Phospholipid/zein hybrid nanoparticles as promising carriers for the protection and delivery of all-trans retinoic acid

Journal:	*ACS Macro Letters*
Manuscript ID	Draft
Manuscript Type:	Letter
Date Submitted by the Author:	n/a
Complete List of Authors:	Gagliardi, Agnese; Universita degli Studi Magna Graecia di Catanzaro, Department of Health Sciences, University of Catanzaro "Magna Graecia" Voci, Silvia; Universita degli Studi Magna Graecia di Catanzaro, Department of Health Sciences, University of Catanzaro "Magna Graecia" Giuliano, Elena; Universita degli Studi Magna Graecia di Catanzaro, Department of Health Sciences, University of Catanzaro "Magna Graecia" Salvatici, Maria; National Research Council, bInstitute of Chemistry of Organometallic Compounds (ICCOM)-Electron Microscopy Centre (Ce.M.E.) Fresta, Massimo; University of Catanzaro, Department of Health Sciences Cosco, Donato; Universita degli Studi Magna Graecia di Catanzaro, Department of Health Sciences, University of Catanzaro "Magna Graecia"

Phospholipid/zein hybrid nanoparticles as promising carriers for the protection and delivery of all-*trans* retinoic acid

Agnese Gagliardi[a], Silvia Voci[a], Elena Giuliano[a], Maria Cristina Salvatici[b], Massimo Fresta[a], Donato Cosco[a,*]

[a]*Department of Health Sciences, University "Magna Græcia" of Catanzaro, Campus Universitario "S Venuta", I-88100, Catanzaro, Italy*

[b]*Institute of Chemistry of Organometallic Compounds (ICCOM)-Electron Microscopy Centre (Ce.M.E.), National Reasearch Council (CNR), via Madonna del Piano n. 10, 50019 Sesto Fiorentino, Firenze, Italy*

KEYWORDS : All-trans retinoic acid, hybrid nanoparticles, phospholipon, zein.

ABSTRACT: A totally biodegradable mixed system made up of phospholipids and zein was developed in order to effectively improve the photostability of all-*trans* retinoic acid, preserving its pharmacological properties. Photon correlation spectroscopy showed that the formulation obtained using phospholipon 85G and zein at a ratio of 7:3 w/w was characterized by an average diameter of less than 200 nm, a narrow size distribution and a significant time- and temperature-dependent stability. The use of specific cryoprotectants such as mannose and glucose favoured the long-term storage of the nanocarriers after the freeze-drying procedure. The nanoparticles increased the stability of the ATRA against photochemical degradation with respect to the free drug and its antitumor effect was preserved as a consequence of the cell uptake of the colloidal systems. The results demonstrate the potential of the proposed hybrid nanosystems to provide a high level of stabilization for sensitive and labile antitumor compounds.

In recent years, the development of hybrid colloidal particles using natural biomaterials (i.e. proteins and lipids) has drawn great interest due to their excellent degree of safety, notable biocompatibility, easy preparation procedure and their versatility in applications as drug delivery systems.[1-4] Indeed, the nanoparticles made up of a single main component often have inherent drawbacks, such as their scarce time- or temperature-related stability, limited drug loading capacity and reduced range of functionality.[5] On the contrary, hybrid nanoparticles are promising systems able to combine the specific features of different materials.[6-10] Among these, proteins are being widely used for the preparation of colloidal particles because they can easily self-assemble into various structures due to the presence of numerous types of functional groups that allow them to interact with other components.[11-13] In particular, zein, a natural protein obtained from corn characterized by a substantial lipophilic nature, has been amply employed in the formation of nanoparticles, favouring the encapsulation of poorly-soluble compounds while avoiding the use of harmful chemical crosslinkers.[14,15] The US Food and Drug Administration (FDA) approved the protein as a GRAS (Generally Recognized as Safe) material for drug delivery applications due to these favourable characteristics.[16,17] Namely, a recent study showed that composite colloidal particles made up of lecithin and zein could significantly enhance the thermal and saline stability of protein-based nanosystems, demonstrating the feasibility of developing useful multicomponent nanoparticles.[18] Moreover, it has been shown that the nanoencapsulation of curcumin in zein-lecithin complexes enhances the stability as well as the functional properties of the hydrophobic drug.[19] In another experimental work, the nanoparticles made up of a blend of zein and rhamnolipid have been shown to optimize the encapsulation efficiency and the bioavailability of curcumin.[20,21] Based on these findings, the aim of this investigation was to exploit the biomimetic and stabilizing activity of several phospholipon® made up of different compositions of phospholipid mixtures and also the peculiar characteristics of raw zein for obtaining hybrid nanoparticles. The physico-chemical, thermal and morphological properties of the resulting systems were investigated and the best formulation was chosen for improving the photostability of all-*trans*-retinoic acid (ATRA), a metabolite of retinol (vitamin A), which generally shows significant degradation when exposed to physical stress. Moreover, the cytotoxicity of ATRA-loaded lipoprotein nanoparticles was investigated with respect to the free form of the drug in order to evaluate whether the colloidal formulation could preserve the pharmacological activity of the compound on different human cancer cell lines. Indeed, ATRA is an effective differentiating agent that exhibits anticancer properties associated with a strong antiproliferative effect that contributes to the noteworthy remission of different types of tumors.[22-24] This phenomenon is related to the activation of retinoic acid receptors (RAR) and the retinoid X receptor (RXR) on the nuclear membranes of cancer cells that are able to regulate the transcription of genes which cause growth inhibition, differentiation and apoptosis.[25-26]

In Table 1 it is possible to observe the composition of the hybrid nanoparticles. The first phase of the characterization was focused on the evaluation of the physico-chemical properties of the systems obtained through nanoprecipitation of the lipid/protein mixture in water. In particular, photon correlation spectroscopy showed a significant variability of the dimensional values of the systems as a function of the mixtures of components used. Specifically, the use of PL90H and PL80H induced the formation of macro-aggregates and large particles with heterogeneous dimensions, as evidenced by the polydispersity index. This phenomenon may be related to the low degree of unsaturation present in the phospholipid structure, a consequent lower structural rigidity and a different interaction with the protein. Inversely, the use of non-hydrogenated lipid mixtures (PL90G and PL85G) showed nanoparticles characterized by a smaller average diameter (Table 1).

