

# Advances in nanocarrier-mediated delivery of chrysin: Enhancing solubility, bioavailability, and anticancer efficacy

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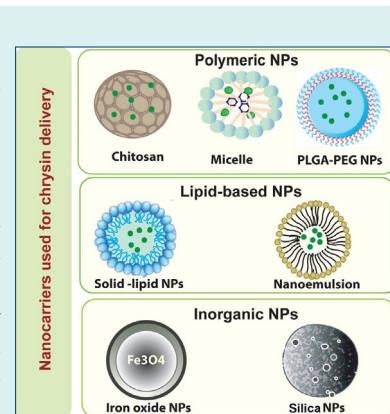
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## Abstract

Chrysin, a natural phytochemical compound found in various plant sources, possesses diverse pharmacological benefits, including anticancer, antioxidant, antidiabetic, neuroprotective, cardioprotective, hepatoprotective, immunoregulatory, and anti-inflammatory properties. Despite its well-documented biological activities, chrysin's low water solubility and bioavailability hinder its clinical development. This review explores the application of nanocarriers as a strategic approach to overcome these challenges and enhance the delivery of chrysin. Nanocarriers, including polymer-based nanoparticles (NPs), lipid-based NPs, and inorganic nanocarriers, have shown promise in improving the solubility, bioavailability, and tumor-targeted delivery of chrysin. The paper discusses chrysin's anticancer effects on different types of human cancers, elucidating its impact on crucial signaling pathways involved in tumorigenesis. The review categorizes and analyzes various nanocarriers, providing insights into their structural properties and drug release profiles. Among the nanocarriers, polymer-based NPs, especially those utilizing PLGA, emerge as promising strategies for chrysin encapsulation, demonstrating improvements in drug release, stability, and bioavailability. Lipid-based NPs and inorganic nanocarriers also exhibit potential in enhancing chrysin delivery. The comprehensive insights provided contribute to a deeper understanding of chrysin's pharmacological properties and its potential clinical applications, offering valuable perspectives for future research and translation into clinical settings. The review underscores the importance of selecting suitable structures for chrysin encapsulation to enhance its physicochemical properties and anticancer effects, paving the way for innovative nanomedicine approaches in cancer therapy.



## Introduction

Chrysin, a natural phytochemical compound, is widely distributed through the higher plants in the form of aglycone or glycoside.<sup>1</sup> Chrysin is widely present in propolis, honey, passion fruit, some mushrooms and other plant sources with the IUPAC name of 5,7-dihydroxy-2-phenyl-4H-chromen-4-one and 5,7-dihydroxyflavone. Several lines of research have reported different pharmacological benefits for chrysin such as anticancer, antioxidant,<sup>2</sup> antidiabetic,<sup>3</sup> neuroprotective,<sup>4,5</sup> cardioprotective, hepatoprotective,<sup>6</sup> immunoregulatory and anti-inflammatory properties.<sup>7</sup> Chrysin is one of the most extensively studied natural compounds due to its remarkable anticancer effects on

different phases of tumorigenesis. While chrysin's known biological activities are promising, this compound has not been moved to clinical use because of low aqueous solubility and poor bioavailability.<sup>8</sup>

Application of nanocarriers is one of the successful strategies for providing higher bioavailability, controlled release profile and tumor-targeted delivery of poorly water soluble anticancer agents.<sup>9-12</sup> Current advancement in the science and technology has led to the use of nanotechnology for amending bioavailability and aqueous solubility of drugs.<sup>13,14</sup> Nanomedicine is the medical application of nanotechnology which includes the development of nanocrystals, nanosuspensions, nanoemulsions,

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microemulsions, liposomes and nanoparticles (NPs) to enhance the solubility of newly discovered drugs.<sup>14</sup> NPs are colloidal particle with size range from 10 nm to 1000 nm which can increase solubility and efficiency of drugs due to their small size and large surface area.<sup>15</sup> Surface modified NPs protect drugs from degradation and reduce their bio distribution which leads to increased biological half-life and reduced side effect.<sup>16</sup> Thus, choosing a suitable structure for chrysin encapsulation can help to enhance its physicochemical properties and anticancer effects. This review elucidates different delivery approaches used for improving the bioavailability of chrysin and tumor-targeted delivery of this natural compound

### Structure and metabolism of chrysin

Fig. 1 demonstrates chemical structure of chrysin. Structurally, chrysin holds 2 benzene rings (A & B) and a heterocyclic ring containing one oxygen. Unlike various flavonoids, chrysin does not share any oxygenation in the B and C ring. Interestingly, the biological function of chrysin has been shown to be associated with its specific structure.<sup>9</sup> A-ring contains 2 hydroxyl groups at 5th and 7th carbon atoms which may be linked with the ability of free oxygen radical scavenging. Moreover, previous studies claimed that the existence of a carbonyl group on C4 and a double bond among C2–C3 atoms may be responsible for antioxidant role of chrysin.<sup>17</sup>

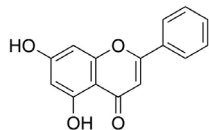


Fig. 1. Chemical structure of chrysin.

