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Vitamin C: One compound, several uses. Advances for delivery, efficiency and stability

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Abstract

Vitamin C (Vit C) is a potent antioxidant with several applications in the cosmetic and pharmaceutical fields. However, the biggest challenge in the utilization of Vit C is to maintain its stability and improve its delivery to the active site. Several strategies have been developed such as: controlling the oxygen levels during formulation and storage, low pH, reduction of water content in the formulation and the addition of preservative agents. Additionally, the utilization of derivatives of Vit C and the development of micro and nanoencapsulated delivery systems have been highlighted. In this article, the multiple applications and mechanisms of action of vitamin C will be reviewed and discussed, as well as the new possibilities of delivery and improvement of stability.

Key words: Ascorbic acid; Drug delivery; Nanoparticles; Liposomes; Microemulsions; Targeting

Vitamin C (Vit C) or Ascorbic acid (AA) is a hydrophilic molecule, composed of six carbons, similar to glucose.[1] In the organisms, Vit C can be found in its reduced form (ascorbic acid or ascorbate) or in its oxidized form called dehydroascorbic acid (DHA), which is a product of two-electron oxidation of ascorbic acid.[2] It has essential physiological and metabolic activities in humans, but is only obtained through diet (humans do not have the enzyme L-gulono-1,4 lactone oxidase, essential for its production). It is known that its deficiency causes scurvy, a disease characterized by tissue fragility, poor healing and capillary fragility. Although the appearance of scurvy is rare nowadays, AA stands out as prominent ingredient in the food, cosmetic and pharmaceutical fields.[3] The molecular structure of AA can be seen in Figure 1 below.

Applications of vitamin C

The Vit C is a potent antioxidant capable of neutralizing oxidative stress through an electron donation/transfer process.[4] Vit C is able to reduce unstable species of oxygen, nitrogen and sulfur radicals in addition to regenerating other antioxidants in the body, such as alpha-tocopherol (Vitamin E). Besides, studies with human plasma have shown that Vit C is effective in preventing lipid peroxidation induced by peroxide radicals.[3]

Also, Vit C favors the absorption of iron, calcium and folic acid, which prevents allergic reactions and a decrease in the intracellular content of Vit C can lead to immunosuppression. Vit C is essential for the synthesis of immunoglobulins, for the production of interferon, and for the suppression of the production of interleukin-18, a regulating factor in malignant tumors. Therefore, Vit C supplementation is recommended during infection and stress.[5]

Another relevant point is that Vit C stimulates the formation of bile in the gallbladder and facilitates the excretion of steroid hormones.[6] It acts directly on collagen biosynthesis and is an enzymatic co-factor for lysyl and prolyl hydroxylases, key enzymes for the stabilization and cross-linking of type I and III collagen fibers. It also plays an important role in the intracellular signaling cascade that leads to fibroblast proliferation.[7,8]

It was reported for the first time in 1969 that venous administration of Vit C in high concentrations could exert a prooxidant action on cancer cells.[9] This theory has been

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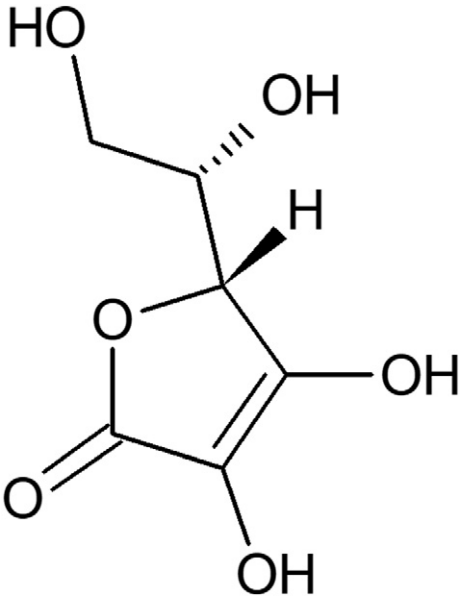

Figure 1. Molecular structure of L- ascorbic acid.

reviewed in recent years. Yun et al[10] reported that Vit C could selectively kill cancer cells by inducing H_2O_2 production. Its selective action is due to the fact that cancer cells have reduced amounts of antioxidant enzymes (such as catalase, superoxide dismutase and glutathione peroxidase) compared to normal cells. However, this theory is still controversial in the scientific community. Initially, because H_2O_2 is a metabolite commonly produced by cells and is generally overproduced under conditions of malignancy. In addition, ascorbic acid could be substituted in parenteral administration by H_2O_2 directly. Thus, more studies are needed to elucidate the relationship between the carcinogenic and anticarcinogenic activity of AA, depending on the concentration and cellular location.[11]

When applied topically, Vit C can neutralize reactive oxygen species (ROS) triggered by exposure to solar radiation and environmental factors such as smoke and pollution.[4] Studies also indicate that Vit C is effective in the treatment of hyperpigmentation, melasmas and sunspots.[8] This activity appears to be related to its ability to interfere with the active site of tyrosinase, the melanogenesis-limiting enzyme. Tyrosinase catalyzes the hydroxylation of tyrosine in 3,4-dihydroxyphenylalanine (DOPA) and leads to the production of a precursor molecule of melanin.[12]In addition, Vit C favors cell differentiation of keratinocytes and improve cohesion dermal–epidermal.[13]

Articles published recently also indicate that Vit C acts on epigenetic mechanisms.[12] The DNA methylation process is an inherited epigenetic modification, involved in gene silencing. Mutations induced by exposure to UV radiation, for example, may alter the methylation process, leading to the silencing of genes involved in processes of cell differentiation and apoptosis. The induction of the apoptotic process after exposure to UV radiation is an important defense mechanism to prevent the onset of malignancies, such as melanoma. Since methylation is a reversible process, studies have been conducted aiming at cell protection.[14]

Ten-eleven translocation (TETs) enzymes catalyze the removal of methylated cytokines from the DNA by their hydroxylation (5-methylcytosine is converted to 5-hydroxymethylcytosine).[12] Experiments performed on stem-embryonic stem cells (ES) reported that AA acts as a co-factor for TETs, leading to an increase of 5-hydroxymethylcytosine.[15,16] The same effect was observed in mouse embryonic fibroblasts cells (MEFs), suggesting that this mechanism can be applied to other cell lines.[17] In 2014, Lin and colleagues[14] investigated the role of AA in epigenetic modulation of human skin cancer cells (A431). The results indicate that AA reduced overall DNA methylation. Specifically, AA reactivated the expression of tumor suppressor genes by increasing the levels of 5-hydroxymethylcytosine *via* TET. In 2015, Gustafson et al[18] showed results consistent with those presented by Lin et al[14]; AA increased the level of 5-hydroxymethylcytosine in melanoma cells.[14,18]

In 2018, Ilíc et al[19] studied the effect of Vit C in the prevention of male infertility. It is known that the maintenance of the genetic material depends, among other factors, of the integrity of sperm cells DNA. The exposition to ROS and apoptotic processes can increase the fragmentation process. The enzyme Deoxyribonuclease I (DNase I) is a Ca^{2+}/ Mg^{2+} dependent endonuclease and is considered a key enzyme for the DNA fragmentation. Studies performed with Site Finder and Molecular Docking techniques showed that Vit C can interact with specific sites of action of DNase I (including H-donor interactions with Asp 168 and Asn 170, and H-acceptor interaction with Asn 170), inactivating it. These results indicate that the use of AA may be useful in preventing damage to seminal DNA.[19]

Vit C also appears to exert important brain functions that go well beyond its antioxidant activity. It acts as an enzymatic cofactor in the biosynthesis of collagen, carnitine, tyrosine and

peptide hormones. It stimulates myelin production, maturation and differentiation of neurons. In addition, studies show that Vit C deficiency is a common factor in the development of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and amyotrophic sclerosis, as well as psychiatric disorders including depression, anxiety and schizophrenia.[2]

Challenges and new possibilities for the stability and delivery of vitamin C

The biggest challenge in the utilization of Vit C is to maintain the stability. Vit C is easily degraded in aqueous medium, at high pH, in the presence of oxygen and metal ions. This process is usually accompanied by a color change in the formulations, which become gradually more yellowish. Several strategies have been developed to limit these processes, among them: controlling the presence of oxygen during formulation and storage, low pH and reduction of water content through the use of anhydrous/nonaqueous formulations.[8,20,21] The addition of preservatives such as antioxidants and anti-chelating agents also prevents the degradation of the Vit C. In this context, molecules such as ferulic acid and sodium metabisulfite have shown good results.[22]

The physicochemical properties of the formulation, such as dielectric constant and viscosity can also impact stability. In general, higher viscosity formulations and multiple emulsified systems offer greater protection against oxidation.[22] Also, in an article published in 2011, Ahmed et al studied the kinetics of photolysis of Vit C in cream formulations. Results showed that the humectant present in the creams can influence the photostability of Vit C. Best stability results were obtained in the presence of palmitic acid and glycerin into the formulation.[23] Additionally, strategies like the utilization of more stable derivatives of Vit C and the development of micro and nanoencapsulated delivery systems have been highlighted in the last years.[8,24] Some of these topics are discussed below.

