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ANTIOXIDANT EFFECT OF CATECHIN LOADED POLYMERIC NANOPARTICLE

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ABSTRACT

Flavonoids are polyphenols compounds a structural class of mainly natural and a products of secondary metabolism of plants. Flavonoids, which can be found in herbs and trees, act as natural chemopreventives and anticancer agents [1]. As natural products, they have major importance for human life and also have a widespread effect such as antioxidant, antinflammatory, anticarcinogen, antidiabetic [2, 3]. However, bioavailability of this polyphenolic antioxidant have limited because of their high water solubility [4], low absorption, permeability, stability, slow dissolution rate, and light-induced decomposition over time in the physiological medium. In recent years, various methods have been tried to overcome the limitations of flavonoids, including its incorporation into micels and nanoparticles.
Keywords: Nanoparticle, catechin, antioxidant activity, polymeric nanoparticle.

KATEŞİN YÜKLÜ POLİMERİK NANOPARTİKÜLLERİN ANTİOKSİDAN AKTİVİTESİ

ÖZET

Flavanoidler bitkilerin doğal ve ikincil metabolizma ürünlerinin başlıca yapısal sınıf oluşturan polifenol bileşiklerdir. Doğal bileşikler olarak, Flavanoidler insan yaşamında büyük öneme sahiptir. Ve bununla birlikte antioksidan, antienflamatuar, antikarsinojen, antidiyabetik gibi geniş bir etkinliğe sahiptir. Ancak, bu polifenolik bileşiklerin biyoyararlanılırlığı aşırı hidrofilik, düşük absorbsiyon, geçirgenlik, stabilite ve çözünürlük göstermeme oranı ve fizyolojik ortamda ışığa maruz kaldığında degrade olmalarından dolayı sınırlanmaktadır. Son yıllarda, Flavanoidlerin etkinliğini sınırlandıran problemlerin üstesinden gelmek için nanopartikül ve misel içerisine yükleme gibi çeşitli metotlar denenmektedir.
Anahtar Sözcükler: Nanopartikül, kateşin, antioksidan aktivite, polimerik nanopartikül.

1. INTRODUCTION

One of the most popular bevarages is Tea plant (Camellia Sinensis), in the World. In addition to that it had been being chosen as a bevarages by the nearly 65% of the world population.

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Because of its health-promoting effects and pleasant aroma, Tea leaf is traditionally distinguished by steeping processed, and based on fermentation degree can be classified as green teas, oolong teas, white teas, yellow teas, black teas and post-fermented teas. Among all of these, however, the most important effects on human health have been observed with the consumption of green tea. Green tea is produced from the unfermented leaves of Camellia sinensis. Green tea harvesting and processing must be to prevent enzymatic oxidation for green tea fabrication. This production method prohibits the enzymatic oxidation of the flavonoids in green tea extracts [5-11]. The chemical composition of green tea is compounds: alkaloids, polyphenols, proteins, amino acids, carbohydrates, chlorophyll, volatile compounds, minerals, fluoride and trace elements, and other undefined compounds. The health-promoting effects of green tea have been highly attributed to the antioxidant properties of the polyphenolic compounds, polyphenols present in green tea are flavonoids. The major flavonoids of green tea are catechins. They consist of (_)-epicatechin, (_)-epicatechin-3-gallate (ECg),3(_)-epigallocatechin, and (_)-epigallocatechin-3-gallate(EGCg), Catechin(-), Catechin(+). Epigallocatechin gallate (EGCG), Epicatechin gallate (ECG), and epigallocatechin (EGC) are the most predominant compounds in green tea EGCG is the major catechin in green tea accounting for 65% of the total. Considering the molecular structures, the substituents at C3 and the B ring take the *cis* form in the cases of catechins [(1)-C, (2)-C, (2)-GC, (2)-Cg, (2)- GCg]. But, for their epimers, the B ring take the *trans* form and the substituents at C3. [6, 12-18]. Catechin has been shown to have anti-inflammatory, antioxidative, anticarcinogenic, chemopreventive and hepatoprotective effect [19-27] .