Accumulating data have revealed that chrysin metabolism does not depend on intestinal oxidation process, which causes poor absorption of this compound. In contrast, sulfation and glucuronidation conjugation pathways catalyze chrysin and lead to a rapid metabolism of this compound. On the other hand, the high affinity of enzymes like UGT1A6, M-PST and P-PST, which contribute to the chrysin metabolism, results in systemic elimination and low plasma concentration of free drug.<sup>18,19</sup> These phenomena are responsible for limited bioavailability, therapeutic efficacy and clinical usage of chrysin.<sup>10</sup> Additionally, poor water solubility of newly discovered compounds such as chrysin is the most common challenge in the drug development process.

### Anticancer mechanism of chrysin

Chrysin has been widely studied for its anticancer effects. Several studies have shown that chrysin has anticancer properties against various types of human cancers such as breast, gastrointestinal, liver, bladder, ovarian, prostate, leukemia respiratory, eye, brain, and skin.<sup>17</sup> The anticancer effects of chrysin have been attributed to the important role of chrysin in modulating cancer related-signaling pathways such as oxidative stress, apoptosis, autophagy, and inflammatory responses. Fig. 2 indicates the important mechanisms for anticancer effects of chrysin on tumor cells. Assessment of chrysin antiproliferative effects on cancer cells has revealed that chrysin inhibits cell growth through different mechanisms. This includes cell cycle arrest, downregulation of cyclin D1 and Telomerase Reverse Transcriptase (TERT), inhibition of DNA methyltransferases and signal transducer and activator of transcription 3 (STAT3) activation, suppression of DNA topoisomerases and histone deacetylase, and expression

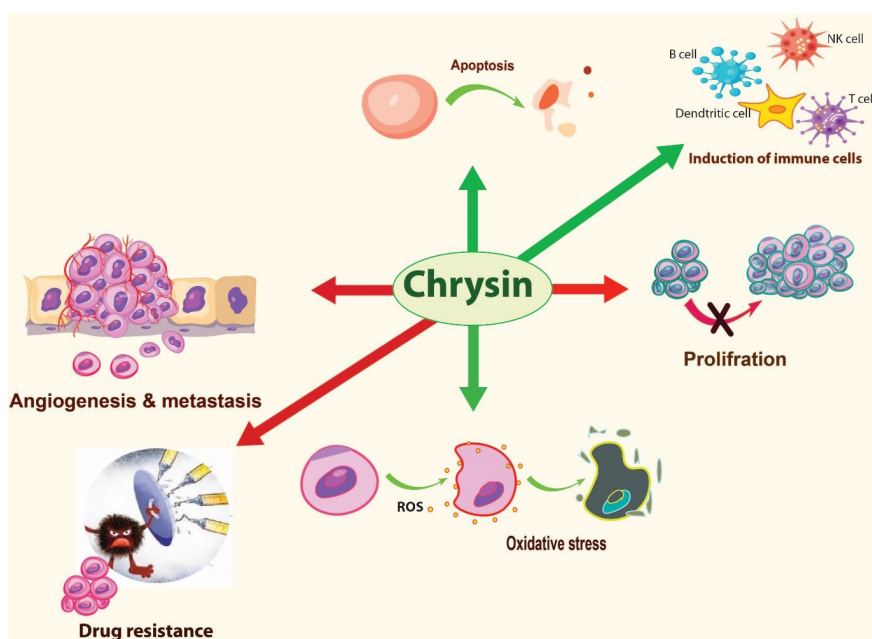


Fig. 2. Important anticancer mechanisms of chrysin.

of peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) mRNA.<sup>20-23</sup>

Several lines of research have confirmed that chrysin can induce apoptosis.<sup>12,24</sup> Apoptosis is considered as a desirable form of death in cancer cells.<sup>25</sup> Chrysin induces apoptosis by different mechanisms including upregulation of apoptosis-associated proteins (P53 protein, caspases-3, caspases-9, Bcl2, Bax),<sup>26,27</sup> activation of Notch1 signaling by cleavage of PARP expression,<sup>28</sup> TNF-related apoptosis-inducing ligand (TRAIL) -mediated apoptosis<sup>29,30</sup> and expression of Skp2 and LRP6.<sup>29</sup> Moreover, chrysin can significantly amplify the autophagy-associated markers such as LC3-II in colorectal cancer cell lines which makes it a potential candidate to induce autophagy in cancer cells.<sup>17</sup>

Although significant antioxidant activities have been recognized for chrysin, it has been revealed that most of polyphenolic compounds including chrysin can induce the production of reactive oxygen species (ROS) and act such a pro-oxidant under certain conditions.<sup>31,32</sup> The capability of chrysin to induce ROS production through oxidative stress changes this compound into a promising candidate to induce immunogenic cell death (ICD) in cancerous cells.<sup>8,33</sup> Induction of ICD is an important strategy for activating anticancer immune responses and immunotherapy of cancer. Furthermore, it has been reported that chrysin can enhance population of immune cells (T- and B cells), promote cytotoxicity of NK cells and macrophage phagocytosis ability in leukemic mice.<sup>34</sup> Other studies report suppressive effects of chrysin on production of inflammatory factors associated with Th2 type inflammatory response which are known to be an undesirable or bad inflammation in the context of cancer immunotherapy. One previous study reported that, treatment of A549 lung cancer cells with chrysin results in inhibitory effects against the release of pro-inflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, IL-10, and tumor necrosis factor alpha (TNF- $\alpha$ ).<sup>35</sup> Another study has shown that chrysin suppresses the expression of inflammatory proteins and genes by affecting the key inflammatory pathways such as JAK-STAT, MAPK, COX-2 and NF- $\kappa$ B.<sup>10</sup>

