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A Review of Encapsulation Using Emulsion Crosslinking Method

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ABSTRACT

Encapsulation is a process to protect active components or other materials in the form of liquids, solids, and gases which are sensitive to the environment using coating materials. Encapsulation can be used for the pharmaceutical, food, beverage, textile, and other industries. The encapsulation method has been developed depending on the active ingredient being protected and its function. The encapsulation method is generally divided into chemical and mechanical methods. This review aims to explain the emulsion crosslinking which is one of the encapsulation methods. This method was easy and simple, just add a crosslink agent to the emulsion and then the microparticles formed were washed, filtered, and dried. This review also reports several encapsulation studies using the emulsion crosslinking method.

Keywords: *crosslinker, emulsion crosslinking, encapsulation, wall material*

1. INTRODUCTION

Encapsulation is a process to protect active ingredients from environmental influences using polymer or biopolymer coatings. The product of Encapsulation is in the form of a powder with a micrometer or nanometer size. Protected materials could be dyes, catalysts, cosmetic medicines, curing agents, even plasticizers. The encapsulation types could be in the form of Monocore (Reservoir), Multicore, Matrix, Coated monocore-type Core-Shell, and Coated matrix (Jayanudin, et al., 2016; Miloševic, et al., 2016).

Encapsulation was first developed around the 1950s, in the manufacture of carbonless copy paper. The need for encapsulation technology in various fields has increased from time to time. Encapsulation methods are also rapidly increased. According to Mishra (2016), there are 36 encapsulation techniques that are divided into chemical and mechanical (physical) processes. The method depends on the physical and chemical properties of the core and shell/coating material. The encapsulation method chosen must be selective, not a matter of trial and error to get the right process. The benefits to be achieved from encapsulation technology such as desired properties, increased product/process, storage stability, etc. (Mishra, 2016).

Emulsion crosslinking is one of a simple encapsulation method and could be used to encapsulate core material of soluble, in-soluble, liquid, and solid. Emulsion crosslinking method also produces both microparticles and nanoparticles (Manjanna, et al., 2010; Mitra and Dey, 2011). This method is related to the interaction between coating (wall) material and crosslinking agents, where the interaction is in the emulsion form and then converted become a powder.

The emulsion crosslinking method had long been developed for the preparation of microspheres in pharmaceuticals, as reported by Thanoo, et al (1992). Until now, the emulsion crosslinking method is still used not only for carriers of drugs but also for fertilizers (Jayanudin, et al 2021). Materials used as coatings or carrier of microcapsules/microspheres can come from nature such as chitosan and also synthetic ones such as polyvinyl alcohol. Information about emulsion crosslinking can be useful for research that will use this method.

The aim of this review was to provide the information about encapsulation using emulsion crosslinking method with various wall materials of microcapsules and types of crosslinking agent.

2. ENCAPSULATION WITH EMULSION CROSSLINKING METHOD

Encapsulation using emulsion crosslinking method is a method that utilizes crosslinked the function group of coating with function group of crosslinking agent. This method makes water in oil emulsion (W/O), which emulsifies the coating solution in the oil phase. To stabilize the emulsion can be used a suitable surfactants. After the formation of a stable emulsion, a crosslinking agent was added to harden the emulsion droplets. The microparticles (microcapsules) formed were filtered and washed using petroleum ether followed by hexane. With this method, the particle size can be controlled (Mitra and Dey, 2011). Fig. 1 shows the encapsulation process using the emulsion crosslinking technique.

