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Review: Preparation of Flavonoid Nanoparticles using the Nanoprecipitation Method

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Abstract

Flavonoids are polyphenolic compounds that have 15 carbon chains, 2 benzene rings and a heterocyclic pyran ring. From the literature study, it is known that flavonoids have various pharmacological activities such as anticancer, antimicrobial, antiviral, antiangiogenic, antimalarial, antioxidant, neuroprotective, antitumor, and antiproliferative agents. However, flavonoids have limited oral bioavailability which may be due to their poor solubility, low permeability, and low stability, which impair their effectiveness as therapeutic agents. One of the efforts to increase solubility is nanoparticle technology where the active compound particles are reduced to the nanometer scale, usually up to 100 nm. Nanoprecipitation is a method of preparing nanoparticles by dissolving the active drug substance and polymer into an organic solvent and then adding an anti-solvent such as water. The advantages of this method are the production is relatively fast, inexpensive, does not require a lot of energy, and does not require emulsion precursors. The purpose of this literature review is to examine the technique of making flavonoid nanoparticles using the nanoprecipitation method, the results of their characterization and evaluation. Based on a literature review that has been carried out on 30 journals, there are 20 flavonoid secondary metabolites that have been prepared into nanoparticles using the nanoprecipitation method. Some of the polymers used were effective in achieving satisfactory particle size, polydispersity index (PDI), Zeta potential and Encapsulation Efficiency (EE%). Thus, the nanoprecipitation method can be used to make flavonoid nanoparticles with optimal formulations to improve the physicochemical properties of flavonoids for drug development in the future.

Keywords: Flavonoid, Nanoparticles, Nanoprecipitation Method, Characterizatio

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1. Introduction

Flavonoids are polyphenolic compounds that have 15 carbon chains, 2 benzene rings and a heterocyclic pyran ring. Based on existing literature studies, flavonoids have various pharmacological activities such as anticancer, antimicrobial, antiviral, antiangiogenic, antimalarial, antioxidant, neuroprotective, antitumor, and antiproliferative agents. One example of a compound that has pharmacological activity is quercetin which has anticancer activity. However, some flavonoid compounds, especially flavonoids which are included in the category of flavonoid aglycones have low solubility, low absorption and short drug residence time [1,2].

In order to overcome these problems, physical and chemical modifications of drugs and various methods were carried out. The methods used were about reducing particle size, formation of co-crystal, formation of salt, solid dispersion, surfactants appliaction, complexation methods, approaches with nanotechnology and so on [3,4]. Several studies have approached nanotechnology to make stable formulas. Nano technology is used to reduce the particle size of a particle so that it will form nanoparticles. Nanoparticles are substances that have a diameter of less than 1000 nm [5]. Utilization of nano technology or the manufacture of nanoparticles can be used for flavonoid modification. One example of the research conducted by Telange et al. which proves that the solubility of Apigenin can be increased by the solubility value of Apigenin-phopolipid Phytosome in water by 22.80 g/mL. The formation of nanoparticles also serves to increase anticancer activity, for example, the formation of quercetin nanoparticles with PLGA polymer which prevents the formation of hepatocellular carcinoma through the protection of the mitochondrial membrane in the liver [6]. The simplest method of forming nanoparticles is Nanoprecipitation.

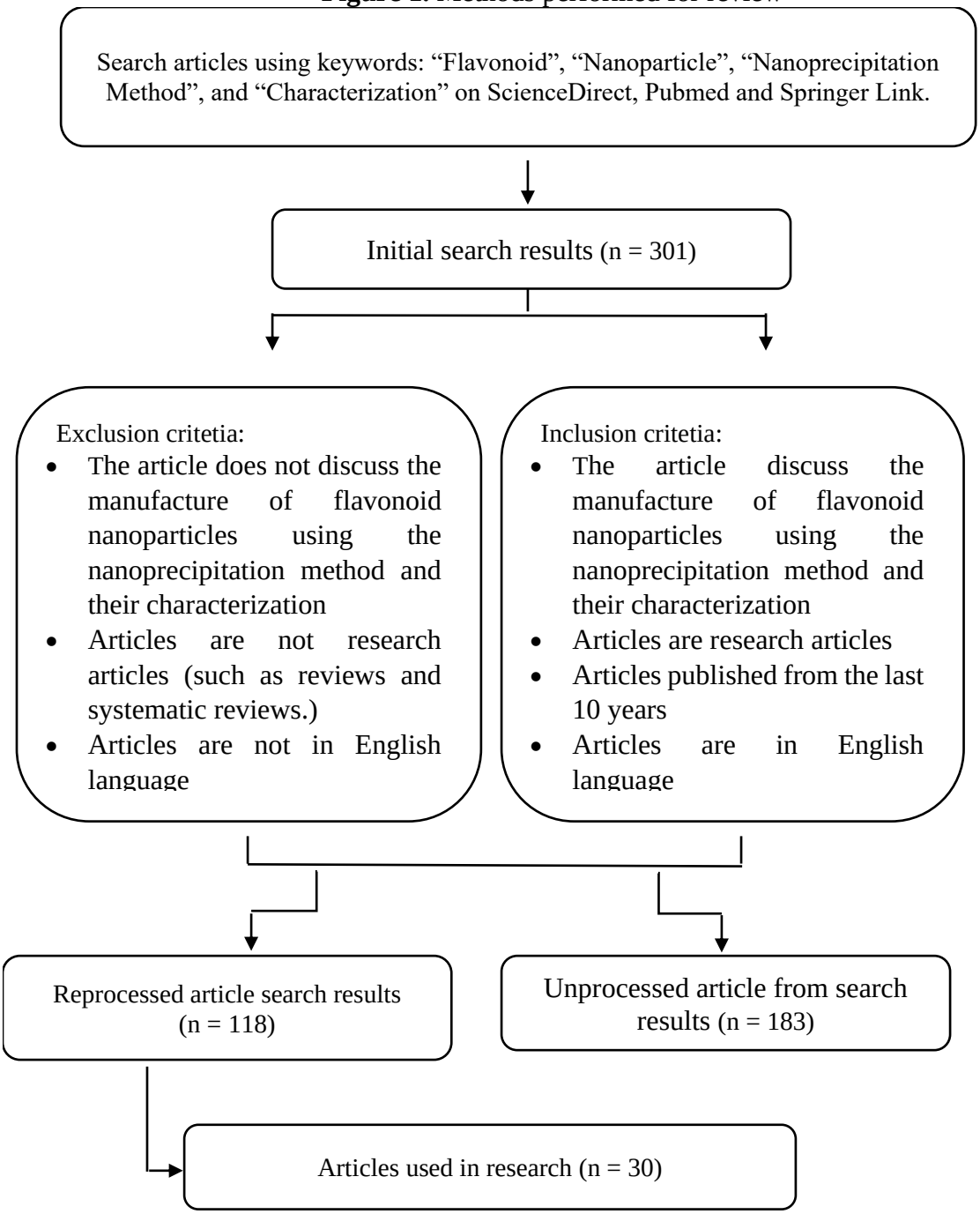
Nanoprecipitation is a method of preparing nanoparticles for hydrophobic active substances developed by Fessi et al in 1989. The principle behind this method is using the Marangoni effect. Overall this method is carried out by making the active drug substance and polymer dissolved in an organic solvent which is then added to an anti-solvent such as water. The advantages of this method are that the production is relatively fast, low cost, does not require a lot of energy, and does not require emulsion precursors. In addition, nanoprecipitation can also produce nanoparticles with particle sizes in the range of 50-300 nm [5,7,8,9].

Based on the literature search, the preparation of nanoparticles with the nanoprecipitation method has been widely carried out on natural materials including flavonoids. Therefore, a literature review was carried out by collecting data on the manufacture of flavonoid nanoparticles with the nanoprecipitation method as a reference for future research on the manufacture of flavonoid nanoparticles.

2. Methods

This review was carried out by conducting a literature search related to the manufacture of flavonoid nanoparticles using the nanoprecipitation method and their characterization on the ScienceDirect, Pubmed and Springer Link sites with specific keywords used to search for qualified articles such as "Flavonoid", "Nanoparticle", "Nanoprecipitation". Method", and “Characterization”. The articles used are articles from the last 10 years in English.

