. The optimal formulation contained 1.8% sodium alginate, 0.432 mg of pectin, and 10% calcium chloride. This formulation achieved a yield of 96.30% and an entrapment efficiency of 95.66%.	(Nagasree et al., 2016)

3.	Ovalbumin	Protein Antigen	1%; 1.5%	0.25M; 0.5M; 1.5M	Ionotropic gelation using aerosolization and drop technique	Ovalbumin-loaded alginate microspheres have maximum loadings and encapsulation effectiveness of about 89%. Ovalbumin-loaded alginate microspheres produced by aerosolization have a smooth and spherical surface with an average particle size of 12 to 30 µm smaller than the drop technique with microsphere sizes made of 1-3 mm.	(Hariyadi et al., 2014)
4.	Resveratrol	Antioxidant	0.5%; 1%	0.5%; 1%	Ionic gelation	Microspheres have an average particle size of 175.52 µm and 244.03 µm, with an entrapment efficiency of over 95%. Increasing $CaCl_2$ concentration resulted in higher entrapment efficiency and smaller particle size of microspheres.	(Ra et al., 2014)
5.	Risedronate Sodium	Osteoporosis drug	3%	2%; 4 %	Emulsification crosslinking method	The yield of Risedronate sodium microspheres is 61.29-89.33%, with entrapment efficiency of around 42.25-62.58% and mucoadhesion strength 68.15-82.24.	(Gedam et al., 2018)
6.	Gliclazide	Antidiabetic	1%	2-10%	Ionic gelation	Glicazide microspheres had spherical shapes and rough surfaces with particle sizes between 752.12 µm-948.49 µm. These microspheres had a drug entrapment efficiency of around 58.12-82.78%. The entrapment efficiency of glicazide microspheres decreased with increasing particle size, but on the other hand, the entrapment efficiency increased with decreasing TSP to alginate ratio and increasing crosslinker concentration.	(Pal & Nayak, 2012)
7.	Olmesartan	Antihypertensive	1% - 2.5%	6%; 10%	Ionotropic gelation method	The formula of microspheres containing sodium alginate 1.75%, ethyl cellulose 250 mg, and calcium chloride 10% showed a spherical shape with a good percentage yield of 98.34% and entrapment efficiency of 97.36%.	(Kumar & Suresh, 2018)
8.	Quercetin	Anti-inflammatory	1% - 2.5%	5.5%	Ionic gelation with aerosolization	Quercetin-loaded alginate microspheres have a percentage of yield 41.33%-76.14%, drug loading <6%, and entrapment efficiency 74.153%-93.805%. They also had particle sizes of less than 2 µm with excellent flow.	(Kalalo et al., 2022)
9.	Cefixime	Antibiotic	3% - 6%	5%	Ionic gelation technique	Microspheres had more spherical shapes, smooth surfaces, good flowing characteristics with particle size 642-720 µm, and efficiency of entrapment 88.30%-89.01%. The entrapment efficiency of microspheres increased with the increase of polymer.	(Vasam et al., 2016)

10.	Aceclofenac	NSAID	1% - 6%	5%	Ionic gelation technique	Microspheres have a particle size that ranges from 650 to 802 µm, with a yield between 88.71% and 96.64%. The drug loading varies from 13.19% to 28.65%, while the entrapment efficiency lies between 51.29% and 89.34%. The entrapment efficiency and particle size of aceclofenac microspheres increased with higher polymer concentration.	(Chakraborty et al., 2012)
11.	Erythropoietin	Glycoprotein hormone	2%	0.5M; 0.75M; 1M	Ionotropic gelation method involving aerosolization	Microspheres have smooth and spherical surfaces with particle size of about 2.86-3.23 µm and mass swelling index at 24h between 1.11-1.25; at 30 h, it was 1.72-2.00 and yield around 77.76-82.97%.	(Hariyadi et al., 2018)
12.	Risperidone	Antipsychotic	1.5%; 3%	5%	Employing cross-linking method	The formed microspheres exhibited good drug loading 78.6-59.9% and encapsulation efficiencies 71.97-72.63%. Increasing the drug-to-alginate ratio increased the percentage of loading.	(Al-Tahami, 2014)
13.	Glutathione	Antioxidant	2%	1M	Ionotropic gelation method involving aerosolization	Alginate microspheres loaded with glutathione and surfactants have been successfully created. Alginate 2% was used to produce the small and spherical microspheres.	(Hariyadi et al., 2018)
14.	Bordetella pertussis	Vaccine	3.8%	8%	Emulsification method	The microspheres, both empty and loaded with *Bordetella pertussis*, have smooth and spherical surfaces. The microspheres containing *Bordetella pertussis* produced under ideal circumstances had an average particle size of 151.1 µm, a polydispersity index of 0.43, an 89.6% loading efficiency, and a 36.3% loading capacity.	(Dounighi et al., 2017)

In Vitro Drug Release of Calcium-Alginate Microspheres for Drug Delivery System

The process by which drug molecules in the drug delivery system are transferred to the external surface and then, as solutes, into the release medium is referred to as drug release (Talevi & Ruiz, 2021). It is a complicated phenomenon that may include one or more processes depending on the delivery system (Bruschi et al., 2015). Diffusion, swelling, erosion, and degradation are common mechanisms by which drugs are released from the microspheres (Asmatulu et al., 2009; Balagani et al., 2011), shown in Figure 3.

Diffusion

Diffusion is the process of moving a substance mass in individual molecules from one area of a system to another where there is a gradient concentration. The spontaneous flow of matter reduces the concentration difference during this process. Mass transfer in diffusion is a kinetic process in non-equilibrium systems (Bruschi et al., 2015). When drug molecules in the system dissolve in body fluids surrounding or contained by the particles and move away from the particles, this is known as diffusion of microspheres (Balagani et al., 2011).

Swelling

The mechanism of release by swelling of the microspheres is initially dry microspheres. Still, when the microspheres are placed in the body in contact with aqueous, the microspheres expand, increasing the pressure and porosity inside of them, allowing drug molecules to escape from the swollen tissue (Balagani et al., 2011; Talevi & Ruiz, 2021). The swelling mechanism has three sequential steps: water diffusion, polymer chain relaxation, and drug dissolution, and diffusion (Wang et al., 2020).