Table 1. Physico-chemical characterization of zein PL-nanoparticles[a]

PL[b]	Composition of the phospholipid mixtures	Zein (%w/w)	Mean sizes (nm)	PDI[c]	Zeta Potential (mV)
80H	Hydrogenated Phosphatidylcholine min. 60.0 % Hydrogenated Lysophosphatidylcholine max. 10 %	10	439±19	0.618±0.083	-30±1
		20	>1000	0.647±0.039	-28±1
		30	536±15	0.408±0.099	-28±1
		40	>1000	0.910±0.155	-35±2
		50	>1000	0.297±0.132	-34±1
		60	>1000	0.609±0.376	-36±1
		70	>1000	0.255±0.284	-33±2
		80	>1000	0.998±0.003	-18±1
		90	>1000	0.650±0.367	-13±1
90H	Hydrogenated Phosphatidylcholine min. 90.0% Hydrogenated Lysophosphatidylcholine max. 4.0 % Oil/triglycerides max. 2.0%	10	>1000	0.890±0.191	-18±1
		20	>1000	0.949±0.089	-10±1
		30	>1000	0.768±0.401	-10±1
		40	>1000	0.563±0.134	-11±1
		50	>1000	0.337±0.193	-23±1
		60	>1000	0.996±0.058	-6±1
		70	>1000	0.943±0.098	-9±1
		80	>1000	0.533±0.095	-10±1
		90	>1000	0.678±0.237	-10 ± 1
85G	Phosphatidylcholine min. 60.0% Lysophosphatidylcholine 3-6%	10	70±1	0.266±0.07	-30±2
		20	74±4	0.288±0.05	-29±1
		30	89±5	0.317±0.023	-24±1
		40	123±2	0.227±0.026	-22±2
		50	812±90	0.626±0.328	-32±1
		60	552±24	0.431±0.136	-28±2
		70	>1000	0.763±0.149	-24±1
		80	>1000	0.656±0.131	-24±1
		90	>1000	0.861±0.241	-26±1
90G	Phosphatidylcholine 94-100% Lysophosphatidylcholine max. 4.0% Tocopherol max 0.3%	10	114±1	0.454±0.02	-31±1
		20	253±1	0.521±0.008	-21±1
		30	240±4	0.528±0.077	-17±1
		40	719±5	0.653±0.057	-24±1
		50	>1000	0.547±0.454	-20±2
		60	>1000	0.405±0.125	-23±1
		70	>1000	0.535±0.105	-9±1
		80	>1000	0.624±0.114	-20±1
		90	>1000	0.876±0.215	-10±1

[a]The concentration of the components of the various formulations are 0.2% w/v.
[b]PL:Phospholipon
[c]PDI: Polydispersity Index.

In particular, the systems prepared using PL90G were of mean sizes ranging between 110 and 250 nm (9:1, 8:2 and 7:3 % w/w), though a wide range of dimensional distribution was maintained. The destabilizing effect exerted by zein on the structure of the hybrid systems was evident because the increase of their average diameter and the polydispersity index was directly proportional to the amount of the protein used. It should be noted that macroaggregates and precipitates were observed when protein concentrations over 60% w/w were used (Table 1), data in agreement with Dai and co-workers.[18]

The use of PL85G promoted a decrease of the mean diameter and PdI of the nanosystems prepared using a protein concentration up to 40%, demonstrating that it is the best phospholipid-derivative to be used for the development of hybrid nanoparticles (Table 1). The evaluation of the surface charges showed a certain variation occurring among the various nanosystems. In particular, the systems prepared using PL85G and PL80H were characterized by a zeta potential of about -30 mV, ideal for their stability over time.[27] However, all the formulations showed negative values as compared to the zein nanoparticles[18,28] demonstrating the huge contribution the phospholipid mixture makes towards the formation of the hybrid nanoparticles. This is a result of the binding between the hydrophilic and lipophilic portions of zein and the polar heads and alkyl chains of the phospholipids, respectively, via hydrogen bonds, hydrophobic effects, and electrostatic interactions, as previously reported.[19]

The use of the sonication process made a significant improvement in the aforementioned parameters, demonstrating the usefulness of this approach to obtain a better homogeneous size distribution of nanosystems.[27,29]Again in this case, the best results were achieved with the formulations prepared using PL85G (Figure 1). The presence of a phospholipid-based coating on the zein nanoparticles was demonstrated by TEM (Figure 1), confirming the results of the surface charge previously discussed (Table 1). For this reason, the next steps of the experimental study were focused on the characterization of the hybrid nanosystems made up of PL85G and zein.

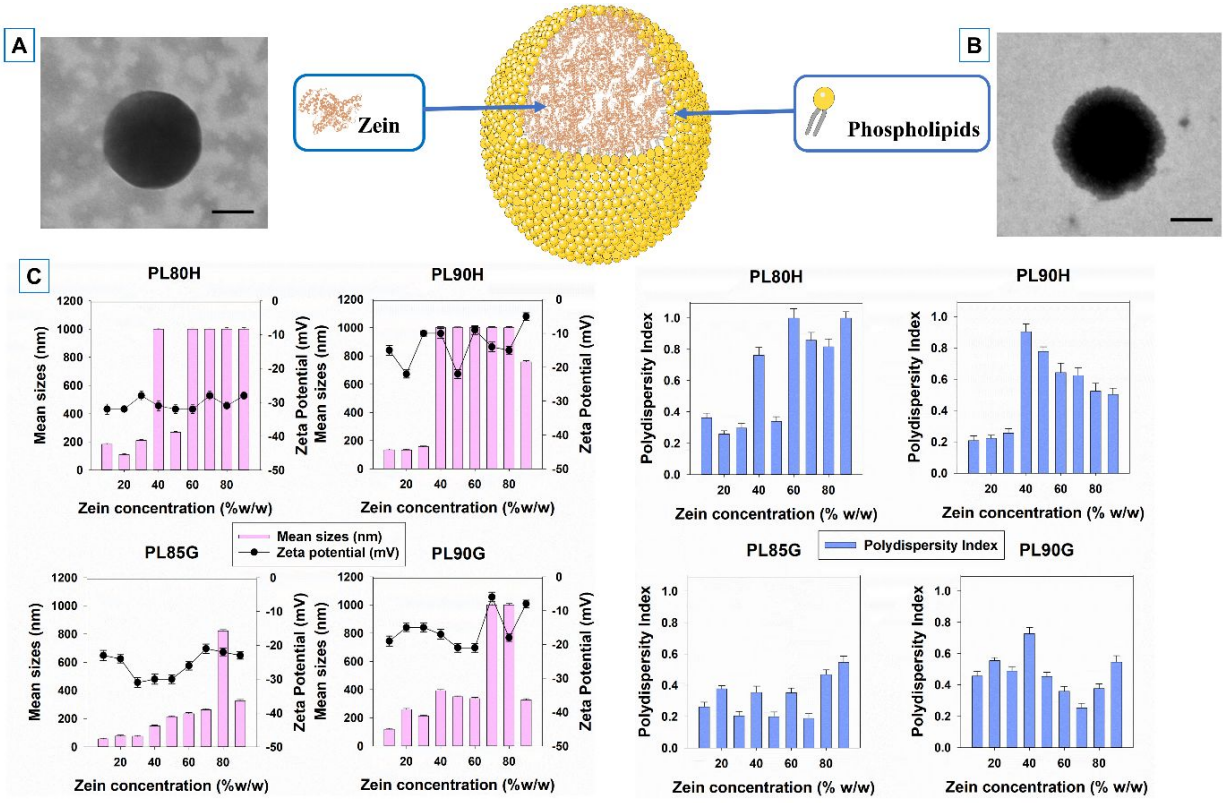


Figure 1. TEM micrograph of A) zein nanoparticles (2 mg/ml of protein) and of B) PL85G/zein hybrid nanosystems (7:3 w/w, 2 mg/ml of materials). Bar=200 nm C) Physico-chemical characterization of phospholipon/zein nanohybrids after the sonication process.