Figure 1. Methods performed for review



3. Results and Discussion

Table 1. Variety of the Nanoprecipitation method used in the preparation of Flavonoid

Flavonoid	Nanoparticle Technologies	Nanoparticle Types	Nanoprecipitation method	Carrier	Solubility/Bioavailability /Dissolution	References
Apigenin	*Apigenin*-	Polimeric	Traditional	PLGA	-	[27]

	loaded PLGA nanoparticles	Nanoparticles	Nanoprecipitation			
	Apigenin-loaded galactose tailored PLGA nanoparticles	Polimeric Nanoparticles	Traditional Nanoprecipitation	Galaktosa dan PLGA	-	[27]
Artocarpin	*Artocarpin Nanoparticle system (Artocarpin: PVP)*	Polimeric Nanoparticles	Heat induced evaporative antisolvent nanoprecipitation	PVP	Solubility: 1400-fold	[28]
Chrysin	Chrysin nanocapsules	Lipid-polymer hybrid Nanoparticles	Traditional Nanoprecipitation	PLGA, Labrafac PG, phosphatidylcholine	-	[29]
Cirsiliol	Cirsiliol-loaded nanocapsules	Lipid-polymer hybrid Nanoparticles	Traditional Nanoprecipitation	PEG-PCL, Span 80, Tween 80	Solubility: 24-fold	[30]
Curcumin	Curcumin encapsulated Chitosan functionalized PLGA Core Shell Nanoparticles	Organic-Inorganic hybrid Nanoparticles	Traditional Nanoprecipitation	PLGA, Kitosan	-	[31]
	PLGA-CTAB curcumin nanoparticles	Polimeric Nanoparticles	Heat induced evaporative antisolvent nanoprecipitation	PLGA, CTAB	-	[32]
Dihydromyricetin	*Nanocapsule suspensions containing Dihydromyricetin*	Polimeric Nanoparticles	Traditional Nanoprecipitation	Eudragit RS100	-	[33]
Diosmin	*Polymer-stabilized diosmin nanosuspension*	Polimeric Nanoparticles	Traditional Nanoprecipitation	HPMC/MC	Dissolution: 2-fold	[34]
Eupafolin	*Eupafolin nanoparticles*	Polimeric Nanoparticles	Traditional Nanoprecipitation	PVA, Eudragit E100	-	[35]
Fisetin	*Fisetin-loaded nanoparticles*	Polimeric Nanoparticles	Traditional Nanoprecipitation	PCL, PLGA-PEG-COOH, Pluronic F127.	-	[12]
Genistein	*Genistein-loaded M-PLGA-TPGS*	Polimeric Nanoparticles	Traditional Nanoprecipitation	M-PLGA-TPGS	-	[26]
Luteolin	Hybrid	Polimeric	Heat induced	PLA, Eudragit	Bioavaialbility: 2.61-fold	[36]

	PLA/Eudragit 100 Luteolin nanoparticles	Nanoparticles	evaporative antisolvent nanoprecipitation	L100, Pluronic F127		
Naringenin	PVP-coated naringenin nanoparticles	Polimeric Nanoparticles	Sonication-assisted nanoprecipitation	PVP	-	[37]
	Naringenin-loaded nanoparticles	Polimeric Nanoparticles	Traditional Nanoprecipitation	Eudragit, PVA	-	[25]
Phloretin	Hydrogel containing polymeric nanocapsules loaded with phloretin	Polimeric Nanoparticles	Heat induced evaporative antisolvent nanoprecipitation	PCL, Span 60, Tween 80,	-	[38]
Poly-puerarin	Poly-puerarin nanoparticles with Paclitaxel	Polimeric Nanoparticles	Traditional Nanoprecipitation	Pluronic F127	-	[39]
Proantosianidin	PLGA nanoparticles loaded with Proamthocianidin-rich Grapeseed Extract,	Polimeric Nanoparticles	Traditional Nanoprecipitation	PLGA	-	[40]
	P. guajava ethyl acetate fraction loaded nanosuspension	Polimeric nanoparticles	Sonication-assisted nanoprecipitation	PVA	Bioavailability: 6,28-fold	[15]
	Starch nanoparticles loaded with Quercetin	Starch nanoparticles	Traditional Nanoprecipitation	Pati Kentang	-	[24]
	MPEG-PLA encapsulated Quercetin nanoparticle	Polimeric nanoparticles	Traditional Nanoprecipitation	MPEG-PLA, Pluronic F-68	Bioavaialability: 1.87-fold	[16]
Quercetin	Quercetin embedded PLA nanoparticles	Polimeric nanoparticles	Sonication-assisted nanoprecipitation	PLA, PVA	-	[41]
	Zinc phthalocyanine-Quercetin loaded lipid-polymer hybrid nanoparticle	Lipid-polymer hybrid Nanoparticles	Heat induced evaporative antisolvent nanoprecipitation	PLGA, soybean lecithin, DSPE-PEG2000	Bioavailability: 3.64-fold	[42]
	Quercetin-loaded Eudragit®	Polimeric nanoparticles	Traditional Nanoprecipitation	Eudragit S100	-	[43]

	S100 Nanoparticles					
	Quercetin-loaded PCL based nanoparticles	Polimeric nanoparticles	Heat induced evaporative antisolvent nanoprecipitation	PCL	-	[44]
	Quercetin conjugated Fe_3O_4 nanoparticles	Magnetic nanoparticles	Sonication-assisted nanoprecipitation	Dextran, Fe_3O_4	-	[11]
Rutin	Rutin nanospheres	Polimeric Nanoparticles	Sonication-assisted nanoprecipitation	Eudragit S100, Poloxamer-188.	-	[45]
Salvigenin	Salvigenin-loaded mPEG-*b*-PLGA with Fe_3O_4	Magnetic nanoparticles	Heat induced evaporative antisolvent nanoprecipitation	mPEG-*b*-PLGA, Fe_3O_4	-	[23]
Silibinin	Silibinin-loaded nanoparticles	Polimeric Nanoparticles	Traditional Nanoprecipitation	PVA, Eudragit E100	-	[46]
Silymarin	Silymarin-loaded lipid polymer hybrid nanoparticles containing chitosan	Lipid-polymer hybrid Nanoparticles	Heat induced evaporative antisolvent nanoprecipitation	PLGA, soybean lecithin, DSPE-PEG2000, Kitosan	-	[47]
	Silymarin-Loaded Eudragit	Polimeric Nanoparticles	Sonication-assisted nanoprecipitation	PVA, Eudragit RS100 & RL100	-	[14]

Table 2. Characterization of Flavonoid Nanoparticles Using Nanoprecipitation Method

Flavonoid	Nanoparticle Technologies	Partikel Size (nm)	PDI	Zeta Potential (Mv)	EE (%)	Activity	References
Apigenin	*Apigenin-loaded PLGA nanoparticles*	110,0	0,041 ± 0,004	-25,0	70,3	Hepatocellular Carcinoma Treatment	[27]
	Apigenin-loaded galactose tailored PLGA nanoparticles	129,0	0,059 ± 0,007	-14,0	75,4	Hepatocellular Carcinoma Treatment	[27]
Artocarpin	*Artocarpin Nanoparticle system (Artocarpin: PVP)*	128,4 ± 0,7	0,266 ± 0.024	-	>99	Hepatocellular Carcinoma Treatment	[28]
Chrysin	Chrysin nanocapsules	176 ± 2,10	0,22 ± 0,01	-6,23 ± 0,18	87,10 ± 6,71	Antiglycemic, Antihyperlipidemic	[29]
Cirsiliol	Cirsiliol-loaded nanocapsules	158.1 ± 12,4	0,19 ± 0,01	−2,6 ± 5.1	53,5 ± 2,1	Anticancer	[30]
Curcumin	Curcumin encapsulated Chitosan functionalized	207,6 ± 2,71	0,165 ± 0,075	+31,9 ± 1,03	75.53 ± 2,09	Alzheimer's Disease Treatment	[31]

