Expert Opinion

Retinoids: new use by innovative drug-delivery systems

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Background: Retinoids represent an old class of bioactives used in the treatment of different skin pathologies (such as acne and psoriasis) and in the treatment of many tumors. Unfortunately, they present several side effects, i.e., burning of skin and general malaise after systemic administration and they are very unstable after exposition to light. *Methods*: One of the most promising new approaches for reducing the side effects of retinoids while improving their pharmacological effect is the use of drug-delivery devices. This review explains the current status of retinoid drug transport, which has been developing over the last few years, explaining the modification of their biopharmaceutical properties in detail after encapsulation/inclusion in vesicular and polymeric systems. *Results/conclusion*: Different colloidal and micellar systems containing retinoid drugs have been realized furnishing important potential advancements in traditional therapy.

Keywords: liposomes, microemulsions, niosomes, retinoids, solid lipid nanoparticles

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1. Introduction

The retinoids are a group of compounds that are chemically related to vitamin A. They fulfill crucial functions in the human body, the most important of which are cell proliferation and differentiation, bone tissue growth, immune function regulation, and tumor-suppressor gene expression [1,2].

Natural retinoids derived from vitamin A (retinol) are introduced into the body via the diet (eggs, milk, butter, fish liver oil, etc.). After uptake by the intestinal cells, retinol is esterified and the formed retinyl ester reaches the blood as very-low-density lipoprotein (VLDL) and then is stored by the liver. After the breakdown of retinyl ester, the retinol is oxidized into retinal by the action of the enzyme alcohol dehydrogenase (ADH). The human body contains three classes of ADH – ADH1, ADH2, and ADH3 – the most active, important, and omnipresent of which is ADH3. Retinaldeide dehydrogenase (RALDH) catalyzes the irreversible oxidation of the retinal to retinoic acid (**Figure 1**). The three different isoforms for this enzyme (RALDH1, RALDH2, and RALDH3) are expressed only in tissues able to produce retinoic acid. Only a small amount of retinol is converted into all-*trans*-retinoic acid (ATRA), which is an important retinoic acid receptor agonist [3]. The retinoid structure consists of a cyclic group and a polar group separated by a ramificate polyen chain. In particular, the alternated sequence of double C=C bonds in the chain is responsible for the color of retinoids (yellow, orange, or red) and represents the molecular section which is easily destabilized after light exposition. If we consider their chemical structures, retinoids can be classified in three different generations (**Figure 1**): i) retinol, retinal, tretinoin (ATRA), isotretinoin (13-*cis*-retinoic acid; 13-*cis*-RA), and alitretinoin (9-*cis*-retinoic acid; 9-*cis*-RA); ii) etretinate and its metabolite acitretin; and iii) tazarotene, bexarotene, and adapalene. The first- and second-generation compounds interact with all retinoid receptors because of their greater degree of flexibility with respect to those of the third generation.

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The activities of vitamin A, and of its natural and synthetic analogs, are mediated by two classes of nuclear hormone receptor: the retinoic acid receptor (RAR), which is activated by ATRA and 9-*cis*-RA, and the retinoid X-receptor (RXR), which is exclusively activated by 9-*cis*-RA. RAR exists in three different receptor isoform: RARα, β, and γ, which are encoded by the *RARA*, *RARB*, and *RARG* genes, respectively. Whereas RARα is expressed ubiquitously throughout adult tissues, RARγ is expressed primarily in skin and RARβ is essentially expressed in epithelial cells, although it possesses a special property because its expression can be induced by RA. Many epithelial tumors do not express the RARβ isoform. For example, most breast cancer cells do not express this gene, which could explain their reduced response to retinoic acid [4].

RXR also exists in three different isoforms, α, β, and γ, which belong to the steroid/thyroid superfamily of transcription factors and which heterodimerize to form the RAR/RXR dimer [5]. Although RA binds with the same affinity to all three RAR subtypes, it does not interact with RXR. On the contrary, 9-*cis*-RA binds the RXR and RAR receptors with similar affinities. The RAR/RXR heterodimers are able to modulate the transcription of target genes after binding with retinoic acid response element (RARE) and to modulate the efficiency of other pathways, although the mechanisms are not completely clear. In the presence of antagonists of the RAR–RXR dimer or in absence of their ligands, target genes are repressed. This is made possible by the presence of histone deacetylase-containing complex (HDAC), which is linked to RAR/RXR heterodimers by corepressors, i.e., nuclear receptor corepressor (NCoR) and silencing mediator for retinoid and thyroid hormone receptor (SMRT). As a consequence, the histone deacetylation and chromatin condensation induce the silencing of the gene. Conversely, when present, agonist ligands bind the receptors, inducing conformational changes in their structure and the dissociation of corepressors. More-over, the receptor conformation variation increases the binding affinity of coactivators such as TIF1, TIF2 (transcriptional intermediary factor-1 or -2) and RIP140 (receptor interacting protein 140) [6,7].

In addition to the nuclear receptor, other proteins that interact with RA are the cellular retinoic-acid-binding proteins (CRABP I and CRABP II). These cytoplasm proteins have low molecular weights and bind retinoids inside the cell, although their function remains unknown. CRAB I is expressed ubiquitously whereas CRABP II is principally present in adult human and rat skin. These proteins are believed to be involved in the distribution and metabolism of RA, thus maintaining controlled levels of free intracellular RA [8].

Retinoids are used in the treatment of many diseases and are efficacious in the cure of different dermatological conditions (inflammatory skin disorders, skin cancers, disorders of increased cell turnover, photoaging, acne, and psoriasis) [9-11]. Retinoids, as previously reported, are important for embryonic development and maintenance of the differentiated tissues. In fact, a low concentration of vitamin A causes malformations, probably as the result of genetic defects of the RAR/RXR complex. For this reason, although they are indicated in pregnancy, the administered dose should be kept low because teratology has been observed at elevated doses [12].

In adults, retinoids are essential for the functionality of various organs, including liver, lungs, nervous system, and immune system; moreover, their property of inducing cell differentiation makes them suitable compounds in anticancer therapy. For this reason, many studies have been conducted in recent years to define their possible application in the field of the treatment of cancer [13,14]. In particular, the antitumor effect of retinoids has been associated with a direct antiproliferative property, as demonstrated in *in-vitro* tests carried out on different cell lines (fibroblastic, melanoma, pancreatic, and lymphoid carcinoma cells) [15,16]. Unfortunately, after prolonged retinoid administration anorexia, skin lesions, hair loss, hepatosplenomegaly, papilloedema, bleeding, general malaise, pseudotumor cerebri, and death may also occur.

In addition, isotretinoin is an efficacious agent in the treatment of a variety of dermatologic conditions, such as the control of sebum production, the regulation of *Propionobacterium acnes* colonization of ductal and skin surfaces, comedogenesis, and local inflammation; however, it does not effectuate gene-regulating activity. Acne therapy with isotretinoin allows a permanent remission of the condition although local irritation of the skin is a derived drawback. The drug is contraindicated during pregnancy because of its potential induction of birth defects [17]. Another retinoid compound used to treat acne is tazarotene, which, although approved by US FDA in 1997 for the treatment of psoriasis, provokes skin irritant reactions that reduce patient compliance [18].

2. Retinoid delivery

One way to avoid destabilization phenomena in the case of retinoids is their encapsulation/complexation in different drug-delivery systems. In fact, as abundantly reported in literature, the use of colloidal carriers (i.e., liposomes, niosomes, nanodisks, solid lipid nanoparticles (SLNs), polymeric micro- and nanoparticles, cyclodextrins, and microemulsions) favors the preservation of the pharmacological activities of the aforesaid compounds and reduces their side effects.

2.1 Liposomes

Liposomes are self-assembled vesicles that occur naturally and can be prepared artificially, as shown by Bangham and collaborators in the mid-1960s [19]. In 2001, Brisaert and coworkers evaluated the loading capacity, release profile, and stability of a conventional liposomal formulation (comprising egg phosphatidyl choline/cholesterol 72 : 28 w/w) containing differing amounts of tretinoin (1 – 6 mg) with the aim of proposing them as suitable systems in the treatment of acne [20]. They showed that the best set of liposomal suspensions, in which all the tretinoin was entrapped in the lipid fraction, consisted of 300 mg lipid and 2 mg tretinoin per milliliter

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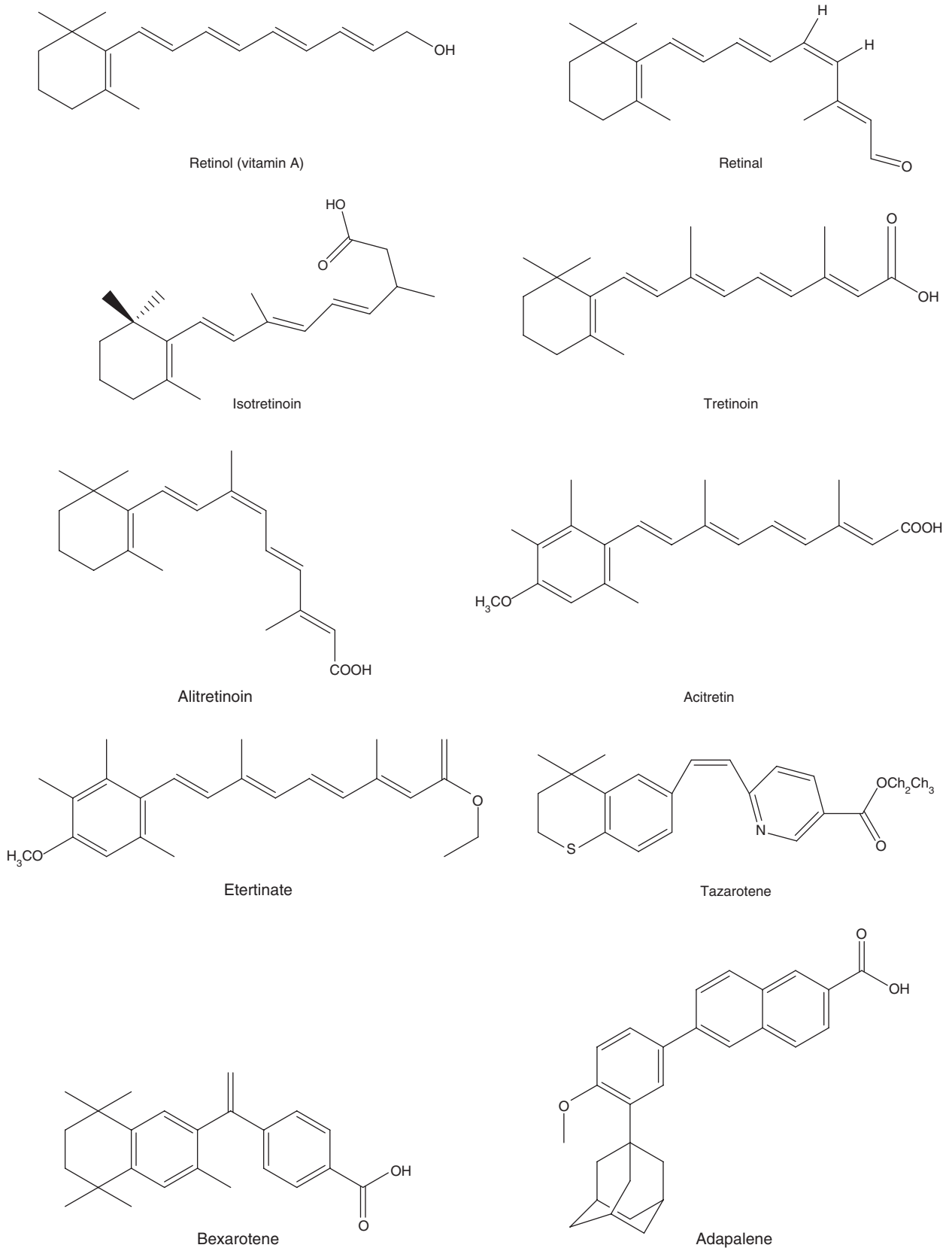