Moreover, the temperature-dependent stability of a formulation is a very important parameter to be evaluated. The analysis was carried out using a Turbiscan Lab® apparatus and the results were reported as the Turbiscan Stability Index (TSI), which correlates the transmittance and backscattering of a sample as a function of time and temperature.[30,31] The colloidal systems prepared with PL85G were incubated at room (25 °C) and body temperature (37 °C) in order to evaluate whether their stability could be affected by the variation of the amount of zein. The results showed excellent stability of the systems, revealing the absence of destabilization phenomena induced by the presence of the protein in the colloidal structure. Furthermore, a decrease in the slope of the TSI profiles following the sonication process of the samples was evident, probably as a consequence of the modulation of interfacial phenomena (Figure S1).

The freeze-drying process can be a useful way to preserve the intrinsic characteristics of the colloidal systems.[32] For this reason, lyophilization studies were carried out on the formulations prepared with PL85G and zein (9:1-5:5 w/w) enriched with various cryoprotectants (Tables S2). All the excipients enabled the systems to be resuspended in water in the same volume of medium initially used, but the ideal concentrations were 5 and 10% w/v. Namely, the addition of excipients below 5% w/v induced structural destabilization of the systems and the presence of aggregates after resuspension, data in agreement with several other studies.[33-37]

Indeed, as shown in table 2, the addition of mannitol (5-10% w/v) and sorbitol (5% w/v) did not promote the preservation of the mean sizes of the colloidal systems made up of PL85G/zein 7:3, while the addition of the other cryoprotectants induced a slight increase of the average diameter (100-200 nm) and the size distribution (Table 2). Moreover, these excipients did not induce significant variations in the surface charges of hybrid nanoparticles, showing negative values of Zeta potential similar to those of the nanosystems before the freeze-drying process. In particular, it was observed that glucose and mannose favour the development of acceptable freeze-dried nanosystems characterized by a long-term storage stability. Considering these results, the formulation prepared using PL85G and zein (7:3 w/w) was selected as the ideal system and was used to encapsulate ATRA in order to preserve the physico-chemical and pharmacological properties of the lipophilic drug.

Table 2. Physico-chemical properties of nanoparticles prepared using PL85G and zein (7:3 w/w) subsequent to the freeze-drying process as a function of different cryoprotectants used.

Sample		Mean sizes (nm)	Polydispesity Index	Zeta Potential (mV)
Zein Nanoparticles		106±1	0.20±0.01	20±1
PL85G Nanoparticles		100±1	0.22±0.01	-30±1
PL85G/Zein 7:3 w/w		79±2	0.214±0.04	-31±1
Glucose	5%	143±7	0.306±0.09	-33±2
	10%	99±4	0.238±0.05	-31±3
Mannitol	5%	>1000	0.618±0.21	-38±5
	10%	>1000	0.594±0.30	-36±6
Mannose	5%	106±8	0.295±0.06	-34±3
	10%	140±15	0.345±0.05	-33±4
Sucrose	5%	160±6	0.412±0.06	-37±2
	10%	132±3	0.361±0.07	-33±1
Sorbitol	5%	401±22	0.538±0.11	-35±4
	10%	223±7	0.337±0.13	-33±3
Trehalose	5%	111±17	0.350±0.10	-31±2
	10%	131±14	0.477±0.09	-34±3

Different amounts of bioactive (0.5, 1 and 2 mg/formulation) were used in order to evaluate the ability of the hybrid systems to retain the lipophilic compound. For this reason, the colloidal formulation prepared with PL85G and zein (7: 3 w/w) required a new PCS study as a function of the amount of ATRA initially used in the preparation phases of the systems. As can be seen in Table S3, the active compound induced a significant increase in the average size of the systems, causing the appearance of aggregates and polydispersed populations. For this reason, the colloidal systems were sonicated resulting in particles characterized by a mean diameter of ~90 nm and a homogeneous size distribution (Figure 2).

The zeta potential of the nanosystems was not affected by the bioactive, probably because ATRA did not significantly influence the protein arrangement. Moreover, the encapsulation rate of ATRA was investigated as a function of the drug/lipoprotein ratio (Figure 2B). Namely, an initial concentration of ATRA equal to 0.1 mg/ml favoured a retention of the bioactive of about 60% (~ 0.3 mg/formulation) in the colloidal structure, while the use of 0.4 mg/ml gave an encapsulation of about 35% of the lipophilic drug (~0.7 mg/formulation). The best results were achieved using 0.2 mg/ml of drug because the amount of bioactive compound effectively retained by the nanosystems was approximately 55% (~0.55 mg/formulation) (Figure 2B). Probably the increased encapsulation of the drug among the hybrid

nanoparticles with respect to the zein nanoparticles[28] is related to the numerous hydrophobic interactions coming about between the lipophilic portion of the bioactive and the lipophilic tail of the phospholipids, as well as the electrostatic interactions and hydrogen bonding occurring between the polar hydroxyl group of the drug and head group of the lipids, as evidenced by Fourier transform infrared spectroscopy in several papers.[27,29,30]

The application of ATRA is limited because of its sensitivity following exposition to external agents such as light. The hybrid nanoparticles provide a great degree of protection of ATRA against UV light-induced degradation in comparison to the free form of the drug in ethanol (Figure 2 panels D and E). In fact, the PL85G/zein nanosystems showed a reduced decrease of absorbance after UV-exposition with respect to an ethanol solution of the drug (from 1.795 to 1.655 and from 1.733 to 1.594, respectively, after a UV exposure of 360 min) (Figure 2, panels D and E). This phenomenon is probably due to the structure of the lipopolymer which contains aromatic and double bond residues and is able to absorb UV light preserving the active compound from photochemical degradation as described in other experimental works. [19,40,41]