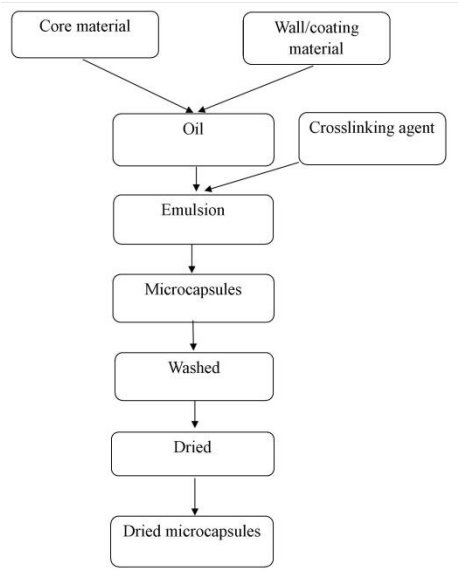
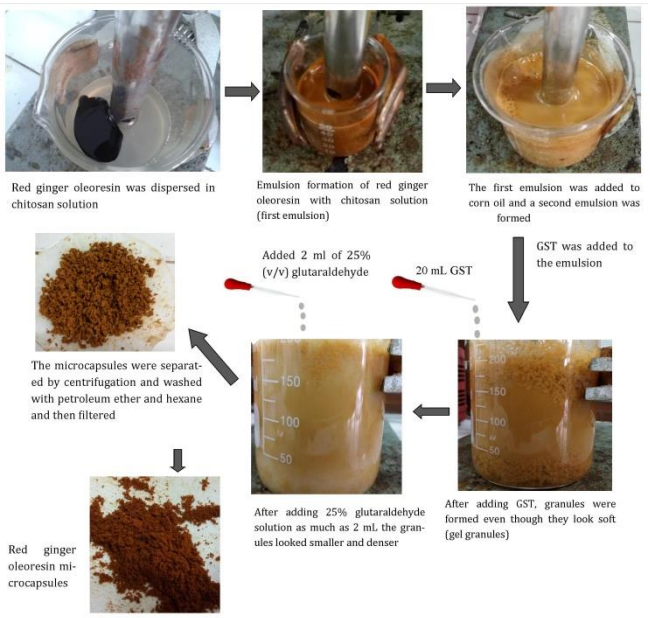


Fig. 1. The encapsulation stage using emulsion crosslinking technique

Figure 1 shows that the initial step of encapsulation using the crosslink emulsion method is to form water in oil (W/O) emulsion. If the main ingredient is oil, the substance will be first emulsified with a coating solution then added into the oil as a continuous phase and stirred to form oil in water in oil (O/W/O) emulsion. After the emulsion is formed, the crosslinking agent is added to form microcapsules dropwise. The cross-linking process caused the coating layer to harden during the solidification process. The continuous phase used is either the combination of heavy and light paraffin, paraffin and petroleum ether, or vegetable oils. Vegetable oils used as the continuous phase are either corn oil, sunflower oil, soybean oil, cottonseed oil, or sesame oil (Campos et al., 2013; Chang and Bodmeier, 1996). The advantage of vegetable oil as a continuous phase is safer and non-toxic, and more stable even without added surfactants (Campos et al., 2013).

The encapsulation method used an emulsion crosslinking had been reported by Jayanudin, et al (2019) with the red ginger oleoresin as core material and chitosan solution as coating. The continuous phase used was corn oil. The stages are as follows: red ginger oleoresin was dispersed into chitosan solution and stirred to form an emulsion. After that, it was added into corn oil and stirred again to form a second emulsion. Glutaraldehyde saturated toluene (GST) was added dropwise into the emulsion and kept stirring. After completing the addition of GST, then proceed by adding a solution of glutaraldehyde with a concentration of 25% (v/v) of 2 mL and keep stirring.



Source: Jayanudin, 2019

Fig. 2. The emulsion crosslinking method for encapsulation of red ginger oleoresin

The addition of a 25% (v / v) glutaraldehyde solution serves to further harden the walls of the microcapsules. The microcapsules formed were separated by centrifuge and then washed using petroleum ether followed by hexane and filtered. The microcapsules were dried in an oven at 65^{o}C.

3. TYPE OF CROSSLINKING AGENT FOR EMULSION CROSSLINKING METHOD

The role of the crosslinking agent for the encapsulation technique with the emulsion crosslinking is very important because it functions to harden the walls of the microcapsule due to the cross linking between the functional groups of coating material and the crosslinking agent. In general, the crosslinking agent used is glutaraldehyde solution or glutaraldehyde saturated toluene (GST). The form of this crosslinking agent reacts evenly because the solubility in the oil medium is uniform to produce microcapsules with a perfectly spherical shape (Thanoo et al., 1992).

Another crosslink agent used is genipin. This crosslinking agent is an alternative to glutaraldehyde because it is toxic and unsafe for consumption. Although according to Campos, et al (2013) the use of glutaraldehyde as a crosslinking agent had no effect because the cross-linking reaction minimizes adverse effects on cell survival.

3.1. Glutaraldehyde

The crosslinking reaction between aldehyde groups (glutaraldehyde) is a covalent bond that forms an imine bonds with amino group (chitosan) and acetal bonds with a hydroxyl group provides efficiency in cross-linking with chitosan (Gonçalves et al., 2005; Campos et al., 2013).