Erosion

Some coating using polymers can be designed to gradually erode over time and release the drug that is

contained within the particles. The polymer erosion begins with a change in the carrier microstructure due to water penetrating it which leads to matrix plasticization so that the coating becomes thin and causes the entrapped drug to escape from the microsphere system (Balagani et al., 2011).

Degradation

Polymer degradation in the microsphere system occurs when the polymer chains are hydrolyzed so that the microspheres that have a significant molecular weight become in the polymer chains (Asmatulu et al., 2009). In general, there are three degradation molecules with a lower molecular weight, and then successfully dissolve the drug molecules entrapped processes associated with the release of drug microspheres: physical methods, chemical processes, and biological processes (Wang et al., 2020).

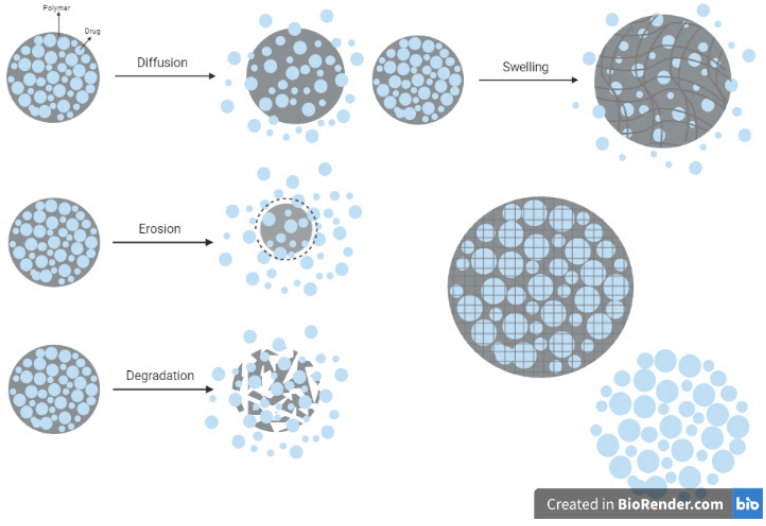

Figure 3. Schematic illustration of the drug release mechanism from the microspheres through diffusion, swelling, erosion, and degradation (Asmatulu et al., 2009).

Drug release of calcium-alginate microspheres follows several kinetics models including zero-order kinetics, first-order kinetics, Korsmeyer-Peppas, and Higuchi (Salome et al., 2013). Drug release is in zero-order kinetics, and time is only considered a function of drug release because the process moves forward at a constant rate regardless of drug concentration. First-order release kinetics describes the drug release from a system where the release rate is dependent on concentration. Both Fickian and non-Fickian drug release from swelling as well as non-swelling polymeric delivery systems were examined using the Korsmeyer-Peppas model. The Higuchi model describes the release of water-soluble and low-solubility drugs, including in semisolid and solid matrices. The Higuchi model is based on several hypotheses, including that the initial concentration of the drug in the formulation is greater than the drugs solubility, that the drug is only dispersed in one dimension, that the substance's particles are smaller than the size of the carrier, that swelling of the system and the drug's dissolution are not significant, that the drug's diffusivity is unaltered, and that sink conditions are reached (Salome et al., 2013; Wang et al., 2020). A summary of *in vitro* drug release studies of calcium-alginate microspheres for drug delivery systems is shown in Table 2.

Table 2. *In Vitro* Drug Release of Calcium-Alginate Microspheres

No.	Drug	Release Medium	*In Vitro* Release	Release Kinetics Profile	Ref.
1.	Ciprofloxacin HCl	Phosphate buffer saline (pH 7.4)	The amount of ciprofloxacin released for 24 hours ranged from 80 to 100%. The release rate was shown to decrease with increasing alginate and $CaCl_2$	Zero order with a mechanism based on non-Fickian diffusion	(Hariyadi et al., 2019)
2.	Gatifloxacin	N HCl (pH 1.2)	The optimum formulation, containing sodium alginate 1.8%, pectin 0.432 mg, and calcium chloride 10%, showed the highest release, 95.21%, after 12 h. This formulation provides a controlled release compared to the innovator product.	Higuchi model with non-Fickian diffusion release mechanism	(Nagasree et al., 2016)
3.	Resveratrol	Phosphate buffer saline (pH 7.4)	In comparison to wet microspheres, freeze-dried resveratrol microspheres showed a slower initial burst release. Dry microspheres only release 30% of their load while more than 60% of their loadings are released by wet microspheres during 30 minutes. Increasing alginate and crosslinker concentrations resulted in a slower release rate for microspheres.	Korsmeyer-Peppas model with anomalous transport mechanism	(Ra et al., 2014)
4.	Olmesartan	N HCl (pH 1.2)	The formulation containing sodium alginate 1.75%, ethyl cellulose 250 mg, and $CaCl_2$ 10% was the optimum formula and showed the highest drug release 96.98% ± 5.28 within 12 hours. Olmesartan microspheres may represent a viable option for sustained drug delivery, providing a secure and efficient method that increases bioavailability.	Zero order with anomalous non-Fickian diffusion	(Kumar & Suresh, 2018)
5.	Aceclofenac	0.1 N HCl (pH 1.2) for 2 hours followed by phosphate buffer (pH 6.8)	Aceclofenac was released slowly in an acidic medium (3.16% to 11.31%) and more rapidly in a phosphate buffer due to the swelling and rapid erosion of sodium alginate in the higher pH environment. Additionally, the release of aceclofenac slowed as the polymer concentration increased, with a sustained release observed for up to 12 hours.	Korsmeyer-Peppas model with Fickian diffusion mechanism	(Chakraborty et al., 2012)
6.	Metronidazole	0.1 M HCl (pH 1.2) and simulated vaginal fluid (SVF pH 4.2)	After 0.5 hours, significant burst releases of metronidazole were seen in SVF, and for the next 4 hours, the drug continued to be released continuously. In contrast, there was no burst effect in 0.1M HCl, and after 3 hours, 80% metronidazole was released and lasted for up to 6 hours. Metronidazole is sustained and released in 0.1M HCl when sodium alginate is converted to insoluble (at acidic pH) alginic acid.	First order with a mechanism based on fiction diffusion in SVF and anomalous transport in HCl	(Szekalska et al., 2015)
7.	Diclofenac Sodium	HCL/NaCl (pH=1.2) and Phosphate buffer saline (pH 6.8)	Diclofenac microspheres did not swell and did not dissolve in acidic media so the cumulative release obtained was only less than 1%. In contrast, in phosphate-buffered saline solution, the release of diclofenac approached 100% within 3 hours. Sustained release of diclofenac is obtained by a first-order kinetics model.	First order with a mechanism based on Fickian diffusion	(Song et al., 2018)
8.	Curcumin	Phosphate buffer saline (pH 7.4).	The formulation containing alginate 4% (w/v) was the optimum formula. The alginate matrix system effectively encapsulated curcumin, resulting in a prolonged drug release pattern over an extended period, with 98.32% of the drug released cumulatively after 672 h. Furthermore, the high Ca^{2+} crosslinker concentration may have contributed to the extended drug release behavior.	Zero order with anomalous non-Fickian diffusion	(Uyen et al., 2020)