Nonaqueous/anhydrous emulsion

Vit C is known as a potent antioxidant for skin care products with skin lightening properties. The development of skin lightening products can be a challenge to formulators since Vit C is highly susceptible to oxidation, especially in water-based systems and when exposed to air. Although the derivatives of Vit C have been developed with greater stability, their efficacy and greater formulation cost have led the cosmetic industry to decrease their quantity in the final products. In addition, it is challenging to design finished products that remain stable for a long period of time, because most contain a relatively high percentage of water. It has been confirmed that water seen as the key reactant for the instability of some actives in emulsions, for example, color changes occur in aqueous solutions or emulsions containing Vit C under normal conditions.[20,25,26]

Due to the instability of Vit C when in contact with water, new strategies and also new formulations are being proposed for the dissolution of the active. Appropriate solvents such as glycol to form one phase, appropriate oil phase such as silicone oil or another oil with a light feel. The Vit C is soluble in polyol solvents, such as propylene glycol, butylene glycol, hexylene glycol, glycerin, polyethylene glycols, glycereth-7, glycereth-26, ethoxydiglycol and ethanol. The advantages of using any of these solvents for nonaqueous emulsions are that they have less oxygen permeability and do not carry water, which prevent discoloration reactions in the formulation. These polyol solvents, appropriate oil phase and surfactants combined together, can form two immiscible phases which result in a nonaqueous or anhydrous emulsion that are heat-stable, non discoloring and also able to deliver the active.[20]

Two studies conducted by Eeman et al, 2014 and 2016 compared the stability studies of water-based benchmark formulations and nonaqueous/anhydrous emulsions containing Vit C. The stability of Vit C was assessed visually and using a UPLC system. The water-based commercial benchmark formulations showed significant evidence of discoloration associated with oxidation, although the glycerin-in-silicone formulation exhibited only slight yellowing effect at 50 °C. Also, the studies showed that nonaqueous/anhydrous systems can stabilize Vit C levels as high as 10% for a much longer period of time compared to a water-based commercial benchmark.[27,28]

Ascorbic acid derivatives

Some modifications have been done to Vit C molecule to improve its stability. One option is to bind ionic salts to the molecule. In this sense, among the most well-known complexes are ascorbyl 2-phosphates, which are formulated with sodium (SAP) or magnesium (MAP) salts and are hydrophilic in character. These structures can be seen in Figure 2. The introduction of a phosphate group in the second position of the cyclic ring of the molecule is effective against oxidation. However, these derivatives do not have direct antioxidant activity and must be converted, *in vivo*, by enzymatic reaction into L-ascorbic acid. Despite being more stable, these derivatives seem to present less permeability through the skin in comparison with ascorbic acid.[8]

Another derivative, ascorbic acid 2-glucoside (AA-2G) was studied by Lin et al, 2016. It is known that the hydroxyl group of carbon 2 of ascorbic acid directly influences its pharmacological activity. Likewise, this site is responsible for the degradation process. The AA-2G molecule has a conjugated glucose in the carbon-2 hydroxyl. This binding results in increased stability of the molecule (protection against degradation at high temperatures, pH and metal ions). When applied topically, AA-2G is hydrolyzed by a cellular α-glucosylase and is converted to L-ascorbic acid. In this study, the AA-2G was incorporated in a microemulsion. The results indicate that the system has higher permeability when compared to commercial emulsions and whitening ability for the skin.[29]

Another possibility is the utilization of lipophilic derivatives of ascorbic acid, such as: ascorbyl 6-palmitate (AA-Pal) and tetra-isopalmitoyl ascorbic acid (IPAA). In 2012, Maia Campos et al[30] verified the chemical stability and clinical efficacy of IPAA in dermatological formulations. The formulations developed showed chemical stability from 6 to 12 months and clinical trials, performed by non-invasive techniques on the skin of

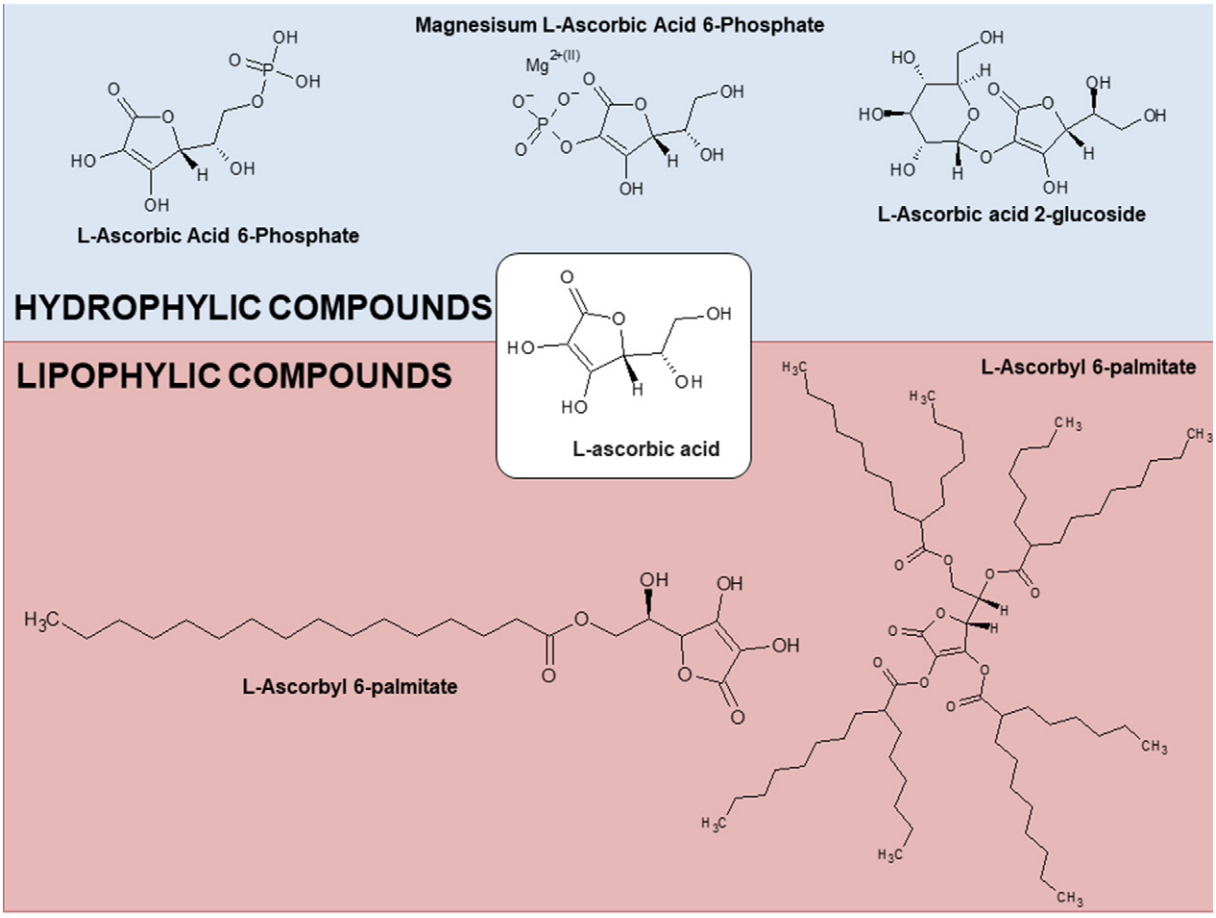

Figure 2. L-ascorbic acid molecule and its derivatives.

human volunteers, indicated moisturizing effects on the stratum corneum and viable epidermis.