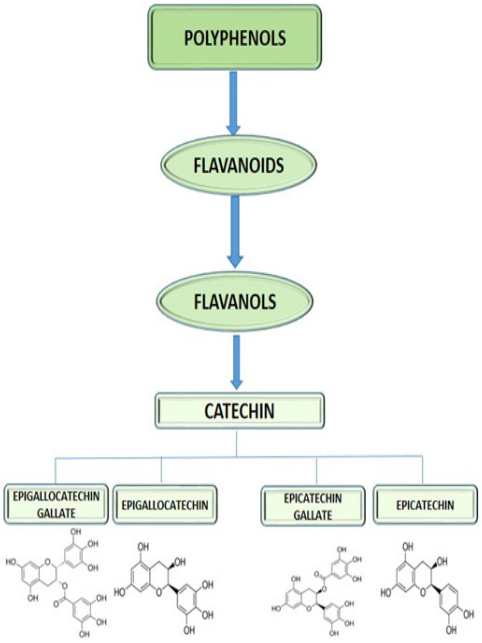

Figure 1. Brief representation of the polyphenols classification with focus on green tea catechins [21].

2. ANTIOXIDANT EFFECT OF CATECHIN

Reactive oxygen species (ROS) are significant in regulating normal cell physiological functions, but when produced in excess lead to the augmented pathogenesis of various diseases.

Antioxidants are substances that prevent oxidation of other compounds. Antioxidant properties depend upon the molecular structure of the compounds. A variety of assays are available to determine antioxidant properties, e.g., radical scavenging. The most frequently used analytical tests are DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant power), ORAC (oxygen radical absorbance capacity), total phenolics content, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline- 6-sulphonic acid), CUPRAC (cupric reducing antioxidant capacity), TRAP (total radical-trapping antioxidant parameter), TEAC (Trolox equivalent antioxidant capacity) and others. Many studies have been made on the effect of antioxidant catechin in recent years. As a result of these studies, Catechin has been observed that it possesses human health-promoting effects. The major emphasis is being placed on metabolic process at the cellular level. Most of the interest has focused on the potential therapeutic effect of oxidant/antioxidant activity concerning to the aging process and degenerative diseases like diabetes, cardiovascular disease and cancer. Catechin shows improved antioxidant actvity in vitro by chelating redox-active transition metal ions and scavenging reactive oxygen and nitrogen species. It may also show activity indirectly as antioxidants owing to inhibition of the redox-sensitive transcription factors, activator protein-1 and nuclear factor-_B, inhibition of "pro-oxidant" enzymes, such as inducible lipoxygenases, nitric oxide synthase, xanthine oxidase and cyclooxygenases; and induction of phase II and antioxidant enzymes such as superoxide dismutases, glutathioneS-transferases. The antioxidant capacities of Catechin is much stronger than many molecules having powerful antioxidant properties. For example, the one-electron reduction potential of epigallocatechin gallate is 550 mV, a value higher than comparable to that of α-tocopherol (480 mV). In vivo studies provide evidence that green tea catechins enhance total plasma antioxidant activity and also structure–activity research data have revealed that the ortho-trihydroxyl group in the B-ring and the galloyl moiety at the 3-position of flavan-3-ol skeleton are the most significant structural features for showing high scavenging ability on free radical [9, 28-31]. The antioxidant activity of catechin on a lipid perooxidation system are of interest because of the chiral structure of catechins. The Catechin showed obvious antioxidant activity on NADPH-dependent lipid peroxidation in rat liver microsomes. Studies *in vitro* taking advantage of a superoxide-generating system showed that catechin readily scavenged superoxide at a high rate. It is also effectively scavenge reactive nitrogen species (RNS). *In vitro*, catechin were shown to scavenge nitric oxide and peroxynitrite. A separate line of evidence also indicates that catechin chelate redox-active transition metals, such as free copper and iron, supporting that these polyphenols may mitigate metal-catalyzed ROS generation. Catechin also increases catalase and glutathione peroxidase, which function to degrade hydrogen peroxide to water. [21, 27, 31-37]. Catechin may protect against obesity by reducing intestinal lipid absorption and also decreases body mass by increasing β-oxidation via the upregulation of the expression of genes from this energy-producing pathway and reduction of the expression of lipogenic genes [38].