Figure 1. Chemical structures of retinoids.

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of buffer at pH = 5 (0.68% w/w). The release of the active compound was evaluated after 48 h by a dialysis technique using a Kontron–Diapack model 4000 apparatus (Kontron Instruments, Zurich, Switzerland) equipped with a Teflon T35 membrane filter, 0.2 µm, (Schleicher and Schüll, Dassel, Germany) in which the donor compartment was filled with 2 ml of the liposomal suspension containing tretinoin and the acceptor compartment with 2 ml of empty liposomal suspension. This procedure was necessary to assure the sink conditions of the experiment because tretinoin is insoluble in water and poorly soluble in buffers [21]. Only 3.6, 2.8 and 1.8% of the active compound was found in the acceptor compartment, respectively, from liposomes containing 100, 200 and 300 mg of lipids per milliliter of buffer, indicating that the release of tretinoin decreases with increasing lipid concentration. Finally, the stability of the formulations containing or not an antioxidant was studied by storing them at differing temperatures and evaluating the drug leakage over time; obtained results showed that the liposomal tretinoin remained stable for at least 3 months if an antioxidant is added and fluctuations in temperature during this period made no difference. Moreover, the effect of light on the active compound in free (solubilized in castor oil) and encapsulated form was evaluated by means of a XM-450 H/V xenon lamp (ORC Lighting Products, Azusa, CA). Brisaert demonstrated that there was no difference in photodegradation between the liposomal suspensions prepared with differing amounts of lipids, and that the vesicular carriers protected the drug more than the castor oil probably because it was less accessible to light.

These results agreed with those obtained by Ioele and coworkers [22]. In particular, they explained the increased photostability of tretinoin and isotretinoin after their incorporation into liposomal vesicles with respect to that of the drugs in an ethanolic solution. They used a xenon lamp as the light source and carried out their photodegradation studies by taking spectrophotometric measurements at increasing time periods. As can be observed in **Figure 2**, the exposure of the RA ethanolic solution to light caused a sharp degradation after just a few seconds with a shift of the maximum absorbance peak from 349 to 346 nm. An identical phenomenon was obtained after the irradiation of isotretinoin. The encapsulation of the active compounds in the liposomal carriers caused a strong reduction of the photodegradation process compared to the ethanolic formulation and, in fact, no shift of the maximum RA peak at 349 nm was observed. Moreover, the photostability of the 13-*cis*-RA-liposome complex improved when compared with that recorded of the drug in the ethanolic solution, but in a lesser measure when compared with ATRA-liposome complex (data not shown).

A few years later, Sinico and coworkers evaluated the influence of liposome composition, size, lamellarity, and charge on the (*trans*)dermal delivery of tretinoin [23]. In particular, in order to study the influence of phase transition temperature (T_c) of the main liposomal component in the active compound delivery, they prepared multilamellar (MLV) or unilamellar (ULV) liposomes using two different commercial mixtures of hydrogenated (Phospholipon® 90H, P90H) or nonhydrogenated soy phosphatydilcholine (Phospholipon® 90, P90) as the main bilayer component. They varied the surface charge by adding stearylamine (SA) or dicetylphosphate (DCP) to the preparations. In particular, the presence of DCP in MLVs, as shown in **Table 1**, caused an improvement in the drug-loading efficiency with respect to the SA formulation, while SA unilamellar vesicles showed an improvement in the amount of tretinoin encapsulated with respect to DCP-ULVs and paradoxically SA-MLVs. This phenomenon could be due to the necessity to utilize more energy during the system preparation to encapsulate the drug when SA is present. It is also interesting to note that, except for P90H MLVs, negatively-charged liposomes showed a higher tretinoin release rate than positively-charged liposomes, probably because the drug is partially ionized at pH = 7 (pK_a = 7.85) and might therefore be more strongly retained in SA vesicles. Subsequently, the difference in skin permeation was evaluated (newborn pig skin in occlusive conditions) between the liposomal tretinoin formulations and three different controls: an oil medium, a hydroalcoholic solution, and a commercial preparation of the drug (Retin-A®, Ortho-McNeil Pharmaceutical, Inc., Titusville, NJ). The results indicated that tretinoin skin permeation occurred rapidly when the drug was encapsulated in positively-charged liposomes and that vesicle size and lamellarity did not affect tretinoin delivery through the pig skin (for each composition the permeation profile was very similar for both multi- or unilamellar liposomal formulations). In particular, **Figure 3** highlights the fact that liposomal formulations do not penetrate intact into the deeper skin layers and suggests that absorption, lipid exchange, and fusion are probably involved in the permeation of vesicles across the skin. This, in turn, suggests that the main advantage of liposomes as a transdermal drug delivery system is due to the drug residence time on the skin surface. Later, they demonstrated that: i) liposomes saturated with tretinoin are capable of significantly promoting drug accumulation in pig skin, although the same does not occur when the vesicular bilayer is not saturated with the active compound; and ii) that P90H formulation gave a higher drug permeation with respect to the corresponding DPPC (dipalmytoilphosphatidylcholine) liposomes [24]. The results on the skin permeation of liposomes after variation of their surface charge are in agreement with those obtained by Montenegro and coworkers in 1996 [25]. This theory was also used by Kitagawa and Kasamaki for the delivery of retinoic acid to the skin [26]. In particular, they evaluated the difference in skin permeation of the drug compound after encapsulation in neutral, made up of DPPC, egg yolk phosphocholine (PC), or dimyristoylphosphatidylcholine (DMPC) and cholesterol and cationic liposomes containing dioleoyltrimethylammonium propane (DOTAP) or dimethyldipalmitylammonium by *in-vitro* studies effectuated with a Franz cell apparatus equipped with excised

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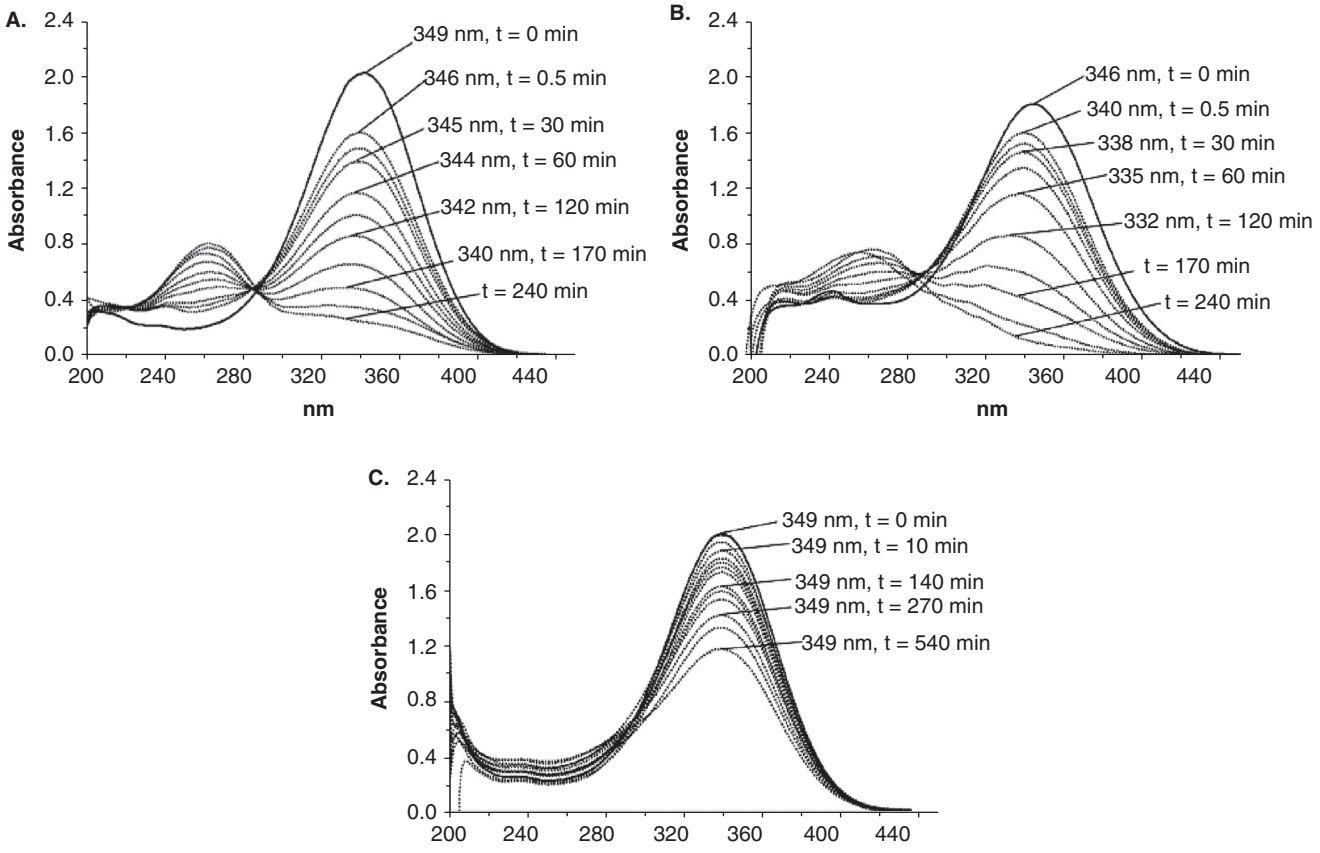

Figure 2. Ultraviolet spectra. A. 35.0 μgmL^{-1} RA in ethanol solution, **B.** 30.2 μg ml^{-1} 13 RA in ethanol solution after different times of light exposure, and **C.** 2.40 μg ml^{-1} RA in liposomes after different times of light exposure [22].