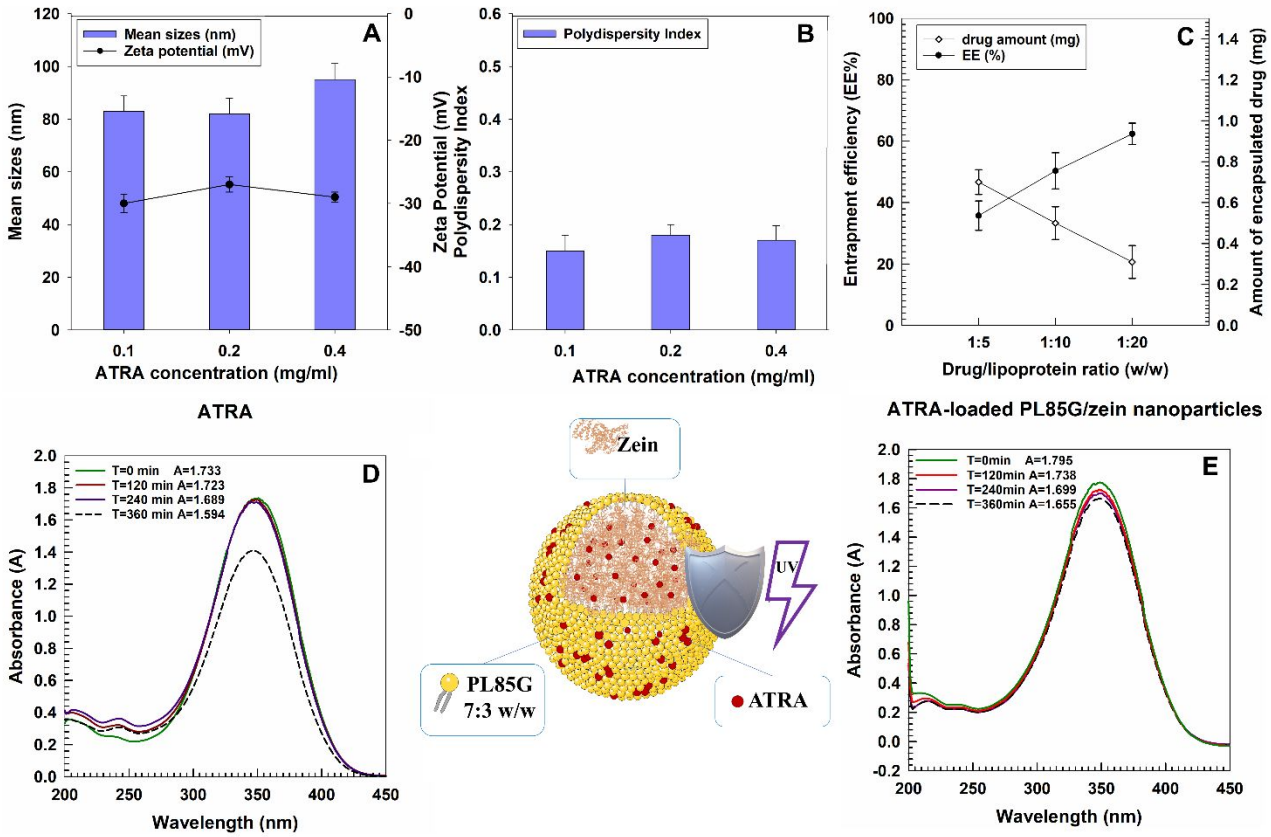


Figure 2. Physico-chemical properties of PL85G/Zein nanoparticles containing ATRA (A,B). Entrapment efficiency of ATRA as a function of the amount of the drug used during the preparation of nanoparticles (C). Photostability of ATRA (drug concentration of 110 µg/ml) as an ethanol solution (D) and entrapped in PL85G/zein nanoparticles (7:3 w/w) (E) as a function of the duration of UV-exposition.

The next phase of the study was focused on the evaluation of the cytotoxic activity of the nanosystems prepared with PL85G and zein (7:3 w/w ratio). Indeed, although both phospholipon® and zein have been approved by the FDA and the EMA for pharmaceutical use, the safe concentration of obtained colloids to be used must be evaluated in order to operate at subtoxic dosages. The investigated cell lines were A549 (human lung cancer) and CaCo-2 (human colon cancer) because they are ideal models upon which ATRA has been shown to exert significant biological effects.[42,43]

Figure 3A shows that the cytotoxic effects of the colloidal formulation were evident only at concentrations of lipoprotein material greater than 200 µg/ml and at incubation times of over 24h. These findings revealed that the hybrid carriers were highly biocompatible. In addition, the toxic concentrations reported above were not reached in the following experiments in order to evaluate the real benefits deriving from the encapsulation of ATRA in the nanosystems. For this reason, the cytotoxic profiles of hybrid systems containing the drug have been compared to those of the free drug (Figure 3B). The pharmacological activity of ATRA was evident at a drug concentration ≥1 µM on both cell lines and the best cytotoxic effects were obtained at a concentration of 10 µM. The nanoencapsulation of the drug within hybrid nanoparticles did

not compromise the pharmacological efficacy of the active compound and increased after 24 h of incubation (Figure 3).

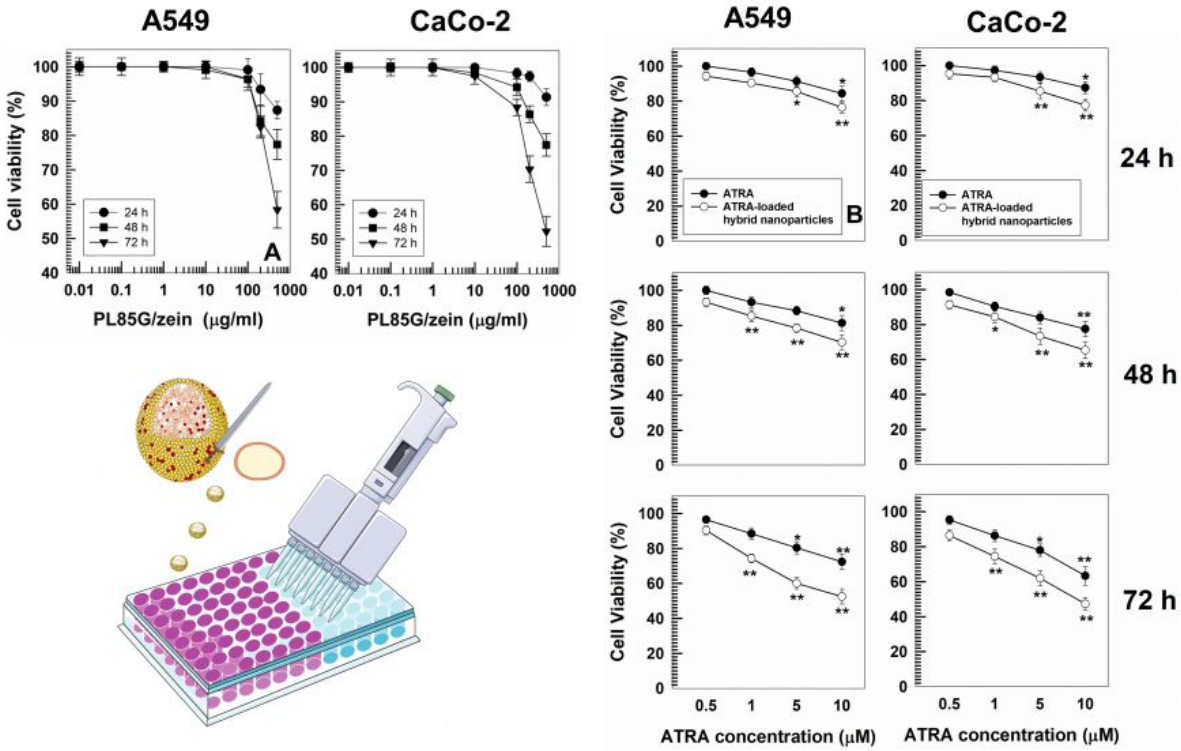


Figure 3. (A) Evaluation of *in vitro* cytotoxicity of PL85G/zein nanoparticles on A549 and CaCo-2 cells as a function of the material concentration and incubation time; (B) *In vitro* cytotoxicity of ATRA and ATRA-loaded PL85G/zein nanoparticles on A549 and CaCo-2 cells as function of the drug concentration and the incubation time.
Results are the mean of three different experiments ± standard deviation. *p < 0.05, **p < 0.001 (with respect to the untreated cells).