The inhibitory effects of chrysin on cancer angiogenesis is another important property of chrysin, which makes this compound an effective anticancer agent. The suppressive effects of chrysin on angiogenesis has been shown to be through the inhibition of inflammatory cytokines (e.g. IL-6), growth factors (e.g. VEGF) and modulation of Jak/STAT3 signaling factor, which plays an important role in induction of angiogenesis and metastasis.<sup>24</sup> Additionally, chrysin can suppress epithelial-to-mesenchymal transition (EMT) and metastasis in cancer cells via down-regulation of Snail and Matrix metalloproteinases (MMPs) (MMP-2 and MMP-9), and inhibition of STAT3 activation, which are involved in invasion of cancer cells.<sup>36,37</sup> Chrysin has been also shown to reverse

multiple drug resistance in cancer cells. Former studies have demonstrated that this compound can enhance the efficiency of chemotherapeutic agents such as cisplatin,<sup>38</sup> 5-FU,<sup>39</sup> doxorubicin,<sup>40</sup> and docetaxel<sup>41</sup> in combination therapies. Down regulation of Nuclear factor erythroid 2-related factor 2 (Nrf2), increasing oxidative stress and suppressing STAT3 activation are some of the important mechanisms by which chrysin reverses multiple drug resistance in cancer cells.<sup>24,38,42</sup>

### Enhancement of chrysin bioavailability

Chrysin is a lipophilic agent with low molecular weight. Its water solubility at pH 7.4 and pH 6.5 is  $0.058 \pm 0.04$  mg/mL and  $0.06 \pm 0.1$  mg/mL, respectively.<sup>43</sup> Studies suggest that for a medium-permeable drug like chrysin at a 1 mg/kg dose, the minimum aqueous solubility (MAS) should be higher than 0.05  $\mu$ g/mL for good absorption. While chrysin's solubility approaches 0.05  $\mu$ g/mL and its permeability is considered medium at a 1 mg/kg dose, animal and human studies using higher doses show poor absorption of the majority of the administered chrysin.<sup>44</sup> This limited absorption at higher doses may lead to a longer T<sub>max</sub> (time to reach peak plasma concentration) for chrysin.<sup>45</sup>

The extremely low oral bioavailability of chrysin has been demonstrated in several studies.<sup>46,47</sup> Evidence reveals that while chrysin acts as a potent agent in *in vitro* studies, its efficacy in *in vivo* studies is not promising. In a study done on human volunteers, it was estimated that oral bioavailability of this compound is less than 1%. In this study, 400 mg of chrysin was used orally.<sup>47</sup> Various factors restricted chrysin's oral bioavailability such as rapid metabolism, low aqueous solubility, moderate permeability.<sup>48</sup> As reported by Walle et al, the major part of chrysin (>90%) is eliminated through the feces and urine as aglycone and chrysin-glucuronide, respectively.<sup>47</sup> Low water solubility or high enterohepatic recycling could be involved in fecal elimination of this compound. However, due to the enterohepatic recycling, chrysin can be recycled and reused in the intestine and this phenomenon results in improved local bioavailability. But the systematic bioavailability of chrysin is still poor.<sup>49</sup> Understanding the factors affecting drugs oral bioavailability is essential for the development and implementation of effective strategies to improve chrysin's therapeutic potential. Enterohepatic recycling allows for some reabsorption of chrysin in the intestine. However, further research is needed to investigate the efficacy and safety of developing metabolic enzyme inhibitors (such as UGT1A1 inhibitors) to potentially boost chrysin's oral bioavailability. Chrysin's low water solubility contributes to poor absorption. Nanocarriers offer a promising approach to increase chrysin's water solubility and absorption. Studies with formulations like chrysin-folate conjugated micelles and lipid-core nanocapsules have shown positive results in

enhancing chrysin absorption and bioavailability.<sup>11,50</sup>

### Nano-based delivery systems for chrysin

Accumulating data underline the importance of encapsulating chrysin in NPs as a promising strategy to overcome its limited bioavailability. Various types of organic and inorganic nanocarriers, have been explored for efficient chrysin delivery. Organic carriers encompass polymeric NPs, polymeric micelles, polymeric nanofibers, and lipid-based NPs. Inorganic options include mesoporous silica NPs, polymer-coated CaCO<sub>3</sub> NPs, and iron oxide magnetic NPs. Notably, co-polymeric NPs emerge as the extensively studied carriers. Polymeric NPs, categorized into nanocapsules and nanospheres, offer versatile options for physically encapsulating active compounds through either entrapment or surface adsorption onto the polymeric shell. Among all applied carriers, copolymeric NPs are the most applied ones. Polymeric NPs are the nanoscale particles subdivided into two main groups including nanocapsules and nanospheres. Physical encapsulation of active compounds in the polymeric NPs can be done through two main routes including entrapping or surface-adsorption on the polymeric core.<sup>51,52</sup>