9.	Zidovudine	0.1 M HCl and Phosphate buffer saline (pH 7.4)	It was discovered that the total percentage of medication released from the various formulations ranged between 84.63% and 97.17%. Zidovudine release from the microspheres was prolonged for 8 to 12 hours. Microspheres produced with sodium alginate and 1% chitosan as the coated polymer showed a satisfactory sustained release profile.	Zero order with a mechanism based on bizarre and super case (II) transport	(Rao & Kanakamn, 2019)
10.	Histone Deacetylase Inhibitor (HDACi)	Phosphate buffer saline (pH 7.4)	The drug release time gradually increased as the concentration of Ca^{2+} increased. At a concentration of 2% crosslinked calcium ions, the duration of drug release was approximately 7 minutes. This duration was extended to about 25 minutes when the calcium ion concentration increased to 10%.	-	(Man et al., 2022)
11.	Gallic Acid and Crocin	Destilled water (pH 6.8)	Gallic acid and crocin, as bioactive substances, were released from microcapsules with rapid kinetics (85% within the first 20 minutes for gallic acid and 75% within the first 50 minutes for crocin) at a pH of 6.8 in distilled water (as a hydrophilic system) with no observed effect of alginate polymer concentration on the release kinetics. The release mechanism is affected by the structure and physicochemical properties of the encapsulated molecules.	The release kinetics of gallic acid and crocin follow the Korsmeyer–Peppas model with the Fickian diffusion mechanism.	(Essifi et al., 2021)

In vitro drug release studies of calcium-alginate microspheres have shown that increasing concentration of polymers and crosslinker causes a decrease in drug release (Hariyadi et al., 2014; Ra et al., 2014; Al-Tahami, 2014; Hariyadi et al., 2019; Kalalo et al., 2022) this occurs due to an increase in the viscosity and bond strength of the crosslinker so that the surface of the formed microspheres is thicker and denser, resulting in a more extended drug release from the microspheres due to a slower rate of diffusion of the loose media into the microspheres, thereby preventing burst release (Ra et al., 2014) and obtaining extended drug release such as risperidone microspheres showed extended drug release for 8 hours in phosphate buffer (Al-Tahami, 2014), controlled drug release (Nagasree et al., 2016), sustained drug release (Szekalska et al., 2015; Kumar & Suresh, 2018; Song et al., 2018; Uyen et al., 2020) and prolonged release (Rao & Kanakamn, 2019).

In Vitro Activity of Calcium-Alginate Microspheres for Drug Delivery System

In vitro activity studies of calcium alginate microspheres were carried out to assess the activity capabilities of drugs encapsulated in the microsphere system compared with conventional preparations. Several studies regarding the use of microspheres as a drug delivery system to increase drug activity are shown in Table 3.

Table 3. *In Vitro* Activity of Calcium-Alginate Microspheres

No.	Drug	Application	*In Vitro* Activity	Ref.
1.	Ciprofloxacin HCl	Antibacterial activity	The *in vitro* diffusion technique was used to test the antibacterial activity. An inhibitory diameter ranging from 15.05 ± 0.07 mm and 15.30 ± 0.36 mm was observed in the ciprofloxacin-alginate formula antibacterial activity test against *Staphylococcus aureus* (ATCC 6538). Similarly, an inhibitory diameter ranging from 15.37 ± 0.38 mm and 15.92 ± 0.28 mm was observed in the antibacterial activity test against *Escherichia coli* (ATCC 8739).	(Hariyadi et al., 2019[a])
2.	Curcumin	Antibacterial activity	The agar well diffusion technique was used to test for antibacterial activity. The microsphere sample displayed an inhibition zone measuring 1.21 cm in diameter on the *Staphylococcus aureus* (ATCC 12600), but no inhibition zone was visible on the *Escherichia coli* (ATCC 25922). Therefore, the in vitro analysis indicates that Cur-AMs were resistant to E. *coli* and susceptible to S. *aureus*.	(Uyen et al., 2020)
3.	Ciprofloxacin HCl	Antibacterial activity	The in vitro diffusion technique was used to test the antibacterial activity. It was shown that the activity of every formula microsphere against *Staphylococcus aureus* (ATCC 25923) was greater than that of ciprofloxacin HCl. However, ciprofloxacin HCl was only effective at alginate polymer concentration greater than 0.75%, according to results for the formula against *Pseudomonas aeruginosa* (ATCC 27853).	(Hariyadi et al., 2019[b])
4.	Nystatin	Antifungal activity	The antifungal activity of the microspheres was tested using the *Candida albicans* (ATCC 10231). In all nystatin-loaded microspheres, fungicidal activity was shown for up to 48 hours. When the commercial product and microspheres were separated for four hours, no statistically significant differences were seen. Nystatin microspheres showed marked fungicidal activity, as evidenced by a drastic reduction in the initial load of *Candida albicans*.	(Martín et al., 2015)
5.	Metformin	Hypoglycemic activity	The hypoglycemic effect was studied in vitro using a glucose uptake assay by *Saccharomyces cerevisiae* cells. Alginate microspheres improve the hypoglycemic activity of metformin, according to the evaluation of its *in vitro* hypoglycemic activity.	(Szekalska et al., 2016)
6.	Astaxanthin	cell proliferation inhibition of hepatoma cells	The growth of THLE-2 cells was not inhibited by astaxanthin. However, after 96 hours of incubation at 40 μM, the development of HepG2 cells was significantly suppressed, with an inhibition rate that could reach 40%. The current investigation showed that astaxanthin encapsulated in calcium alginate could inhibit the division of HepG2 cells, but had little to no influence on the proliferation of THLE-2 cells.	(Zhang et al., 2020)
7.	Doxorubicin and $NaHCO_3$	Anticancer activity	The cytotoxicity of alginate microspheres with or without $NaHCO_3$ and, or with or without Dox was assessed using two cell lines generated from hepatocellular carcinomas: Huh-7 [Tumor Protein 53 (TP53)-positive)] and Hep-3B (TP53-deficient). The results showed that cell viability decreased in microspheres containing Dox with a relatively high $NaHCO_3$ ratio in both time- and dose-dependent manners.	(Pan et al., 2021)