Table 1. Diameter and entrapment efficiency (E%) of tretinoin incorporating liposomes [23].

Composition	E%		Average size (nm ± S.D.)	
	MLVs	ULVs	MLVs	ULVs
P90/SA	71.80 ± 5.1	93.03 ± 1.7	598 ± 67	297 ± 74
P90H/SA	70.01 ± 2.1	91.91 ± 1.9	1163 ± 84	205 ± 53
P90/DCP	97.32 ± 1.8	75.98 ± 2.5	536 ± 49	293 ± 53
P90H/DCP	96.75 ± 2.2	78.53 ± 2.6	993 ± 122	135 ± 56

DCP: Dicetylphosphate; P90: Soy phosphatidilcholine; P90H: Hydrogenated soy phosphatidilcholine; SA: Stearylamine.

guinea-pig dorsal skin. The results highlighted the fact that liposomes consisting of DOTAP, egg yolk PC, and retinoic acid at a molar ratio of 2 : 2 : 1 induced a 3.7-fold increase in the incorporation of the drug into the skin with respect to the egg yolk PC liposomes without DOTAP (4 : 1 molar ratio) and that no significant difference was observed when either DMPC or DPPC was used instead of egg yolk PC as well as when dimethyldipalmitylammonium was used instead of DOTAP.

Similar technological considerations were used to deliver ATRA in systemic tumor therapy. In fact, Kawakami and coworkers realized a cationic liposomal system containing the drug (DOTAP/cholesterol 1 : 1 molar ratio) after which they evaluated its technological parameters and cytotoxicity on nonsmall cell lung carcinoma (NSCLC, A549 cells) with respect to ATRA-loaded conventional liposomes (DSPC/cholesterol 3 : 2 molar ratio) [27]. As can be observed in **Table 2**, the mean size of the two formulations and the drug loading capacity were very similar while the surface charge varied between − 3 and 50 mV. Moreover, the greatest cytotoxic effects on the aforesaid carcinoma cells (resistant to the differentiation phenomena induced by administration of the free ATRA) were obtained after encapsulation of the

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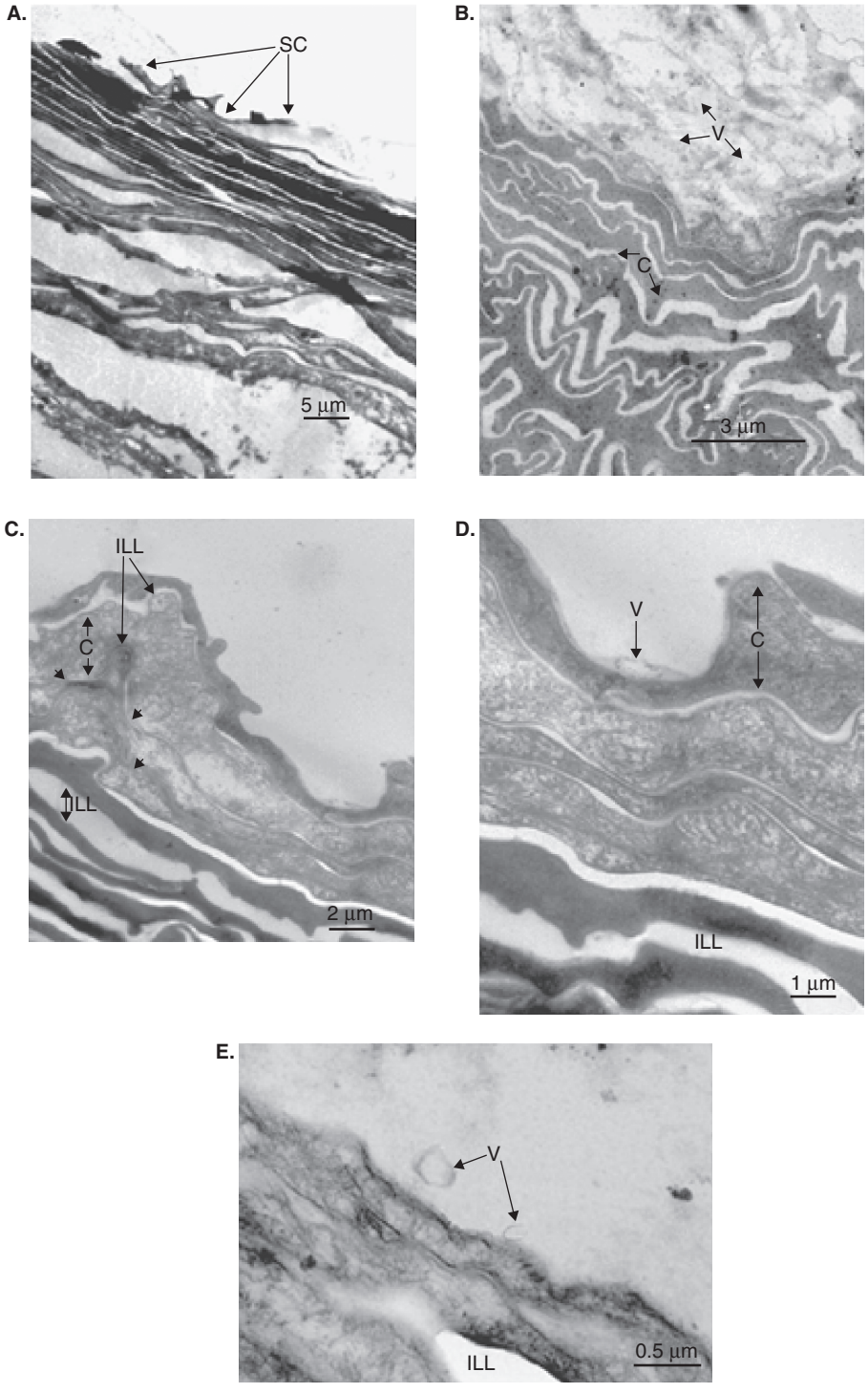

Figure 3. Transmission electron micrographs of pig skin treated with negatively charged liposomes or phosphate-buffered saline (PBS) solution (control) in occlusive conditions. A. Overview of control pig skin incubated for 1 h with PBS. **B.** Pig skin after 1 h of treatment with P90H/DCP liposomes; aggregates of vesicles (V) can be seen intact on the SC surface and corneocytes (C) are swollen and less electron dense than the control, whereas intercellular lipids (ILL) are larger and have an irregular appearance. **C.** Overview of the pig skin treated with P90/DCP liposomes (1 h). Outermost corneocytes are very swollen and not as dark as the control. Cellular cornified envelope (arrows) can also be observed. Inner SC layers show intercellular lamellar lipid regions (ILL1) larger than those of the upper four SC layers. **D.** Same sample as **Figure 4C** at higher magnifications. One liposome is spread on the corneocyte surface and is fusing with it. **E.** Outermost SC after 9 h of treatment with P90H/DCP liposomes. Two liposomes are still adhering to the pig skin surface [23].
C: Corneocytes; ILL: Intercellular lamellar lipid; V: Vesicles.

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lipophilic compound in the cationic system. In particular, at a concentration 1 μM and after 48 h treatment (MTT-test), the DOTAP colloidal ATRA reduced the cell viability by ~ 40% as compared to the free drug; very slight effects were obtained after the drug's inclusion in DSPC-cholesterol vesicles. A possible explanation for this phenomenon could be the better cellular uptake of the DOTAP system; in fact, the use of tritiated ATRA showed that, after 6 h incubation, the greatest intracellular presence of the active compound occurred with the encapsulated cationic vesicles. Moreover, the evaluation of the apoptotic effects of the different ATRA formulations by cytometric flow analysis and DNA fragment extraction highlighted the fact that DOTAP liposomes induced the most relevant DNA mutation. Finally, the TIG3 mRNA expression evaluation (a retinoic-acid-inducible class II tumor-suppressor gene down-regulated in several human tumors and malignant cell lines) showed that mRNA was not induced by free ATRA in A549 cells at a concentration of 1 μM, whereas the amount of TIG3 mRNA increased after treatment with the same drug concentration incorporated into DOTAP-cholesterol liposomes. These observations provided evidence that TIG3 induction by ATRA incorporated in DOTAP/cholesterol liposomes induced cytotoxicity and/or apoptosis in A549 cells.

Following these excellent results, the same research group evaluated the biodistribution of the two types of liposomes containing [^{3}H]-ATRA after injection into the tail vein of ddY mice, and studied their *in-vivo* pharmacological effects on a pulmonary metastasis model after implantation of CT-26 cells in CDF_1 mice [28]. The experiments showed that the lung was the major distribution organ for ATRA loaded in DOTA-cholesterol liposomes regardless of the dose of drug used, suggesting that the distribution profiles of the compound incorporated in DOTAP-cholesterol liposomes were not affected by the dose of ATRA but by the characteristics of the cationic liposomes. This trend explained why the number of lung cancer nodules decreased in mice treated with the cationic formulation (0.585 mg/kg of drug) with respect to those treated with the same ATRA concentration in free [dissolved in 5% HCO-60 (Nicco Chem. Co., Tokyo, Japan)] and DSPC-cholesterol liposomal form (**Figure** 4).

Another procedure for transporting ATRA was based on its encapsulation in liposomes containing a sterylglucoside (SG) mixture made up of β-sitosterol (49.9%), campesterol (29.1%), stigmasterol (13.8%), and brassicasterol (7.2%) [29]. This drug-delivery device was able to efficiently transport the encapsulated drugs to the liver [30], which is why Shimizu and coworkers evaluated the antitumoral effects of ATRA-loaded SG-liposomes in mice with hepatic metastasis of a M5076 tumor. The technological parameters of SG-colloids showed that the carrier mean sizes were about of 170 nm, and that increasing the drug concentrations (from 1 to 9% of total lipid composition) decreased the loading efficiency of the system. After a single intravenous administration, the mice treated with liposomal ATRA (0.585 mg/kg) showed a higher survival rate than those inoculated with the free drug or saline solution. It was suggested that liposomal ATRA was more effective for prolonging survival time than free ATRA because of an increase in activity due to liver targeting, alteration of metabolism rates and decrease of active compound toxicity due to altered pharmacokinetics.