In terms of nanocarrier evaluation, drug loaded NPs are usually characterized for their structural properties, which include morphology, size distribution, Zeta potential (ZP), polydispersity index (PDI), stability, encapsulation efficiency (EE), loading capacity and drug release status. Concerning the size of NPs, it can be affected by various parameters such as drug/carrier ratio and the synthesis method. It is typically assessed by SEM or DLS technology. Furthermore, PDI, an important property of nanocarriers, indicates their dispersion homogeneity. While PDI usually ranges from 0 to 1, nanoparticles with a PDI greater than 0.7 are considered polydisperse, and those with a PDI less than 0.2 (close to zero) are considered monodisperse.<sup>53</sup> ZP is another important structural property and stability indicator of NPs. A higher ZP, regardless of its charge, prevents the aggregation of NPs, while a lower value causes agglomeration and particle growth.<sup>54</sup> Drug release status is considered as another key parameter to evaluate the safety, efficacy, and behavior of a nanoformulation in the body.<sup>55</sup> In the following sections, chrysin-loaded nanocarriers are classified and their characteristics are discussed in details.

### Polymer-based NPs

Polymeric NPs are colloidal particles capable of encapsulating therapeutic compounds. Polymers used in NPs can be obtained from natural sources (e.g., gelatin, albumin, dextrin, and chitosan), pseudo synthetic (e.g., polyamino acids) or synthetic (e.g., Polycaprolactone, polylactic acid, polylactic-co-glycolic acid) origin.<sup>56</sup> Additionally, applied synthetic polymers may have linear,

di-block, tri-block and cross-linked structure.

Polymeric NPs have demonstrated promising results in chrysin delivery due to their several advantages such as simple encapsulation, lower toxicity, high loading efficiency and controlled release.<sup>51</sup> Polymers and copolymers applied for chrysin encapsulation include PCL-PEG-PCL,<sup>57</sup> PLGA-PEG,<sup>58-63</sup> PLGA-PEG-PLGA,<sup>39</sup> methoxy polyethylene glycol (mPEG)- $\beta$ -polycaprolactone,<sup>64</sup> chitosan<sup>65</sup> and PLGA.<sup>66-68</sup>

PLGA-based polymers are the most widely used for chrysin delivery, particularly PLGA polymer and its di-block (PLGA-PEG) and tri-block (PLGA-PEG-PLGA) copolymers. PLGA-based polymers loaded with anticancer agents, are reported to have enhanced anticancer activity on diverse cancer cell lines. Moreover, this type of nanocarriers have been found to cause macrophage repolarization into anti-inflammatory type and exhibit a neuroprotective role against kindling-induced epilepsy.<sup>60,68</sup> In addition, di and triblock amphoteric PLGA copolymers were applied for co-delivery of chrysin with other anticancer agents. Previous studies reported that co-encapsulation of chrysin with curcumin in PLGA-PEG NPs or 5-fluorouracil in PLGA-PEG-PLGA NPs, led to synergistic *in vitro* anticancer effects on SW480 and HT29 colon cancer and MDA-MB-231 breast cancer cell lines.<sup>39,59,61</sup>

All studied chrysin-loaded PLGA-based polymers, exhibited a relatively high optimized EE ranging from  $85.52 \pm 0.10\%$ <sup>54</sup> to  $99.89\%$ .<sup>39</sup> Thus, it can be concluded that, PLGA-based polymers provide effective encapsulation for drugs with low aqueous solubility such as chrysin.

Although the smallest reported size range for chrysin-loaded PLGA-PEG and PLGA-PEG-PLGA NPs is approximately 40-75 nm.<sup>63</sup> Sulaiman et al reported a minimum size range of 30-70 nm for chrysin-loaded PLGA NPs.<sup>67</sup> Based on previous reports, the use of di-block and tri-block copolymers of PLGA resulted in NPs with larger size range when compared to PLGA alone. Furthermore, all the optimized PDI values for PLGA and PLGA-PEG NPs were near zero and ranged 0.132<sup>60</sup> to 0.22.<sup>54</sup> This contrasts with the large PDI value of 0.62 reported for triblock PLGA-PEG-PLGA NPs reported by Khaledi et al.<sup>39</sup> However, all of these values indicate that PLGA-based nanocarriers were monodisperse and had an acceptable size distribution.

Release profile of chrysin from all PLGA-based NPs depicted a biphasic pattern with a burst release of surface bounded drug in the first step followed by a slower release as the second phase. Various mechanisms can contribute to the sustained release of chrysin, including the hydrolysis of polymeric matrix, pore diffusion, and swelling of the nanocarriers.<sup>61</sup> Comparing the release profile of two different studies revealed that chrysin shows higher release amounts from lipophilic PLGA polymer alone ( $72.05 \pm 3.71\%$  at 22 hours) than from hydrophilic

PEG containing PLGA copolymer (65% during 120 h).<sup>60,68</sup> Greater lipophilicity of the copolymer may increase the rate and amount of release due to better diffusion of lipophilic chrysin from polymeric matrix. Supporting this claim, Siddhardha et al reported a total release of  $90.5 \pm 0.50\%$  for chrysin from chitosan lipophilic core in 10 h, while the most reported release amount from PEG containing copolymers was 65% during 120 h.<sup>60</sup> However, this result may also relate to the physicochemical properties and ratio of applied copolymers.