3. THE ENCAPSULATION OF CATECHIN

A number of in vitro and in vivo studies have been demonstrated to support that consumption of Catechin showed antioxidant effect and offered various benefits for human health. For example, it has been reported to show anti-cancer [39], anticarcinogenic activity [40], anticataract activity [41], antidiabetic activity [42], antiapopototic activity [16] , antibacterial activity [43], antimutagenic activity [44], antioxidative activity [45], antiproliferative activity [46]. Although catechin has important activities for human metabolism, its low membrane permeability and low stability in the presence of oxygen, alkaline pH, and high temperature, compromises its bioavailability and also the oral bioavailability of catechin is known to be low with a bioavailability of less than 2—5%. Therefore, there is an urgent need to improve effective methods for efficient delivery of this molecule to the required sites. To overcome this problem, the most suitable system is drug—carrier system. Because of their favorable characteristics as a

biodegradable drug reservoir. Among the various drug-carrier systems, nanoencapsulation has emerged as a key and an efficient delivery process [32, 47, 48]. Because of the inherent ability of antioxidants to begin free radical scavenging activity, their formulation as NPs will enhance their efficiency, However, Some type of polymeric carrier molecules have pro-oxidant properties, When They accumulate in tissues such as liver and brain and others. Toxicities have been observed in tissue. This side effect of nanoparticular carrier system can be prevented by using biocompatibility and biodegradable carrier molecules such as poly lactic-co glycolic acid, poly lactic acid or chitosan, that can be degraded by hydrolytic or lysosomal degredation of matrix polymers [47].

4. NANOPARTICULAR SYSTEM

The history of nanotechnology traces back to 1959 when physicist Richard Feynman (1960) recognized the potential of manipulating individual atoms and molecules at the nanometer scale while the 1980 and 1990 are start of development nanotechnology [47, 49]. Nanoparticles are subnanosized colloidal carrier system and the particle size ranges from 10-1000 nm in diameter. They are produced from synthetic, semisynthetic or natural polymers [50]. Nanoparticle delivery system has been commonly used in pharmaceutical industry to improve absorption of bioactive compounds [51]. High biodegradability and poor stability of phenolic phytochemicals in the body play efficient roles in their low absorption rate. Nanoparticles can also protect phenolic phytochemicals against the oxidation and degradation in the body by nanoencapsulation [51, 52]. Polymers, used as nanomaterial, can be classified as natural or synthetic. Natural polymers, such as polysaccharides and proteins, have positive properties, such as biodegradability and biocompatibility. However, a number of advantages are reported for synthetic polymers when compared with natural polymers, including the extremely controlled and stable degradation features and high reproducible mechanical and physical feature such as tensile strength, elastic modulus and degradation rate [53].

5. POLYMERIC NANOPARTICLES

Polymeric nanoparticles (NP) are colloidal systems, adsorbed or chemically coupled to a orbicular polymer matrix [54]. Polymeric nanoparticles which have been used as particulate carriers, have been extensively studied on medicine and medical field [55]. The usage of polymeric nanoparticles (NPs) can increase the efficiency and selectivity of active compounds, thus the usage of polymeric nanoparticle, have been shown widely on medical and medicine field [56]. Polymer-based nanocarriers are potentially used as carriers for drugs, proteins, and DNA to targeted cells and organs. Their size at the nanometer level provides influential permeation through cell membranes and stability in the blood stream and also provide high thermodynamic stability to the system and it can easily permeate through various biological barriers [57]. There are two conformations of polymeric nanoparticles. The first of these is nanocapsule. İt consists of a core, in which the drug can be entrapped. The second of these is nanosphere. İt is a nanoparticle made of entangled [58]. Usually, two primary strategies are used for nanoparticles synthesis: the dispersion of preformed polymers and the polymerization of monomers [56]. Polymeric nanoparticles can be also made directly from natural and synthetic polymers and by desolvation of macromolecules [55]. Usually, PLA, PLGA [54], ethylcellulose (EC) [55], cellulose acetate phthalate [3], poly (E-caprolactone) (PCL) [56-58], and poly (h-hydroxybutyrate) (PHB) are used in nanoparticle synthesis [59, 60].