Díaz and collaborators devised another strategy for actively delivering ATRA to the liver a few years after these studies when they realized a liposomal system containing glycolipids (galactosyl-sphingosine) with the aim of utilizing it in the treatment of human hepatocellular carcinoma [31]. The idea behind this strategy is to take advantage of the presence of the asialoglycoprotein receptor (ASGPR), present mainly in hepatocytes, which is also able to recognize low molecular weight glycolipids [32]. Thus, Díaz *et al.* tested ATRA and 13-*cis*-RA-loaded galactosyl-liposomes on two different cell lines, HepG2 and Hep3B, and evaluated its cytotoxicity with respect to the free drug, a mixture of ATRA and galactosyl-sphingosine and a liposomal formulation containing glucosyl-sphingosine (carbohydrate not specifically recognized by ASGPR). The results highlighted the fact that liposomal systems containing the drugs did not improve the cytotoxic effects of the retinoids and the authors explained this phenomenon on the basis of the observation that free hydrophobic drugs permeate plasmatic membranes very rapidly and liposome endocytosis cannot compete in terms of internalization times. In fact, it was demonstrated that retinoid transfer across and between phospholipid bilayers occurs in less than 30 s [33].

Another application of retinoids in experimental tumor therapy was the use of ATRA in the treatment of acute promyelocytic leukemia (APL). In particular, the oral administration of the drug in patients affected by this pathology causes a complete remission of the disease (80% of the total treated subjects). Unfortunately, due to the cytochrome P450 contained in the cellular microsomes, the plasmatic metabolism of the compound is very fast and so it is rapidly eliminated [34,35]. The oral ATRA formulation, Vesanoid® (Hoffmann La Roche, Inc., Nutley, NJ), which is made up of a soft-gelatin capsule containing the drug in a mixture of glycerin and soybean oil, induces the ATRA syndrome as principal side effect (headache, hypertriglyceridemia, nausea, vomiting) and is normally associated with a standard chemotherapeutic cycle because ATRA monotherapy is not able to maintain long-term remissions in the patients with APL [36]. In 1998, Parthasarathy and Metha evaluated the metabolism of free ATRA with respect to a liposomal formulation of the drug made up of DPPC and SA [37]. First, they isolated rat liver microsomes and incubated them with the [^{3}H]ATRA formulations; the quantification of the amount of intact drug after incubation showed that only 38% of the free drug was recovered intact as compared to 58% of encapsulated form. In the same study, the differentiation properties of the compound were evaluated on embryonal teratocarcinoma cells (F9), and great morphological changes were obtained by using both the formulations. The F9 cells were also used

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Table 2. The zeta potential, mean particle size and loading capacity of ATRA incorporated in DSPC/cholesterol or DOTAP/cholesterol liposomesa [26].

Formulations	Zeta potential (mV)	Particle size (nm)	Loading capacity (%)
ATRA incorporated in DOTAP-cholesterol liposomes*	51.2 ± 0.8	132 ± 57.3	97.5 ± 0.38
ATRA incorporated in DSPC-cholesterol liposomes*	-3.11 ± 0.3	115 ± 40.0	95.6 ± 0.42

*ATRA was mixed at the molar ratio of 1:100 of the total lipid content of the liposomes.
Each value represents the mean ± S.D. of at least three experiments.
DOTAP: Dimethyldipalmitylammonium; DSPC: Distearoylphosphatidilcholine.

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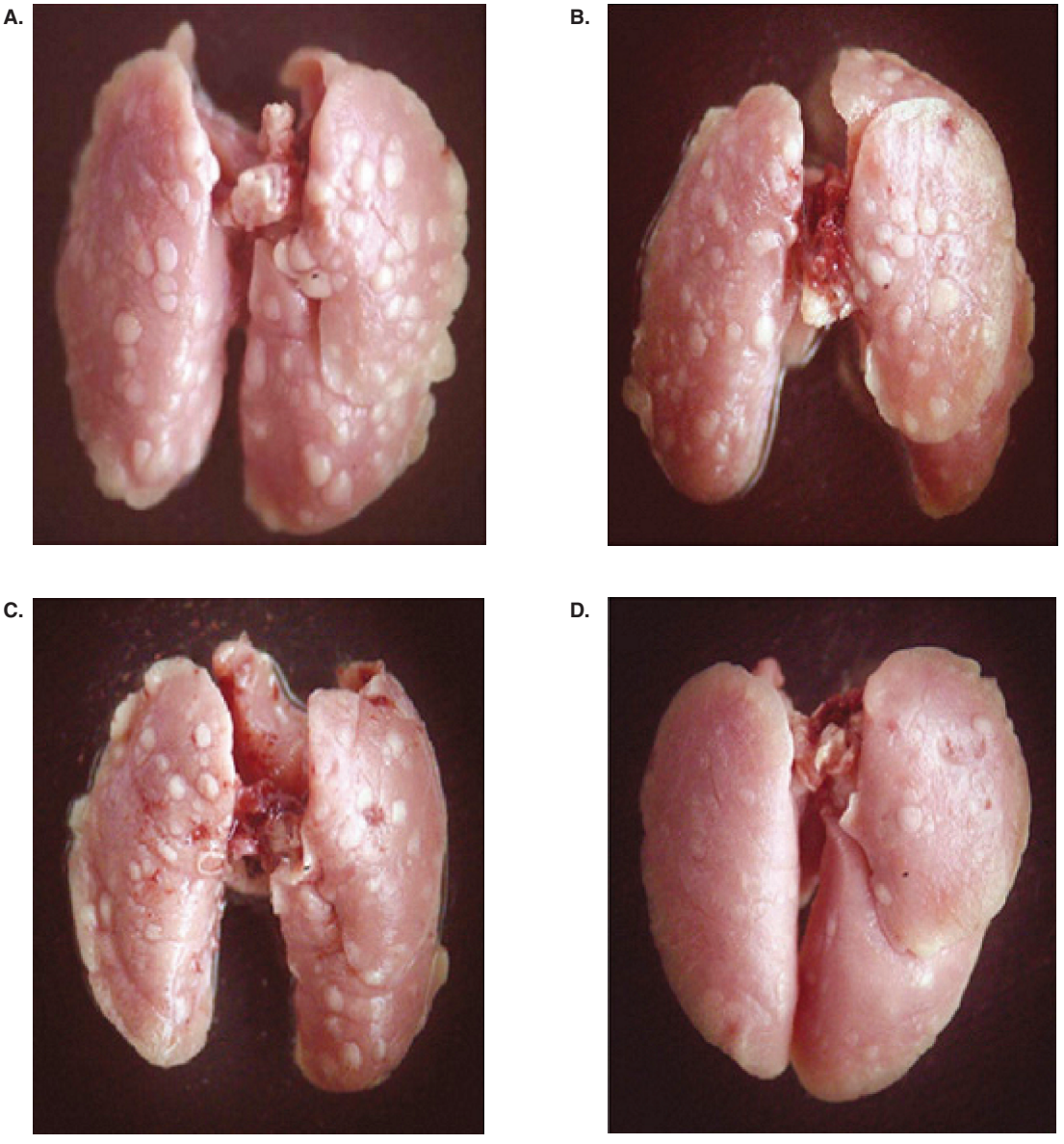

Figure 4. A. Typical morphology of lung treated with saline; **B.** ATRA dispersed in HCO-60; **C.** ATRA incorporated in DSPC/cholesterol liposomes; and **D.** DOTAP/cholesterol liposomes. ATRA was injected at a dose of 0.585 mg/kg weight [28].

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to demonstrate the protective action of the liposomes towards the drug; in particular, the cells were pretreated with 10 μM of liarozolo (an imidazole-compound that inhibits P450-dependent metabolism) for 30 min and then with free ATRA or liposomal ATRA for 6 h obtaining an existence of metabolites of about 55 and 29%, respectively. It is interesting to note that cells untreated with liarozolo but only with liposomal ATRA caused a metabolite amount of ~ 52%.

This study, in association with the aforesaid advantages deriving from the encapsulation of an active compound in a drug delivery system, permitted the use of a commercial intravenous (i.v.) formulation of liposomal ATRA (ATRAGEN®, Aronex Pharmaceuticals, Inc., The Woodlands, TX) on 69 patients with APL without the association of chemotherapy. These patients received the formulation on a daily basis until a complete remission was achieved or for a maximum of 56 days [38]. The dosage used was of 90 mg/m^2 (two-fold higher than that used with Vesanoid) and the results were very encouraging, provoking a visible ATRA syndrome in only 26% of the patients. Other advantages in comparison with the oral ATRA treatment of APL were: i) i.v. ATRA can be administered more reliably to small children who cannot easily swallow capsules; ii) the i.v. formulation would be the only reliable way of delivering ATRA to patients with APL who are unconscious or intubated, as it is extremely difficult to administer the contents of ATRA capsules via a nasogastric tube; iii) absorption of oral ATRA could be less consistent in patients who receive oral ATRA in association with chemotherapeutic agents because it results in vomiting and damage to the intestinal mucosa; and iv) a more stable blood concentration produced by liposomal ATRA may permit more efficient maintenance therapy than that obtained by the use of an oral one. Subsequently, Westervelt and collaborators demonstrated that ATRAGEN and arsenic trioxide did not synergize to produce higher remission rates in immunocompetent APL mice, but the combination slightly improved the long-term survival of SCID mice. This study reinforces the fact that liposomal ATRA is also effective monotherapically [39].

These results were confirmed by the evaluation of the pharmacokinetic profile of ATRA administered after oral (Vesanoid) and i.v. liposomal formulation (ATRAGEN) on healthy patients [40]. Subjects were clinically administered relevant doses of oral ATRA (45 mg/m^2 every day) or L-ATRA (90 mg/m^2 every other day) for 15 days. The quantification of the amount of the drug showed a significant decrease in concentrations of ATRA in plasma after oral administration while a constant quantity of the compound was measurable after i.v. L-ATRA treatment, which suggests that a liposomal carrier may prevent the rapid decrease in plasma drug concentrations and its clearance from the bloodstream. The explanation of the phenomenon was attributed to the fact that liposomal ATRA decreases the metabolism of the drug by the hepatic microsomes in rats that were continuously treated by i.v., while microsomes isolated from animals that received oral ATRA showed a significant improvement in the metabolism of the active compound [41].