Crosslinking reactions mostly occur between molecules. However, it can also occur intramolecularly. Intramolecular crosslinking is a cross-linking reaction to form a Schiff base by amino and aldehyde. The mechanism of cross-linking reaction between chitosan and glutaraldehyde has not been described in detail. According to Monteiro and Airoldi (1999) there are three different structures used to interpret the crosslinking reaction of glutaraldehyde and chitosan, namely:

1. There is only one Schiff base formation, with one aldehyde functional group from the glutaraldehyde, the other aldehyde groups remain free, and are usually used for subsequent reactions.
2. A crosslinking is formed with only one glutaraldehyde molecule and two chitosan to produce two Schiff bases involving the two aldehyde groups of the glutaraldehyde molecule
3. The cross-linking is formed not only with one glutaraldehyde molecule, but the polymerization of the glutaraldehyde, as a result it forms a larger cross-linking chain.

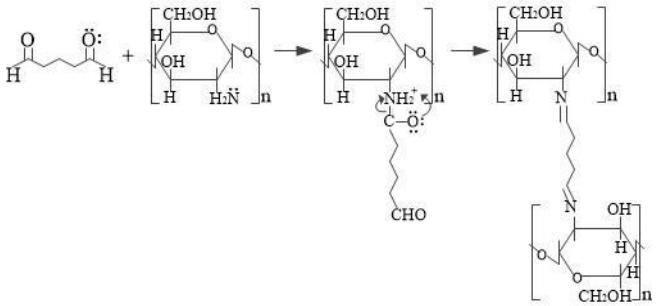


Fig. 3. The chitosan and glutaraldehyde crosslinking reaction mechanism

Figure 3 shows the electrophilic carbon atom of the aldehyde and ketone which can be the target of nucleophilic attack by amines. The end result of this reaction is a compound in which the C = O double bond is replaced by the C = N double bond. This type of compound is known as imine or Schiff base.

The Crosslinking agent of glutaraldehyde can be modified to become glutaraldehyde saturated toluene (GST). This type of crosslinking agent was first used by Longo et al. (1982), GST was made by mixing glutaraldehyde and toluene with a volume ratio of 1: 1 then stirred at a speed of 2000-3000 rpm for 1 hour, the mixture was allowed to stand overnight for the equilibrium process. The mixture would separate into two layers; the GST solution was on the top layer.

A uniform crosslinking process on the droplet surface was desirable to produce a perfectly spherical geometry, so that GST was chosen as the crosslinker instead of glutaraldehyde, because its solubility in the oil medium would be uniform for the cross-linking of the droplet surface.

Solidification of the droplet surface by crosslinker would improve the shape and surface morphology of the microspheres, further crosslinking can be done by adding glutaraldehyde so that the surface hardening process was achieved (Thanoo et al., 1992; Jayanudin, et al., 2019).

3.2. Genipin

Genipin is the result of extraction from the Gardenia jasminoides Ellis plant. Genipin is a natural cross-linking agent for collagen, gelatin, protein and chitosan. Genipin is used as a crosslinking agent because of its biocompatibility and can produce stable and biocompatible products. Genipin is also considered to be a crosslink agent for biomedicine aplication, fixation of biological tissues as bio prostheses (Muzzarelli, 2009; Butler, et al., 2003; Sung, et al., 1999; Manickam, et al., 2014). Genipin is easily soluble in methanol, ethanol, acetone, and slightly soluble in water. This crosslinking agent has 5000-10,000 times less cytoxicity levels than other crosslinkers (Nishi, et al., 1995; Manickam, et al., 2014).

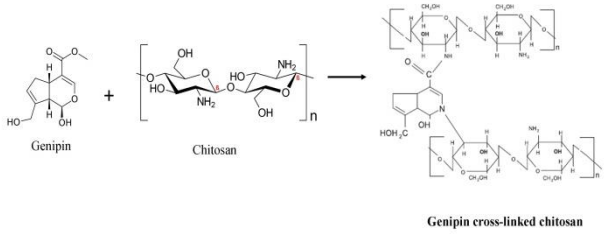


Fig. 4. The reaction of chitosan with genipin

Figure 4 shows chitosan cross-linked genipin. The mechanism of the genipin crosslinking is quite complicated; Zhu and Park (2007) proposed a mechanism based on the genipin ring opening reaction. The step begins when the amino group through a nucleophilic attack on the olefin carbon atom of the genipin. Further covalent grafting of genipin to a polymer with an amino group by a two-step reaction. The aldehyde group is formed as a result of an unstable intermediate which is then attacked again by another amine group from another polymer which forms another covalent bond that produce a cross-link (Pal, et al., 2013).