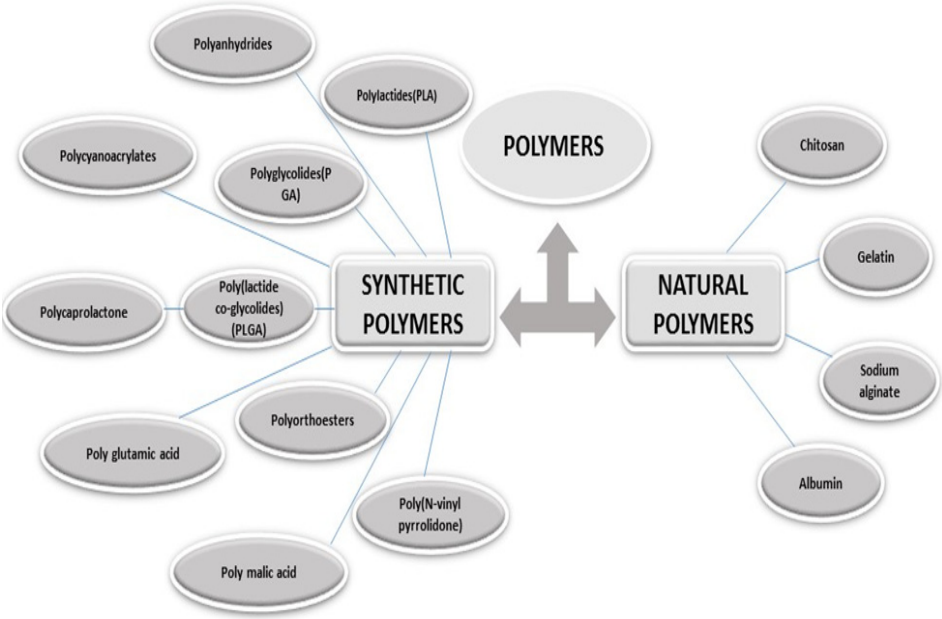

Figure 2. Polymers used for preparation of polymeric nanoparticles [59].

In despite of these advantages, some properties of nanoparticles limits its effectiveness. For example; their different size and surface properties area can cause to particle aggregation in addition to that it is difficult to make the physical circulation of nanoparticles in liquid and dry forms. To obtain high loading capacity in hydrophilic molecules needs to seriously challenging due to partitioning of molecules from the organic phase into the external phase solidification of particles. Thus, the improving the existing methods or development of new methods so as to enhanced the loading capacity of hydrophilic molecules in PLGA nanosphere has attracted great interest. Advancements in the field nanoparticular carrier systems can increase the number of effective molecules entering the pharmaceutical industry in the near future. [60-63].

6. CHARACTERIZATION OF NANOPARTICLE

In order to understand and predict the system performance in the body, compherensive characterization of a nanoparticle system is essential [64].

6.1. Particle Size and Polydispersity Index (PDI)

The size of nanoparticles is a significant parameter as the nanoparticles need to pass through biological barriers including; blood walls, blood brain barrier, moreover, it has to be directly delivered into the cells. Polydispersity index (PDI) has been used as a measurement of particle size homogeneity of the produced nanoparticles. The PDI is associated with the width of vesicle size distribution. While a value of PDI less than 0.3 indicates a homogenous vesicle population. The measurement of the correlation function was analyzed and the diffusion coefficient was obtained [58, 65-68].