The favorable pharmacokinetic profile of liposomal ATRA, together with its pharmacological effect, suggest that this formulation offers potential advantages over oral ATRA and may be highly effective as a monotherapy for the long-term treatment of APL and other retinoid-sensitive tumors [42].

2.2 Niosomes

Niosomes are nonionic surfactant vesicles, formed from the self-assembly of nonionic amphiphiles in aqueous media. Niosomes present a bilayer structure similar to that of liposomes. Niosomes are able to entrap hydrophilic and hydrophobic molecules and can be used as a drug-delivery system. The great availability of surfactants, plus their low cost and stability, has led to the investigation of these colloidal carriers as an alternative to conventional liposomes [43]. In 2002, Fadda and collaborators started a detailed study in an attempt to identify the best niosomal ATRA formulation with the aim of using it in the treatment of skin pathologies responsive to retinoids [44]. In particular, they prepared several vesicle formulations with different techniques (i.e., the film method, sonication, and extrusion) and using different types of surfactants (Span® 40, Span® 60, Brij® 30, Triton® CG 110); in order to carry out an appropriate comparison, tretinoin liposomal formulations made from hydrogenated and nonhydrogenated soy phosphatidylcholine (P90H and P90) were also prepared and characterized. Moreover, all formulations contained cholesterol and dicetyl phosphate (DCP) was also incorporated into the bilayer to prevent aggregation of the vesicles. The results obtained showed that vesicle sizes were dependent on the method of vesicle preparation, the composition of the bilayer and the drug load. Specifically, vesicles prepared by the film method (multilamellar) were always larger than the others with empty niosomes that were smaller than the corresponding empty liposomes. It was interesting to note that tretinoin-incorporated multilamellar vesicles were always smaller than the corresponding empty vesicles and this trend was especially evident in liposomal formulations probably because the amphiphilic drug intercalates into the lipid bilayer, composed of the double chain surfactant and cholesterol. Moreover, none of the ATRA niosomes made up of Span® 60 showed any appreciable difference in size with respect to the empty ones and they were generally larger than Span® 40 niosomes, although the difference was slight. In addition, the drug-loading capacity was very high in all formulations; Span® 60 niosomes evidenced an improvement in the active compound entrapment compared with Span® 40 vesicles, confirming the hypothesis of Uchegbu and Florence that the efficiency capacity may be correlated to the hydrophobicity of the alkyl chain of the sorbitan esters [45]. Moreover, by reducing the carrier dimension by extrusion or sonication techniques, the difference in ATRA entrapment between the two Span® niosomes became more evident. Likewise, the formulations made

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up of Brij® 30 (polyoxyethylene (4) lauryl ether) and Triton® CG 110 (octyl/decyl polyglucoside) also presented an elevated drug entrapment percentage that was not, however, affected by the preparation method used or vesicular mean sizes.

The ATRA release profiles of different formulations were evaluated by means of vertical Franz diffusion cells (Rofarma, Milan) equipped with a silicone membrane (Perthèse®), utilizing a mixture of water-ethanol (50 : 50 v/v) as the receiver medium because of the very low aqueous solubility of the drug. Multilamellar P90 liposomes showed the fastest drug release profile with respect to the other formulations, whereas Triton CG 110 vesicles appeared to possess the most stable bilayers. In addition, large unilamellar Span niosomes and unilamellar Brij® 30 vesicles showed the fastest ATRA release compared to the other formulations.

Subsequently, the same research group studied the stability of ATRA encapsulated in the aforesaid carriers after UV light exposition by a lamp set at 366 nm (Min UVIS, Desaga, GmbH, Germany) [46]. The active compound, in fact, after solubilization in methanol and UV exposition (10 min) degraded rapidly, generating several isomers the most important of which was isotretinoin (HPLC analysis). Moreover, after 1 h of UV light irradiation, only about 40% of the initial concentration was still present, whereas after 8 h the intact compound was ~ 10%. Conversely, ATRA encapsulation in the different delivery systems favored the improvement of its half-life (from 3- to 25-fold). In particular, liposomes were always more efficient than niosomes in the photoprotection of the drug and PL90H vesicles showed a retinoid half-life more than 25 times that of the free drug. Niosomal formulations slightly improved the stability of the active compound (three to seven times longer than the free drug); the best results were obtained with Brij® 30 and Triton® CG 110 niosomes, whereas Span® vesicles showed very little improvement of ATRA stability, probably because of the low stability of the bilayer in which the estereal bond of the monomers can be easily hydrolyzed. The authors evaluated the drug stability after fluorescent light exposition (420 nm) for 21 days. It had been previously reported that ATRA instability is situated around 420 nm and not at the wavelength of its maximum absorption ($\lambda = 350$ nm) [47]. In this case, the best results in terms of ATRA photodegradation inhibition were furnished by Brij® 30 niosomes, which provided a constant degradation value of approximately 6- to 11-fold lower than that obtained after drug solubilization in methanol. The incorporation into P90H and Triton® CG 110 vesicles reduced the ATRA degradation by three- to five-fold with respect to the solvent. It is interesting to note that the ATRA-loaded PL90 formulation had a half-life shorter or very close to that of the free drug, probably due to oxidation phenomena and to the rapid drug release from the carriers that simplify the exposition of the active compound to daylight and air.

A few years later, a series experiments was conducted with the aim of evaluating the *in-vitro* skin permeation and regional distribution of ATRA after encapsulation in the best aforementioned formulations [48]. In particular, different types of alkyl polyglucoside [APGs, i.e., octyl-decyl polyglucoside (Oramix CG 110®, Seppic, Italy; OrCG110) and decyl polyglucoside (Oramix NS10®, Seppic, Italy; OrNS10) and Brij® 30] were used to obtain niosomal systems while P90 liposomes were used for an appropriate comparison. SA and DCP were added to obtain positively or negatively charged vesicular formulations. All systems were formulated in multilamellar and unilamellar forms with two different lipid/TRA molar ratios in order to obtain saturated and unsaturated ATRA formulations, which were both diluted to obtain a treinoin concentration of 0.2 mg/ml. The transdermal permeation profile of encapsulated ATRA was carried out using vertical diffusion Franz cells equipped with newborn pig skin in occlusive conditions and in comparison with a commercial cream formulation of the drug (Retin-A®, Janssen-Cilag, Milan) as a control. After 9 h, the permeated dose of retinoic drug increased in this order: OrNS10 < Br30 < P90 < OrCG110 vesicles both in MLV and ULV formulations (differences statistically irrelevant). Moreover, positively charged vesicles always had a higher permeation rate than the negatively charged ones. In addition, ATRA skin accumulation provided by OrNS10 and Brij® 30 DCP vesicles was found to be higher than that resulting from OrCG110 systems and the control; more specifically, unilamellar formulations showed a better regional drug distribution on the skin probably as a consequence of instability due to their disintegration after contact with skin lipids of the stratum corneum. On the contrary, multilamellar structures, as demonstrated by Verma and collaborators [49], formed a thick lipid layer on the skin that increased the tretinoin diffusion pathway. It was interesting to note that negative vesicles showed the best ATRA skin accumulation, whereas permeation was highly promoted by the cationic ones, as previously reported. In particular, the greatest amount of the drug was accumulated in the stratum corneum skin compartment, through all types of vesicular system.

Furthermore, when nonsaturated ATRA systems were applied to pig skin, similar or lower results in drug permeation and retention were obtained with respect to the control formulation. In conclusion, Professor Fadda's research team evidenced the fact that alkyl polyglucosides represent an interesting class of amphiphiles for niosome formation. Moreover, they are able to improve both transdermal and cutaneous delivery of tretinoin although Oramix NS10® and Brij® 30 vesicles and showed the best cumulative amount of the drug compound inside the skin.

Niosomes were also used to deliver ATRA in the lungs. In particular, different nonionic surfactant associations (i.e., Span and Tween) were combined to obtain a vesicular formulation compatible with a nebulization process with the aim of curing lung cancers [50]. Optimum drug encapsulation and nebulization efficiency were obtained with combinations of Span 20 + Tween 80 and Span 60 + Tween 80 using an ATRA concentration

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of 1 mg/ml. For the aforementioned reasons, SA was added to the formulations but, although it favored a high encapsulation efficiency, it unfortunately caused poor nebulization efficiency. After the aerosolic process, the drug loading capacity was about 70% and 58% for the formulations made up of Span 20–Tween 80 and Span 60–Tween 80 respectively with average mass aerodynamic diameters of ~ 3.5 µm.

2.3 Solid lipid nanoparticles

Solid lipid nanoparticles (SLN) are nanoparticles with a solid lipid matrix presenting an average diameter in the nanometer range. They were introduced in 1991 to overcome the problems deriving from the encapsulation of lipophilic compounds into nanoemulsions, which lose them easily; their particular structure, in fact, allows their drug release to be controlled because its mobility is much slower in a solid lipid than in an oil [51]. General ingredients include solid lipids, emulsifiers, and water. Lipids include triglycerides, partial glycerides, fatty acids, steroids, and waxes. All excipients are generally recognized as safe substances, so a wide variety of compounds can be used for formulation purposes. Considering the physicochemical properties of ATRA, SLNs could be a suitable system for its stabilization. In fact, ATRA-SLN powder was used to protect the compound from damage resulting from light exposure and to improve its pharmacological activity [52]. SLNs (made up of tricaprin as solid lipid, egg phosphatidilcholine, Tween 80 and DSPE-PEG) were prepared by the melt-homogenization method and then lyophilized. The loading capacity of SLN powder was 95 ± 11%, 91 ± 4%, 76 ± 7% and 44 ± 9% when 1, 2.5, 4.5, and 6 mg/g of ATRA were initially added, showing the decrease in the load efficiency with the increase in the initial concentration of the compound. When SLNs were prepared with 2.5 mg/g of ATRA and then freeze-dried, the drug was loaded at the concentration of 2.1 – 0.1 mg/ml and the yield was >90%. Moreover, the powder exhibited a particle size of 246 – 38 nm, a polydispersity index of about 0.190 – 0.025 and a zeta potential of -38 mV after reconstitution in distilled water. The stability tests showed that intact ATRA in a methanol solution decreased rapidly during incubation at room temperature during light exposure (less than 30% after 1 h), whereas the rate of ATRA degradation in 1% Tween 80 solution was slightly slower (30% of intact drug in the1% Tween 80); conversely, ATRA encapsulation in SLN prevented destabilization with a percentage of intact compound (after 7 h) of about 60% and 95% for dispersion or powder, respectively. Moreover, if the samples were stored at 4°C, approximately 50% of ATRA remained intact in the case of 1% Tween 80 solution and SLN dispersion whereas SLN powder showed a great drug stability for 3 months (~ 90% of intact compound). The anticancerogenic activity of free ATRA (solubilized in DMSO) and SLN powder of the drug was evaluated on HL-60, MCF-7, and KB-cells; the obtained results highlighted the fact that there were no significant differences between the two formulations on the three cellular cytotoxic profiles, confirming the hypothesis that the SLN preparation technique did not mutate the pharmacological activity of the compound. Moreover, as intravascular haemolysis was observed in cancer patients after ATRA treatment [53], the two formulations were also tested *in vitro* on red blood cells (RBCs) and it was shown that the ATRA-loaded SLN formulation induced a lower RBC toxicity with respect to the free drug; in particular, the ED_{50} (the concentration of ATRA required for 50% hemolysis) was lower than 20 µg/ml in free form, as compared to > 100 µg/ml in the form of SLN powder.