Stability of Calcium-Alginate Microspheres for Drug Delivery Systems

Pharmaceutical product stability is a complex set of processes for developing the effectiveness, quality, and safety of pharmaceutical product formulations. According to ICH, based on temperature and humidity, the stability test is divided into four climate zones: Zone I (temperature climate), Zone II (subtropical and Mediterranean climates), Zone III (hot and dry climate), and Zone IV (hot and humid environment). Generally, stability carried out on calcium alginate microspheres includes long-term/real-time stability and accelerated (WHO, 2018). Factors that affect drug stability include temperature, humidity, pH, excipients, oxygen, and light (Zothanpuii et al., 2020). The stability of calcium-alginate microspheres for drug delivery is shown in Table 4.

Table 4. Stability Study of Calcium-Alginate Microspheres

No.	Drug	Method	Stability Result	Ref.
1.	Ciprofloxacin HCl	The accelerated stability test was conducted for 28 days at 0, 7, 14, 21, and 30 days in a room at 25±2°C and an oven at 40±2°C with RH 75±5%.	After 30 days of storage, it was confirmed that all the microspheres were stable because there had been no appreciable changes to their morphology, organoleptic, and drug content.	(Hariyadi et al., 2020)
2.	Gatifloxacin	According to ICH guidelines, the stability study was conducted under various conditions and stored in a stability chamber. Accelerated stability tests were run for 6 months at 40°C and 75% RH.	Stability studies were conducted over 6 months to measure the yield percentage, entrapment efficiency, and in-vitro drug release profile. The results indicate that the optimized formulation is stable and maintains its original properties.	(Nagasree et al., 2016)
3.	Risedronate Sodium	The effect of temperature and humidity on the microspheres was tested during a 30-day stability test that was conducted at 4°C and 45% RH.	Microspheres kept at room temperature lose their stability over time, but those kept at 4°C retain their strength. This is indicated by the decreased entrapment efficiency and different drug release patterns after storage at room temperature for 30 days.	(Gedam et al., 2018)
4.	Olmesartan	Following ICH recommendations, the stability test of the optimum formulas was performed under various circumstances. The accelerated stability test was conducted for 6 months in a stability chamber at 40±2°C with RH 75±5%.	Stability test results show minor variations in yield percentage, entrapment efficiency, and cumulative percent of drug released so that the optimized formulation is stable and retains its original properties.	(Kumar & Suresh, 2018)
5.	Quercetin	The stability of quercetin-loaded calcium alginate microspheres was tested for 28 days at two different temperatures 25°C±2°C and 40°C±2°C, RH 75±5%.	The findings revealed that particle size increased at both 25°C and 40°C. However, the four formulas still meet the inhalation delivery requirement of a particle size of >6 µm. Because quercetin was degraded during storage under the influence of temperature and storage time, thereby decreasing the drug loading and entrapment efficiency of the microsphere.	(Kalalo et al., 2022)
6.	Aceclofenac	Following ICH guidelines, the formulation of microspheres aceclofenac was tested for stability. Six sets of microsphere samples were prepared, sealed in tubes, and stored for 30 days in a room at 25±2°C with RH 60±5% and under accelerated conditions at 40±2°C with RH 75±5%.	The optimized alginate microsphere formulation had a shelf life of 3.57 years at room temperature and 1.96 years under accelerated conditions. The texture of the microspheres had barely changed after 150 days. Within 180 days, the drug loading in the accelerated condition dropped to 97.55%, whereas good stability was seen in the case of room temperature (98,61±3.26%).	(Chakraborty et al., 2012)
7.	Glutathione	Storage in an oven for five days at 50, 60, 80°C and 75%RH was required for the glutathione microspheres stress test.	According to the results of the stress test, glutathione belonged to the first-order. Furthermore, the glutathione is more stable with surfactants than glutathione alone.	(Hariyadi et al., 2018)
8.	Zidovudine	According to ICH guidelines, the microspheres produced in this study were kept in HDPE containers for 3 months at 40°C/75% RH. Next, the drug loading was characterized.	The accelerated stability test findings indicated that the microsphere formulation was stable. This is demonstrated by the fact that the percentage of drug loading in the microsphere formulation has not changed significantly.	(Rao & Kanakamn, 2019)

Based on the results of research on the stability of calcium-alginate microspheres that have been developed for drug delivery, it can be concluded that drug stability can be increased and maintained when the drug is encapsulated in a matrix such as calcium-alginate microspheres with a preparation that uses sodium alginate as a polymer and $CaCl_2$ as a crosslinker.

In Vivo Studies of Calcium-Alginate Microspheres for Drug Delivery Systems

An *in vivo* study tests drugs or chemicals in living organisms, such as animals or humans. This method aims to determine the effect of drugs or chemicals on the organism, including possible side effects. *In vivo* testing is often used to develop new drugs, including the development of calcium-alginate microspheres for drug delivery systems. A summary of the *in vivo* studies of calcium-alginate microspheres is shown in Table 5.