A plausible explanation for the improved cytotoxic effect of ATRA could be attributed to the enhanced cellular uptake promoted by the colloidal systems, which allowed the bioactive to concentrate itself in the cytosol, maximizing its biological effects as was described with the use of many other polymeric carriers.[44,45] In this regard, the incubation of fluorescent nanosystems (containing rhodamine-DHPE) with A549 cells confirmed their high degree of interaction with biological substrates; in fact, a massive staining of the cytosol was obtained after just 3 h incubation as a result of the great uptake of hybrid nanoparticles (Figure 4). The Z-stack analysis demonstrated the successful internalization of the systems in the cells, confirming that they are able to effectively deliver the encapsulated active compounds.

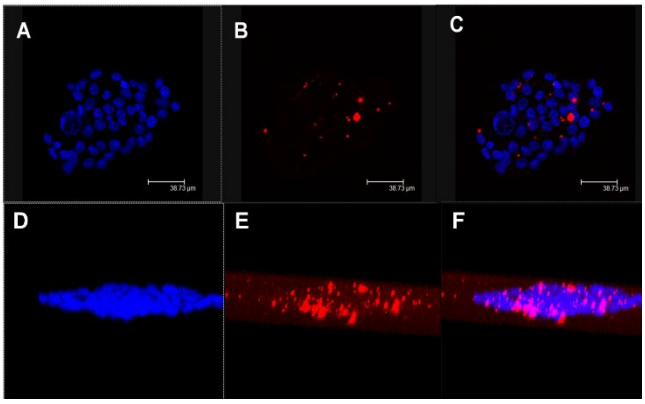


Fig. 4. CLSM micrographs of A549 cells incubated with rhodamine-DHPE-labelled PL85G/zein nanoparticles for 3 h. 2D (upper), Z-stack (right). Panel A,D: Hoechst filter; panel B,E: TRITC filter; panel C,F: overlay. No auto-fluorescent phenomena were observed.

In conclusion, the hybrid nanosystems made up of PL85G and zein showed promising features for a useful delivery of ATRA. In conclusion, the hybrid nanosystems made up of PL85G and

zein showed promising features for a useful delivery of ATRA. The lipoprotein structure preserved the lipophilic active compound from UV-related degradation maintaining its antitumoral pharmacological efficacy. The hybrid nanosystems herein described are innovative carriers for the delivery of photo-sensitive, poorly-water-soluble drugs. This is because they favour the administration of entrapped compounds in polar media thus avoiding the use of organic solvents and their potential degradation due to various types of external stimuli.
.

ASSOCIATED CONTENT

Experimental details and supporting figures. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Donato Cosco- Department of Health Sciences, University "Magna Græcia" of Catanzaro, Campus Universitario "S Venuta", I-88100, Catanzaro, Italy,
email: donatocosco@unicz.it

Author Contributions

A.G., M.F. and D.C. designed the experiments, analyzed data and prepared the manuscript. A.G., S.V. and E.G. prepared the nanosystems and evaluated their physico-chemical characteristics. A.G. and D.C. performed the experiments of cell viability and contributed to the analysis of the results. M.C.S. performed the TEM experiments. A.G, D.C and M.F. acquired data and provided a critical revision. M.F. and D.C. contributed reagents and materials. All authors discussed the results and approved the final version of the manuscript.

Funding Sources

This research was funded by a grant from the Italian Ministry of University and Research (PRIN2017, prot. n. 20173ZECCM_003).

Notes

The authors report no declarations of interest.

ACKNOWLEDGMENT

The authors are grateful to Lynn Whitted for her language revision of this article.

ABBREVIATIONS

ATRA (All-*Trans* Retinoic Acid) FDA (Food and Drug Administration) GRAS (Generally Recognized As Safe) RAR (Retinoic Acid Receptors) RXR (Retinoid X Receptor) PCS (Photon Correlation Spectroscopy) PL (Phospholipon) PDI (Polydispersity Index) TSI (Turbiscan Stability Index) EMA (European Medicines Agency)

REFERENCES

1. Spasojević, L.; Bučko, S.; Kovačević, D.; Bohinc, K.; Jukić, J.; Abram, A.; Požar, J.; Katona, J. Interactions of zein and zein/rosin nanoparticles with natural polyanion gum arabic. Colloids Surfaces B Biointerfaces 2020, 196, 111289.

2. Voci, S.; Gagliardi, A.; Fresta, M.; Cosco, D. Antitumor Features of Vegetal Protein-Based Nanotherapeutics. Pharmaceutics 2020, 12, E65.

3. Vatankhah, Z.; Dehghani, E.; Salami-Kalajahi, M.; Roghani-Mamaqani, H. Seed's morphology-induced core-shell composite particles by seeded emulsion polymerization for drug delivery. Colloids Surfaces B Biointerfaces 2020, 191, 111008.

4. Kang, S.; He, Y.; Yu, D.-G.; Li, W.; Wang, K. Drug–zein@ lipid hybrid nanoparticles: Electrospraying preparation and drug extended release application. Colloids Surfaces B Biointerfaces 2021, 201, 111629.

5. Chen, X.; Chen, Y.; Zou, L.; Zhang, X.; Dong, Y.; Tang, J.; McClements, D.J.; Liu, W. Plant-based nanoparticles prepared from proteins and phospholipids consisting of a core–multilayer-shell structure: Fabrication, stability, and foamability. J. Agric. Food Chem. 2019, 67, 6574–6584.

6. Hong, S.S.; Thapa, R.K.; Kim, J.H.; Kim, S.Y.; Kim, J.O.; Kim, J.K.; Choi, H.G.; Lim, S.J. Role of zein incorporation on hydrophobic drug-loading capacity and colloidal stability of phospholipid nanoparticles. Colloids Surfaces B Biointerfaces 2018, 171, 514-521

7. Liu, G.; Huang, W.; Babii, O.; Gong, X.; Tian, Z.; Yang, J.; Wang, Y.; Jacobs, R.L.; Donna, V.; Lavasanifar, A. Novel protein–lipid composite nanoparticles with an inner aqueous compartment as delivery systems of hydrophilic nutraceutical compounds. Nanoscale 2018, 10, 10629–10640.

8. Sun, C.; Liu, F.; Yang, J.; Yang, W.; Yuan, F.; Gao, Y. Physical, structural, thermal and morphological characteristics of zeinquercetagetin composite colloidal nanoparticles. Ind. Crops Prod. 2015, 77, 476–483.