The technological parameters of ATRA-loaded SLNs already discussed were essentially in agreement with those obtained by Lim and Kim [54]. In particular, the smallest mean particle size of SLNs was obtained with 50 mg/g surfactant mixture composed of 54 : 46% (w/w) egg phosphatidylcholine : Tween 80 (154.9 nm). The addition of a PEG agent, EggPC : DSPE-PEG : Tween 80 (48 : 6 : 46% w/w), allowed for a Z-potential value of -38.18 mV, whereas the presence of 2.4% of ATRA (calculated on the amount of lipid matrix) did not change the stability profile of the formulation. Moreover, the freeze-drying procedure slightly increased the mean size and polydispersity index of SLNs which varied from 181.8 nm and 0.173 to 265.2 nm and 0.200, respectively.

Recently, SLNs containing tretinoin were realized through a solvent emulsification–diffusion technique with the aim of proposing them as a suitable formulation for topical application [55]. Different solid lipids, i.e., glyceryl monostearate (GMS), atomized glyceryl behenate (Compritol 888 ATO), tripalmitin (Dynasan 116), and a mixture of glyceryl stearate, ceteraryl alcohol, cetyl palpitate and cocoglyceride (Cutina CBS), were used to determine the best tretinoin solubilization property. The best result was furnished by GMS, a biocompatible compound that solubilized 10 mg tretinoin in only ~ 78 mg of lipids. In addition, it was shown that the particle size, the polydispersity index, and the drug entrapment efficacy of SLN increased with the improvement in the GMS content in the final dispersion. The ability of the system to protect the drug from light exposure was evaluated by means of the method previously described by Ioele and coworkers [22], obtaining, after 3 h exposure, a residual intact tretinoin of about 70% in the case of SLN but only 12% when the compound was solubilized in methanol. Successively, the tretinoin-loaded SLN was put into gel form using Carbopol® ETD 2020 (Noveon Chemicals, Mumbai, India) and the resulting formulation was compared with a commercial one (Retino-A® cream, Janssen-Cilag, India). This was done to study skin irritation phenomena after topical application on the hair free skin of White New Zealand rabbits. In fact, one of the major disadvantages associated with tretinoin therapy is skin irritation (erythema), which strongly limits its utility and acceptability by the patients. The study showed that the SLN-tretinoin induced less irritation than the marketed drug formulation after just 24 h, and that the inflammation continued to increase over time with the Retino-A® cream formulation

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but did not augment in the case of SLN-based tretinoin gel. In addition, the comparison between the two formulations based on their capacities to promote skin permeation of the drug (by using Franz cell apparatus equipped with rat skin) demonstrated that there was no great difference between the tretinoin permeation profiles. This confirmed the potential advantage in topical delivery of retinoid drugs by SLNs, mostly because of improved patient acceptance.

In the same period, Liu and collaborators realized an isotretinoin-SLN formulation with the aim of obtaining specific skin targeting of the drug [56]. They used the hot homogenization method and Precirol ATO 5 (Gatteffosse, France) and soybean lecithin as the lipid phase. The amount of lecithin, surfactant (Tween 80), and water was varied to obtain five isotretinoin systems with a mean size of about 50 nm, a polydispersity index of ~ 0.2, and a Z-potential of -15 mV. The *in-vitro* permeation studies, carried out using vertical diffusion Franz cells equipped with full-thickness abdomen skin from rats, evidenced the fact that not all SLN formulations favored the transdermal passage of the drug with respect to a control made up of an ethanolic solution of the active compound. The quantification of isotretinoin entrapped in skin showed that only the SLN formulations made up of 3% (w/w) Precirol ATO 5, 4% (w/w) soybean lecithin, and 4.5% (w/w) Tween 80 could significantly increase the accumulative uptake of isotretinoin in skin. This demonstrated that the components of the colloidal systems influenced the accumulation of drug in skin even though the targeting mechanism is unclear.

At last, it is interesting to report a novel SLN formulation made up of highly purified natural solid lipids (stearine fractions), realized by microemulsion techniques and used for the topical delivery of tretinoin [57]. After gelification, it showed lesser skin irritancy, greater skin tolerance, occlusivity and slow drug release with respect to the commercial Retino-A® cream (Janssen-Cilag, India). This work put in evidence the possibility to obtain SLNs by means of low cost lipids that could improve treatment efficacy and patient compliance.

2.4 Polymeric systems

Polymeric micelles are nano-delivery systems formed through self-assembly of amphiphilic block copolymers in an aqueous environment. The three most widely studied block copolymer classes are characterized by their hydrophobic blocks, and are poly(propylene oxide), poly(L-amino acid)s and poly(ester)s [58]. Micro- and nano-polymeric particles are solid colloidal suspensions in which the mean particle size is > 1 µm or < 1 µm, respectively. These systems can be classified in capsules and spheres as a consequence of their morphology; more specifically, spheres are usually characterized by a porous matrix in which the drug are contained, whereas capsules are formed by a core containing drugs surrounded by a shell [59]. Polymeric systems were also used to efficaciously deliver ATRA to hepatocytes. For example, biotinylated poly(ethylene glycol) nanoparticles conjugated with lactobionic acid containing a galactose moiety (BEL) were designed with the aim to taking advantage of the presence of ASGPR on the liver surface cells (see section 2.1) [60]. The particles, which have a mean size of ~ 40 nm, are negatively stained, and are characterized by a spherical shape, showed an ATRA loading content of 36.2 wt% and a prolonged drug release (about 1 month) after a pseudo zero-order kinetic. More recently, ATRA-loaded poly(*N-p*-vinylbenzyl-4-*o*-β-D-galactopyranosyl-d-gluconamide) (PVLA) nanoparticles have been used as hepatocyte-specific targeting candidates [61]. In fact, PVLA is known to interact with hepatocytes because galactose residues in the side chain of PVLA are recognized by liver ASGPR. ATRA-loaded PVLA nanoparticles, prepared by a dialysis method, caused an improvement in the mean size of the micellar system (from ~ 7 nm to ~ 60 nm) and the presence of the retinoic drug was confirmed through ^{1}H-NMR and wide-angle X-ray diffractometry. To demonstrate the effective delivery of PVLA in mouse hepatocytes, cells were incubated for 30 min with empty PVLA, ATRA solubilized in DMSO and ATRA-loaded PVLA. An RT-PCR analysis was then effected, with the aim of evaluating the *CYP26* gene expression induced by the drug (for inhibition of the specific interaction, isolated hepatocytes were preincubated in the presence of 20 mM galactose as a competitive inhibitor). The test proved that expression of mouse CYP26 gene was induced by ATRA as well as ATRA-loaded PVLA nanoparticles in liver cells in the absence of a competitive inhibitor but not preincubated in the presence of galactose (**Figure 5**). At the same time, the interaction between the carriers (stained with fluorescein isothiocyanate; FITC) and hepatocytes was demonstrated by cytometric flow analysis; in particular, it was possible to observe that the fluorescent intensity of hepatocytes stained with FITC-PVLA nanoparticles in the absence of a competitive inhibitor was stronger than in the presence of galactose or the control (**Figure 5**).

Recently, a new method of encapsulating retinol and solubilizing it in an aqueous solution was discovered [62]. Briefly, retinol was solubilized in ethanol and then dropped into an aqueous solution of water-soluble chitosan (MW = 18,000; KITTOLIFE Co., Korea). The formulation was then sonicated, subjected to rotary evaporation, and finally lyophilized. Different retinol : chitosan weight ratio associations were realized, obtaining an increase in particle size (from 70 to 250 nm) and Z-potential according to the increase in drug content. Moreover, the water solubility of retinol was increased over 28 µg/ml according to the incrementation of the concentration of chitosan, indicating that it was improved at least more than 1600-fold since the aqueous solubility of retinol is known to be 0.06 µM. FT-IR (Fourier transform infrared spectroscopy) and 1H-NMR studies confirmed the presence of drug in the particles which was over 60% in all formulations. In addition, it is well known that nanoparticles have to be lyophilized in the presence of lyoprotectant agents such as saccharides to avoid aggregations after reconstitution