A study conducted by Segall and Moyano, 2008 compared the stability of Vit C derivatives such as ascorbyl palmitate, sodium ascorbyl phosphate and magnesium ascorbyl phosphate in o/w emulsions for cosmetic application. The stability study was performed during 18 and 30 months and behavior *versus* time of ascorbyl palmitate, sodium ascorbyl phosphate and magnesium ascorbyl was examined.

The results showed that sodium ascorbyl phosphate and magnesium ascorbyl phosphate kept its stability to nearly 60-70% even after 365 days of storage in the dark at ambient temperature, whereas ascorbyl palmitate already showed great instability with no detected HPLC peaks after the same time. Furthermore the results showed that when butylhydroxytoluene is added to the formulation, it favors the long-term chemical stability of Vit C derivatives. Between Vit C derivatives, it seems that sodium ascorbyl phosphate is more stable than magnesium ascorbyl phosphate in the long-term studies. The conclusion of this study demonstrates that phosphate ester of Vit C formulations are more stable than ascorbyl palmitate formulations. In particular, esterification with palmitic acid in sixth position reduces the hydrolysis of ascorbic acid but does not guarantee satisfactory stability levels in the finished products. Instead, the introduction of phosphoric group in second position protects the molecule from break-up of the enediol system, thus confirming phosphate ester of Vit C as stable derivatives of Vit C that may be easily used in cosmetic products.[31]

Synergistic antioxidant systems

The synergistic use of antioxidant molecules is a natural process in human organisms. The skin, for example, uses mainly Vit C to protect cellular aqueous compartments and Vitamin E (Vit E) to protect lipid structures. After the oxidation process, Vit E can be regenerated in the cell membrane by Vit C.[32,33]

From this premise, Vit C was combined with Vit E for a topical formulation. The authors report that the combination of Vit C 15% and Vit E 1% promoted greater protection against erythema and prevented the formation of thymine dimers in DNA.[31] In a previous study, ferulic acid, a potent ubiquitous plant antioxidant, was incorporated into the system. The addition of 0.5% ferulic acid increased the stability of Vit C by 90% and doubled the photoprotective capacity. The authors report that ferulic acid can interact favorably with pro-oxidative intermediates or act as a main

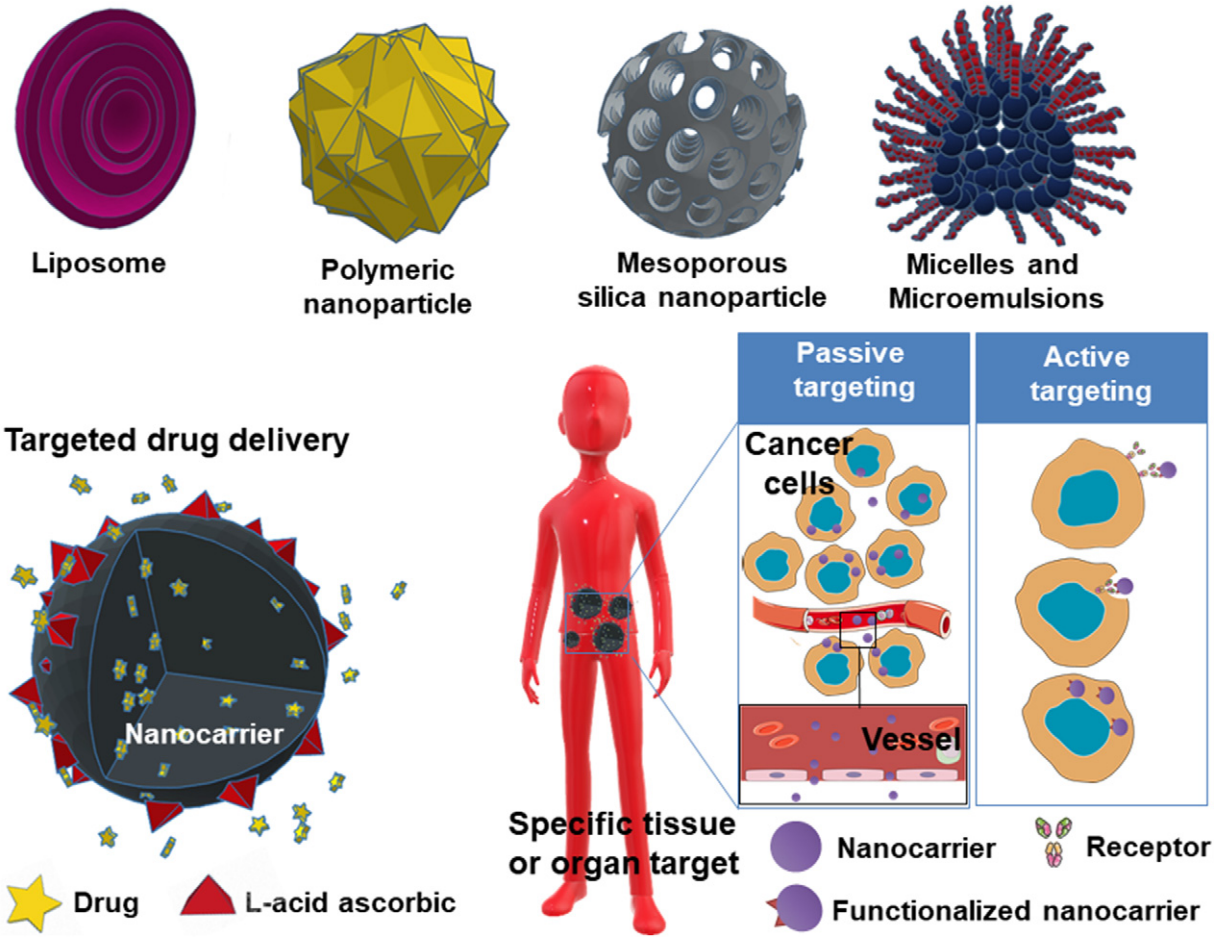

Figure 3. Some of drug delivery systems used to encapsulate Vit C and their mechanisms to improve drug targeting: due passive targeting, where the nanoparticles are target to a specific area of body and due active targeting, where nanoparticles interact directly with the cancer cells.

substrate. The increase in photoprotection can be explained as a consequence of the higher antioxidant activity of the system.[33,34]

In 2016, a serum containing Vit C, Vit E and ferulic acid was tested in humans after a treatment with fractional ablative laser for facial skin rejuvenation. Ablative lasers have been used with success to induce neocollagenesis and to enhance drug permeation. In this work, the objective was to evaluate if the utilization of the serum immediately after the treatment could improve wound healing. Fifteen healthy men and women of ages 30-55 were treated with the serum on one side of the face and with excipients to the other side, 2 min after the laser treatment. Results showed a decrease in edema *versus* vehicle and a faster wound healing.[35]

In another work, the physico-chemical stability of a formulation containing 1% of retinyl palmitate, ascorbyl tetraisopalmitate and tocopheryl acetate, alone or in combination, was determined by HPLC with UV detection. When combined, the degradation rate was slightly lower than when the antioxidants were alone. The authors support the theory that an intermolecular interaction of vitamins derivatives may reduce the reactivity of molecular oxygen, thus increasing their own stability in the formulation.[36]

Vit C loaded delivery systems

Many studies have shown that Vit C has been incorporated successfully into different delivery systems, in order to improve the treatment outcomes, targeting or protecting the molecule from chemical degradation. Several of these are described below and can be seen in Figure 3 below.

Nano and microparticles

Nanoparticles (NPs) produced from natural polymers have been extensively employed in the pharmaceutical and food industries. These systems have low toxicity, are biocompatible and biodegradable. In this context, the chitosan nanoparticles are highlighted. Chitosan is a cellulose-like molecule, consisting of two repeat units N-acetyl-d-glucosamine and d-glucosamine, linked by (1–4)-β-glycosidic linkage. Among its therapeutic applications, the following can be emphasized: improvement in the dissolution of drugs with low solubility in water, control of the drug release, improvement of drug targeting, protection against the degradation of the encapsulated compound, and increase of the absorption.[37]

Chitosan NPs are usually produced by dissolution in acetic acid. However, this process affects the biocompatibility at acidic pH. In 2018, Elshoky et al[24] developed and characterized

Table 1
Summary of Vit C loaded delivery systems.