9. Dave, V.; Srivastava, P.; Sharma, S.; Bajaj, J.; Tak, K. PEGylated PLA-Phospholipon 90G complex hybrid nanoparticles loaded with etoricoxib for effective treatment pain relief potential. Int. J. Polym. Mater. Polym. Biomater. 2019, 69, 640-652.

10. Yang, S.; Dai, L.; Sun, C.; Gao, Y. Characterization of curcumin loaded gliadin-lecithin composite nanoparticles fabricated by antisolvent precipitation in different blending sequences. Food Hydrocoll. 2018, 85, 185–194.

11. Voci, S.; Fresta, M.; Cosco, D. Gliadins as versatile biomaterials for drug delivery applications. J. Control. Release 2021, 329, 385-400.

12. Xue, J.; Zhang, Y.; Huang, G.; Liu, J.; Slavin, M.; Yu, L.L. Zein-caseinate composite nanoparticles for bioactive delivery using curcumin as a probe compound. Food Hydrocoll. 2018, 83, 25–35.

13. Liu, C.; Li, M.; Yang, J.; Xiong, L.; Sun, Q. Fabrication and characterization of biocompatible hybrid nanoparticles from spontaneous co-assembly of casein/gliadin and proanthocyanidin. Food Hydrocoll. 2017, 73, 74–89.

14. Yu, X.; Wu, H.; Hu, H.; Dong, Z.; Dang, Y.; Qi, Q.; Wang, Y.; Du, S.; Lu, Y. Zein nanoparticles as nontoxic delivery system for maytansine in the treatment of non-small cell lung cancer. Drug Deliv. 2020, 27, 100–109.

15. Gagliardi, A.; Froiio, F.; Salvatici, M.C.; Paolino, D.; Fresta, M.; Cosco, D. Characterization and refinement of zein-based gels. Food Hydrocoll. 2020, 101, 105555.

16. Gagliardi, A.; Voci, S.; Paolino, D.; Fresta, M.; Cosco, D. Influence of Various Model Compounds on the Rheological Properties of Zein-Based Gels. Molecules 2020, 25, 3174.

17. Yuan, Y.; Li, H.; Zhu, J.; Liu, C.; Sun, X.; Wang, D.; Xu, Y. Fabrication and characterization of zein nanoparticles by dextran sulfate coating as vehicles for delivery of curcumin. Int. J. Biol. Macromol. 2020, 151, 1074–1083.

18. Dai, L.; Sun, C.; Wang, D.; Gao, Y. The interaction between zein and lecithin in ethanol-water solution and characterization of zein–lecithin composite colloidal nanoparticles. PL0S One 2016, 11, e0167172.

19. Dai, L.; Sun, C.; Li, R.; Mao, L.; Liu, F.; Gao, Y. Structural characterization, formation mechanism and stability of curcumin in zein-lecithin composite nanoparticles fabricated by antisolvent co-precipitation. Food Chem. 2017, 237, 1163–1171.

20. Dai, L.; Li, R.; Wei, Y.; Sun, C.; Mao, L.; Gao, Y. Fabrication of zein and rhamnolipid complex nanoparticles to enhance the stability and in vitro release of curcumin. Food Hydrocoll. 2018, 77, 617–628.

21. Dai, L.; Zhou, H.; Wei, Y.; Gao, Y.; McClements, D.J. Curcumin encapsulation in zein-rhamnolipid composite nanoparticles using a pH-driven method. Food Hydrocoll. 2019, 93, 342–350.

22. Siddikuzzaman; Guruvayoorappan, C.; Berlin Grace, V.M. All trans retinoic acid and cancer. Immunopharmacol. Immunotoxicol. 2011, 33, 241–249.

23. Giuli, M.V.; Hanieh, P.N.; Giuliani, E.; Rinaldi, F.; Marianecci, C.; Screpanti, I.; Checquolo, S.; Carafa, M. Current Trends in ATRA Delivery for Cancer Therapy. Pharmaceutics 2020, 12, 707.

24. Cristiano, M.C.; Cosco, D.; Celia, C.; Tudose, A.; Mare, R.; Paolino, D.; Fresta, M. Anticancer activity of all-trans retinoic acid-loaded liposomes on human thyroid carcinoma cells. Colloids Surfaces B Biointerfaces 2017, 150, 408-416.

25. di Masi, A.; Leboffe, L.; De Marinis, E.; Pagano, F.; Cicconi, L.; Rochette-Egly, C.; Lo-Coco, F.; Ascenzi, P.; Nervi, C. Retinoic acid receptors: from molecular mechanisms to cancer therapy. Mol. Aspects Med. 2015, 41, 1–115.

26. Costantini, L.; Molinari, R.; Farinon, B.; Merendino, N. Retinoic acids in the treatment of most lethal solid cancers. J. Clin. Med. 2020, 9, 360.

27. Radwan, S.A.A.; El-Maadawy, W.H.; Yousry, C.; ElMeshad, A.N.; Shoukri, R.A. Zein/phospholipid composite nanoparticles for successful delivery of gallic acid into ahscs: Influence of size, surface charge, and vitamin a coupling. Int. J. Nanomedicine 2020, 15, 7995.

28. Gagliardi, A.; Paolino, D.; Iannone, M.; Palma, E.; Fresta, M.; Cosco, D. Sodium deoxycholate-decorated zein nanoparticles for a stable colloidal drug delivery system. Int. J. Nanomedicine 2018, 13, 601–614.

29. Yousry, C.; Fahmy, R.H.; Essam, T.; El-Laithy, H.M.; Elkheshen, S.A. Nanoparticles as tool for enhanced ophthalmic delivery of vancomycin: a multidistrict-based microbiological study, solid lipid nanoparticles formulation and evaluation. Drug Dev. Ind. Pharm. 2016, 42, 1752–1762.

30. Giuliano, E.; Paolino, D.; Cristiano, M.C.; Fresta, M.; Cosco, D. Rutin-Loaded Poloxamer 407-Based Hydrogels for In Situ Administration: Stability Profiles and Rheological Properties. Nanomaterials 2020, 10, 1069.

31. Gagliardi, A.; Cosco, D.; Udongo, B.P.; Dini, L.; Viglietto, G.; Paolino, D. Design and Characterization of Glyceryl Monooleate-Nanostructures Containing Doxorubicin Hydrochloride. Pharmaceutics 2020, 12, 1017.

32. Fonte, P.; Soares, S.; Sousa, F.; Costa, A.; Seabra, V.; Reis, S.; Sarmento, B. Stability study perspective of the effect of freeze-drying using cryoprotectants on the structure of insulin loaded into PLGA nanoparticles. Biomacromolecules 2014, 15, 3753–3765.

33. Carpenter, J.F.; Prestrelski, S.J.; Arakawa, T. Separation of freezing-and drying-induced denaturation of lyophilized proteins using stress-specific stabilization: I. Enzyme activity and calorimetric studies. Arch. Biochem. Biophys. 1993, 303, 456–464.