4. PARAMETERS AFFECTING THE EMULSION CROSSLINKING METHOD

The success of the encapsulation process can be determined by several parameters such as: yield, encapsulation efficiency, characterization of microparticles, stability, and release. The yield determines the number of microcapsules produced and the encapsulation efficiency indicates the amount of active material that the polymer can overlay.

The following are some of the factors that affect encapsulation efficiency, yield and particle size:

a. Concentration of polymer

Increased polymer concentration causes increased encapsulation efficiency (Mehta et al, 1996; Rafati et al, 1997). The high viscosity and solidification rate of the dispersion phase will reduce the porosity of the microparticles (Schlicher et al, 1997). The effect of high polymer concentrations on encapsulation efficiency is caused by two ways, namely precipitates faster of polymer and blocking drug diffusion and also delaying drug diffusion in polymer droplets (Rafati, et al., 1997). High polymer concentrations can result in larger particle sizes (Bhardwaj et al, 1995).

b. Dispersion phase to continuous phase ratio (DP / CP)

The encapsulation efficiency and particle size increase with increasing volume of the continuous phase. An example is the encapsulation efficiency increased twice against a decrease in the ratio of the dispersion to the continuous phase (FD/FK) from 1/50 to 1/300 (Mehta et al, 1996; Jyothi, et al., 2016). The large volume of the continuous phase provides a high concentration gradient of the organic solvent across the boundary by diluting the solvent leading to rapid compaction of the microparticles (Jyothi et al., 2010). The particle size increases with increasing volume of the continuous phase due to the fast solidification process. The increase in the ratio of the dispersion phase to the continuous phase (FD / FK) causes the porosity of the particles to increase by analyzing a specific area (measured by BET and scanning electron microscope (SEM) (Jeyanthi et al., 1997).

c. Volume of crosslinking agents

The volume of the crosslinking agent represents the amount of the crosslinking agent added to encapsulation process. The volume of the crosslink agent will affect the yield, the particle size, and the release of core material from the microcapsules. Based on research conducted by Ofokansi et al (2013), the yield of microparticle was irregular with the volume of GST. The largest yield from 25 mL GST was 99.19% and the smallest yield from 15 mL GST was 68.27%. The same thing also happened to the effect of GST volume on particle size, and percentage release, where the results were irregular (Ofokansi et al., 2013). In contrast to the research conducted by Panchal et al. (2012) which showed that the increase in GST volume, yield also increased. Increasing the volume of GST produces a denser matrix due to increased crosslinking reaction with chitosan and reduces the rate of drug release and can increase entrapment efficiency.

d. The ratio between the core material and the polymer (coating)

The ratio between the core material and the polymer shows the change in the amount or concentration of the polymer used to coating the core material which causes an increase in the droplet of the emulsion and the continuous phase as a result of an increase in the distribution of the amount of core material into a continuous phase. Based on research conducted by Biswal et al. (2011), in general, an increase in the ratio of the core material to the polymer will increase the amount of yield and the efficiency of the encapsulation. Increasing the ratio of the core material to the polymer will increase the viscosity of the polymer solution; the diffusion of the drug will be small through the polymer membrane. After the polymer solution is solidified, the core material did not easily come out of the polymer layer and thus the encapsulation efficiency will be high. Minemoto et al. (2002) said that the encapsulation efficiency decreased because the coating material was insufficient to cover all the core materials.

5. TYPES OF WALL MATERIALS FOR EMULSION CROSSLINKING

Encapsulation with the emulsion crosslinking method is a technique for dispersing an aqueous solution in a polymer containing the core material in an immiscible organic solvent in small droplets. The droplets are then dripped with a crosslinker to form covalent bonds to harden the microcapsules/microspheres. Crosslinkers used such as formaldehyde, terephthaloil chloride, glutaraldehyde, genipin, etc.).

Wall materials that have been developed based on several studies that have been reported were chitosan, polyvinyl alcohol, bovine casein, polymethyl methacrylate or polyoxyethylene-polyoxypropylene block copolymer, and poly (lactic-co-glycolic acid (Manjanna, et al., 2010; Longo, 2010). , 1982; Latha and Jayakrishnan, 1994; Hassani, et al., 2018).

The material that has been widely used in recent years is chitosan which can also be combined with polyvinyl alcohol. Chitosan is a biodegradable, biocompatible, and non-toxic biomaterial so it is safe for use in the pharmaceutical and agricultural fields (Saikia et al, 2015).