	PLGA Core Shell Nanoparticles						
	PLGA-CTAB curcumin nanoparticles	81,05 ± 3.85	0,107	+31,8	69,1	Breast Cancer Treatment	[32]
Dihydromyricetin	*Nanocapsule suspensions containing Dihydromyricetin*	160 ± 5,0	0,120 ± 0,05	+8,5 ± 1,5	-	Photoprotection	[33]
Diosmin	*Polymer-stabilized diosmin nanosuspension*	316 ± 5,55	0,41 ± 0,04	-	-	-	[34]
Eupafolin	*Eupafolin nanoparticles*	90,8	-	-	-	Acute Renal Injury Treatment	[35]
Fisetin	*Fisetin-loaded nanoparticles*	198,7 ± 6,0	0,158 ± 0,02	-	74,78 ± 1,9	Antioxidant	[12]
Genistein	*Genistein-loaded M-PLGA-TPGS*	225,7 ± 2,5	0,169	-14,2 ± 0,7	97,66	Liver Cancer Treatment	[26]
Luteolin	Hybrid PLA/Eudragit 100 Luteolin nanoparticles	452,23 ± 22,4	-	0,92 mV ± 0,04	71,02 ± 14,6%	-	[36]
Naringenin	PVP-coated naringenin nanoparticles	110	-	-	99,93	Antioxidant	[37]
	Naringenin-loaded nanoparticles	90	-	-	-	Anticancer	[25]
Phloretin	Hydrogel containing polymeric nanocapsules loaded with phloretin	252 ± 12,01	1,68 ± 0,11	>-1	>99	Antitumoral	[38]
Poly-puerarin	Poly-puerarin nanoparticles with Paclitaxel	70,26	0,15	−23,1	91,3	Anticancer	[39]
Proantosianidin	PLGA nanoparticles loaded with Proamthocianidin-rich Grapeseed Extract,	132,5 ± 12,2	-	–26,7 ± 3,8	65,2 ± 6,1	Dentin Degradation Resistance	[40]
Quercetin	*P. guajava ethyl acetate fraction loaded nanosuspension*	241,32 ± 1,25	0,224 ± 0,011	-22 ± 0,4	92,85 ± 2,23	Antihyperglycemic	[15]
	Starch nanoparticles loaded with Quercetin	91,2 – 154,5	0,276 – 0,41	-	-	Antioxidant	[24]
	MPEG-PLA encapsulated Quercetin nanoparticle	155,3 ± 3,2	0,2 ± 0,05	−3,14	-	Breast Cancer Treatment	[16]
	Quercetin embedded PLA nanoparticles	46 ± 4	-	-	62±3	Anticancer	[41]
	Zinc phthalocyanine-Quercetin loaded lipid-polymer hybrid nanoparticle	174,8	0,331	−30 ± 10	10 ± 4	Photodynamic Anticancer	[42]
	Quercetin-loaded Eudragit® S100 Nanoparticles	66,8 ± 2,3	-	-5,2 ± 2,4	41,8 ± 9,1	Colon Cancer Treatment	[43]
	Quercetin-loaded PCL based nanoparticles	215,9 ± 2,9	0,094	-12,9 ± 0,35	66,32 ± 0,4	-	[44]
	Quercetin conjugated Fe_3O_4 nanoparticles	72	-	+6,14	81,6	Breast Cancer Treatment	[11]

Rutin	Rutin nanospheres	130,30 ± 35,29	0,29 ± 0,10	-22,90 ± 5,18	98,10 ± 0,50	Colon Carcinoma Treatment	[45]
Salvigenin	Salvigenin-loaded mPEG-*b*-PLGA with Fe_3O_4	57 ± 2	0,168 ± 0,03	−33 ± 1,2	82 ± 1,6	Anticancer	[23]
Silibinin	Silibinin-loaded nanoparticles	120	-	+4,6	79,0 ± 2,4	Oral Carcinoma Treament	[46]
Silymarin	Silymarin-loaded lipid polymer hybrid nanoparticles containing chitosan	286,5 ± 23,8	0,226 ± 0,008	45,3 ± 8,9	97,05 ± 0,01	Hepatoprotective Agent	[47]
	Silymarin-Loaded Eudragit	84,70	0,38 ± 0,01		83,45	Hepatoprotective Agent	[14]

The formation of flavonoid nanoparticles increases the oral bioavailability of insoluble active flavonoid components via particle reduction which resulting in increase of surface are. Nanoparticles formation also led to the increase of saturation solubility and dissolution rate. [10,11,12,13].

Particle size and polydispersity index (PDI) influence the physicochemical properties of nanoparticles such as dissolution speed, saturation solubility, and physical stability. Polydispertity index also describes the uniformity of particle shape. The effective nanosuspension size for absorption in the intestine ranged from 100 to 500 nm. The nanosuspension size is in the range of 100 - 200 nm. The ideal particle size for in-vivo treatment is less than 200 nm. Nanoparticles are said to be homogeneous if their polydispersity index is less than 0.4. PDI values greater than 0.4 indicate particle aggregation [14,15,16,17,18].

Zeta potential is related to the electrostatic potential that develops at an indistinct boundary between the nanoparticles and the solution. Nanoparticles have is stable and have positive or negative charge if the zeta potential value is greater than +30 mV or less than -30 mV for positive charge and negative charge respectively [15,19,20].

Encapsulation Efficiency (EE%) is the percentage of drug trapped into the nanocarrier matrix which refers to the total drug input. A good Efficiency of Encapsulation has a value of >80% [21,22,23].

Preparation of nanoparticles with the Nanoprecipitation method is carried out by adding a dilute polymer solution to a non-solvent or vice versa which is then followed by polymer deposition at the nanoscale [14,24,25,26].

The traditional Nanoprecipitation method is prepared in large volumes of solution by adding an anti-solvent to a solvent containing hydrophobic molecules or vice versa drop by drop under mixing. In small-volume mixing processes, this process produces particles instantaneously with a narrow size distribution often at the nanoscale. Anti-solvents must be miscible with solvents containing polymers or drugs. Solutions with large volumes are difficult to control the results with the stirring process [48].

The Heat induced evaporative antisolvent nanoprecipitation (HIEAN) method begins by dissolving the active

substance in an organic solvent at a low boiling point. Then, the resulting solution is added to the heated aqueous solution. The increased temperature of the heated aqueous solution ensures rapid evaporation of organic solvents and results in a high degree of saturation and rapid precipitation of the drug in the form of suspended particles. Stabilizers added to a heated aqueous solution adsorb onto the surface of newly formed particles and reduce the surface energy and prevent particle growth [49, 50].

The nanoprecipitation method approach with the help of sonication is applied to reduce the particle size of the active substance which is not soluble in water. Ultrasonication into the carrier containing solution is carried out to coat and stabilize the nanoparticles. The propagation of ultrasound into a liquid medium results in alternating cycles of compression and contraction resulting in vacuum bubbles which accumulate energy and then release it violently or it is also called cavitation. Cavitation can trigger and accelerate various chemical reactions including the formation of nanoparticles [51].

The nanoparticles that are often made by this method are polymeric nanoparticles. Polymeric nanoparticles have more stability in the gastrointestinal tract than other colloidal carriers. Polymeric nanoparticles have other advantages such as maintaining effect on target tissues, solubility for intravascular delivery and protection from enzymatic degradation, especially in gastric acid. The polymers that are often used are polymers from the polyester family such as PLGA, PLA and PCL [14,24,25,26].

Based on the results of the literature research that has been carried out, 301 articles were obtained which were then processed into 30 articles. The article research process was carried out using indexed databases such as ScienceDirect (n = 24), PubMed (n = 4) and SpringerLink (n = 2). The results of literature research can be found in Table 1.

3.1 Apigenin

Apigenin was prepared into two different nanoparticles, the first nanoparticle using PLGA as a carrier while the second nanoparticle using Galactose-PLGA as a carrier. Both nanoparticles use the same method. The manufacture of nanoparticles starts from dissolving the polymer (PLGA or Galactose-PLGA) and Apigenin in acetone. The mixtures were poured into ultrapure water containing poloxamer 188 [27].