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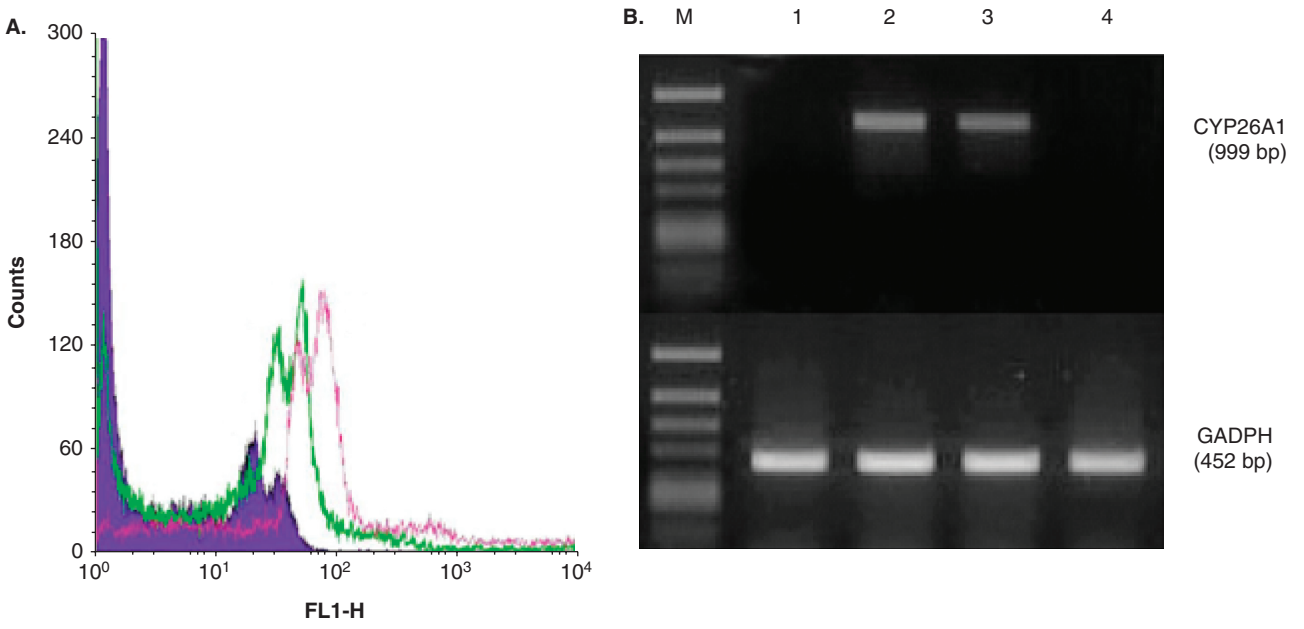

Figure 5. A. Flow-cytometric analysis of hepatocytes. Hepatocytes were untreated (A) or treated with FITC-PVLA nanoparticles for 30 min in the absence (C) or presence of galactose as a competitive inhibitor (B). **B.** RT-PCR analysis of mouse ***CYP26A1*** gene expression in hepatocytes. Hepatocytes were treated for 30 min with PVLA nanoparticles (lane 1), ATRA (lane 2), and ATRA-loaded PVLA nanoparticles in the absence (lane 3) or presence of galactose as a competitive inhibitor (lane 4) [61].

in water; the retinol-water soluble chitosan particles, on the contrary, were easily reconstituted without cryoprotectants and without any variation of either mean diameter or Z-potential of the systems. The stability of the drug was also evaluated by HPLC; in particular, retinol was extracted from the particles by using water/EtOH solvent mixtures and was analysed, obtaining an identical retention time value with respect to the original drug.

Successively, ATRA was incorporated in innovative polymeric micelles made up of the amphiphilic grafted copolymer *N*-phthaloylchitosan-grafted poly(ethylene glycol) methyl ether (PLC-g-mPEG), synthesized using chitosan with four different degrees of deacetylations (DD) (80, 85, 90, and 95%) [63]. The systems were obtained by codissolving PLC-g-mPEG and ATRA in DMSO, putting the mixture in a dialysis bag (membrane : Spectra/Por® 12,000–14,000 MWCO, Spectrum Laboratories, USA) and dialyzing against distilled water overnight (dialysis method). The mean particle sizes of the micellar ATRA ranged from 80 to 160 nm in diameter, which were larger than that of the bare micelles (50 – 100 nm) (**Figure 6**). It was interesting to note that increasing the percentage of DD dramatically increased the amount of ATRA incorporated into the micelles and, in fact, PLC-g-mPEG systems of 90 and 95% DD, with 5% initial ATRA, showed the highest incorporation efficiency (98% yield). This phenomenon could be due to the hydrophobic interaction between the drug and *N*-phthaloylchitosan units and represented a novel incorporating procedure of the bioactives, which does not need chemical conjugation. Moreover, PLC-g-mPEG systems with 95% DD assured a very slow drug-release profile (50% after 96 h) and the best ATRA protection from UV light after 6 h exposition (half-life of about 19 h).

In 2008, tretinoin loaded poly-ε-caprolactone nanocapsules with oil core were realized [64]. The systems, obtained by interfacial deposition of preformed polymers, were prepared by using two different oily phases: capric/caprylic triglyceride mixture (CCM) and sunflower seed oil (SFO). In brief, an organic solution composed of tretinoin, CCM or SFO, sorbitan monooleate, poly-ε-caprolactone, and acetone was added to an aqueous solution containing polysorbate 80 under moderate magnetic stirring. Successively, the acetone was eliminated and the aqueous phase concentrated by evaporation under reduced pressure. Nanoemulsions (previously described formulations without polymers) were prepared with the aim of evaluating the influence of poly-ε-caprolactone. A technological parameter evaluation showed that drug-loaded nanocapsules and nanoemulsions had similar mean diameters (210 – 230 nm), acidic pH values (5.60 – 6.90), and negative Z-potentials (between -4.45 and -8.40 mV), as well as polydispersity indices below 0.25, indicating an adequate homogeneity of these systems; moreover, the active compound loading capacity of carriers was > 99% (0.5 mg/ml). The stability of tretinoin solubilized in methanol or encapsulated in nanosystems was appraised by exposing them to UV light for 1 h and it was shown that the half-life ($t_{1/2}$) of free drug solubilized in organic solvent was about 40 min, whereas that of tretinoin nanocapsules and nanoemulsions was ~ 90 and ~ 80 min, respectively. The improved drug $t_{1/2}$ in formulations

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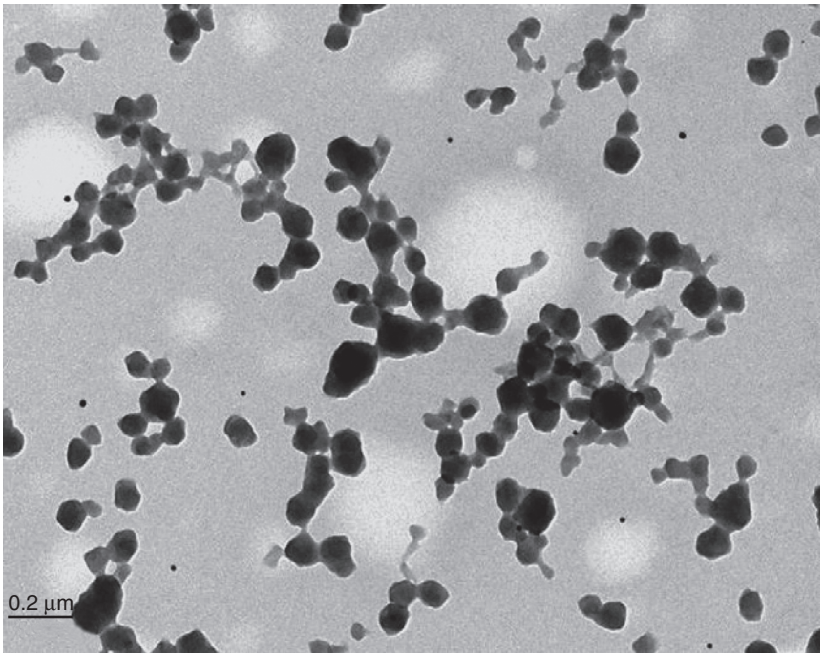

Figure 6. TEM micrographs of all-*trans* retinoic acid (ATRA)-loaded polymeric micelles forming from *N*-phthaloylchitosan-grafted mPEG polymer (magnification 50,000×) [63].

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containing the polymer was attributed to the ability of the poly-ε-caprolactone to reflect and scatter UV light.

2.5 Other carriers

Although the principal drug-delivery systems used to modulate the solubility, stability, and pharmacological parameters of the retinoid drugs are essentially represented by vesicular and polymeric colloidal systems, over the last decade other carriers have been used. For example, ATRA-microemulsion systems, made up of distearoylphosphatidylethanolamine-*N*-poly(ethyleneglycol) 2000, phosphatidylcholine, and soybean oil, were realized with the aim of obtaining a parenteral formulation of the drug [65]. For this purpose, pharmacokinetic tests were conduced on male Sprague–Dawley rats inoculated with free ATRA sodium salt (containing sodium hydroxide; NaOH) and drug-loaded microemulsion formulation. The plasmatic concentration showed that there was no difference between the two samples; the advantage of the lipidic system derived from the consideration that sodium ATRA in NaOH solution cannot be a pharmaceutically acceptable parenteral formulation due to its irritability. At the same time, the antitumoral effect of free ATRA (solubilized in DMSO) and microemulsion containing the active compound on HL-60 and MCF-7 cells was investigated and, also in this case, there was no great variation between the two formulation in terms of dosage as far as cytotoxic effects were concerned.

Another way of modifing biopharmaceutical properties of retinoids is their complexation into cyclodextrins, a family of cyclic oligosaccharides composed of α-(1,4) linked glucopyranose subunits that are able to carry out chemical reactions that involve intramolecular interactions where covalent bonds are not formed between interacting molecules, ions, or radicals [19]. Recently, 13-*cis*-retinoid acid (13-*cis*-RA) was complexed in α (α–CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) [66]; in particular, drug/cyclodextrin inclusion complexes in the respective phosphate buffer solution (pH = 5.9, 7.4, 8.1) were prepared by adding an excess of 13-*cis*-RA (10 mg/ml) to different concentrations of cyclodextrin solutions (HP-β-CD: 0, 0.01, 0.02, 0.03, 0.05, 0.10, 0.15, 0.20, 0.30 M; α-cyclodextrin: 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.08, 0.10, 0.12 M), and shaking the obtained suspension for 8 days. The products obtained demonstrated that the apparent solubility of the active compound in the presence of HP-β-CD increased more than that in the presence of α-CD at all pH values, probably because the cavity of HP-β-CD is more suitable to include 13-*cis*-RA molecule than that of α-CD. This hypothesis was also supported by inclusion-docking studies on the molecule (**Figure** 7). Moreover, at low concentrations of HP-β-CD, a 1 : 1 inclusion complex is formed, whereas at high cyclodextrin concentrations, the formation of a 1 : 2 (drug/CD) inclusion complex is favored. Stability studies effectuated on 13-*cis*-RA solubilized in methanol or complexed with HP-β-CD, after exposition of the samples to a light source (three fluorescent tubes of 6 W each), showed that the degradation constant of the drug contained in the CD cavity was more than seven times lower than that of uncomplexed 13-*cis*-RA.