System	Materials	Drug	Results	Test	Ref
Nanoparticles	Chitosan	Ascorbic acid	No internalization of the NPs associated to ascorbic acid into the cells. Ascorbic acid prevents cellular uptake and improves biocompatibility of chitosan nanoparticles.	*In vitro*	38
	N-trimethyl chitosan and N-triethyl chitosan	Ascorbic acid	Particles size increasing with ascorbic acid concentration. Different drug release profiles with pH values. Chitosan derivatives influence on profile drug release. Cellular uptake increases with derivatives chitosan carriers.	*In vitro*	39
	N-acyl chitosan	Ascorbic acid	N-acyl side chain lengths affect the loading and drug release profiles. Loading of drug leads the decrease in diameter and zeta potential of NPs.	*In vitro*	40
	Chitosan	Ascorbic acid	Measurements of the *in vivo*/*in vitro* release rate of drug encapsulation extended the shelf life.	*In vitro* *In vivo*	41
	Mesoporous silica	Ascorbic acid	Drug release control.	*In vitro*	45
	Mesoporous silica	Ascorbic acid	Causes the significant arrest in the G1 phase and the down-regulation of stemness genes. Differentiation of human ES cells into cardiomyocytes.	*In vitro*	46
	Hydroxyapatite	Ascorbic acid	Control of drug release.	*In vitro*	48
	Ascorbic acid-PLGA	Violacein	System was observed to be 2 folds more efficient as an antitumoral compared with free violacein.	*In vitro*	49
	Glyceryl behenate	Ascorbic acid	Nanoencapsulation improve anticancer effects of ascorbic acid. No damaging on NIH/3T3 normal cells was evidenced.	*In vitro*	50
	MAA-PEGMA	Ascorbic acid	Increase in stability and permeation.	*In vitro*	51
Liposomes	Soybean phosphatidylcholine, cholesterol, chitosan.	Folic acid, and ascorbic acid	High encapsulation efficiencies. Chitosan coating can efficiently improve the physical stability. Antioxidant activity of coated LPs is higher compared to non-coated.	*In vitro*	61
	Phosphatidylcholine and cholesterol	Palmitoyl ascorbate and doxorubicin	Intracellular concentration of drug-loaded LPs. Antitumor ability, but no changes of body weight and reduced damages to tissues.	*In vitro* *In vivo*	62
	Soybean phosphatidylcholine, cholesterol	Palmitoyl ascorbate and docetaxel	LPs entered the cells successfully and the co-delivery of drugs was more effective in inhibiting tumor growth than either palmitoyl ascorbate or docetaxel individually.	*In vitro*	63
	Soybean phosphatidylcoline and sodium cholate	Sodium ascorbate	Ability of skin penetration. Improves antioxidant capacities and decreases anti-inflammatory chemicals on skin irradiated UVA/UVB compared to drug in solution.	*In vitro*	64
	Soybean phosphatidylcoline,cholesterol, medium-chain fatty acids	Ascorbic acid	Use of high pressure microfluidization provides LPs formation. Ability of control release.	*In vitro*	65
	Distearoylphosphatidylcholine, cholesterol	Ascorbic acid Potassium antimony (III) tartrate hydrate	Single dose resulted in 47%, 33% and 47% reduction of parasitic levels liver, spleen and bone marrow, respectively. LPs in association with ascorbic acid alleviates the major tissue alterations promoted by antimonial drugs.	*In vivo*	66
	Soybean phosphatidylcholine, cholesterol and Tween® 80	Ascorbic acid	LMP LPs improves their stability compares to HMP or non-coated LPs.	*In vitro*	

	System	Materials	Drug	Results	
					67
			HMP and LMP LPs improved the permeation of drug 1.7-fold and 2.1-fold after 24 h, respectively, in comparison with non-coated LPs.		
	Phosphatidylcholine, 1,2-distearoyl-sn-glycero-phosphoethanolamine-N-(poly[ethylen e glycol]2000) cholesterol.	Epirubicin Ascorbic acid	The antitumor activity of the encapsulated drug was confirmed and shows tumor-growth inhibition over 40% .	*In vitro*	68
				In vivo	
	L-α-dipalmitoyl phosphatidyl choline	Cyclophosphamide, ascorbic acid	Liposomal formulation doped with Vit C to diminish the potential side effects of the drug.	*In vivo*	69
	Egg phosphatidylcholine, cholesterol, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (poly (ethylene glycol))-2000]	Palmitoyl ascorbate	Treatment of cancer cells with LPs enhanced anticancer activity.	*In vitro*	70
			Liposomal killing of cancer cells are unaffected by PEGylation.	*In vivo*	
			Predominant deposition of drug in the liver, spleen, and lungs and the tumor site.		
			LPs slowed growth of tumors.		
	Egg phosphatidylcholine, cholesterol, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (poly (ethylene glycol))-2000].	Paclitaxel and Palmitoyl ascorbate	Palmitoyl ascorbate LPs targeted and killed cancer cells *in vitro.*	*In vitro*	71
			Increased accumulation of the liposomes in tumor.	*In vivo*	
			Anti-tumor activity.		
Microemulsions	Decyl glucoside, propylene glycol and oils	Ascorbic acid	Ascorbic acid cutaneous delivery was achieved (1.5–3-fold).	*In vitro*	74
			Penetration-enhancing ability depends on oil type MEs.		
			MEs is less cytotoxic and promote tissue antioxidant activity.		
Micelles	Polyethylene glycol-phosphatidylethanolamine	Palmitoyl ascorbate	Exhibited anti-cancer activity in cancer cell lines both *in vitro* and *in vivo.*	*In vitro*	75
				In vivo	

Table 1: Abbreviation list
Ascorbic acid-PLGA: Poly lactic-co-glycolic acid
HMP LPs: low methoxyl pectin liposomes
LMP LPs: high methoxyl pectin liposomes
LPs: liposomes
MAA-PEGMA: polymer synthesized from metharcrylic acid (MAA) and poly(ethylene glycol)
MEs: microemulsions
NPs: nanoparticles

chitosan NPs using two dissolution agents, acetic acid and AA and compared their properties, including their cytotoxicity and cellular uptake in human colon carcinoma (CaCo-2) cells. Dynamic light scattering technique was used to verify the particle size distribution. AA NPs showed a bigger average particle size when compared to acetic acid NPs. However, average particle size can be managed through the modification of the concentration of ascorbic acid. Zeta potential remained identical in both situations and images obtained by Transmission Electronic Microscopy (TEM) confirmed the spherical shape of both NPs. Cellular uptake and internalization of chitosan nanoparticles were investigated by confocal laser scanning microscopy (CLSM). CaCo-2 cells were incubated with the NPs and after 24 h at 37 °C, acetic acid NPs were found in cytoplasm and the nucleus while the AA NPs were found attached to the cell membrane. This result suggests that AA NPs could be used to target cell membrane receptors, such as glucose-specific receptors. This study and the others studies presented in this session of the article are summarized in Table 1.

Jang et al[38] developed NPs with N-trimethyl chitosan (TMC-NPs) and with N-triethyl chitosan (TEC-NPs) by ionic gelation with sodium tripolyphosphate (TPP) anions to enhance the permeability of AA. Both NPs maintained hydrophobic characteristics and avoid agglomeration. Encapsulation efficiency for AA was ~40% with TMC-NPs and varying from 18 to 55% with TEC-NPs. Drug release rates of AA were also different between the NPs: while TMC-NPs exhibited an initial burst release, TEC-NPs exhibited a controlled release, increasing rapidly after 2 h. To study the permeation of AA from the NPs, Caco-2 cells were treated with 6 different conditions: control, AA alone, an AA mixture with TMC, an AA mixture with TEC, AA-loaded TMC NPs, and AA-loaded TEC NPs. The degree of AA permeation was assessed by applying calcium-sensitive fluorescent dye to treated Caco-2 cells. Results were obtained by CLSM. It was reported that cells treated with AA-loaded TMC and TEC NPs exhibited the highest AA permeation followed, in descending order, by AA with TEC, AA with TMC, AA, and the control. These results suggest that AA-loaded TMC and TEC NPs can enhance the permeation of AA and could be interesting for drug delivery.