Previous studies showed that pH can affect the drug release in addition to other parameters. For instance, Firouzi-Amandi et al reported a faster and higher drug release from PEG-PLGA nanocarriers in lower pH conditions.<sup>60</sup> In contrast, Khaledi et al reported a pH-independent release for chrysin from PLGA-PEG-PLGA triblock copolymers.<sup>39</sup> This difference may be attributed to different materials and methods applied in each research.

Temperature is another factor affecting release behavior. A previous study reported an increase of chrysin release in higher temperatures.<sup>39</sup> This can be a useful element for drug delivery systems in thermotherapy of cancer.

Almost all chrysin-loaded PLGA-based nanocarriers exhibit a slightly negative to neutral surface with  $-6.3 \pm -3.5$  mV as the highest reported value for ZP.<sup>60,61</sup> However, Zhang et al, reported  $-29.20 \pm 0.24$  mV for chrysin-loaded PLGA NPs, which highly differs from others. This may be seen due to the different method of preparation, drug-polymer ratio, pH of solution and type and concentration of stabilizer.<sup>69</sup> Moreover, optimized ZP for MPEG-PCL reported by Kim et al was lower than most of the PLGA-based nanocarriers, which may return to the carboxylic groups of PLGA causing more negative surface charge.<sup>64</sup>

Polymeric micelles applied for chrysin include chrysin-loaded folate conjugated PF127-F68 mixed micelles (CH-MM)<sup>11</sup> and P(HEMA-LA-MADQUAT) micelles.<sup>70</sup> All applied micelles have been assessed for enhanced anticancer effects. Besides, *in vivo* pharmacokinetic studies for CH-MM were carried on female Wister rats by Baidya et al<sup>11</sup> Additionally, Davaran et al evaluated P(HEMA-LA-MADQUAT) micelles for co-delivery of MTX and chrysin<sup>70</sup>

Pharmacokinetic observations showed an increase in  $C_{max}$ ,  $AUC_{0-\infty}$  and half-life of chrysin encapsulated in CH-MM formulations in comparison with free drug. Furthermore, significant oral bioavailability improvement for CH-MM formulation may be attributed to its higher water solubility and controlled release from mixed micelles.<sup>11</sup>

Compared to CH-MM, P(HEMA-LA-MADQUAT) micelles exhibited lower critical micelle concentration (CMC) and ZP values. While lower CMC values may increase the stability of micelles even after dilution, a lower ZP (negative or positive) can decrease the stability. Moreover, P(HEMA-LA-MADQUAT) micelles indicated

a positive ZP because of the cationic MADQUAT moiety of copolymer. However, CH-MM had a negative ZP, which may be attributed to the carboxylic acid groups of folic acid.

While EE values of CH-MM ranged from 52 to 89% and showed strong dependence to polymer amounts used, no significant difference was observed in EE values of optimized formulations of CH-MM and P(HEMA-LA-MADQUAT) micelles. Furthermore, optimized formulations of both micelles have also resulted in almost the same size. However, Baidya et al reported a connection between micelle size and amounts of applied polymers as increasing the amount of polymers can increase the micelle size.<sup>11,70</sup>

Chrysin-loaded P(HEMA-LA-MADQUAT) micelles showed a pH-independent and temperature-dependent release profile, similar to what reported for PLGA-PEG-PLGA triblock copolymers by Khaledi et al.<sup>39</sup> According to the release profile of drug, chrysin exhibited a higher release rate and amount from CH-MM (about 75.4% during 24 h) when compared to P(HEMA-LA-MADQUAT) micelles (59.9% release over 6 days). The observed difference in drug release from two micellar formulations might be related to the differences in the lipophilicity of applied polymers.<sup>39,70</sup> However, both micellar formulations of chrysin demonstrated a controlled release with a biphasic pattern. Compared to free chrysin, CH-MM released a greater amount of chrysin in a controlled manner. This is because the drug is encapsulated within the hydrophobic micellar core with a large surface area.<sup>11</sup>

### **Lipid-based NPs**

Lipid-based NPs have been applied widely for drug encapsulation due to their advantages compared to polymeric NPs. These structures have demonstrated improvements in the problems related to large-scale production and cytotoxicity. Lipid-based NPs can be further categorized into 5 groups including liposomes, lipid nano emulsions (LNEs), solid lipid NPs (SLNs), lipid NPs (LNPs) and nanostructured lipid carriers (NLCs). Advancements in the formulation of SLNs have yielded several advantages over traditional lipid-based formulations like liposomes and nanoemulsions. These advantages include enhanced stability, improved drug protection, and the feasibility of large-scale production. This progress can be attributed to the utilization of solid lipids (SL) instead of oily phases in emulsions. Moreover, SLNs offer the potential for a controlled release pattern for both hydrophilic and hydrophobic drugs, further enhancing their versatility.<sup>71</sup> In contrast to SLNs, NLCs are characterized by the development of an amorphous solid matrix formed through a blend of solid and liquid lipids. The inclusion of liquid lipids and the amorphous lattice in NLCs facilitates efficient drug loading, resulting in a higher drug payload compared to the crystalline solid