Table 5. *In Vivo* Studies of Calcium-Alginate Microspheres

No.	Drug	Pharmaceutical Applications	*In Vivo* Study	Ref.
1.	Gliclazide	Antidiabetic	Gliclazide microspheres were used in in vivo investigations comparing pure gliclazide solution to diabetic albino rats caused by alloxan. When pure gliclazide solution is administered, blood glucose levels can drop sharply for up to 2 hours before swiftly returning to normal. In contrast, when gliclazide microspheres are administered, the most significant drop in blood glucose levels is observed 4 hours after oral administration and the decrease in blood glucose levels lasts 12 hours. These *in vivo* studies demonstrate that gliclazide microspheres effectively lower blood glucose levels when administered orally. These in vivo investigations show the gliclazide microspheres have significant hypoglycemic effects when taken orally. They may be beneficial for the extended systemic absorption of gliclazide to sustain appropriate blood glucose levels and improve patient adherence.	(Pal & Nayak, 2012)
2.	Aceclofenac	Anti-inflammatory	Aceclofenac microspheres, pure drug, and marketed formulations were tested for their anti-inflammatory, based on their ability to prevent rat hind paw edema caused by carrageenan. The anti-inflammatory action of the pure medication is remarkably rapid (88.71 ± 7.78% inhibition in 4 hours) when compared to the marketed product (74.47 ± 3.64 % inhibition in 8 hours) and optimized alginate microsphere formulation (84.41 ± 4.82 % inhibition in 8 hours). Studies conducted *in vivo* have demonstrated that aceclofenac microspheres had a much stronger and longer-lasting anti-inflammatory effect than pure aceclofenac.	(Chakraborty et al., 2012)
3.	Nystatin	Antifungal	According to *in vivo* research, nystatin was not detected in the bloodstream, indicating the treatment's safety. The levels of nystatin that were kept in the mucosa were more than sufficient to have a fungicidal impact that was successful and without causing tissue damage. The resulting levels of nystatin kept in the microsphere-retained porcine mucosa were 4.73 ± 0.18 µg/g tissue/cm^2. The concentration of nystatin that was retained in the mucosa was six times higher than the 0.78 µg/mL, or 0.79 µg/g, minimum inhibitory concentration (MIC).	(Martín et al., 2015)
4.	Carvedilol	Pharmacokinetics Deposition and Clearance	A pharmacokinetics investigation showed that the area under the curve (AUC) of carvedilol microspheres increased. Following intranasal delivery of carvedilol microspheres and carvedilol solution intravenously, the AUC was approximately 215.83 ± 18.56 and 54.06 ± 6.45 µg h/ml, and Cmax values of microspheres were 64.85 ± 4.15 µg h/ml. Carvedilol microspheres had a relative bioavailability of 67.87%, suggesting that nasal administration enhances carvedilol absorption from alginate microspheres in rabbits. As demonstrated by pharmacokinetic studies, the gamma scintigraphy showed that the microspheres cleared more slowly and stayed in the nasal cavity longer than the lactose powder (61.55% of the alginate microspheres and 87.36% of the lactose powder were cleared from the nasal cavity after 4 hours). This implies that the nasal mucosa's sustained and improved medication absorption is provided by the microspheres.	(Patil et al., 2012)
5.	Mesalamine	Anti-inflammatory	In vivo research was carried out on rats with inflammatory colonic lesions, and a decrease in the ulcer index was observed. The colon damage score for healthy controls was 0.0 ± 0.0, colitis controls was 5.0 ± 0.0, and the scores for those treated with conventional mesalamine and mesalamine microspheres were 2.2 ± 0.8 and 1.6 ± 0.6, respectively. Compared to other groups, the mesalamine microsphere formulation intended for the colon demonstrated a notable decrease in ulcer index. Moreover, tissue sample histological analysis verified the outcomes. The amount and severity of histological indications of cell damage were significantly reduced in the mesalamine microsphere formulation. There were no indications of bleeding or ulceration, and the colon looked normal.	(Patole & Pandit, 2018)

6.	Vascular Endothelial Growth Factor (VEGF)	Angiogenesis protein for Revascularization	Adipose tissue transplantation receptors were created using BALB/c nude mice. Mice were given subcutaneous implants of adipocytes combined with VEGF-CA microspheres in their dorsum. In comparison to the other groups, the mass and microvascular density of the grafts in the VEGF-CA microspheres were observed to be statistically higher in a time-dependent way. Adipose graft neovascularization was markedly enhanced by VEGF-CA microspheres, which also increased adipocyte survival.	(Ding et al., 2015)
7.	Dendritic Cell	Tumor Vaccine	Tumor lysates, live tumor cells, a recombinant MIP-3a adenovirus, and BCG were all encapsulated in an alginate microsphere (PaLtTcAdMIP3a). PaLtTcAdMIP3a was employed as a model vaccination to evaluate its antitumor activity. PaLtTcAdMIP3a was injected into tumor-bearing mice, and the results demonstrated antitumor immunity in CT26, Meth A, B16-F10, and H22 models, both therapeutically and prophylactically, without increasing side effects. The antitumor activity was partially eliminated with the depletion of $CD8^+$ and $CD4^+$ T cells. Additionally, the number of $CD4^+CD25^+FOXP3^+$ regulatory T cells (Treg) in the tumor tissues decreased. In contrast, the number of IFN-g-producing $CD8^+$ T cells increased significantly in both the spleen and the tumor tissues. These findings strongly imply that microspheres might be a helpful vaccination for tumor models.	(Huang et al., 2015)
8.	Isoniazid	Pharmacokinetics	Microspheres were prepared by encapsulating isoniazid into a calcium alginate-piperine matrix (INH-CaSP Ms) to improve the encapsulation efficiency and oral bioavailability of isoniazid. The oral bioavailability results showed that, compared to pure INH, INH-CaSP Ms significantly increased the oral bioavailability of INH by raising the Cmax, Tmax, t1/2, and AUC values.	(Telange et al., 2022)

CONCLUSION

This review study explores a range of studies on the development of calcium alginate microspheres for drug delivery, primarily focusing on characteristics, drug release, activity, stability, and in vivo studies. Calcium-alginate microspheres have advantages over other formulations because alginate when compared to other polysaccharides, has a more remarkable ability to form gels that are not affected by temperature so that it has excellent thickening and gelling properties and provides the highest mucoadhesiveness and is capable of multiple chemical modifications with $CaCl_2$. It has been determined in those studies that various formulations of calcium alginate-based microspheres possess diverse characteristics. Additionally, it has been shown that specific formulations exhibit a steady-slow release profile, thereby preventing burst release, and the microsphere formulation increased the drug activity through *in vitro* studies with good stability when the drug is encapsulated in a matrix such as calcium-alginate microspheres.