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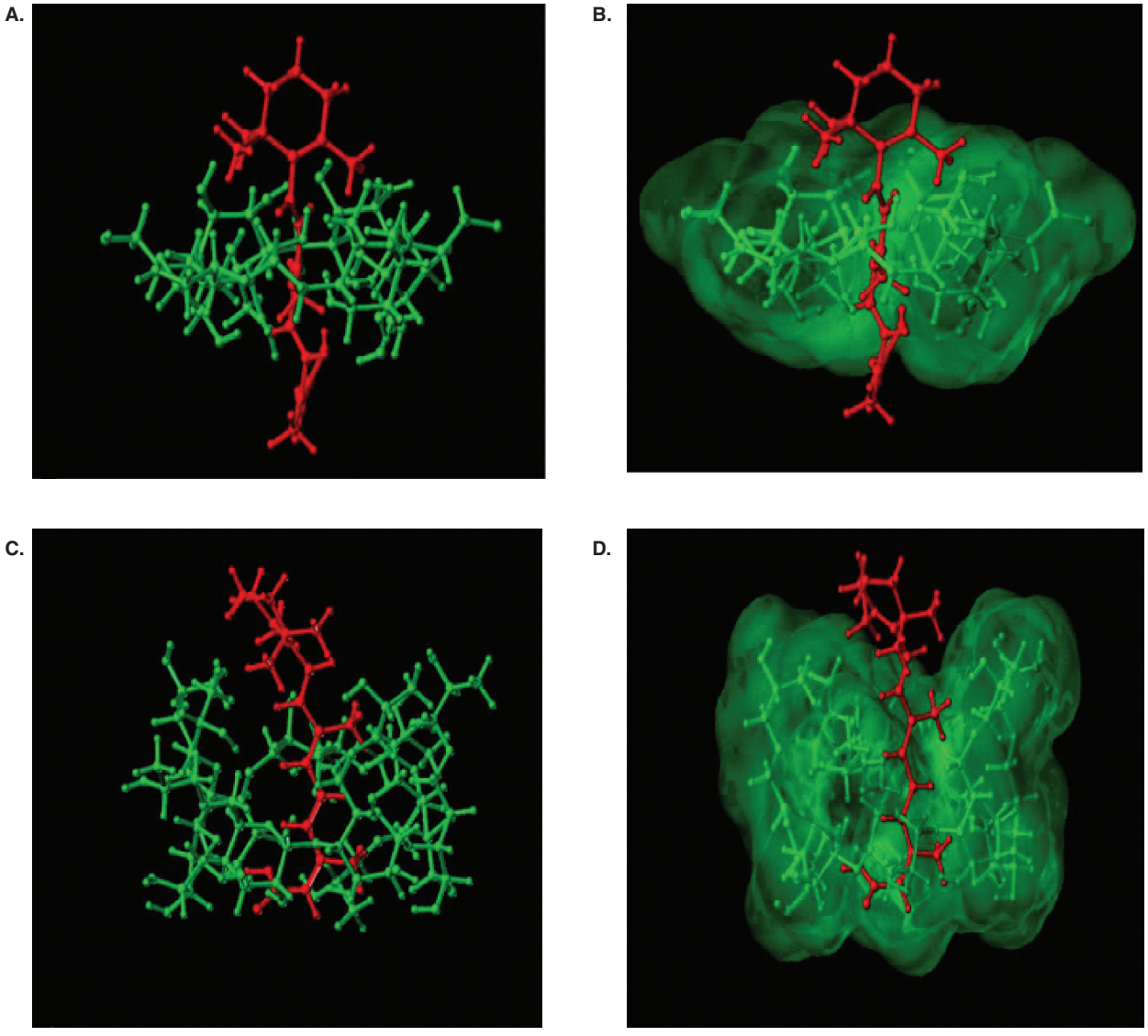

Figure 7. Modeling of docking of 13-*cis*-RA. In α-CD: (**A**) without and (**B**) with electron surface. In HP-β-CD: (**C**) without and (**D**) with electron surface [66].

Retinoic-acid-loaded β-CDs were used to treat acne vulgaris with the aim of reducing the characteristic side effects such as erythema, xerosis, and burning sensation of the commercial formulation [67]. Patients affected by the pathology were treated for 3 months with retinoic acid contained in a commercial product, in a β-CD complex in a hydrogel base and in a β-CD in a moisturizing base and the best results in terms of acne regression and reduction of side effects were furnished by the drug complexed in the cyclodextrin formulations.

Another example of an innovative formulation of ATRA for external treatments of photodamaged skin was realized, favoring the nucleation of inorganic minerals around the drug molecule and obtaining novel ATRA micelles. In more detail, the systems were prepared using boundary-organized nanoscale reaction droplets: initially, ATRA was mixed with a solution of Tween 80 and NaOH to obtain a stable suspension of drug sodium salt. Successively, $CaCl_2$ (Ca^{2+} was attracted by the negative surface of the suspension) and Na_2CO_3 were added, obtaining the formation of a $CaCO_3$ shell [68]. ATRA micelles contained in Vaseline® cream were tested on ddY mouse epidermis for 4 days to evaluate their efficiency in treating hyperpigmented lesions, and were then compared with the effect obtained with a Vaseline® cream of free drug. Histological examination revealed a more than two-fold increase in epidermal thickness than when using ATRA Vaseline® cream, and an overproduction of hyaluronan and increased expression of mRNA for heparin-binding epidermal growth factor-like growth factor (HB-EGF) from keratinocytes (HB-EGF is known as one of the hallmarks of turnover of keratinocytes).

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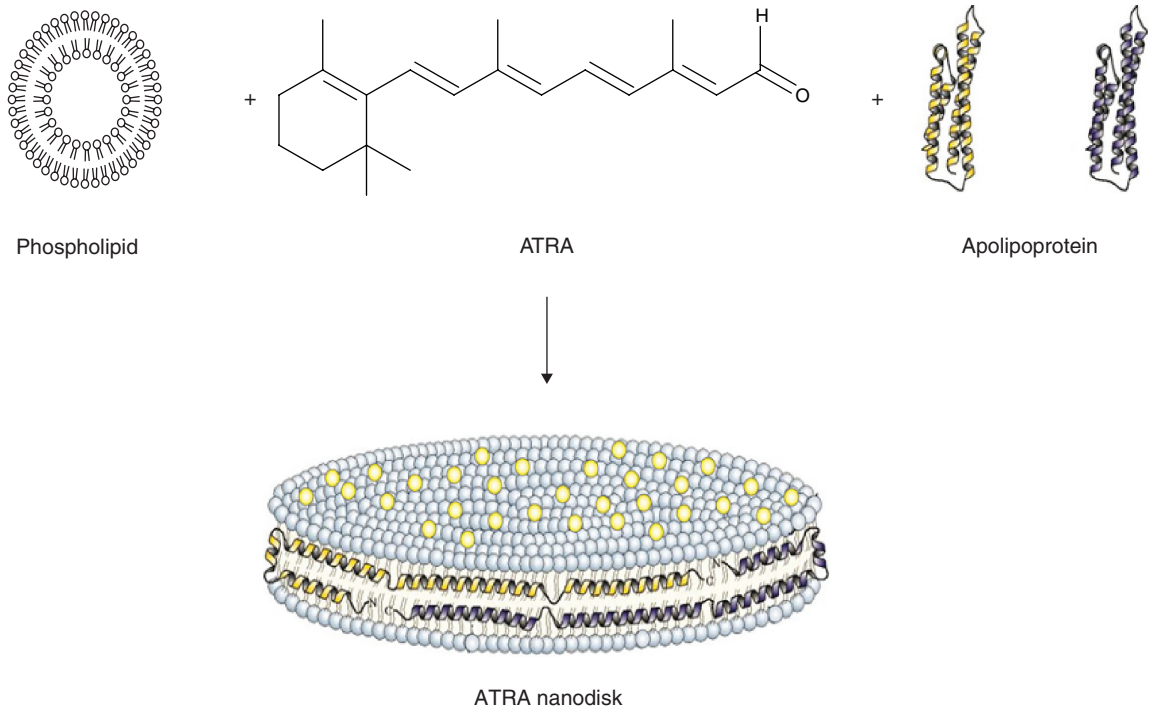

Figure 8. ATRA-ND formulation scheme and structure model. Phospholipid vesicles, ATRA, and apoE3-NT were combined to form ATRA-ND. Lipid interaction converts apoE3-NT from a soluble four-helix bundle into an open, extended, lipid-bound conformation. ATRA-ND comprise a disk-shaped phospholipid bilayer in which ATRA molecules (yellow dots) are integrated. The edge of the ND bilayer is stabilized by the apolipoprotein 'scaffold'. Component images are not to scale [69].

Finally, it is interesting to note the idea of a formulation of nanodisks (ND) containing ATRA [69]. These are nanoscale, disk-shaped, phospholipid bilayers, stabilized by association of apolipoprotein molecules (**Figure 8**). In this case, they were made up of dimyristoylphosphatidylcholine, dimyristoylphosphatidylglycerol, and apoE3-NT. In particular, the lipid/drug molar ratio was of 5.5 : 1. The mean sizes of the systems were about 8 – 20 nm and the efficiency of drug entrapment was higher than 85%. ATRA-NDs were tested on cultured HepG2 human hepatoma cells and compared with free drug, with the aim of evaluating the variation of the cytotoxicity of retinoids. It was shown that ATRA-ND inhibited hepatoma cell growth more effectively than the free compound. Further studies are in progress to demonstrate whether the phenomenon is related to an improvement of the drug uptake into the cells.

3. Conclusion

The use of innovative drug-delivery systems represents a development in retinoid-based therapy. Commercial products, such as ATRAGEN (which received orphan drug designations from the USA FDA for T-cell non-Hodgkin's lymphoma and acute and chronic leukemia [70]), showed an improvement of the biopharmaceutical properties of the drug and a reduction of the most important side effects. Moreover, the possibility of modulating the technological parameters of the described carriers allows the targeting of retinoid compounds in most of our body compartments.

4. Expert opinion

Retinoids are an important class of drug characterized by a chemical instability and severe side effects. An innovative way to avoid the destabilization phenomena of these drugs is represented by their encapsulation/complexation in different drug-delivery systems. In particular, the use of liposomes permits us to reduce drastically the photodegradation of unstable retinoids. The versatility of this kind of carrier is really useful; in fact, the use of cationic liposomes guarantees an increased activity both when applied topically and systemically.

SLN present good characteristics both in loading capacity (depending on the amount of added drug) and in photostability. The possibility of obtaining a targeted carrier by means of modified polymers completes this formulative aspect. It is our opinion that the choice of a suitable carrier, the exact formulation, and the realization of the drug-delivery system will result in a noticeable improvement in the therapeutic use of this class of drug.

Declaration of interest

The authors declare that they have no conflicts of interests concerning the preparation of this manuscript.

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