Nanoparticles with PLGA produced have particle size of 110 nm, polydispersity index 0.041 ± 0.004, Zeta Potential -25.0 mV and Efficiency of 70.3%. Nanoparticles with Galactose-PLGA produced a particle size of 129.0 nm, polydispersity index 0.059 ± 0.007, Zeta Potential -14.0 mV and encapsulation efficiency of 75.4%. The low polydispersity index value indicates that the nanoparticles show the same size between each other. The zeta potential value of nanoparticles made with Galactose-PLGA is smaller than using only PLGA. This is due to the PLGA galactosylation. The negative zeta potential also aids the uptake of the reticuloendothelial system (RES) [27].

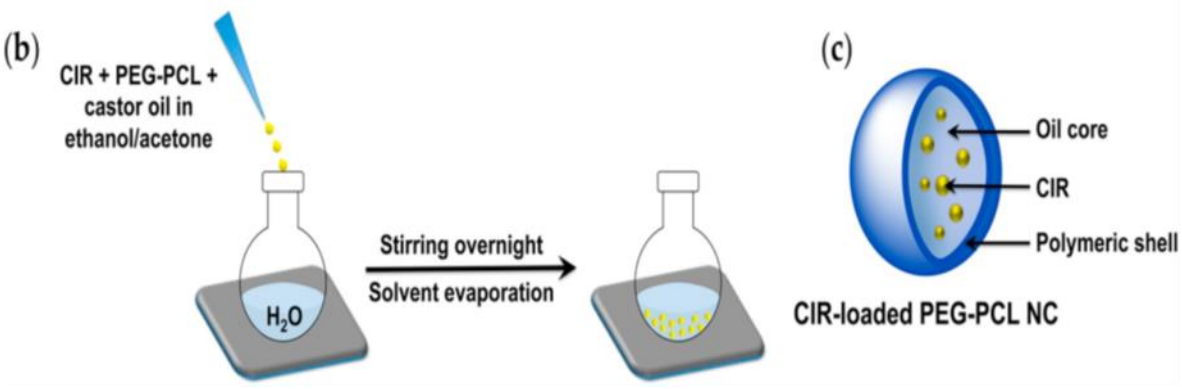
3.2 Artocarpin

Artocarpine which has an autophagic effect was made into naooparticles with an artocarpine: PVP nanoparticle system with a ratio of 1:10. The steps start from dissolving artocarpine in ethanol to form organic phase. The aqueous phase is prepared by adding PVP in ultrapure water. The organic phase was then injected into the aqueous phase. The resulting nanoparticles had a particle size of 128.4 ± 0.7 nm, a polydispersity index of 0.266 ± 0.024, an encapsulation efficiency above 99% and a water solubility of 1400 times that of free Artocarpin. The particle size below 200 nm makes administration of the drug in the body more effective. The PDI value indicates the absence of agglomerates. Formation of intermolecular hydrogen bonds between the OH bonds of artocarpin and the CO bonds of PVP increase solubility [28].

3.3 Chrysin

Chrysin nanocapsules were made using Labrafac PG as oil, PLGA as polymer and Phosphatidylcholine (PC) and Tween 80 as surfactants. The resulting nanocapsules had a particle size of 176 ± 2.10 nm, a polydispersity index of 0.22 ± 0.01, a Zeta Potential of -6.23 ± 0.18 mV, and an encapsulation efficiency of 87.10 ± 6.71%. The obtained small particle size nanocapsules provide a high mucosal adhesion ability on the gastrointestinal surface, which ensures a longer retention time. The PDI values of the nanocapsules showed a good size distribution. The increase of polymers and surfactants caused an increase in the particle size of the nanocapsules. This is due to an increase in the viscosity of the polymer solution, making it difficult for the emulsification process to become smaller droplets. Increasing the concentration of tween 80 decrease particle size at a constant amount of polymer and drug. This is due to a reduction in the interfacial tension between dispersed organic phase and dispersion medium. The zeta potential value obtained is too low but tween 80 which is reported to stabilize nanoparticles with a steric effect will provide protect nanocapsules stability even though their zeta potential value is low [29].

3.4 Cirsiliol



Picture 1. Production of Cirsiliol nanocapsules [30]

Nanocapsules containing Cirsiliol were prepared using the modified nanoprecipitation method by adding oil and lipophilic surfactants to the organic phase and adding hydrophilic surfactants to the aqueous phase. The method produces colloidal balls with an oil-filled core. The organic phase consisted of castor oil, cirsiliol, PEG-PCL and Span 80 which were dissolved in a mixture of acetone and ethanol while the aqueous phase consisted of Tween 80 dissolved in water. PEG is used as a carrier because PEG has a slow degradation due to its high crystallinity level, thereby prolonging drug release and maintaining drug stability. To improve PEG performance, PCL and PEG chains were combined by polymerizing CL ring opening using mPEG as a macroinitiator and stannous octoate as a catalyst. The resulting nanocapsules have a particle size characteristic of 158.1 ± 12.4 nm, a polydispersity index of 0.19 ± 0.01, a Zeta Potential of 2.6 ± 5.1 mV, an encapsulation efficiency of 53.5 ± 2.1% and an increase in solubility of 24 times that of ordinary Cirsiliol [30].

3.5 Curcumin

1. Curcumin encapsulated Chitosan functionalized PLGA Core Shell Nanoparticles

Core/Shell nanoparticles are suitable for drugs that target the brain. In addition, this type of nanoparticles can also protect the drug from P-gp efflux and external degradation [31].

In this study, used the organic phase consisting of curcumin and PLGA dissolved in acetone and the aqueous phase consisting of pluronic F127 dissolved in water. PLGA used as core material because it is biocompatible, biodegradable and non-toxic. This polymer can also be used in conjunction with other polymeric materials. After the nanoparticle solution is formed, chitosan is added to the solution so that electrostatic interactions occur which will produce Curcumin encapsulated Chitosan functionalized PLGA Core Shell Nanoparticles. Chitosan is able to increase the residence time of intranasal drugs. This is because the positive charge interacts electrostatically with the mucin. Chitosan also has the ability to form a gel by absorbing water [31].

The characterization results showed that the nanoparticles made had a particle size of 207.6 ± 2.71 nm, a polydispersity index of 0.165 ± 0.075, a Zeta Potential of +31.9 ± 1.03 mV, and an encapsulation efficiency of 75.53 ± 2.09%. Particle size and polydispersity index increased with increasing concentration of curcumin in the formulation. Particle size also increased by coating chitosan against PLGA [31].

2. PLGA-CTAB curcumin nanoparticles

Curcumin will be used as an anticancer by making nanoparticles by applying PLGA as carrier and hexadecyltrimethylammonium bromide (CTAB) as a surfactant. PLGA and curcumin were added to the mixture of Acetone: Ethanol (17:3) to make an organic phase while CTAB was dissolved in water to make an aqueous phase. PLGA is a polymer with negatively

charged molecules which is often used as a carrier in the manufacture of nanoparticles. However, positively charged nanoparticles move more easily from the endosome to the cytosol so that their bioavailability is better than neutral or negatively charged nanoparticles. To produce positively charged nanoparticles, CTAB which works as a surfactant as well as a molecular charge modifier is added during the nanoparticle manufacturing process [32].

The characterization results showed that the nanoparticles made had a particle size of 81.05 ± 3.85 nm, a polydispersity index of 0.107, a Zeta Potential of +31.8 mV, and an encapsulation efficiency of 69.1%. The small particle size may be due to the addition of CTAB. The results of the Polydispersity Index stated that no aggregates were formed. The zeta potential obtained indicates that the nanoparticles are stable enough so that there is no aggregate formation. The results of encapsulation efficiency are influenced by the presence of PLGA which has a low molecular weight [32].

3.6 Dihydromyricetin

Dihydromyristetin nanocapsules are made by preparing a solution of Eudragit first and then adding Dihydromyristetin. To make hydrogel, 1.5% Hydroxyethylcellulose, methylparaben and propylene glycol were added. Hydroxyethylcellulose works as a thickening agent. The resulting nanocapsules had a particle size of 160 ± 5.0 nm, a polydispersity index of 0.120 ± 0.05 and a Zeta Potential of 8.5 ± 1.5 mV. The nanoparticles made have a narrow particle size distribution but their stability is still questionable because the zeta potential value is close to zero. Zeta Potential with a positive charge is needed in order to provide electrostatic attraction with a negative zeta potential on human skin, causing an occlusive effect on the skin [33].