Cho et al[39] studied the formation of AA NPs composed by the following types of N-acyl chitosan: propionyl chitosan, hexanoyl chitosan, nonanoyl chitosan, lauroyl chitosan, pentadecanoyl chitosan and stearoyl chitosan. Chitosan NPs were positively charged in the range of 10.2-28.9 mV and this value decreased after ascorbate acid loading (from 5.9 mV to 18.4 mV). Mean diameter of NPs ranged from 444.2 to 486.6 nm, after loading of ascorbic acid, these diameters decreased to a range of 215.6 to 288.2. This effect may be explained by the fact that ascorbic acid acts as a cross-linker and increases the inter- and intra-molecular interactions of chitosan NPs. Loading efficiencies of NPs were 55-67% and slightly increased when propionyl chitosan and lauroyl chitosan were used. *In vitro* drug release experiments showed that Vit C in N-acyl chitosan presents controlled release properties at pH 1.3 and pH 7.4. Release rate of Vit C load is reduced with increasing the length of acyl side chain.

Alishahi et al[40] developed NPs for encapsulation of Vit C using chitosan with different molecular masses. Chitosan was reacted with sodium tripolyphosphate (TPP) and the effect of chitosan molecular weight (65-450 kDa) on the average particle size, zeta potential values and nanoparticle yields were evaluated. It was observed that increasing molecular weight, increased average particle size and zeta potential. However, the best encapsulation of Vit C (~60%) was achieved with low molar chitosan (110 kDa). It could be explained because low molar chitosan contains shorter chitosan fragments which make its free amino groups easier to protonate. As a consequence, Vit C can be better absorbed through ionic interactions. Vit C is very sensitive to temperature, oxygen, and light. In this study, the shelf-life was evaluated up to 20 days in rainbow trout (*Oncorhynchus mykiss*). Up to 20 days, encapsulated Vit C exhibited more of 90% of content, whereas free Vit C showed ~40%. The *in vitro* release profiles of Vit C from chitosan NPs were investigated at 37 °C for 100 h in two medium conditions: 0.1 M HCl and PBS. At 100 h of essay, only 30% of Vit C was released in 0.1 M HCl, while the release rate in PBS medium was 75%. This phenomenon can be explained by weakening of electrostatic interaction between complexes and the NPs at the neutral pH, what resulted in a faster release. *In vivo* release of NPs was investigated using rainbow trout (*O. mykiss*), as a model. The results were the same as with the *in vitro* study and thus confirmed the conclusions.[40]

Mesoporous silica nanoparticles (MSNs) are promising delivery systems in the biotechnology field. MSNs are solid structures with hundreds of empty channels (mesopores) arranged in an orderly 2D arrangement, similar to a honeycomb. The channels act as reservoirs for the drug of interest and can have their size, volume and alignment controlled during the preparation process.[41] Basically, MSNs are synthesized from a precursor of silica and surfactants. In this process, the surfactants are used above their critical micellar concentration (CMC) where they will form micelles. The silica precursor will condense around the polar portion of the micelles, giving rise to silica walls. Then, the surfactant is removed and the MSNs are obtained.[42]

The microspheres are stable and have interesting structural properties, such as: high surface area, controlled pore volume, and two functional surfaces (outer particle and interior pore).[40] In addition, it is possible to develop MSNs capable of releasing the drug in a targeted way and at a specific time using external factors such as light, pH, electrical current or mechanical stimuli.[43]

MSNs were designed for the delivery of Vit C.[44] Pore volume was determined by N_2 adsorption–desorption isotherms and values obtained were 0.7909 to 0.6969 $cm^3\ g^{-1}$ which decreased with drug loading. Drug release studies showed during the first 30 min, a burst release of drug, 28.6%, 62.0% and 57.1% into simulated gastrointestinal fluid, simulated intestinal fluid, and simulated body fluid media respectively.[44]

Another study showed that MSNs were able to deliver ascorbic acid for differentiation of human embryonic stem cells into cardiomyocytes. Cell viability and apoptosis of human ES cells treated with NPs exhibited no obvious apoptosis. The same treatment for 14 days exhibited changes of cell cycle in human ES cells and caused a profound G1 cell cycle arrest. Treatments with drug alone showed ~46% cells at G0/G1 cell cycles, while

ascorbic acid-loaded silica NPs showed ~70% at G1 cell cycle. Moreover, the delivery of ascorbic acid inhibited the expression of stemness genes (OCT4 and SOX2) in human ES cells; the use of nanocarriers enhanced the inductive effect of ascorbic acid by effectively transporting these drug molecules into the cells. On the other hand, the treatment induced the differentiation of human ES cells into cardiomyocytes. Percentage of beating cells and the beating frequency (beats per min) were 40% and 65% to free drug and entrapped drug in NPs ($P < 0.05$), and 20 and 30 beats per min ($P < 0.05$) respectively.[45]

Ceramic materials such as silica, zirconia, calcium phosphates, among others, have been used for various biotechnological applications. Among their characteristics, the following stand out: high mechanical resistance, favorable interaction with human tissues and low or nonexistent toxicity. In the field of bone tissue engineering, hydroxyapatite-based materials have great potential due to their osteoconductive properties.[46] Hydroxyapatite nanoparticles are 3D structures with interconnected pores that were used to investigate the control release of ascorbic acid. NPs were synthetized by co-precipitation method and ultrasonic treatment for their formation and then characterized.[47] The average diameter was about 140 nm and average pore diameter of about 15 nm. The method for production of NPs influences the drug release. The authors used sonication energies at 75, 105, 150 W and the released amount of Vit C at 200 min was 50, 40 and 35%, respectively. However, further studies have to be performed in order to evaluate their use for bone engineering.

Poly lactic-co-glycolic acid (PLGA) NPs were prepared by following the nanoprecipitation method. Viability was performed using HL-60 cells and the results showed that violacein loaded NP-ascorbic acid and free violacein showed cellular survival of 30% and ~45%, respectively; Thus, at concentration of 2 μM of violacein, NP-ascorbic acid-violacein was 2 fold more potent compared to free violacein.[48]

Ascorbic acid was loaded into solid lipid nanoparticles (SLNs) made from glyceryl behenate, as the lipid matrix, prepared by the hot homogenization method.[49] Ascorbic acid loaded SLNs exhibited a size of about 200 nm, a negative zeta potential (−19 to −25 mV), and an encapsulation efficiency of ~90%. Drug release studies showed controlled rate of release up to 96 h. Cytotoxicity of SLNs was performed using H-Ras 5RP7 and NIH/3T3 cells, in which the maximum cytotoxic effect of ascorbic acid-loaded was observed at 25 μM/mL and showed 41% cell viability at 72 h ($P < 0.001$) whereas free drug exhibited 58.7% cell viability at the same time of exposure ($P < 0.01$) in H-Ras 5RP7 cells. The percentage of apoptosis in drug loaded nanocarrier (25 μM/mL) treatment group was 51.3% at 72 h and non-loaded drug group was 25%, and the caspase-3 levels found were ~70% and 40% for drug loaded or not loaded into NPs, respectively. Confocal images of cells confirm the *in vivo* study data, where apoptotic morphologies were recorded, most notably by chromatin condensation and morphological holes. Moreover, TEM images of cells showed cell rounding, chromatin condensation, nucleus fragmentation and cell membrane shrinkage.[49]

Hydrogels are vehicles composed of networks of polymers, capable of absorbing large amounts of water or aqueous solvent. Some chemical groups present in their chains can be made responsive to environmental conditions, such as pH, temperature, ionic force, among others. This behavior can be used for topical drug delivery. More specifically, anionic gels possess chemical groups that can become ionized when the pH of the medium increases above the pKa of the hydrogel. In these conditions, there will be a significant swelling of the polymer network and consequent release of the encapsulated compound.-[50] This system was used for the microencapsulation of ascorbic acid for skin delivery. Ascorbic acid was incorporated in the copolymer MAA-PEGMA, synthesized from metharcrylic acid (MAA) and poly(ethylene glycol) (PEGMA, Mw 360) *via* dispersion photopolymerization. The formulation was kept below pH 5 an under these conditions, the polymer network is collapsed and the Vit C cannot be released from the hidrogels particles. However, when the formulation comes into contact with the skin (pH ~ 6), the pH increased over the pKa of the hydrogel leading to the ionization of the carboxylic groups of the MAA. As a consequence, the mesh size increase and the Vit C is abrupt release. The incorporation of ascorbic acid into the hydrogel microcapsules resulted in the protection of ascorbic acid against degradation and its maintenance at high temperatures such 70 °C for 5 days.[50]