Furthermore, *in vivo* studies demonstrated increased drug effectiveness on calcium-alginate microspheres compared with conventional formulations.

ACKNOWLEDGMENTS

The authors thank the Faculty of Pharmacy Universitas Airlangga for the facilities and research support.

AUTHOR CONTRIBUTION STATEMENT

Determination of the Subject (A, DMH), Literature Research (A, DMH, MR), Preparing the Study Text (A), Reviewing the Text (MR, DMH).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Adrian, G., Mihai, M., & Vodnar, D. C. (2019). The Use of Chitosan, Alginate, and Pectin in the Biomedical and Food Sector- Biocompatibility, Bioadhesiveness, and Biodegradability. *Polymers*, *11*(1837), 7–11.

Al-Tahami Alkhaled Pharma, K. (2014). Preparation of Alginate Microspheres for the Delivery of Risperidone. *Yemeni Journal for Medical Sciences*, *8*, 19–23.

Amiruddin, Muh. Agus Syamsur Rijal, & Dewi Melani Hariyadi. (2023). Effect of CaCl2 Crosslinker Concentration On The Characteristics, Release, and Stability of Ciprofloxacin HCl-Alginate-Carrageenan Microspheres. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, *10*(3), 312–323.

Asmatulu, R., Fakhari, A., Wamocha, H. L., Chu, H. Y., Chen, Y. Y., Eltabey, M. M., Hamdeh, H. H., & Ho, J. C. (2009). Drug-Carrying Magnetic Nanocomposite Particles for Potential Drug Delivery Systems. *Journal of Nanotechnology*, *2009*(January), 1–6.

Balagani, P. K., Chandiran, I., B.Bhavya, & Manubolu, S. (2011). Microparticulate drug delivery system: a review. *Indian Journal of Pharmaceutical Science & Research*, *1*, 19–37.

Batista, P. S. P., de Morais, A. M. M. B., Pintado, M. M. E., & de Morais, R. M. S. C. (2019). *Alginate: Pharmaceutical and Medical Applications BT - Extracellular Sugar-Based Biopolymers Matrices* (E. Cohen & H. Merzendorfer (eds.); pp. 649–691). Springer International Publishing.

Bruschi ML. (2015). Main mechanisms to control the drug release. *Strategies to Modify the Drug Release from Pharmaceutical Systems*, 37–62.

Chakraborty, S., Khandai, M., Sharma, A., Khanam, N., Patra, C., Dinda, S., & Sen, K. (2012). Preparation and in vitro in vivo evaluation of aceclofenac loaded alginate microspheres: An investigation of effects of polymer using multiple comparison analysis. *Current Drug Delivery*, *9*(5), 495–505.

Ching, S. H., Bansal, N., & Bhandari, B. (2017). Alginate gel particles – A review of production techniques and physical properties. *Critical Reviews in Food Science and Nutrition*, *57*(6), 1133–1152.

Choukaife, H., Doolaanea, A. A., & Alfatama, M. (2020). Alginate nanoformulation: Influence of process and selected variables. *Pharmaceuticals*, *13*(11), 1–35.

Dhamecha, D., Movsas, R., Sano, U., & Menon, J. U. (2019). Applications of alginate microspheres in therapeutics delivery and cell culture : Past, present and future. *International Journal of Pharmaceutics*, *569*(May), 118627.

Ding, S. L., Zhang, M. Y., Tang, S. J., Yang, H., & Tan, W. Q. (2015). Effect of calcium alginate microsphere loaded with vascular endothelial growth factor on adipose tissue transplantation. *Annals of Plastic Surgery*, *75*(6), 644–651.

Dounighi N, Shahcheraghi F, Razzaghi-Abyaneh M, Nofeli M, Z. H. (2017). A New Vaccine Delivery Vehicle and Adjuvant Candidate: Bordetella pertussis Inactivated Whole Cells Entrapped in Alginate Microspheres. *Current Pharmaceutical Design*, *23*(12), 2665–2672.

Essifi, K., Brahmi, M., Berraaouan, D., Ed-Daoui, A., El Bachiri, A., Fauconnier, M. L., & Tahani, A. (2021). Influence of Sodium Alginate Concentration on Microcapsules Properties Foreseeing the Protection and Controlled Release of Bioactive Substances. *Journal of Chemistry*, *2021*.

Fadhilah, S., Aisyah, N., Mohd, N., & Mat, K. A. (2019). Sodium alginate film : the effect of crosslinker on physical and mechanical properties. *IOP Conference Series: Materials Science and Engineering*, *209*, 1–6.

Fernando, I. P. S., Lee, W., Han, E. J., & Ahn, G. (2020). Alginate-based nanomaterials: Fabrication techniques, properties, and applications. *Chemical Engineering Journal*, *391*.

Frent, O. D., Vicas, L. G., Duteanu, N., Morgovan, C. M., Jurca, T., Pallag, A., Muresan, M. E., Filip, S. M., Lucaciu, R., & Marian, E. (2022). Sodium Alginate — Natural Microencapsulation Material of Polymeric Microparticles. *International Journal of Molecular Sciences Review*, *23*(12108), 1–24.

Gedam, S., Jadhav, P., Talele, S., & Jadhav, A. (2018). Effect Of Crosslinking Agent On Development Of Gastroretentive Mucoadhesive Microspheres Of Risedronate Sodium. *International Journal of Applied Pharmaceutics*, *10*(4), 133–140.

Hariyadi, D., & Hendradi, E. (2020). *Optimization Performance and Physical Stability of Ciprofloxacin HCL- Ca Alginate Microspheres : Effect of Different Concentration of Alginate*. 89–94.