34. Lee, M.K.; Kim, M.Y.; Kim, S.; Lee, J. Cryoprotectants for freeze drying of drug nano-suspensions: effect of freezing rate. J. Pharm. Sci. 2009, 98, 4808–4817.

35. Varshosaz, J.; Eskandari, S.; Tabbakhian, M. Freeze-drying of nanostructure lipid carriers by different carbohydrate polymers used as cryoprotectants. Carbohydr. Polym. 2012, 88, 1157–1163.

36. Alkilany, A.M.; Abulateefeh, S.R.; Mills, K.K.; Bani Yaseen, A.I.; Hamaly, M.A.; Alkhatib, H.S.; Aiedeh, K.M.; Stone, J.W. Colloidal stability of citrate and mercaptoacetic acid capped gold nanoparticles upon lyophilization: effect of capping ligand attachment and type of cryoprotectants. Langmuir 2014, 30, 13799–13808.

37. Wang, L.; Ma, Y.; Gu, Y.; Liu, Y.; Zhao, J.; Yan, B.; Wang, Y. Cryoprotectant choice and analyses of freeze-drying drug suspension of nanoparticles with functional stabilisers. J. Microencapsul. 2018, 35, 241–248.

38. Svensson, F.R.; Lincoln, P.; Nordén, B.; Esbjörner, E.K. Retinoid chromophores as probes of membrane lipid order. J. Phys. Chem. B 2007, 111, 10839–10848.

39. El-Mezayen, N.S.; El-Hadidy, W.F.; El-Refaie, W.M.; Shalaby, T.I.; Khattab, M.M.; El-Khatib, A.S. Hepatic stellate cell-targeted imatinib nanomedicine versus conventional imatinib: a novel strategy with potent efficacy in experimental liver fibrosis. J. Control. release 2017, 266, 226–237.

40. Luo, Y.; Teng, Z.; Wang, Q. Development of zein nanoparticles coated with carboxymethyl chitosan for encapsulation and controlled release of vitamin D3. J. Agric. Food Chem. 2012, 60, 836–843.

41. Xiao, J.; Nian, S.; Huang, Q. Assembly of kafirin/carboxymethyl chitosan nanoparticles to enhance the cellular uptake of curcumin. Food Hydrocoll. 2015, 51, 166–175.

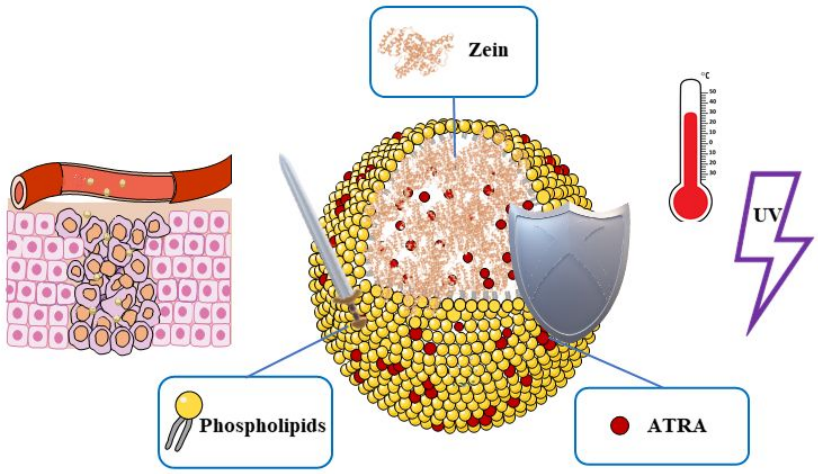
42. Akita, T.; Horiguchi, M.; Ozawa, C.; Terada, H.; Yamashita, C. The Effect of a Retinoic Acid Derivative on Cell-Growth Inhibition in a Pulmonary Carcinoma Cell Line. Biol. Pharm. Bull. 2016, 39, 308–312.

43. Hessel, S.; Lampen, A. All-trans retinoic acid enhances the transport of phase II metabolites of benzo [a] pyrene by inducing the Breast Cancer Resistance Protein expression in Caco-2 cells. Toxicol. Lett. 2010, 197, 151–155.

44. Mura, S.; Fattal, E.; Nicolas, J. From Poly(Alkyl Cyanoacrylate) to Squalene as Core Material to Design Nanomedicines. J. Drug Target. 2019, 27, 470-501.

45. Gagliardi, A.; Paolino, D.; Costa, N.; Fresta, M.; Cosco, D. Zein-vs PLGA-based nanoparticles containing rutin: a comparative investigation. Mater. Sci. Eng. C 2021, 118, 111538.

Table of Contents artwork



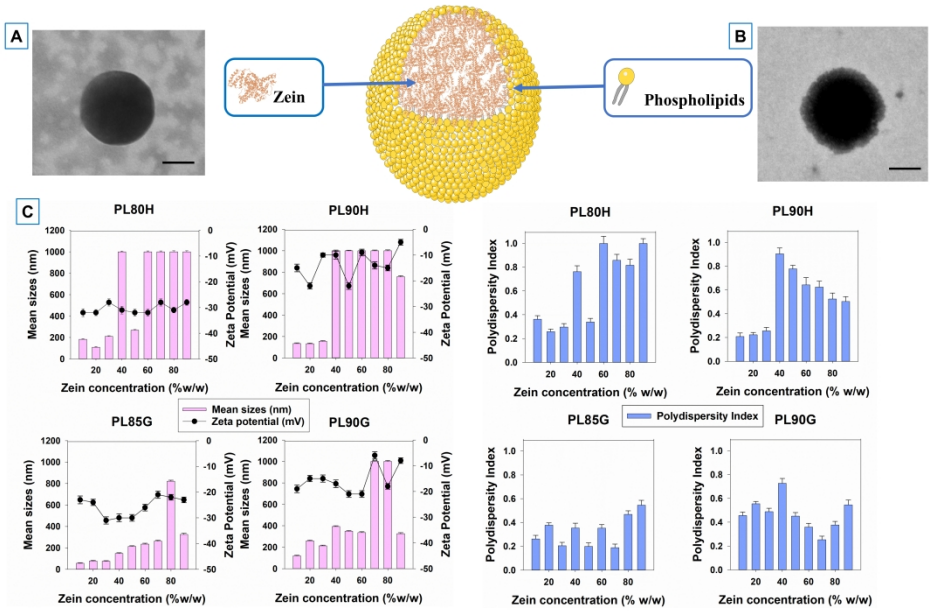


Figure 1. TEM micrograph of A) zein nanoparticles (2 mg/ml of protein) and of B) PL85G/zein hybrid nanosystems (7:3 w/w, 2 mg/ml of materials). Bar=200 nm C) Physico-chemical characterization of phospholipon/zein nanohybrids after the sonication process.