6. SUMMARY OF SOME ENCAPSULATION RESEARCHES USING EMULSION CROSSLINKING METHOD

Encapsulation using the emulsion crosslinking method was started when Longo et al., 1982 made glutaraldehyde in toluene or glutaraldehyde saturated toluene (GST). This emulsion crosslinking method is an easy and simple method. The following are some studies that used the emulsion crosslinking method with the glutaraldehyde saturated toluene (GST) and Genipin as crosslinking agents.

1. Longo et al (1982): preparation of albumin microspheres from hydrophilic and hydrophobic polymers.

Glutaraldehyde saturated tolune (GST) was first reported by Thanoo et al., 1982 as a cross-linking agent for making hydrophobic albumin microspheres

from polymethylmethacrylate polymers or hydrophilic polymers, namely polyoxyethylene-polyoxypropylene block copolymer. Serum albumin was slowly dripped in a polymer solution in a mixture of chlorophome and toluene) then glutaraldehyde saturated toluene (GST) was dropped and produced microparticles. The resulting particle size is between 3-150 μm.

2. **Thanoo, et al (1992): Theophylline, Griseofulvin and Aspirin coated in microsphere chitosan**
   Chitosan microspheres contain Theophylline, Griseofulvin and Aspirin. The active ingredients are dispersed in a chitosan solution and then put in a mixture of light and heavy paraffin liquid with a ratio of 1: 1. While stirring, glutaraldehyde saturated toluene (GST) was added, after completion of the addition of GST then continued with the addition of 5 mL of glutaraldehyde solution at a concentration of 25% (v/v). The resulting encapsulation efficiency was 63.6 - 82.9% for Theophylline, 40.9 - 96.1% for Griseofulvin, and 63.6 - 89.5% for Aspirin. The resulting microsphere chitosan has a spherical geometric shape with a smooth surface. Increasing the volume of GST decreased the active ingredient release by almost 50%.

3. **Latha and Jayakrishnan (1994): preparation of bovine casein microspheres to protect Theophylline**
   Theophylline in bovine casein microspheres. The preparation was done by mixing theophylline in casein solution and dispersing it in a mixture of dichloromethane and hexane containing polyurethane and stirring, then adding 5-10 mL of GST and still stirring for 1 hour. The microcapsules formed were washed in a dichloromethane / hexane mixture in a 1: 1 ratio, then washed again with acetone and continued with sodium bisulfite. Bovine casein microspheres produced an encapsulation efficiency of 64-82%, and the size of the microspheres (microparticles) is between 180-1200 μm. Theophylline release reaches 100% after 300 minutes.

4. **Jameela and Jayakrishnan (1995): chitosan microspheres loaded mitoxantrone**
   Chitosan microspheres containing mitoxantrone were prepared by dispersing the active ingredient in chitosan solution and also containing 2% NaCl. The mixture was dispersed in a continuous phase, namely a mixture of paraffin and petroleum ether. While stirring, 1.5 mL of GST was added followed by the addition of 0.8 mL of glutaraldehyde solution and kept stirring for 1.5 hours. Then it was washed with petroleum ether and followed by methanol, sodium bisulfite and ends with acetone and after that it was dried. The resulting microsphere chitosan is spherical with a smooth surface. The resulting ecapsulation efficiency is 16-40%. The resulting particle size is between 75 - 300 μm. Mitoxantrone released from 25-60% for 1 mount.

5. **Campos et al (2013): preparation of chitosan-PVA microspheres**
   Microparticles of chitosan-polyvinyl alcohol (PVA) were cross-linked with GST. Microparticle preparation carried out was dissolving chitosan with a mixture of acetic acid and methanol in a 2: 1 ratio. The addition of methanol aims to accelerate the evaporation of water in the microcapsules. The polymer solution (chitosan) was slowly added to the continuous phase (corn oil) and stirred to form an emulsion, then dropping GST. The resulting microparticles were washed with petroleum ether and distilled water and then dried. The resulting average microparticle size was 16 ± 11 μm with the following calculations 10% of the sample population is 32 μm, 50% is above 16 μm and 90% is above 1 m. In this study, although using glutaraldehyde was toxic, the results revealed no adverse effects on cell survival suggesting that its toxicity was likely minimized by the crosslinking reaction.