6.2. Zeta Potential Determination

Zeta potential is an important parameter that can be used to to investigate surface charge and surface adsorption of particles and other surfaces in contact with a liquid and to estimate the long term stability of suspensions and emulsions. Particle size and surface charge differences and different cell lines have important effects on the cellular uptake of nanoparticles, and several cycles are included in the cellular uptake process, moreover, the nanoparticle system performance in the body, can be determined by surface charge of nanoparticles [64, 69, 70]. However, small particles have enormous specific surfaces; they may not be present in wet as individual particles. On the contrary, they often exist in aggregation or agglomeration. Therefore, one needs to disperse them into individual. Among the three existing methods for zeta potential determination of suspended particles, electrophoretic light scattering (ELS), acoustic and electroacoustic [69, 71-74]

6.3. Particle morphology (SEM)

The surface nature of nanoparticles can effectually affect the interfacial interaction between polymer matrix and particles [75]. Particle morphology in general refers to the external shape and surface texture of a particle. In addition, it can refer to the internal structure if the particle is porous or contains voids [76]. Various analyses were performed to characterize the phase composition and morphology of the nanoparticles [75]. A scanning electron microscope has been developed mainly for producing an image of high resolution by detecting secondary electrons and backscattered electrons generated from a specimen at a low accelerating voltage in a separate or synthesis fashion [77].

6.4. Entrapment Efficiency (EE)

The entrapment efficiency (EE) is very important physicochemical characteristics of nanoparticle. Encapsulation efficiency can be determined using direct or indirect methods. Both The amount of drug encapsulated is measured in indirect methods and the amount lost drug is measured in indirect methods, In order to estimate really the amount of drug encapsulated [78, 79]. To create successful nanoparticle formulations is calculated the quantity of drug incorporated in the nanoparticles for different formulations used to synthesis nanoparticle and compared the influence of different experimental formulations on the incorporation efficiency of drug in the nanoparticles [58, 80]. To improved entrapment efficiency of drug substance can be modified by changes such as; the amount of the model drug substance, selecting solvent for synthesis, volume ratio of the outer and inner phases, the pH values of the outer and inner phases, and, lastly, by the addition of salt to the aqueous phases. The encapsulation efficiency is calculated by the following formula. [81].

Encapsulation efficiency (%) = (Amount of drug in the nanoparticles / Initial amount of drug) x 100 [82].

6.5. Drug Release

Drug release from nanoparticles may occur by diffusion through the particle, by desorption from the surface or after degradation [64]. Nanocapsules are vesicular systems in which a drug substance is confined to a cavity surrounded by a polymer membrane, which releases the drug by controlled diffusion or erosion from the core across the polymeric membrane or matrix. The membrane coating serves as a barrier to release, thus, the entrapped drug interacts with polymer, the solubility and diffusivity of drug in polymer membrane becomes the determining factor in drug release [83]. In order to develop a successful nanoparticulate system, both polymer

biodegradation and drug release are the two significant consideration factors. Drug release rate generally depends on: desorption of the surfacebound/ adsorbed drug; solubility of drug; drug diffusion through the nanoparticle matrix; nanoparticle matrix erosion/degradation; and combination of erosion/diffusion process therefore, solubility, diffusion and biodegradation of the matrix materials control the release process [62, 84]

6.6. Methods of Polymeric Nanoparticle Production

In order to develope the stability of the polymeric nanoparticles. They can be fabricated using various synthesis techniques. The encapsulation techniques widely are used include the following; [55, 85].