Liposomes

Liposomes (LPs) are vesicles composed of amphiphilic molecules. They have been studied since the 1960s when Alex Bangham discovered that the hydration of a lipid film gave rise to closed spherical structures of microscopic size. This process usually produces multilamellar vesicles (MLVs), which are concentric lipid bilayers separated by aqueous compartments[51] Since then, several methods have been developed for the production of unilamellar liposomes of different sizes, ranging from 30 nm to 100 μm.[52,53] Examples, of such methods include sonication, extrusion, microfluidization, high-press homogenization, reverse phase evaporation, among others.[51,54–56]

Several components can be employed in the production of liposomes. Usually, phospholipids and cholesterol are used as major components, but other compounds such as surfactants, ethanol, terpenes and polymers have also been used to modify some liposomal membrane properties.[53] Due to their structural similarity to cell membranes, liposomes are widely used in basic science to elucidate the properties of membranes and their lipid components.[57,58] As a delivery system, they are interesting because they can carry both hydrophilic molecules (in their aqueous interior) and hydrophobic molecules (in the lipid bilayer). In addition, liposomes exhibit low toxicity and are biocompatible. Several classes of drugs such as antibacterial agents, antifungals, immunosuppressives, antineoplastic agents, corticoids, local anesthetics, retinoids, among others have already been successfully encapsulated in this type of carrier system.[59]

Jiao et al[60] designed LPs coated with chitosan for delivery of ascorbic acid and folic acid. Encapsulation efficiency has a behavior to entrap for both drugs, for ascorbic acid, the encapsulation reached around 80%, whereas, for folic acid this value was ~90%. On the hand, non-coated LPs were ~35% and 65% for ascorbic acid and folic acid, respectively. Studies for

antioxidant properties of the LPs were conducted and a higher antioxidant capacity was reported for coated LPs. Stability studies were performed, and the leakage ratio of drugs was assessed. For coated or non-coated LPs, at 4 °C, the results showed a leakage of 10% at 40 days of storage. However, chitosan coated LPs showed values of leakage less than 5%. At 25 °C, coated LPs showed leakage values less than 10%, but non-coated LPs showed values between 30 and 40%. This may be due to the fact that at the lower temperature the fluidity of the membrane may be decreased and this would inhibit the fusion of LPs while the coating on surface of LP may have promoted resistance to diffusion. In this way, the system developed for the antioxidant defense system is used in the food industry and for cosmetic purposes.

Yang et al[61] developed LPs for co-delivery of a palmitoyl ascorbate and doxorubicin (DOX). The chemotherapeutic agent DOX has been used in the treatment of several types of cancer. Its mechanism of action is based on its ability to intercalate with DNA, interfering in the repair processes, and generate increased production of reactive oxygen species (ROS), leading to DNA damage. Since palmitoyl ascorbate has been used to treat some types of cancer (due your efficacy in in suppressing the proliferation of cancer cells and DNA synthesis) and also can reduce the toxicity caused by DOX (*via* sweeping the peroxidative products) this synergetic system was developed. At high concentrations, the LPs were shown to increase ROS production and induce apoptosis. It has also been shown that the combined use of ascorbic acid with chemotherapeutic agents, such as DOX, enhanced the effectiveness of the treatment. Morphological studies showed homogeneous spherical multilamellar structures, and the size was in agreement with data recorded using dynamic light scattering (91 to 137.5 nm). Stability studies show some slight changes in particle sizes of LPs at 4 °C and 30 days. Release of DOX from LPs was significantly affected by pH. At pH 5.0 (pH inside tumor) the release was faster, indicating that LPs were destroyed in tumor site, and thus have a leakage of drug. In contrast, at pH 7.4 the drug is released slowly from the LP. Cytotoxicity effects were evaluated on human breast adenocarcinoma (MCF-7) cells, human liver hepatocellular carcinoma (HepG2) cells, and human lung carcinoma (A549). These studies show that co-delivery LPs have synergistic effects and anticancer properties. Cellular uptake, endocytosis pathway and intracellular localization were studied in MCF-7 cells. Drug uptake from LPs containing palmitoyl ascorbate was 2.5-fold greater than drug-LPs and 5.4-fold greater than drug in solution. LPs can be endocytosed *via* two pathways: macropinocytosis and clathrin-mediated endocytosis. Besides, it was reported that LPs co-delivery with PA have ability for internalization into cells. The pharmacokinetic study in rats after intravenous administration showed AUC values of 429.19 ± 93.51, 1224.57 ± 171.36, and 13,755.24 ± 2607.48 μg/L.h for drug solution, drug-LP and co-association LPs respectively. These results suggested that palmitoyl ascorbate played a key role in prolonging drug retention *in vivo*, since the plasma half-life was also prolonged. The weights and sizes of the tumor when treated with palmitoyl ascorbate and doxorubicin LPs were reduced by 2-fold and 4-fold compared to drug-LPs and drug in solution, respectively.[61]

Another study shows palmitoyl ascorbate LPs enhanced synergistic antitumor efficacy of docetaxel. *In vitro* drug release shows ability of the carriers to control of the release of drug. Particles size was reported to be around 150 nm and encapsulation efficiency was about 90% for both drugs. Cell uptake was performed using MCF-7 cells, HepG2 cells and human prostate cancer cells (PC-3). The results suggested that the LPs entered the cells successfully and the co-delivery of drugs was more effective in inhibiting tumor growth than either palmitoyl ascorbate or docetaxel alone.[62]

Liposomes were prepared with soybean lecithin and sodium cholate for skin delivery of sodium ascorbate. The final concentration of sodium ascorbate was 100 ± 11 mg/mL resulting in an encapsulation efficiency of 40%. Penetration studies were performed using Franz cells and fluorescence microscopy. Results showed that Vit C LPs improved skin penetration of Vit C. Finally, an *in vitro* study was conducted to evaluate antioxidant and anti-inflammatory properties in human skin exposed to UVA/UVB radiation. Skin was preincubated with LPs and drug in solution for 20 h. After washing, the skin surface was irradiated with UVA/UVB intensity of 50 J/cm^2 for 2 h and incubated in Franz cells for 24 h. The antioxidant capacity was measured by Trolox assay that measures the ability of antioxidants to scavenge 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) radical, a blue-green chromophore that decreases in its intensity in the presence of antioxidants. It was observed that in contrast to ascorbate solution, the LPs prevented the effects of UVA/UVB radiation and avoid the increase of pro-inflammatory cytokines, such as factor alpha (TNFα) and interleukin (IL)-1β. These LP formulations showed promising skin penetration, and antioxidant and anti-inflammatory properties against UVA/UVB photodamage.[63]

Li et al[64] reported the preparation of LPs composed of soybean phosphatidylcoline and cholesterol for the co-delivery of Vit C and medium-chain fatty acids by the double emulsion method or double emulsion-dynamic high pressure microfluidization. Results showed that LPs prepared by the double emulsion-dynamic high pressure microfluidization exhibited higher entrapment efficiency of medium-chain fatty acids, relatively higher entrapment efficiency of Vit C, a lower average size diameter and better storage stability at 4 °C for 90 days than those prepared by double emulsion method. *In vitro* drug release studies show a relatively rapid release observed from 2 to 10 h, followed by a slower release rate after 10 h. These authors suggest that double emulsion-dynamic high pressure microfluidization could be a potential approach for the preparation of LPs that encapsulate Vit C.

Castro et al[65] developed and evaluated the efficacy of trivalent antimonial-loaded LPs, in association with ascorbic acid for treatment of *Leishmania infantum*. These LPs were composed by distearoylphosphatidylcholine and cholesterol and prepared by the extrusion method. LPs showed a median size of 222.5 nm, polydispersion index of 0.214 and 15% encapsulation efficiency. *In vivo* performance was carried out in isogenic BALB/c mice infected with *L. infantum* (C43 strain). Administration of the formulation, as a single dose, resulted in a reduction in parasite burden of organs. However, the administration of ascorbic acid with either free drug or drug entrapped in

Table 2
The utilization of Vit C as a targeting molecule.