3.7 Diosmin

Diosmin made nanoparticles with two nanoprecipitation methods, acid-base neutralization method and anti-solvent precipitation method. The polymers used as stabilizers were Hydroxypropylmethylcellulose (HPMC) and methylcellulose (MC) [34].

The step of the acid-base neutralization method in a nutshell starts with dissolving Diosmin in 1 N NaOH. Diosmin base solution was dropped into 0.1 N HCl containing polymer stabilizer, HPMC or MC. For certain formulations, it is necessary to remove excess stabilizer and then remove it by ultracentrifugation. The supernatant was discarded and then re-dispersed in water [34]. For the antisolvent precipitation method step, Diosmin was dissolved in DMSO then added to an aqueous solution containing 0.15% polymer, HPMC or MC. Stirring was maintained until a homogeneous milk suspension was obtained [34].

The nanoparticles with the best characterization results had an MC concentration of 14% with the addition of 53% mannitol accompanied by spray-drying and using an acid-base neutralization method. The best nanoparticles had a particle size of 316 ± 5.55 nm and a polydispersity index of 0.41 ± 0.04. The anisolven method produces

a higher PDI than the acid-base neutralization method. The higher concentration of stabilizer was reported not to significantly affect the particle size, but the nanoparticle formulation of the precipitation took longer [34].

3.8 Eupafolin

In order to overcome the poor solubility of Eupafolin, a research was conducted on the manufacture of Eupafolin nanoparticles. The reason why Eupafolin is made into nanoparticles is because in previous studies Eupafolin nanoparticles have the potential to create new strategies in dealing with acute kidney injury. In this study, Eupafolin nanoparticles were prepared using the nanoprecipitation method with solvent evaporation. The organic phase consisted of Eupafolin, Eudragit and 95% alcohol while the aqueous phase consisted of PVA and water. Eudragit E100 and PVA were chosen as polymers because of their non-toxic, water-soluble and often used for oral treatment. The resulting nanoparticles have a particle size of 90.8. Nanoparticles of this size are effectively used for in-vivo treatment [35].

3.9 Fisetin

In this study, fisetin nanoparticles were made from an organic phase containing Poly-(ε-caprolactone) (PCL), PLGA-PEG-COOH block copolymer and fisetin in acetonitrile and the aqueous phase only consisted of water. Before fabricating nanoparticles, Sechi et al. do the PLGA-PEG-COOH conjugation first. PLGA polymer was chosen to be a polymer because PLGA can protect the active substance from degradation, reduce side effects and form sustained drug release types. To improve the performance of PLGA, modification of the PLGA surface was carried out with poly(ethylene glycol) (PEG). PEG can prevent the opsonins binding, prolongs circulation time in blood, facilitate tissues targeting and reduces PLGA's absorption by Rapid Reticuloendothelial System (RES). PCL is suitable to be used as a PLGA partner, this is because PLGA and PCL will form hydrophobic core. In addition, PCL is permeable and non-toxic [12].

The characterization results showed a particle size of 198.7 ± 6.0 nm, a polydispersity index of 0.158 ± 0.02, and an encapsulation efficiency of 74.78 ± 1.9%. The particle size of the PLGA-PEG-COOH nanoparticles is larger than that of ordinary PLGA, this is because of hydrophilic PEG chains present in external aqueous phase. The nanoparticles showed a narrow and unimodal particle distribution based on the obtained PDI values. PCL results in better encapsulation due to its high affinity for Fisetin. The affinity decreases with increasing concentration of PLGA-PEG-COOH which is more hydrophilic [12].

3.10 Genistein

Genistein nanoparticles are made using PLGA, this is because PLGA is part of the polyester family which has good biocompatibility and biodegradability. However, PLGA and other polyester families has disadvantage. Liver and RES can easily eliminate and absorbed PLGA. This can be solved by the addition of d-a-tocopheryl polyethylene glycol 1000 succinate (TPGS). In order to obtain nanoparticles with higher

drug content and higher drug entrapment efficiency, modification of the molecular shape of the nanoparticles was carried out into stars. Mannitol was used as the core of the star polymer molecule in this study because it has good chemical stability and is suitable for use in the formulation of anticancer tablets. The modified polymer was named Mannitol-core PLGA-TPGS (M-PLGA-TPGS) [26].

The organic phase consisted of M-PLGA-TPGS alongside Genistein dissolved by acetone while the aqueous phase consisted of water. The characterization results showed particle size of 225.7 ± 2.5 nm, polydispersity index of 0.169, Zeta Potential of -14.2 ± 0.7 mV and encapsulation efficiency of 97.66 %. The size of the nanoparticles is in the size range that facilitates drug accumulation in tumor blood vessels increased of drug permeation and retention. Star-shaped copolymers reduce particle size. The PDI value indicates a relatively narrow size distribution. Zeta potential is negative which caused by ionized carboxyl groups from the PLA and PGA. The encapsulation efficiency is influenced by the binding affinity PLGA which has star-shaped form and hydrophobic Genistein [26].

3.11 Luteolin

In order to make biodegradable luteolin nanoparticles, a biodegradable polymer was chosen, polylactic acid (PLA). Eudragit L100 is used in conjunction with PLA as a polymer. This is because Eudragit L100 is a pH-dependent polymer that is resistant to gastric acidity and is soluble in intestinal fluids. This polymer is suitable for the active substance delivered to the large intestine. In this research, two kinds of organic phases were made. The first organic phase has the composition Luteolin and Eudragit L100 in Ethyl Alcohol. The second organic phase is PLA in Dichloromethane. The aqueous phase is Pluronic F127 which acts as an emulsifier and is dissolved in HCl pH 4 [36].

The resulting nanoparticles have a particle size of 452.23 ± 22.4 nm, Zeta Potential of 0.92 ± 0.04 mV, and encapsulation efficiency of 71.02 ± 14.6%. The higher concentration of total polymer (combination of PLA and EUD100) led to the production of larger nanoparticles. This is due to an increase in the viscous force which opposes the breakdown of the particles. However, the viscous force increases encapsulation efficiency. The presence of PLA should make the zeta potential negatively charged. The formation of a PF127 layer on the particle surface makes the surface charge a less negative value [36].

3.12 Naringenin

1. PVP-coated naringenin nanoparticles

Naringenin nanoparticles were prepared by a simple nanoprecipitation method using polyvinylpyrrolidone (PVP) as a hydrophilic carrier. PVP is a strong hydrophilic polymer which has advantages such as delaying the crystallization of compounds by forming adduct molecules, non-toxic and uncharged. PVP coating hydrophobic surfaces increase biocompatibility and reduce complement activation. The manufacturing stage starts from dissolving Naringenin in ethanol to make the organic phase. The organic phase was

rapidly injected into the aqueous solution containing the PVP. The injection process was carried out alongside sonication of mixture. The particle size obtained is 110 nm with encapsulation efficiency of 99.93%. The nitrogen atom coordinated with naringenin provides stability and prevents agglomeration [37].

2. Naringenin-loaded nanoparticles

In another study, naringenin nanoparticles were fabricated using Eudragit E and PVA. Eudragit E and PVA were used as polymers/carriers simultaneously. Eudragit E was chosen because Eudragit has the function of increasing the solubility of drugs that are poorly soluble in water and have a dimethylamino group base site that is ionized in gastric juice. Naringenin nanoparticles were made by dissolving Naringenin and Eudragit® E in ethanol to make an internal organic phase. The phase is rapidly injected into the external aqueous phase containing PVA. The particle size obtained is 90 nm. Nanoparticles do not form agglomerates or adhesions. Judging from the particle size, these nanoparticles can cross the vascular endothelium and accumulate at the tumor site through the EPR effect because the nanoparticle size is less than 400 nm [25].

3.13 Phloretin

Phloretin is made into Hydrogel preparations containing Floretin-charged polymer nanocapsules. The core shell of the nanocapsule can resist degradation caused by ultraviolet light. PCL is used as a polymer because it has advantages such as excellent drug loading capacity, controlled release rate and slower degradation compared to poly(glycolic acid) and other polymers [38].