505x308mm (300 x 300 DPI)

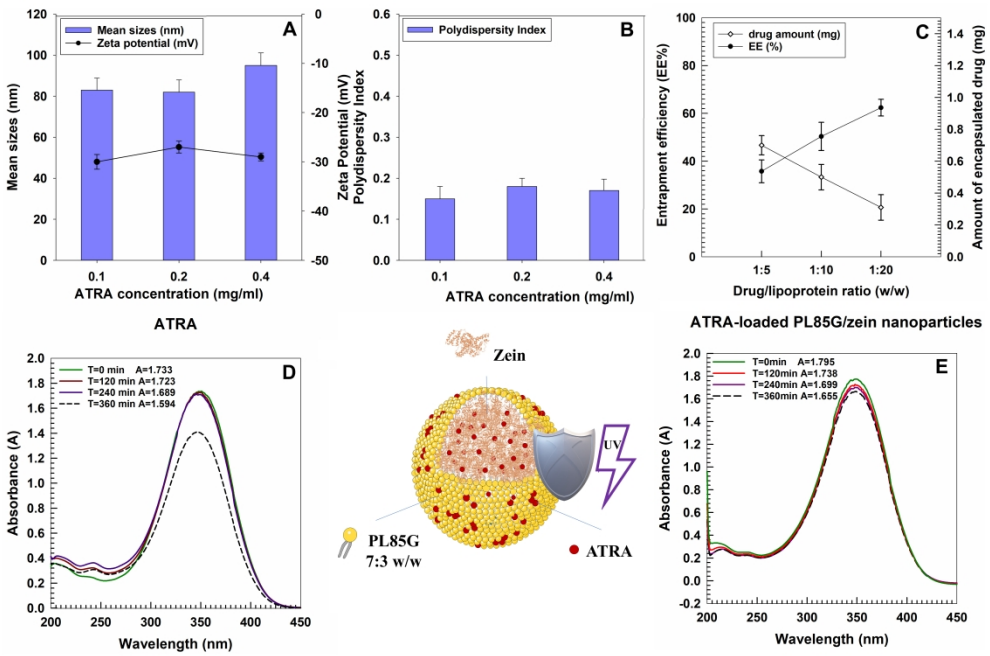


Figure 2. Physico-chemical properties of PL85G/Zein nanoparticles containing ATRA (A,B). Entrapment efficiency of ATRA as a function of the amount of the drug used during the preparation of nanoparticles (C). Photostability of ATRA (drug concentration of 110 μg/ml) as an ethanol solution (D) and entrapped in PL85G/zein nanoparticles (7:3 w/w) (E) as a function of the duration of UV-exposition.

500x351mm (300 x 300 DPI)

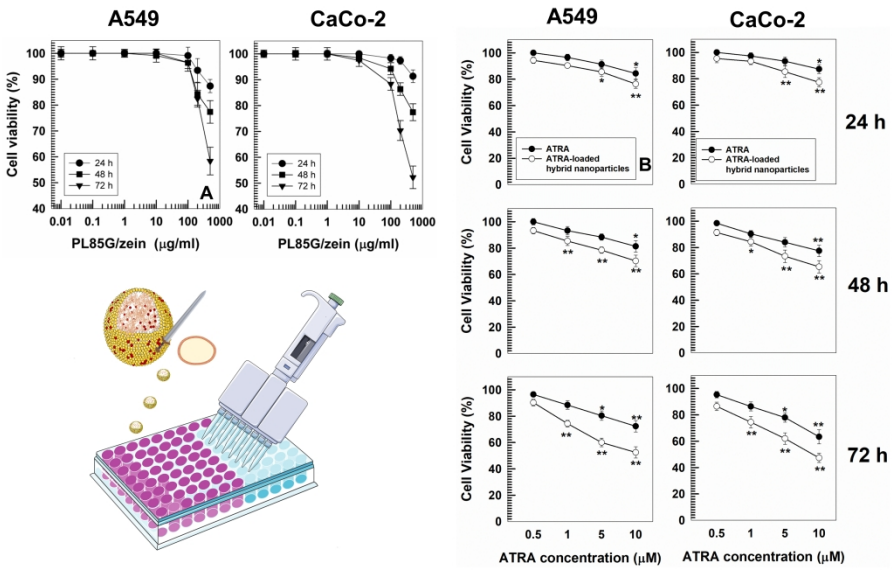


Figure 3. (A) Evaluation of in vitro cytotoxicity of PL85G/zein nanoparticles on A549 and CaCo-2 cells as a function of the material concentration and incubation time; (B) In vitro cytotoxicity of ATRA and ATRA-loaded PL85G/zein nanoparticles on A549 and CaCo-2 cells as function of the drug concentration and the incubation time.
Results are the mean of three different experiments ± standard deviation. *p < 0.05, **p < 0.001 (with respect to the untreated cells).

453x290mm (300 x 300 DPI)

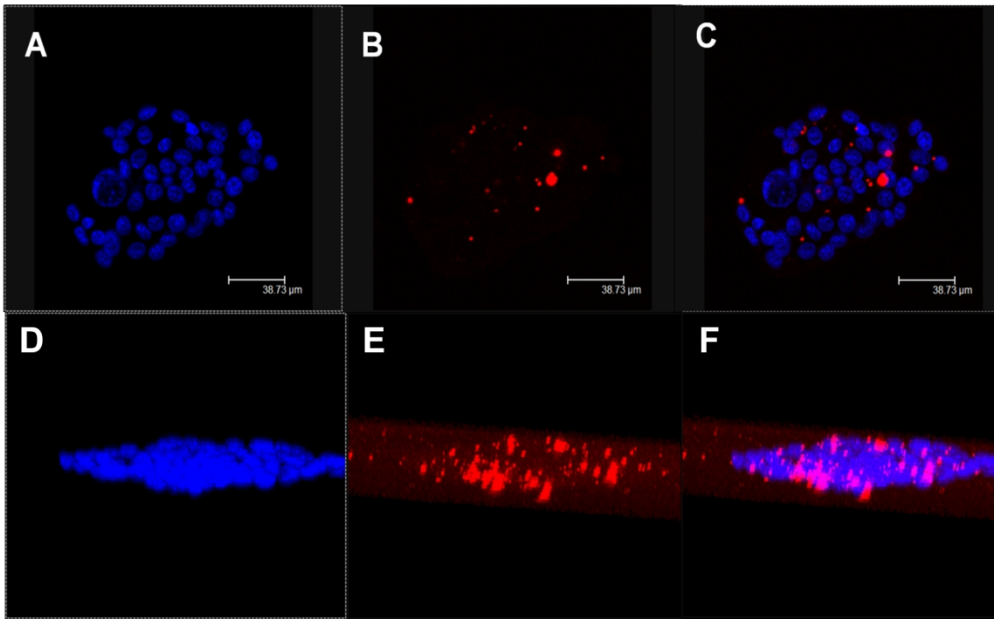


Fig. 4. CLSM micrographs of A549 cells incubated with rho-damine-DHPE-labelled PL85G/zein nanoparticles for 3 h. 2D (upper), Z-stack (right). Panel A,D: Hoechst filter; panel B,E: TRITC filter; panel C,F: overlay. No auto-fluorescent phenomena were observed.

161x99mm (300 x 300 DPI)