	Materials	Drug	Results	Test	Ref.
Targeting	Metallic NPs	Ibuprofen	No cytotoxicity effects on bEnd.3 cells. Ability of drug release in plasma and brain.	*In vitro*	77
	Polymeric NPs	Paclitaxel	At 20% of conjugation on surface was able to internalize in Caco-2 cells NPs internalized into cells *via* caveolae-mediated pathway Biodistribution and perfusion study demonstrated that conjugated NPs accumulated in the villi and penetrated to the basolateral side.	*In vitro* *In vivo*	78
	Polymeric NPs	Galantamine	Improves concentration of drug on NIH/3 T3 cells overexpressing receptor to ascorbic acid. Sustained action for pharmacological assay was achieved High distribution of the drug to the brain.	*In vitro* *In vivo*	79
	Polymeric NPs	Dehydrocrotonin	Good loading capacity and narrow size distribution Increase cytotoxicity when compared to free drug Increase the induction of apoptosis	*In vitro*	80

liposomes did not reduce the parasite levels. On the other hand, drug-loaded LPs in association with ascorbic acid alleviated the major tissue alterations promoted by the antimonial, such as pronounced reduction in the inflammatory infiltrate and associated hyperemia when compared to treatment with drug entrapped in LPs only. Thus, the association of ascorbic acid with LPs represents a better alternative to reduce the toxic effects of potassium antimony (III) tartrate hydrate.

Zhou et al[66] proposed pectin-coated LPs for skin drug delivery. LPs were prepared by thin film evaporation combined with dynamic high pressure microfluidization. Two approaches were evaluated, the coating with high methoxyl pectin (HMP) and low methoxyl pectin (LMP). The entrapment efficiency profiles were around 50% of Vit C and it was independent of pectin concentration and molecular weight. However, the diameter and polydispersity index of LPs increased with the increase of pectin concentration. In addition, the zeta potentials of LPs decreased with the increase of pectin concentration. Morphology was assessed by AFM and TEM techniques and showed a small size and well-distributed particles for non-coated LPs, whereas larger and irregular particles with globule-like structure were formed for the coated LPs. Physical stability was monitored for 10 weeks and showed a leakage ratio up to 10% for coated or non-coated LPs at 25 °C or 4 °C, except for non-coated LPs at 25 °C (~50%). Thus, LMP coated LPs showed better physicochemical stability. *In vitro* skin permeation studies shows a higher permeation of Vit C entrapped into LMP coated LPs (40.1 ± 4.7 μg/cm^2) compared to HMP (32.2 ± 5.2 μg/cm^2) or non-coated LPs (19.2 ± 3.2 μg/cm^2).

LPs were formulated with hydrogenated soy phosphatidylcholine, 1,2-dis tearoyl-*sn*-glycero-phosphoethanolamine-N-(poly[ethylene glycol]2000) (DSPE-PEG 2000) and cholesterol for a co-delivery of epirubicin and ascorbic acid. In this study, the authors evaluated the influence of pH on the encapsulation efficiency. A gradual increase in drug encapsulation with increasing external pH was observed. At pH > 7.0 the drug loading achieved 90%. MCF-7 cells viability shows ascorbic acid gradient shows superior cytotoxicity. *In vivo* antitumor activity was evaluated in mice inoculated with the 4T-1 murine mammary cancer cell line and showed a reduction on tumor growth of 50% at 18 days of treatment. Antitumor activity presented to epirubicin-encapsulated lipossomes with an ascorbic acid against 4T-1 murine mammary cancer was evaluated and showed ability to decrease cell proliferation.[67]

Tohamy et al[68] studied the application of Vit C entrapment into LPs and evaluated the alleviation of genotoxic effects of cyclophosphamide. Toxicological effects were evaluated in actual age male albino Swiss mice. Clastogenic and cytotoxic effects were observed to the cyclophosphamide treatment. A significant decrease of polychromatic to normochromatic erythrocytes ratio when compared to the group treated with cyclophosphamide-loaded LPs and Vit C. Amelioration of S-transferase activity was observed with treatment of co-encapsulated LPs.

Palmitoyl ascorbate was encapsulated into LPs for anticancer therapeutic effects.[69] *In vitro* cytotoxicity of 4T1 cells and MCF-7 cells shows that palmitoyl ascorbate LPs has ability to kill the cells. *In vivo*, palmitoyl ascorbate LPs were modified with polyethylene glycol (PEG-PA LPs). However, PEGylation process did not alter the cytotoxicity profile. Biodistribution of PEG-PA LPs showed predominant deposition in the liver, spleen, and lungs. The distribution of PEG-PA LPs in tumor-bearing mice was studied and formulations showed accumulation in tumors. 4T1 mouse mammary tumor model was used and showed that PEG-PA LPs significantly ($P < 0.05$) slowed growth of tumors.[69]

Sawant et al[70] developed palmitoyl ascorbate-modified LPs for paclitaxel co-delivery with antiproliferative activity. Egg phosphatidylcholine and cholesterol were used to prepare LPs. These LPs possessed the ability to kill cells in *in vitro* studies and decreased ROS production in the cellular microenvironment. The effect of paclitaxel-encapsulated LPs palmitoyl ascorbate-modified LPs provided a platform with enhancing the anti-tumor properties of ascorbate.[70]

Microemulsions and micelles

Over the last few decades, emulsified systems have gained more visibility in area of drug delivery. Emulsions are colloidal

systems, typically composed of oil, water and surfactants and are examples of complex formulations employed to optimize the delivery of many cosmetic and pharmaceutical actives. In general, they can be classified by the diameter of their droplets into: macroemulsions (400 nm), nanoemulsions (100-400 nm) and microemulsions (10-100 nm).[71]

Microemulsions were introduced in the 1940s and seem to be advantageous for a number of reasons. These systems are transparent (due to the smaller diameter of their droplets), optically isotropic and have thermodynamic stability. In addition, the microemulsions present low interfacial tension, large interfacial area and solubilizing properties for both hydrophilic and hydrophobic drugs. When applied on the skin, the emulsions interact with the stratum corneum, promoting structural modifications in the lipid layers. These changes favor the partition coefficient of the drugs and favor the permeation.[72]

Pepe et al[73] designed decyl glucoside-based microemulsions composed of decylglucoside used as surfactant and propylene glycol as the co-surfactant, oil phase consisted of isopropyl myristate and monoglycerides (monocaprylin, monolaurin or monoolein) and water loaded or not with ascorbic acid at 0.2% (w/w). Skin penetration assays were performed and showed that larger amounts of ascorbic acid were delivered, but the effect of monocaprylin was weaker and very similar to monoolein. In addition, monolaurin failed to significantly increase ascorbic acid delivery into the stratum corneum and viable skin layers. Skin electrical resistance measurements were performed and all formulations were reported to increase the skin electrical resistance, suggesting that their ability to disrupt the skin barrier does not depend only on the amphiphilic effects, but on the combination of components (oily phase): monocaprylin had the strongest effect (3.4-fold decrease), followed by monoolein (2.5-fold decrease), and monolaurin (1.9-fold decrease). Antioxidant capacity of the microemulsion-treated skin was evaluated and showed unloaded MEs; the antioxidant activity of the skin increased 5.6 times for ascorbic acid-loaded MEs. Thus, these overall results reinforce the importance of the molecular structure of oily phase types and formulation design for delivering compounds into the skin.