Hariyadi, D. M., & Hendradi, E. (2019a). Effect of Polymer Concentration on Micromeritics, Kinetics, and Activity of Ciprofloxacin HCl-Alginate Microspheres. *Asian Journal of Pharmaceutics*, *13*(4), 349–355.

Hariyadi, D. M., Hendradi, E., & Kurniawan, T. D. (2019b). Alginate Microspheres Encapsulating Ciprofloxacin HCl : Characteristics, Release and Antibacterial Activity. *International Journal of Pharma Research and Health Sciences*, *7*(4), 3020–3027.

Hariyadi, D. M., Hendradi, E., Purwanti, T., Fadil, F. D. G. P., & Ramadani, C. N. (2014). Effect of cross-linking agent and polymer on the characteristics of ovalbumin loaded alginate microspheres. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(4), 469–474.

Hariyadi, D. M., Purwanti, T., & Adilla, S. (2018). Influence of crosslinker concentration on the characteristics of erythropoietin-alginate microspheres. *Journal of Pharmacy & Pharmacognosy Research*, *6*(4), 250–259.

Hariyadi, D. M., Rosita, N., & Nugrahaeni, F. (2018). Formulation, characteristic evaluation, stress test, and effectiveness study of matrix metalloproteinase-1 (MMP-1) expression of glutathione-loaded alginate microspheres and gel. *Pharmaceutical Sciences*, *24*(4), 304–312.

Hasnain, S., Jameel, E., Mohanta, B., Dhara, A. K., Alkahtani, S., & Nayak, A. K. (2020). Chapter 1. Alginates: sources, structure, and properties. In *Alginates in Drug Delivery*. INC.

Huang, F. Y., Huang, F. R., Chen, B., Liu, Q., Wang, H., Zhou, S. L., Zhao, H. G., Huang, Y. H., Lin, Y. Y., & Tan, G. H. (2015). Microencapsulation of tumor lysates and live cell engineering with MIP-3α as an effective vaccine. *Biomaterials*, *53*, 554–565.

Jain, K. K. (2020). *An Overview of Drug Delivery Systems* (Vol. 2059).

Kadam N. R. and Suvarna. (2015). Microsphere: A Brief Review. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *05*(47), 13–19.

Kalalo, T., Miatmoko, A., Tanojo, H., Erawati, T., Hariyadi, D. M., & Rosita, N. (2022). Effect of Sodium Alginate Concentration on Characteristics, Stability and Drug Release of Inhalation Quercetin Microspheres. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, *9*(2), 107–114.

Kumar, K. R., & Suresh, G. (2018). Development and Characterization of Alginate Microspheres Containing Olmesartan by Ionotropic Gelation Method. *International Journal of Pharmaceutical Sciences and Drug Research*, *10*(4), 335–341.

Lee, K. Y., & Mooney, D. J. (2012). Alginate: Properties and biomedical applications. *Progress in Polymer Science (Oxford)*, *37*(1), 106–126.

Łętocha, A., Miastkowska, M., & Sikora, E. (2022). Preparation and Characteristics of Alginate Microparticles for Food, Pharmaceutical and Cosmetic Applications. *Polymers*, *14*(18).

Loquercio, A., Castell-Perez, E., Gomes, C., & Moreira, R. G. (2015). Preparation of Chitosan-Alginate Nanoparticles for Trans-cinnamaldehyde Entrapment. *Journal of Food Science*, *80*(10), N2305–N2315.

Mali, K. K., Dias, R. J., Ghorpade, V. S., & Havaldar, V. D. (2010). Sodium alginate microspheres containing multicomponent inclusion complex of domperidone. *Latin American Journal of Pharmacy*, *29*(7), 1199–1207.

Man, J., Wang, X., Li, J., Cui, X., Hua, Z., Li, J., Mao, Z., & Zhang, S. (2022). Intravenous Calcium Alginate Microspheres as Drug Delivery Vehicles in Acute Kidney Injury Treatment. *Micromachines*, *13*(4), 1–9.

Manjanna, K. M., Kumar, T. M. P., & Shivakumar, B. (2010). Calcium alginate cross-linked polymeric microbeads for oral sustained drug delivery in arthritis. *Drug Discoveries & Therapeutics*, *4*(2), 109–122.

Martín, M. J., Calpena, A. C., Fernández, F., Mallandrich, M., Gálvez, P., & Clares, B. (2015). Development of alginate microspheres as nystatin carriers for oral mucosa drug delivery. *Carbohydrate Polymers*, *117*, 140–149.

Mishra, M. (2015). Handbook of Encapsulation and Controlled Release. In *CRC Press* (Issue 11).

Nagasree, K., Chowdary, G. V, Kumar, C. B. M., Reddy, T. R. M., Bhikshapathi, D. V. R. N., & Nagasree, K. (2016). *Design and evaluation of sodium alginate microspheres loaded with gatifloxacin*. *8*(4), 361–370.

Pal, D., & Nayak, A. K. (2012). Novel tamarind seed polysaccharide-alginate mucoadhesive microspheres for oral gliclazide delivery : in vitro – in vivo evaluation. *Drug Delivery*, *19*(January), 123–131.

Pan, C. T., Yu, R. S., Yang, C. J., Chen, L. R., Wen, Z. H., Chen, N. Y., Ou, H. Y., Yu, C. Y., & Shiue, Y. L. (2021). Sustained-release and pH-adjusted alginate microspheres-encapsulated doxorubicin inhibit the viabilities in hepatocellular carcinoma-derived cells. *Pharmaceutics*, *13*(9).

Patil, J. S., Kamalapur, M. V., Marapur, S. C., & Kadam, D. V. (2010). Ionotropic gelation and polyelectrolyte complexation: The novel techniques to design hydrogel particulate sustained, modulated drug delivery system: A review. *Digest Journal of Nanomaterials and Biostructures*, *5*(1), 241–248.

Patil, S. B., Kaul, A., Babbar, A., Mathur, R., Mishra, A., & Sawant, K. K. (2012). In vivo evaluation of alginate microspheres of carvedilol for nasal delivery. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *1*, 249–255.