6. **Ramachandran et al (2011): preparation of microspherical chitosan filled with ranitidine.**
   Chitosan microspheres containing ranitidine were prepared by the following procedure: ranitidine was mixed with chitosan which was dissolved using acetic acid containing NaCl. The mixture is dispersed in liquid paraffin and petroleum ether containing sorbiton sesquioleate then stirred, and then 10 mL of GST are added, after 30 minutes of stirring then added 25% glutaraldehyde solution and continue to stir for 1-3 hours. Chitosan microspheres were washed with petroleum ether, acetone, sodium metabisulfate, and water. After that, the chitosan microspheres were dried in the oven. Chitosan microspheres containing ranitidine yield an efficiency of 53 ± 2 - 84 ± 3%, the particle diameter was 125 ± 18–236 ± 14 μm. Ranitidine released from microsphere chitosan reached 74% for 24 hours.

7. **Ofokansi et al, (2013): glutaraldehyde saturated toluene (GST) as a crosslinking agent for ibuprofen loaded chitosan microparticles**
   Ibuprofen was dispersed in chitosan solution. The mixture of chitosan and ibuprofen solutions was added into a continuous phase (a mixture of heavy and light paraffin solutions containing span 80). The mixture was stirred to form an emulsion then dropped GST which was made from a mixture of toluene and glutaraldehyde solution with a volume ratio of 1: 1, then stirred and allowed to stand overnight and the top layer was taken as GST. The emulsion was dripped with GST. The resulting microparticles were washed with petroleum ether followed by acetone and then dried. The size of the microparticles was 100.05 ± 8.82 - 326.70 ± 10.43 μm, yield (69.2 - 99.2%), and showed a greater water absorption capacity in SIF (122.2%) than in SGF

(60%). The drug in the microparticles contained 12.90 ± 1.89-23.32 ± 0.97%, and the ibuprofen released in the SIF medium reached 93.6% while the SGF medium was lower than SIF.

8. **Jayanudin, et al (2019): preparation of red ginger oleoresin microcapsules**
   Red ginger oleoresin in microcapsules with chitosan as wall material which was cross-linked GST. The preparation was to mix the chitosan solution with red ginger oleoresin. The mixture stirred to form an emulsion, and then added to the continuous phase (corn oil). The next step was added GST to the emulsion followed by the addition of glutaraldehyde. The microcapsules of red ginger oleoresin were filtered, washed, and dried. Red ginger oleoresin microcapsules have a smooth surface with an encapsulation efficiency of 83.1% and a yield of 98.93%, while the particle size ranges from 75.61 ± 11.8 μm to 178.65 ± 40.7 μm.

9. **Luo, et al., 2015: preparation of salidroside microspheres with genipin as crosslinking agent**
   Salidroside (Sal) in chitosan microspheres (CsM) was crosslinked with genipin (Gp) and then analyzed its release in vitro. Sal-loaded CsMs (Sal-CsMs) have an almost spherical shape with a smooth surface. The particle size of the Sal-CsMs ranged from 0.56-5.01 μm, and the encapsulation efficiency and loading capacity exceeded 77.58% and 23.29%, respectively.

10. **Hussain, et al (2012): urea loaded chitosan microspheres with genipin as crosslinking agent**
    Chitosan microspheres were filled with urea and use genipin as a crosslinking agent. Changes in chitosan and genipin concentrations determine urea loading and Entrapment efficiency. The result were 44.90 ± 0.1 - 269.54 ± 0.0% for urea loading and 87.3 ± 1.1 - 99 ± 0.5% for Entrapment efficiency. The release rate of urea was influenced by the concentration of urea, chitosan, and the crosslinker. The release rate increased due to the increase in urea concentration, while the release rate decreased due to the increase in the concentration of chitosan and crosslinking agents.

Based on reference tracing, there were still many encapsulation processes that used the emulsion crosslinking method using either glutaraldehyde, GST, or genipin as the crosslinking agent. Various wall materials were also used, such as chitosan, alginate, gelatin, and others.

7. CONCLUSION

The results of the literature review described many core materials for pharmaceutical, food, and even agriculture using the emulsion crosslinking method with various wall materials and crosslinkers. In general, the wall materials used are chitosan and glutaraldehyde or in the form of glutaraldehyde saturated toluene (GST) and genipine as crosslinking agents. The success of this method depends on the formation and stability of the emulsion. The resulting microcapsules or microspheres were perfectly spherical with a smooth surface. This shows that the emulsion crosslinking method was proven to be effectively applied for microcapsules formation.

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