matrix of SLNs.<sup>71</sup>

Lipid-based NPs applied for chrysin include sodium oleate-based nanoemulsions,<sup>46</sup> chrysin phospholipid complex loaded SLNs (Ch-PC-SLNs) and chrysin-loaded SLNs (Ch-SLNs).<sup>72</sup> Komath et al<sup>72</sup> employed ten different types of lipid NPs for chrysin featuring a size range of 309-944 nm and Zeta potential values ranging from -20 to -47. Among the various formulations, the soya lecithin formulation (ch-sln10) exhibited the most negative Zeta potential and the smallest size. This formulation demonstrated the ability to stabilize chrysin, resulting in improved dispersibility, oral absorbance, and cellular uptake.<sup>46</sup> Moreover, Dong et al. applied 21 different pharmaceutical excipients (PEs) to create chrysin-loaded nanoemulsions, assessing the effects of PEs on chrysin glucuronidation. Sodium oleate-based nanoemulsion was found to be the most potent inhibitor with a mean particle size of 83.2 nm and the ZP value of -43.7.<sup>46</sup>

According to Komath et al loading chrysin phospholipid complex in SLNs caused significantly improved EE compared to Ch-SLN1 which didn't contain any phospholipid complex.<sup>72</sup> The presence of additional phospholipids, serving as surfactants, is likely the primary factor in stabilizing the formulation. This contributes to achieving an encapsulation efficiency exceeding 90%, reducing the particle size to  $309 \pm 2$  nm, and enhancing the overall particle size distribution. Sodium oleate-based nanoemulsions showed an EE of 89.5 %, which was also higher than CH-SLN10 with 80.2% EE value.<sup>46</sup>

While the release kinetics of chrysin from Ch-PC-SLNs followed a zero-order model ( $R^2=0.99$ ), indicating time-dependent release, Ch-SLN10 displayed Higuchi kinetics ( $R^2=0.97$ ) with a biphasic release pattern. However, both Ch-SLN10 and Ch-PC-SLNs could provide the sustained release of chrysin. Furthermore, due to the phospholipid complex of chrysin, Ch-PC-SLN depicted a slower release as compared to Ch-SLN10 (17 h to release 50% of chrysin for Ch-SLN10 and 28 h for Ch-PC-SLN).<sup>72</sup> A sustained release was also reported for chrysin from SO-NE formulation; however, the Ch-PC-SLN exhibited the slowest release among all Lipid-based NPs applied for chrysin.<sup>46</sup>

### Inorganic nanocarriers

Inorganic drug nanocarriers are widely used in drug delivery studies to improve bioavailability. Biocompatibility and biodegradability are important elements to be sure about biosafety and *in vivo* use of these carriers. Inorganic carriers such as metals, metal oxides, silica, carbon-based materials may cause toxicity and their use in clinical application is limited due to their difficult degradation process. On the other hand, biomineral-based carriers containing calcium carbonate, calcium phosphate and calcium silicate demonstrate high biocompatibility and biodegradability.<sup>73</sup> Several inorganic nanocarriers have been developed for chrysin delivery,

including mesoporous silica NPs (MSNP),<sup>74</sup> amino acid modified iron oxide magnetic NPs (IONPs)<sup>75</sup> and CaCO<sub>3</sub> NPs (CCNPs) coated by multilayer polymer (MLNP).<sup>76</sup>

While, MSNP formulations have primarily been studied to evaluate nose-to-brain delivery of chrysin, MLNP structures have been evaluated for their anticancer effects. Co-drug delivery studies have been conducted for co-encapsulation of chrysin with cisplatin and curcumin in MLNP and MSNP, respectively. In the study done by Menhath et al loading chrysin into cisplatin-loaded MLNP caused higher tumor regression in tumor-induced hamsters. Besides, the dual drug-loaded MLNP also showed improvements in terms of cell apoptosis, ROS levels and mitochondrial membrane damage. Furthermore, amino acid modified IONPs have been investigated to assess whether they are appropriate delivery carriers for drugs like chrysin. To this purpose, hemolysis assay and cytotoxicity studies indicated that IONPs are biocompatible formulations, consequently.<sup>74-76</sup>

Among all tested inorganic nanocarriers, chrysin-loaded MSNPs showed the lowest release percentage during 24 h ( $9.4 \pm 0.6\%$  and  $16.8 \pm 0.8\%$  at pH=5.5 and pH=7.4, respectively). Both chrysin-loaded MSNP<sup>74</sup> and MLNP<sup>76</sup> formulations demonstrated a pH-sensitive biphasic release pattern. While MSNP formulations indicated a lower release rate and amount in acidic pH, MLNP formulations showed higher release results in acidic pH. This may be attributed to protonation of the nanomaterial in MLNPs, which leads to de-stabilization and higher erosion of NPs and results in higher amounts of drug release at tumor site. However, the hydrophilic character of polymer layers around CCNPs enhanced water permeation, layer erosion, and the diffusion of chrysin through the swollen matrix.