Micelles were prepared using polyethylene glycol-phosphatidylethanolamine for incorporation of palmitoyl ascorbate. A co-culture of cancer cells and GFP-expressing non-cancer cells was used to determine the specificity of these micelles and exhibited anti-cancer activity in cancer cells. *In vivo* anti-cancer activity was studied in female Balb/c mice bearing a murine mammary carcinoma (4T1 cells) and showed good effects for killing tumor cells and this mechanism of cell death was caused by the generation of ROS.[74]

Drug targeting

Nanotechnology has provided the possibility to improve drug delivery to a specific body area or cell type, as can be seen in Figure 3 above.[75] Recently, the use of ascorbic acid has been reported to deliver therapeutic agents to the targeted regions of body. Wang et al[76] developed NPs for deliver ibuprofen to the brain through Na^+-dependent Vit C transporter 2 (SVCT2) and glucose transporter 1 ($GLUT_1$). NPs were synthetized using Fe3O4 and many steps were attempted to achieve ascorbic acid inclusion on the NP surface. The results suggested that NPs prepared had limited toxicity on murine brain endothelial cells (bEnd.3 cells). Drug disposition was carried out plasma and brain homogenate and presented the brain homogenate the amount of the model drug selected, ibuprofen released was ~20%,. Additional studies should be accomplished for more understanding of targeting ability and therapeutic effects *in vitro* and *in vivo*. This study and others presented in this section of the article are summarized in Table 2.

Table 3
A summary of conditions and quantify of studies for ascorbic acid obtained on Clinical Trials Database (https://clinicaltrials.gov). Data retrieved on August 5th, 2019.

Condition	Number of study
Skin Diseases	33
Skin Diseases, Eczematous	1
Skin Diseases, Genetic	2
Skin Manifestations	2
Skin Neoplasms	2
Skin Ulcer	4
Wound Infection	2
Wounds and Injuries	46
Pancreatic Cancer	18

Luo et al[77] developed NPs for targeting on sodium-dependent Vit C transporter 1 (SVCT1). In this study, these authors exploited the use of ascorbate-conjugated NPs for oral drug delivery. In general, the results showed that conjugation of 20% ascorbate to the surface of poly(lactic-co-glycolic acid) (PLGA) NPs might achieve a maximum internalization in Caco-2 cells. This internalization was mediated the transport predominantly by caveolae-mediated endocytosis. *In situ* permeability studies were conducted using small intestinal perfusion method in rats and these results showed effective membrane permeability (P_{eff}) and the absorption rate (Ka) were higher in the duodenum, jejunum and ileum to ascorbate-conjugated NPs than non-conjugated NPs (~2.6-fold compared to these). Also, rats were treated orally with NPs and biodistribution in the GI tract was assessed. The modification of ascorbate on the surface of NPs can be able to penetrate on mucus layer, then recognize and internalize by enterocytes with the assistance of receptor of ascorbate, and then enter the systemic circulation.

The use of SVCT2 can be exploited to brain delivery, according to Gajbhiye et al.[78] Ascorbic acid-grafted PLGA-b-PEG NPs and the uptake cellular were performed into VCT2 expressing NIH/3T3 cells. The concentration of galantamine for targeted NPs (2.692 ± 0.382 μg) was 3.55 and 6.53 times higher than PLGA (0.758 ± 0.045 μg) and PLGA-b-mPEG NPs (0.412 ± 0.025 μg; non-targeted), respectively at 3 h. *In vivo* pharmacodynamic studies were performed in albino rats using the Morris Water Maze Test for memory assessments. Results showed higher therapeutic and sustained action by drug loaded ascorbate conjugated to PLGA-b-PEG NPs than free drug and drug loaded PLGA, as well as PLGA-b-mPEG NPs (non-conjugated with ascorbate). Biodistribution studies showed that drug was found in a higher concentration to the brain when loaded to ascorbate conjugated to PLGA-b-PEG NPs compared

to GLM-PLGA-b-mPEG (3.3 folds), to PLGA NPs (2.8 folds), and to free solution (19.4 folds). Hence, the authors suggested that targeting of bioactives to the brain by ascorbic acid grafted PLGA-b-PEG NPs is a promising approach.

Dehydrocrotonin NPs with L-ascorbic acid 6-stearate were synthetized for targeting into tumors.[79] Particle characterization showed a size distribution of 100-140 nm and drug loading of 81-88%. Drug release shows drug has a total solubilization up to 6 h of analysis. In contrast, the NPs show shows a gradual release of drug until 72 h, with a total recovery of 60%. Cytotoxicity assay was performed into HL-60 cells and showed IC_{50} values of 190, 200, and 140 μM for free drug, drug loaded NPs, and drug loaded dehydrocrotonin NPs with L-ascorbic acid 6-stearate, respectively. Caspase-8 activation was higher to drug loaded dehydrocrotonin NPs with L-ascorbic acid 6-stearate compared to drug loaded-NPs without L-ascorbic acid 6-stearate ($P < 0.05$).[78]

Patents and clinical trials concerning ascorbic acid

Over the last decade several fundamental findings have translated into intellectual property with a very real possibility for their future exploitation. Thus, ascorbic acid synthesis was described in literature using microorganisms; also new technologies and products are available in the global market and these industrial processes and products were patented.[80]

The utilization of Vit C in cosmetics or nanoformulations for topical purposes is growing and it demonstrates huge potential.[81] Several patents have been filed by cosmetic industries in the last years. In 2004, a patent was published claiming to increase the solubility, stability and photoprotection of a single phase AA solution. The invention comprised Vit C, a cinnamic derivative (such as p-coumaric acid, ferulic acid, caffeic acid, sinapinic acid) and a combination of solvents (glycol ether, alkanediol and water). The application is active until 2025.[82] Another patent, also filed for a cosmetic industry, claimed a formulation composed of AA or a derivative, a silicone compound and an essential oil to increase the stability of Vit C at room temperature.[83]

In 2008, a liposome preparation method for the encapsulation of Vit C was patented. The liposomes are composed by at least one phospholipid, a sphingolipid, Vit C or derivatives and permeations enhancers (such as: butylene glycol, propylene glycol, hexylene glycol, and polyethylene glycol, and mixtures thereof). The unilamellar liposomal suspension obtained has a mean size between 50 nm to 290 nm. The method includes combining the liposomal suspension with a cosmetically suitable matrix to form a cosmetic formulation. The patent is active until 2031.[84]

Clinical trials are experiments or observations in clinical research, which human participants are designed to new treatments generating data on safety and efficacy.[85] Clinical trials with ascorbic acid have been performed; a total of 1127 studies recorded on Clinical Trials Database (https://clinicaltrials.gov) applied to several illness; 626 are completed, 207 are in recruiting or not recruiting status, 34 studies have been suspended or withdrawn, and 73 studies are awaiting enrollment. All these studies were applied to 848 conditions, among cancers, inflammatory and infectious skin diseases and other conditions. A summary was described in Table 3.

One study using nanotechnological approaches was found. This study aims the bioavailability of AA loaded into liposomal formulation as diet supplement (NCT02606773). Studies with biomedical bias have shown a great potential of encapsulation of this vitamin in nanocarriers systems and the results are reinforced by *in vitro* data and animal models. However, regulatory issues still limit their clinical use.

Conclusion

Vit C has been widely studied and applied in the recent decades as an antioxidant and as a cofactor in the synthesis of collagen. However, studies show that Vit C is a versatile molecule that can be utilized in many fields of science. Topically, Vit C is effective in the treatment of hyperpigmentation, differentiation of keratinocytes, prevention of skin photodamage and can also improve cohesion in the dermal-epidermal junction. Systemically, Vit C acts in epigenetics such as in the synthesis of immunoglobulins, in the production of interferon and in tumor regulatory factors. Studies also discuss its efficacy as a pro-oxidant in cancer cells, its role in neurodegenerative diseases and in treatments for male infertility. In addition, Vit C has been successfully used to promote the delivery of therapeutic agents to the body.

However, Vit C's instability and its hydrophilic character have always limited its utilization. In the recent years, some drug delivery systems were developed and they seem to overcome these limitations through better encapsulation and targeting delivery. Besides, the studies carried out have allowed a better understanding of this molecule, which resulted in the development of more stable derivatives with different chemical properties. The problem with using the latter is that the molecules become entrapped within the lipophilic stratum corneum and do not penetrate the rest of the skin layers well. Many of these issues can be solved with using carriers particularly in the nano-sized range to penetrate into the skin.

Another point to highlight is that although nanoformulations are promising, there are regulatory and industrial issues that still limit their clinical use. But these problems are being circumvented by regulatory agencies, so that these products will become more available in the next years. Therefore, it is possible to conclude that although the utilization of Vit C is still a challenge for scientists, several strategies are available to improve its stability, systemic delivery and skin permeation.

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