Phloretin-charged polymer nanocapsules were prepared by mixing an organic phase consisting of copaiba oil, sorbitan monostearate (Span 60), PCL and Floretin in acetone:ethanol (8:1). The hydrophilic phase contains polysorbate 80 (Tween 80) and water. Before making hydrogel with the addition of Lecigel® 1%, Nanocapsules were evaluated first. The evaluation results showed a particle size of 252 ± 12.01 nm and a polydispersity index of 1.68 ± 0.11. The zeta potential is negatively charged and close to zero, due to the presence of polysorbate 80 at the particle/water interface. The highest concentration of drug that can be put into nanocapsules with maximum encapsulation efficiency (>99%) is phloretin 0.2 mg/Ml. The size distribution is fairly narrow because of the polydispersity index results obtained [38].

3.14 Poly-puerarin

Poly-puerarin nanoparticles with Paclitaxel were prepared by using the nanoprecipitation method. Poly-puerarin serves as a carrier. Poly-puerarin is obtained from the modification of Puerarin with unsaturated olefins via acryloyl chloride through free radical polymerization. Azodiisobutyronitrile (AIBN) is used as an initiator in the puerarin modification process. Poly-puerarin nanoparticles were prepared by nanoprecipitation and used as a carrier for loading paclitaxel. Before making nanoparticles, first Poly-puerarin was dissolved in DMSO. The first oil phase was

prepared by dissolving Paclitaxel in DMSO at concentration. The second oil phase was prepared by dissolving Stabilizer F-127 in a different DMSO. The added buffer is saline phosphate buffer [39].

In this study, there were two variations of the formula where there was a difference in the percentage of paclitaxel used. Satisfactory results were obtained from 10% paclitaxel where the particle size characterization results were 70.26 nm, polydispersity index 0.15, Zeta Potential 23.1 mV and encapsulation efficiency of 91.3%. Due to the intermolecular forces, increasing the concentration of paclitaxel and puerarin did not increase the particle size. The hydrophobic drug Paclitaxel increases the amount of the hydrophobic compound of the nanoparticle complex which helps the formation of a dehydrated and compact core. Zeta potential is negatively charged due to the strong electronegativity of glucose groups, ester bonds and other functional groups of poly-puerarin molecules [39].

3.15 Proantosianidin

Proanthocyanidins in grape seed extract bind to type I collagen, inhibit matrix metalloproteinase (MMP) activity and decrease the rate of dentin demineralization. To make a drug that can reduce the level of demineralization of tink with gradual drug release, Proanthocyanidin in the extract was prepared in the form of nanoparticles with different compositions of the ratio of PLGA nanoparticles loaded with grape seed, which have PLGA/Grape Seed Extract ratios of 100:25, 100:50, and 100:50. 75 w/w, synthesized by a modified nanoprecipitation technique [40].

The characterization results showed a particle size of 132.5 ± 12.2 nm, Zeta Potential –26.7 ± 3.8 mV and encapsulation efficiency of 65.2 ± 6.1%. The nanoparticles are uniformly spherical. The resulting particle size aids drug delivery through the exposed dentinal tubules of the demineralized dentin. The OH functional group present in the extract structure makes the zeta potential negatively charged [40].

3.16 Quercetin

1. Psidium guajava ethyl acetate fraction loaded nanosuspension

The ethyl acetate fraction from Psidium guajava was made into nanoparticles because this fraction has antihyperglycemic activity. The fraction contains Quercetin which has low solubility. The organic phase was made by dissolving the ethyl acetate fraction in ethanol while PVA was dissolved in water. PVA works as a surfactant. The characterization results showed that the nanoparticles had a particle size of 241.32 ± 1.25 nm, a polydispersity index of 0.224 ± 0.011, a Zeta Potential of -22 ± 0.4 mV, and an encapsulation efficiency of 92.85 ± 2.23%. The particle size is in the range of 100-500 nm so that absorption in the intestine will be more effective. The polydispersity index obtained shows the uniformity of particle shape in the nanoparticles. The optimal amount of PVA provides protective, electrostatic stability to the particles and reduces agglomeration. Zeta potential in the range of 20-40 mV indicates the presence of electrostatic stability [15].

2. Starch nanoparticles loaded with Quercetin

Quercetin nanoparticles are made by utilizing starch made from corn, potatoes or beans. Starch is a natural polymer that is biodegradable, inexpensive and abundant. However, starch has low solubility and is sensitive to temperature and humidity. For this reason, modifications are made with nano technology. Starch nanoparticles containing quercetin were prepared by dissolving quercetin into a solution of NaOH/urea/H2O (0.8 : 1: 98.2) containing starch. After that, HCl was added. NaOH/urea/H2O solution is used as a solvent because it can dissolve cellulose. Urea has the function of preventing the assemblage of starch molecules [24].

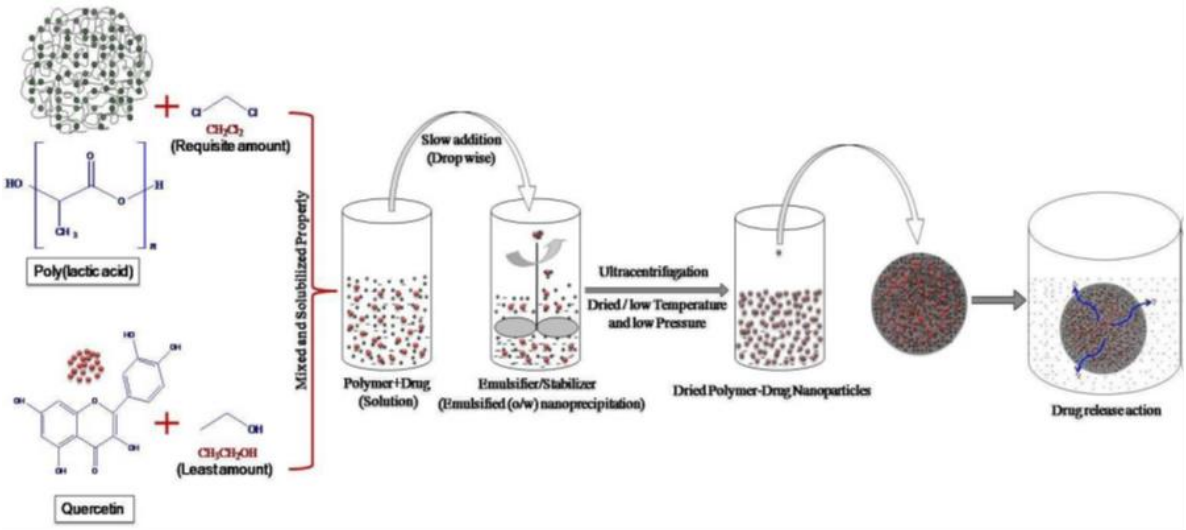
The results showed that the suitable starch to be used as a polymer was potato starch. This is because corn starch and peanut starch form aggregates after lyophilization, while potato starch has a uniform nanofiber-like structure. Potato starch nanoparticles have a particle size of 91.2 – 154.5 nm and a polydispersity index of 0.276 – 0.41 [24].

3. MPEG-PLA encapsulated Quercetin nanoparticle

Methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) polymer is used as a carrier to make quercetin nanoparticles which will be used as an agent to treat breast cancer. The reason why a modified form of PLA is used is because the nanoparticles with the PLA polymer are rapidly cleared from the circulatory system after systemic injection. The copolymerization of PLA with Polyethylene glycol (PEG) can improve these limitations. In addition, the charge of the nanoparticles will shift to a neutral charge, the neutral charge helps the interaction of polymer nanoparticles with the cell membrane. MPEG provides a liquid membrane on the outside of the nanoparticles to resist hydrophobic drug release for drugs that function as sustained-release doses [16].

The organic phase consisted of MPEG-PLA and quercetin in acetonitrile. The organic phase was added to a solution containing pluronic F-68. The resulting nanoparticles had a particle size of 155.3 ± 3.2 nm, a polydispersity index of 0.2 ± 0.05 and a Zeta Potential of 3.14 mV. Particle sizes below 200 nm suggest that nanoparticles can promote accumulation at tumor sites. Polydispersity index below 0.4 indicates that the nanoparticles are in homogeneous condition. The Zeta Potential value obtained states that the nanoparticles can avoid RES [16].