A pH-sensitive release pattern has also been observed for amino acid modified IONPs with higher release results under acidic conditions.<sup>75</sup> Comparing to L-aspartic acid coated IONPs, L-arginine coated ones indicated higher release amount in acidic and physiological conditions. This general behavior may be related to acidic nature of L-aspartic acid and basic structure of L-arginine, which can affect the interactions with hydrophobic molecule of chrysin. Moreover, the amount of released drug in 24 h was above 30% in both amino acids modified formulations, which was higher compared to the MSNP formulation.<sup>74</sup>

Considering the potential for nonspecific cellular uptake and cytotoxicity in NPs smaller than 100 nm, silica NPs with a size of  $283.5 \pm 8.3$  nm can induce endocytosis without causing additional cytotoxic effects. Furthermore, negative ZP of silica NPs ( $-30.8 \pm 0.3$  mV) is not considered an issue for cellular interaction owing to their mesoporous structure.<sup>74</sup> In multilayer polymer-coated CCNP formulation, the ZP value for chrysin-loaded CCNPs increased from -3.6 mV to almost +15 mV after deposition of layer-by-layer polymers on CCNPs.

Due to the positive charge induced by multilayers of polymer (especially poly diallyldimethyl ammonium chloride polymer), endocytosis was reported as the main route of internalization of chrysin-loaded MLNPs without accumulating on cell membrane. The size of MLNPs, with a moderate increase, was evaluated 250-300 ± 20 nm due to assembled layers.<sup>76</sup> The reported ZP for chrysin-loaded IONPS increased to -3.87 mV and -2.12 mV for L-arginine coated NPs and L-aspartic acid ones, respectively. This slightly negative charge can decrease nonspecific contact with plasma and blood cells with electrostatic interactions for IONPS.<sup>75</sup>

Table 1 shows chrysin-loaded nanocarriers and their characteristics.

## Conclusion

Nowadays, identifying natural and safe compounds with low side effects is the goal of medical science for treatment of diseases. This paper provides a comprehensive review of the potential applications of chrysin, a natural phytochemical compound, with a focus on its remarkable anticancer effects. Despite the recognized pharmacological benefits of chrysin, its limited aqueous solubility and bioavailability have hindered its progress to clinical trials. The paper highlights the promising strategy of utilizing nanocarriers, particularly nanomedicine, to overcome these challenges and enhance the delivery of chrysin. The structure and metabolism of chrysin are discussed, emphasizing the unique features that contribute to its biological activities. Chrysin's anticancer

effects have been extensively studied, demonstrating its impact on various signaling pathways associated with cancer development. From cell cycle regulation and apoptosis induction to immune response modulation and inhibition of angiogenesis, chrysin exhibits multifaceted actions against different types of cancers.

To address the limitations of chrysin's poor bioavailability, various nanocarriers, including polymer-based NPs, lipid-based NPs, and inorganic nanocarriers, have been explored. The review categorizes and discusses these nanocarriers, emphasizing their structural properties, drug release profiles, and potential advantages in enhancing the therapeutic efficacy of chrysin. The encapsulation of chrysin in nanocarriers, especially polymer-based NPs like PLGA, has shown promising results in terms of improved drug release, stability, and bioavailability. Lipid-based NPs and inorganic nanocarriers have also demonstrated potential in enhancing the delivery of chrysin, with considerations for biocompatibility and controlled release.

In conclusion, the utilization of nanocarriers presents a promising avenue to overcome the limitations of chrysin and unlock its full therapeutic potential. Further research and development in this field may pave the way for the clinical application of chrysin in the treatment of various cancers. The comprehensive insights provided in this review contribute to a deeper understanding of chrysin's pharmacological properties and its nanocarrier-based delivery approaches, offering valuable perspectives for future studies and clinical translation.

**Table 1.** Summary of drug delivery systems for chrysin

Applied polymer	Size (nm)	EE (%)	PDI	ZP (mV)	Effect	Release profile	Ref
<b>Polymeric NPs</b>							
PLGA-PEG	SEM: 30-60 DLS: 70-300	99.89	-	-	Anticancer activity against breast cancer cells	-	77
PLGA-PEG	30-130	-	-	-	Synergistic anticancer effects of co encapsulated Curcumin and Chrysin NPs on colorectal cancer cells	-	59
PLGA-PEG	233 ± 10.3	85.25	0.148	-6.3 ± 3.5	Anticancer activity of Curcumin and Chrysin co-delivery on breast cancer cells	~60% at ~100 h	61
PLGA-PEG	DLS: 235 SEM: 205	88	0.132	-6.3 ± 3.5	Peritoneal macrophage repolarization into anti-inflammatory phenotype	65% during 120 h	60
PLGA-PEG	-	-	-	-	Effect on metalloproteinase gene Expression in mouse 4T1 tumor model ( <i>in vivo</i> )	-	62
PLGA-PEG	40-75	98.6	-	-	Antigrowth effect on AGS human gastric cancer cells	-	63

Table 1. Continued.