Patole, V. C., & Pandit, A. P. (2018). Mesalamine-loaded alginate microspheres filled in enteric-coated HPMC capsules for local treatment of ulcerative colitis: in vitro and in vivo characterization. *Journal of Pharmaceutical Investigation*, *48*(3), 257–267.

Prasad, B. S. G., Gupta, V. R. M., Devanna, N., & Jayasurya, K. (2014). Microspheres as Drug Delivery System – A Review. *Journal of Global Trends in Pharmaceutical Sciences*, *5*(3), 1961–1972.

Ra, A., Yong, C., Chun, G., Keun, B., & Park, D. J. (2014). Preparation of alginate – CaCl 2 microspheres as resveratrol carriers. *J Mater Sci.*, *49*, 4612–4619.

Ramteke K.H, V.B, J., & S.N., D. (2012). Microspheres: as carriers used for novel drug delivery systems. *IOSR Journal of Pharmacy (IOSRPHR)*, *2*(4), 44–48.

Rao, P. S., & Kanakamn, V. (2019). Formulation and evaluation of Zidovudine alginate microspheres. *Journal of Drug Delivery and Therapeutics*, *9*(5-s), 57–64.

Rowe et al. (2009). Handbook of Pharmaceutical Excipients. Sixth Edition. In *Pharmaceutical Press* (Issue 1).

Salome, A. C., Godswill, C. O., & Ikechukwu, I. O. (2013). Kinetics and mechanisms of drug release from swellable and nonswellable matrices: A review. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *4*(2), 97–103.

Saripilli Rajeswari1*, Teella Prasanthi1, Navya Sudha1, Ranjit Prasad Swain1, S. P. and V. G. (2017). Natural Polymers : A Recent Review. *World Journal of Pharmacy and Pharmaceutical Sciences*, *6*(8), 472–494.

Skrzypczak, K. M. D., & Witek, B. L. A. (2019). Preparation of hydrogel composites using Ca 2 + and Cu 2 + ions as crosslinking agents. *SN Applied Sciences*, *1*(6), 1–15.

Solanki, N. (2018). *Microspheres are an innovative approach to drug delivery system*s. 56–58.

Song, P., Wu, Y., Zhang, X., Yan, Z., Wang, M., & Xu, F. (2018). Preparation of Covalently Crosslinked Sodium Alginate/Hydroxypropyl Methylcellulose Ph-Sensitive Microspheres for Controlled Drug Release. *BioResources*, *13*(4), 8614–8628.

Sosnik, A., & Seremeta, K. P. (2015). Advantages and challenges of the spray-drying technology for producing pure drug particles and drug-loaded polymeric carriers. *Advances in Colloid and Interface Science*.

Szekalska, M., Puciłowska, A., Szymańska, E., Ciosek, P., & Winnicka, K. (2016). Alginate: Current Use and Future Perspectives in Pharmaceutical and Biomedical Applications. *International Journal of Polymer Science*, *2016*.

Szekalska, M., Winnicka, K., Sosnowska, K., & Amelian, A. (2015). Evaluation of Alginate Microspheres with Metronidazole Obtained by The Spray Drying Technique. *Acta Poloniae Pharmaceutica- Drug Research*, *72*(3), 569–578.

Szekalska, M., Wroblewska, M., Sosnowska, K. (2016). Influence of Sodium Alginate on Hypoglycemic Activity of Metformin Hydrochloride in the Microspheres Obtained by the Spray Drying. *International Journal of Polymer Science*, 1–12.

Talevi, A., & Ruiz, M. E. (2021). Drug Release. In *The ADME Encyclopedia* (pp. 1–7). Springer International Publishing.

Tandale, S. R., Mole, M. M., & Gawade, V. R. (2020). *Review on Novel Drug Delivery System of Microsphere: Type, Material, Method of Preparation and Evaluation of Microsphere: Method Evaluation*".

Tecante, A., & Núñez, C. (2012). Solution Properties of κ -Carrageenan and Its Interaction with Other Polysaccharides in Aqueous Media. In *Rheology In Tech* (pp. 241–264).

Telange, D. R., Pandharinath, R. R., Pethe, A. M., Jain, S. P., & Pingale, P. L. (2022). Calcium Ion-Sodium Alginate-Piperine-Based Microspheres: Evidence of Enhanced Encapsulation Efficiency, Bio-Adhesion, Controlled Delivery, and Oral Bioavailability of Isoniazid. *AAPS PharmSciTech*, *23*(4).

Thanh Uyen, N. T., Abdul Hamid, Z. A., Thi, L. A., & Ahmad, N. B. (2020). Synthesis and characterization of curcumin-loaded alginate microspheres for drug delivery *Journal of Drug Delivery Science and Technology*.

Uyen, T. N. T., Ain, Z., Hamid, A., Xuan, N., Tram, T., & Ahmad, N. B. (2019). Fabrication of alginate microspheres for drug delivery : a review. *International Journal of Biological Macromolecules*.

Vasam, M., Sriharsha, S. N., & Bhukya, S. (2016). Composition, formulation, and in-vitro evaluation studies of cefixime microspheres. *International Conference on Electrical, Electronics, and Optimization Techniques, ICEEOT 2016*, 3391–3396.

Verma, S. (2019). Microsphere A Novel Drug Delivery System. *Research Chronicle in Health Sciences*, *5*(July).

Wang, S., Liu, R., Fu, Y., & Kao, W. J. (2020). Release Mechanisms and Applications of Drug Delivery Systems for Extended-Release. *Expert Opinion on Drug Delivery*.

World Health Organization. (2018). Q1F Stability testing of active pharmaceutical ingredients and finished pharmaceutical products.

Zhang, X., Li, W., Dou, X., Nan, D., & He, G. (2020). Astaxanthin Encapsulated in Biodegradable Calcium Alginate Microspheres for the Treatment of Hepatocellular Carcinoma In Vitro. *Applied Biochemistry and Biotechnology*, *191*(2).

Zothanpuii, F., R, R., & K, S. (2020). A Review on Stability Testing Guidelines of Pharmaceutical Products. *Asian Journal of Pharmaceutical and Clinical Research*, *13*(October), 3–9.