4. Quercetin embedded PLA nanoparticles



Picture 2. Production of Quercetin embedded PLA nanoparticles [41]

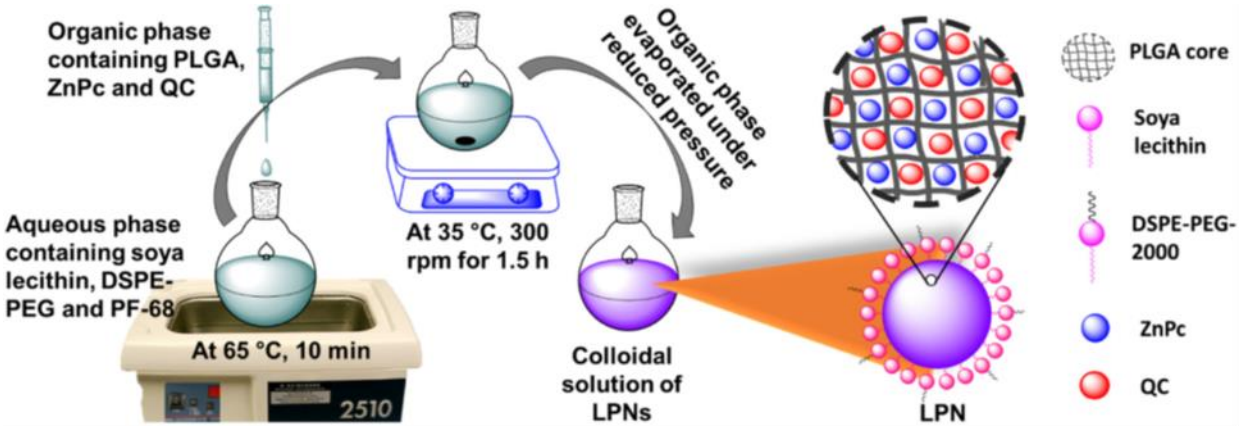
Quercetin nanoparticles with continuous release drug function were prepared using the emulsion (o/w) nanoprecipitation method. PLA is used as a carrier while PVA is used as a stabilizer. Quercetin and PLA allow the formulation of sustained-release drugs. This is due to the delayed diffusion and strong interaction between Quercetin and PLA. In the manufacture of nanoparticles, Quercetin is dissolved in ethanol and then added PLA which has been dissolved in dichloromethane. After that, the mixture was added with PVA dissolved in water [41].

In this study, a variant of the formulation was made with the concentration of PLA, PVA and changing temperature. The best nanoparticles were produced from the formulation with 10% Quercetin, 2% PVA and 20 mg/ml PLA and the manufacturing temperature was at 25oC. The nanoparticles had a particle size of 46 ± 4 nm and an encapsulation efficiency of 62 ± 3 %. The size of the nanoparticles increased with increasing PLA concentration. The particle size gradually decreased with increasing PVA concentration while the encapsulation efficiency did not change drastically. The lower particle size at higher temperatures is due to their high mobility [41].

5. Zinc phthalocyanine-Quercetin loaded lipid-polymer hybrid nanoparticle

Quercetin can function as photodynamic therapy by making Lipid-polymer hybrid nanoparticle (LPN) which encapsulates Zinc phthalocyanine and Quercetin. Zinc-phthalocyanine works as a second generation photosensitizer while Quercetin as an anticancer agent. Both substances must accumulate in the tumor site. The LPN structure consists of a non-aqueous polymer core, a single or multiple lipid layer, and a polyethylene glycol (PEG) conjugated lipid layer. The polymer core encapsulates most of

the Quercetin and Zinc-phthalocyanine. The lipid layer facilitates absorption by cells. The PEG-conjugated lipid layer increases the circulation time of the active substance in the body [42].



Picture 3. Production of Lipid-polymer hybrid nanoparticle [42]

LPN made a modified nanoprecipitation method using PLGA as a carrier. In this method PLGA diffusion occurs between two different liquid phases. The organic phase was prepared by dissolving Quercetin, Zinc-phthalocyanine and PLGA in Acetone. The aqueous phase was prepared by dissolving PF-68, soy lecithin and DSPE-PEG in a mixture of ethanol and water. The resulting nanoparticles had a particle size of 155.3 ± 3.2 nm, polydispersity index 0.2 ± 0.05, Zeta Potential 3.14 mV and encapsulation efficiency of 10 ± 4 % for Quercetin and 55 ± 5% for Zinc-phthalocyanine. Particle sizes below 200 nm prove that the preparation is effective for use by the intravenous route. The concentration of Lecithin and Zinc-phthalocyanine did not affect the particle size while the increase in the concentration of Quercetin and PLGA increased the particle size. The presence of a single layer of DSPE-PEG increased the stability of the preparation [42].

6. Quercetin-loaded Eudragit® S100 Nanoparticles

Quercetin which will be used for colon cancer drug manufacture of Quercetin non-particles with Eudragit S100 polymer as a carrier because other studies have proven an increase in the bioavailability and stability of the drug [43].

The material used consists of an organic phase and an aqueous phase. The organic phase consisted of Eudragit S100 and Quercetin dissolved in ethanol. Eudragit S100 is suitable for drugs targeted against the colon. This is due to the carboxyl group of the methacrylic acid moiety contained in Eudragit S100. The carboxyl group will ionize upon contact with neutral or alkaline pH. This results in a negative repulsion between the carboxylate side groups causing the polymer to lose its charge. This carboxyl group also makes the polymer insoluble in the stomach [43].

The resulting nanoparticles are colloidal dispersion like milk which is stable. This nanoparticle has a particle size of 66.8 ± 2.3 nm, Zeta Potential -5.2 ± 2.4 mV and Encapsulation Efficiency of 41.8 ± 9.1%. A negatively charged zeta potential is generated due to the presence of sodium lauryl sulfate added by to Eudragit S100. The encapsulation efficiency obtained indicates that some of the quercetin may have been

partitioned into the aqueous phase during the nanoparticle formation process [43].

7. Quercetin-loaded PCL based nanoparticles

Polycaprolactone (PCL) is used as a carrier for the manufacture of quercetin nanoparticles with various formulation variants. PCL was chosen because of its high permeability, biodegradability and biocompatibility, non-toxic, non-mutagenic and suitable for controlled release drugs. The organic phase contains PCL and quercetin dissolved in acetone. The aqueous phase contains Pluronic F-127 as a stabilizer which is dissolved in water. The resulting nanoparticles have a particle size of 215.9 ± 2.9 nm, polydispersity index 0.094, Zeta Potential 12.9 ± 0.35 mV and encapsulation efficiency of 66.32 ± 0.4%. Increasing the concentration of Pluronic F-127 increased the particle size. Increasing the concentration of PCL increases the viscosity of the organic phase. The increase in viscosity increases the diffusion resistance of the drug into the aqueous phase and enhances the encapsulation of the drug into nanoparticles [44].

8. Quercetin conjugated Fe3O4 nanoparticles

The Nanoprecipitation method controls the particle diameter and the unique magnetic properties of the magnetite nanoparticles. However, this method has limitations in the manufacture of magnetic nanoparticles where the agglomeration and distribution of particles is not uniform, these limitations can be overcome by the addition of urea. The manufacturing process begins with dissolving quercetin in acetone and dissolving magnetite nanoparticles coated with dextran in dimethyl sulfoxide (DMSO) and deionized water. After that, the magnetite nanoparticle solution was added with N-hydroxy succinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). Then the mixture was added to the quercetin solution and the pH was adjusted with KOH. Urea functions as an aggregation controller and stabilizer to produce prism-shaped particles. Dextran is a surfactant which is used to control the shape of spherical aggregates and to protect the phase change of nanoparticles [11].

The best nanoparticles have a particle size of 72 nm, Zeta Potential 6.14 mV and Efficiency of 81.6%. Particle size occurs due to the conjugation of quercetin to Fe3O4 through the EDC/NHS reaction. The zeta potential is positively charged due to the strong electrostatic interaction between the drug and the protonation of magnetite through the carboxyl molecule and the presence of anionic ions from the drug molecule. Positive charge can increase drug penetration into tumor cells [11].