Applied polymer	Size (nm)	EE (%)	PDI	ZP (mV)	Effect	Release profile	Ref
PLGA	DLS; 115.79 ± 2.84 TEM: 87	93.27 ± 1.2	0.152 ± 0.006	-29.20 ± 0.24	Neuroprotective role against kindling-induced epilepsy through Nrf2/ARE/HO-1 pathway	72.05% ± 3.71% at 22 h	68
PLGA	30-70	92	-	-	Anti-oxidant activity and DNA protective effects against H <sub>2</sub> O <sub>2</sub> , Anticancer activity against breast and ovary cancer cells	~90% at 50 h	67
PLGA	From 145.70 ± 2.44 to 284.00 ± 17.00	From 75.62 ± 7.13 to 89.67 ± 0.93	From 0.17 ± 0.02 to 0.33 ± 0.04	From -6.23 ± 0.18 to -0.04 ± 0.17	Anti-hyperglycemic activity and anti-hyperlipidemic activity	Maximal 52.84 ± 5.17% and minimal 17.19 ± 0.00% at 24 h	54
PLGA-PEG-PLGA	40	97.5	0.62	-12.8 for dual drugs	Synergistic anticancer activity of co encapsulated Chrysin and 5-FU NPs on HT29 cancer cells	12% in the first 5 h~62% over 96 h	39
PCL-PEG-PCL	SEM: 70-300	99.89	-	-	Anticancer activity against breast cancer cell line T47D	-	57
MPEG-PCL	DLS: 77	46.96 ± 1.12	0.148	-2.22	<i>In vivo</i> and <i>In vitro</i> anticancer activity against A549 cells	50% in 37°C and pH = 7.4 at 48 h	64
Chitosan	TEM: 130–341 DLS: 355	80.86 ± 0.30	0.487	-	Antibiofilm activity against <i>Staphylococcus aureus</i>	~90.5% ± 0.50% within 10 h	65
<b>Inorganic Nano Carriers</b>							
Mesoporous silica NPs	283.5 ± 8.3	-	0.31 ± 0.11	-30.8 ± 0.3	Nose-to-brain olfactory drug delivery	9.4 ± 0.6% at Ph = 5.5 16.8 ± 0.8% at pH = 7.4 at 24 h	74
CaCO <sub>3</sub> NPs coated by multilayer Polymer (MLNPs)	250-300 ± 20	-	-	-5.12 (after the absorption of layers)	Anticancer activity of Chrysin and Cisplatin co-delivery on oral carcinoma cells	84% in 80 h and acidic pH 62% in 80 h and neutral pH	76
Chrysin-loaded amino acid modified iron oxide magnetic NPs	F@Arg @Chrysin NPs: 18.75 ± 2.40 F@Asp @Chrysin NPs: 19.86 ± 2.22	-	-	F@Arg@ Chrysin NPs and F@Asp@ Chrysin NPs shifted to -3.87 and -2.12 mV, respectively	Drug delivery and MRI contrast agent applications	F@Asp@ Chrysin: ~40% in 48 h F@Arg@ Chrysin: ~ 50% in 48 h	75
<b>Lipid-based NPs</b>							
Sodium oleate-based Chrysin loaded nano-emulsion	83.2	89.5	-	-43.7	Enhancing oral absorption and systemic exposure of chrysin of	~ 80% in 50h	46
Chrysin-Phospholipid complex loaded SLNs(Ch-PC-SLNs)	309 ± 2	Ch-PC-SLNs: > 90	-	Ch-PC-SLN: -42 ± 0.76	Anticancer activity against MCF-7 cell line	50% at 28 h	72
<b>Micelles</b>							
Chrysin-Loaded Folate Conjugated PF127-F68 Mixed Micelle (CH-MM)	67.6	86.3	-	CH-MM: -21.6	Oral bioavailability and anticancer activity - Active targeting of micelles by attachment of folic acid	75.4% at 24 h	11
MTX@ Chrysin-loaded P(HEMA-LA-MADQUAT)	Dual drug loaded NPs:199	86.5 (for Chrysin)	-	Micelles: + 7.6 After dual drug loading: -2.7	Anticancer activity against MCF-7 cells	34.8% at pH = 5.4 at 41 °C 39.9% at pH = 7.4 at 37 °C, over 6 days	70



## Review Highlights

### What is the current knowledge?

- Chrysin is a natural phytochemical compound which has remarkable anticancer effects.
- Despite the potential pharmacological benefits, limited aqueous solubility and bioavailability of chrysin have hindered its progress to clinical trials.

### What is new here?

- Utilizing nanocarriers, particularly nanomedicine, could overcome limited aqueous solubility of chrysin and enhance its delivery.
- The encapsulation of chrysin in polymer-based NPs like PLGA, has shown promising results in terms of improved drug release, stability, and bioavailability.

### Authors' Contribution

**Conceptualization:** Ommoleila Molavi.

**Data curation:** Sevda Jafari, Ommoleila Molavi.

**Formal analysis:** Sevda Jafari, Ommoleila Molavi.

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**Methodology:** Sevda Jafari, Ommoleila Molavi.

**Project administration:** Ommoleila Molavi.

**Resources:** Sheida Dabiri, Sevda Jafari.

**Supervision:** Ommoleila Molavi.

**Validation:** Sevda Jafari, Ommoleila Molavi.

**Visualization:** Sevda Jafari, Ommoleila Molavi.

**Writing-original draft:** Sheida Dabiri.

**Writing-review & editing:** Sevda Jafari, Ommoleila Molavi.

### Competing Interests

Ommoleila Molavi acts as the Editor of the *BioImpacts* journal. It is hereby declared that Ommoleila Molavi's contribution to this study has not influenced the review process or affected its acceptance.

### Ethical Statement

The authors declare no ethical issues to be considered.

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