1. Emulsification and Solvent Evaporation – Extraction Method

Emulsification and Solvent evaporation is an effective method for production of the nanopartıcular system. The single and double emulsion solvent evaporation methods are widely used techniques for nanoparticle synthesis. Double emulsion-solvent evaporation method has been devoleped from single emulsion-solvent evaporation method [86]. Emulsification-solvent process is generally done in two stage. In the first stage of preparation nanoemulsion, polymer are dissolved in organic solvent and then, a small amount of substance is added in polymer solution. Prepared organic phase is poured into the water phase containing a stabilizing agent. Homogeneously polymer solution dispersed into nanodroplets is derived with the help of high energy sonication or homogenization. In the second stage the organic solvent in polymer solution is evaporated either by increasing the temperature under pressure or by continuous stirring. The evaporation of organic solvent process is resulted in polymer precipitation as nanoparticles [87]. The solidified nanoparticles collected with the help of ultracentrifugation and washed with distilled water to remove the redundant materials. Finally, solution including the solidified nanoparticle are lyophilized to obtain dry form of nanoparticles [55, 56]. Characteristics of nanoparticle such as size, surface charge have been observed to be effected by synthesis parameters like the amount of substance to be loaded, polymer concentration, the type of homogenizer and also the type of stabilizer. In order to synthesize a smaller size nanoparticles usually a high-speed ultrasonication or homogenization may be used [57].

2. Nanoprecipitation Method

Nanoprecipitation method is also known as solvent displacement method. It is generally suitable for lipophilic molecules due to the miscibility of the solvent with the aqueous phase, and it is not an effiective method to encapsulate water-soluble molecules. A polymer poly lactide-co-glycolide (PLGA) and a stabilizer polyethylene glycol (PEG)-5000 defined as a base of the nanoprecipitation technique The nanoprecipitation technique consists on three steps: First, the target actives and polimer are added to each other in a organic solution; second, this mixture is added drop by drop to an aqueous solution, including a surfactant,; third, in order to eliminate the organic solvent, the resulting dispersion of nanoparticles is vacuum evaporated. Finally, in order to obtain the particles, centrifugation and filtration process are applied. [57, 88].

3. Salting out Method

The salting out is a purificiation method. It is depended on to the water miscible solvent separation from aqueous solution with the help of a salting out effect. The salting out method can be assumed that originated from the emulsification/solvent diffusion [55]. The temperature increase does not need for salting out; for this reason, might be useful for heat sensitive

substances process [89]. First of all, polymer and drug are dissolved in a solvent like dichlorometan (DCM). Afterwards, mixed solution is emulsifed an aqueous gel including the salting-out agent and colloidal stabilizer like polyvinylalcohol or hydroxyethylcellulose. With the help of required volume of water or aqueos solution in order to increase the acetone diffusion into aqueous phase, thus inducing the formation of nanospheres, oil/water emulsion is diluted. The choose of the saltingout factor is significant, because it can play an important role in the encapsulation efficiency of the active substance. [90].

4. Super Criticial Method

In the süper critical method, the molecule and polymer are dissolved in a co-solvent, in this method. In order to get a good drug encapsulation and stay away from any initial burst effect, it is important to mix two compounds in the same co-solvent. Afterwards, this mixture is sprayed in to a rapid flow of supercritical fluid. One of the most used supercritical fluid is carbon dioxide. Due to the transition a two phase system (co-solvent and SC-CO2) to the supercritical fluid system of one-phase homogeneous, the molecule and polymer precipitate into nanoparticles at the mixing point. Owing to solvent become miscible, precipitation and production of particles form consists. This effect named as an anti-solvent effect. The miscible solvents in SCF are passed through a filter and dry nanoparticles can be collected form this filter [91].

7. RESULTS

Catechin loaded PLGA nanoparticles have been successfully synthesized. . Due to high hydrophilic property of its, Catechin-loaded PLGA nanoparticles were done with double emulsion solvent evaporation method (w/o/w) widely used for encapsulation of hydrophilic molecules. However, a low entrapment efficiency has been obtained with Catechin as it's well known that the entrapment of hydrophilic molecules substances inside the polymer is a great challenge. To observe effect of experimental parameters on entrapment efficiency and particle size in particle synthesis, several parameters such as Catechin amount and PLGA amount have been changed. As a result of, changed parameters have not significant effected on particle size while these parameters have significant effected on entrapment efficiency. Due to the controlled release speficitaion of nanoparticular carrier system, encapsulated Catechin showed longer antioxidant activity prolong time than free Catechin. With the results obtained, our data indicate that nanoparticles can prevent Catechin against the oxidation/degradation and also be a basic strategy for both enhancing its bioavailability and antioxidant effect.

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