3.17 Rutin

Rutin was made into nanospheres which were prepared by the nanoprecipitation method. Eudragit S100 functions as a polymer and Poloxamer-188 as a stabilizer. Nanosphere is a particle matrix in which the entire mass of solid and drug particles is physically and uniformly dispersed. Eudragit S100 was used as a polymer because it does not degrade below pH 7. In other words, this polymer is insoluble in the pH of the stomach and intestines, but soluble in the pH of the large intestine (pH > 7) [45].

Nanosphere was prepared by dissolving Eudragit S100 and rutin in a sealed bottle containing methanol to make the organic phase and dissolving Poloxamer-188 in distilled water. The characterization results show that the nanosphere has a particle size of 130.30 ± 35.29 nm, polydispersity index 0.29 ± 0.10, Zeta Potential -22.90 ± 5.18 mV and encapsulation efficiency of 98.10 ± 0.50%. The high encapsulation efficiency is due to rutin has a high affinity for organic

solvents that dissolve the dissolved polymer and the interaction between rutin and Eudragit S100 which indicates the formation of intermolecular hydrogen bonds. The polydispersity index value obtained indicates that the nanoparticles are stable without any agglomerates. The negative charge on the zeta potential measurement was attributed to the free acrylic acid group of Eudragit S100 as an anionic polymer [45].

3.18 Salvigenin

In order to overcome its poor bioavailability, a study was conducted on the preparation of Salvigenin loaded mPEG-b-PLGA coated with iron oxide (Fe3O4) nanoparticles. PLGA is used as a polymer because PLGA has various intrinsic physical and chemical properties such as monomer ratio (PL/GA), drug loading potential, and controllable size and shape of drug polymer particles. The addition of iron oxide particles into the polymer nanocarrier is very important because it allows the nanoparticles to be delivered by a magnetic field to the tumor site [23].

Before making Salvigenin nanoparticles, Fe3O4 nanoparticles must be made first with the main ingredients FeCl3.6H2O and FeCl2.4H2O with a molar ratio of 2:1, ammonia solution as a precipitation agent and oleic acid as a surfactant. In the process of making salvigenin nanoparticles, the organic phase was prepared by dissolving mPEG-b-PLGA and Salvigenin in acetone. Fe3O4 solution was dissolved in different acetone. Then the two solutions are mixed. After that it was added to 20 mL of distilled water drop by drop. After the addition of the complete mixture, the acetone was removed under vacuum with a rotary evaporator [23].

The characterization results showed that Salvigenin loaded with mPEG-b-PLGA coated with iron oxide nanoparticles had a particle size of 57 ± 2 nm, a polydispersity index of 0.168 ± 0.03, Zeta Potential 33 ± 1.2 mV and an encapsulation efficiency of 82 ± 1.6%. Nanoparticles are said to be stable and positively charged based on the characterization results [23].

3.19 Silibinin

Silibinin nanoparticles are prepared using the nanoprecipitation method and utilizing Eudragit and PVA. Eudragit was used as a polymer and PVA was used as a stabilizer. The reason for using Eudragit is because Eudragit is a positive copolymer which has a base site for ionized tertiary amine groups in gastric juice which can make nanoparticles easily soluble in the stomach [46].

Silibinin nanoparticles have the composition Silibinin:Eudragit:PVA (1:10:10;w/w/w). The organic phase was prepared by dissolving Silibinin into ethanol in a closed glass bottle. The aqueous phase contains PVA. The characterization results showed that Silibinin nanoparticles had a particle size of 120 nm, Zeta Potential of 4.6 mV and encapsulation efficiency of 79.0 ± 2.4%. Small particle size < 200 nm so that drug accumulation in tumor cells increases. The positive charge is suitable for drugs that are absorbed by charged cellular membranes [46].

3.20 Silymarin

1. Silymarin-loaded lipid polymer hybrid nanoparticles containing chitosan

Silymarin was prepared into lipid-polymer hybrid nanoparticles. This type of nanoparticle utilizes liposomes and polymer nanoparticles. These nanoparticles are shaped like a shell core consisting of a polymer core and a phospholipid wall. The polymer used is chitosan. This is because Chitosan has an amino group that can change the charge to be positive when in a weak acid state. The positive charge allows the compound to adsorb mucin on the intestinal

mucosa through electrostatic interactions [47].

The lipid-polymer hybrid of Silymarin nanoparticles was prepared using a two-step nanoprecipitation method. In this method, lipid-polymer hybrid nanoparticles were obtained by incubating the nanoparticle dispersion with chitosan solution after evaporation of the organic solvent. The aqueous phase was prepared by dissolving soybean lecithin and DSPE-PEG 2000 in ethanol. The Organic Phase was prepared by dissolving PLGA and silymarin in a mixture of acetonitrile-methanol. Chitosan solution was prepared by dissolving chitosan in 0.5% acetic acid and then filtering through a 0.8 m membrane [47].

After being characterized, the lipid-polymer hybrid nanoparticles Silymarin with Chitosan had a particle size of 286.5 ± 23.8 nm, a polydispersity index of 0.226 ± 0.008, a Zeta Potential of 45.3 ± 8.9 mV and an encapsulation efficiency of 97.05 ± 0.01%. The addition of chitosan increases the particle size and changes the zeta potential charge from negative to positive. In addition, the oral bioavailability of silymarin in this form was 14.38 times higher than that of nanoparticles without chitosan [47].

2. Silymarin-Loaded Eudragit

Silymarin is manufactured in the form of Silymarin Loaded Eudragit Nanoparticles. This is because Eudragit Polymer has the ability to form positively charged nanodispersions, moderate bio-adhesive strength, and has no irritating effect on the mucosal surface. Silymarin Loaded Eudragit Nanoparticles were prepared by the nanoprecipitation method. First, an organic phase was made with a polymer composition of silymarin and Eudragit with a constant ratio of Eudragit RS 100 & Eudragit LS 100 (1:1 w/w) dissolved in acetone. The aqueous phase having the composition of deionized water containing PVA. PVA is a synthetic polymer that is soluble in water and functions as a stabilizer. Eudragit RS100 has very low water permeability, while Eudragit RL100 has high water permeability. To improve the quality of the drug release rate, these multiple polymers were used concurrently [14].

In this study, nine formulations were made with three different concentrations of PVA (1, 2, 3% w/v) and three different organic/water phase ratios (1:6, 1:10, 1:20 v/v). The optimal formulation chosen was a drug/polymer ratio of 1:1 and stirring for 5 minutes at 480 rpm on a magnetic stirrer followed by homogenization for 30 minutes at 23.5 krpm. The optimal formulation has characterization results with a particle size of 84.70 nm, a polydispersity index of 0.38 ± 0.01, and an encapsulation efficiency of 84.35 %. In addition, the oral bioavailability of silymarin in this form was 14.38 times higher than that of nanoparticles without chitosan. When the PVA concentration was increased, the particle size and polydispersity index increased at low organic phase ratios to water (1:6) while decreasing at high organic phase ratios to water (1:10 and 1:20). Moreover, at higher organic to water phase ratio and as PVA concentration increased, particle size and entrapment efficiency decreased [14].

4. Conclusion

Based on the literature review that has been done, there are various nanoprecipitation methods that have been applied to the preparation of flavonoid nanoparticles from 20 prepared secondary metabolites of flavonoids. These methods consist of Traditional Nanoprecipitation, Heat induced evaporative antisolvent nanoprecipitation, and Sonication-assisted nanoprecipitation. The most frequently used method is Traditional Nanoprecipitation.

The types of flavonoid nanoparticles which were produced are polymeric nanoparticles, lipid-polymer hybrid nanoparticles, magnetic

nanoparticles, organic-inorganic hybrid nanoparticles and starch nanoparticles. Polymeric nanoparticles are a type of nanoparticle that is often produced.

Nanoparticles with the smallest particle size were obtained from research on production of Quercetin nanoparticles using PLA as a carrier and PVA as a stabilizer using the Sonication-assisted nanoprecipitation method. PLGA in the manufacture of Apigenin nanoparticles using the Traditional Nanoprecipitation method produced the lowest polydispersity index value. The use of PVP and PCL in research on the manufacture of Artocarpin, Naringenin and Floretin nanoparticles resulted in an encapsulation efficiency of greater than 99%.

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