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Vitamin C Based Nanostructures: Potential Utility in Ocular and Transdermal Therapy

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REVIEW

Alkyl vitamin C derivatives (ASCn) combine in their structure a lipophilic and a hydrophilic moiety and exhibit properties of typical surfactant molecules. Self-assembly properties of ASCn depend on the length of *n*-alkyl fatty chain. ASCn start to aggregate at temperatures (CMT, Krafft point) at which the solubility reaches the critical micellar concentration (CMC). Above this temperature, ASCn can aggregate in micelles or gel phase, depending of alkyl side chain. Upon cooling, for less soluble derivatives (ASC12, ASC14 and ASC16) liquid–crystal structures named coagels are obtained. They are able to solubilize insoluble and unstable drugs, protect them from any possible aggressive environment and promote their permeation through skin and mucosa. These systems possess very interesting properties making ASCn coagels promising pharmaceutical platforms for drug delivery. Results from investigations about all these properties will be described and analyzed in the present review with particular emphasis on the use of these systems for drug administration through ocular and transdermal routes.

Keywords:

CONTENTS

1. INTRODUCTION

The use of active surface compounds (surfactants) in pharmaceutical technology has been widely explored. They are commonly utilized as emulgent, solubilizing agent, suspension stabilizers, wetting agent, etc. In these cases, they are incorporated in the formulation in relative low concentrations where surfactant molecules remain disaggregated in a subcolloidal estate at the interface. However, depending on chemical structure, concentration and temperature, surfactants are able to form supramolecular aggregates with very particular properties when the concentration is raised.[1]

Solubility as well as molecular size defines the self assembly characteristics of the surfactants. It is well known that surfactants at determined concentrations (minimal aggregation concentration) begin to form aggregates as consequence of the increment of interactions between molecules. As concentration is raised, the interactions between adjacent structures are increased leading to the coalescence of the system in larger structures usually denominated liquid crystals. The formation of such structures can be evidenced through noticeable changes in viscosity, conductivity, birefringence and X-ray diffraction patterns. The different liquid crystal systems formed from surfactant-solvent interactions are defined as lyotropic liquid crystals (LLC).[2]

Polar lipids have been widely investigated as lyotropic liquid crystal precursors. Among the most studied compounds are fatty acid esthers such as monoglycerides and glycerates.[3, 4] In recent years, LLC systems have received considerable attention because of their high potential as drug vehicles. Among these systems, reversed cubic, hexagonal and lamellar mesophases are the most important and they have been extensively investigated for their ability to sustain the release of a wide range of bioactives, from low molecular weight drugs to proteins, peptides and nucleic acids.[5] Besides, these systems have shown promissory pharmaceutical performance for drug administration through different routes.[6–9]

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From several years ago we have been studying a group of polar lipids consisting of alkyl vitamin C derivatives (ASCn).[10–14] Their amphiphilic nature allows these compounds to form aggregates, mainly lamellar mesophases. By mean of these studies we were able to evaluate the potential utility of this new liquid crystal system, which has evidenced very interesting properties as pharmaceutical carrier. In this review, we focus on the description of the general properties of these systems and the results concerning their potential use for drug administration through ophthalmic and dermal routes.

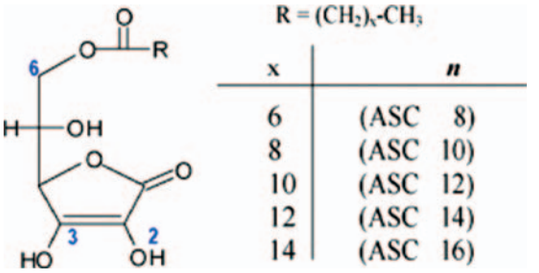

Fig. 1. Schematic chemical compositions of 6-O-alkyl ascorbic acid derivatives. Reproduced with permission from [24], S. Palma, et al., *Il Farmaco.* 58, 1271 (**2003**). © 2003.

2. CHARACTERISTICS OF ASCn

2.1. Physicochemical Properties

Ascorbic acid (AA) is one of the most powerful natural antioxidants but due to its poor solubility in hydrophobic media, its usefulness is limited to aqueous environments.

Ascorbyl-6-O-alkanoates (ASCn) retain the same radical-scavenging properties of ascorbic acid and their antioxidant efficiency is comparable to other natural reducing agents, such as carotenes, polyphenols, and tocopherols.[15] ASCn are obtained through the estherification of hydroxyl group in position 6 of AA with fatty acids of variable length chain (Fig. 1).

The ASCn-water systems give liquid crystals on heating, which, on cooling, become gels with lamellar structure that exhibit sharp X-ray diffraction patterns and optical

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Palma (38 years old), who trained in pharmacy, teaches Pharmaceutical Technology and Community Pharmacy at the Faculty of Chemical Sciences, National University of Córdoba, Argentina. Since 2005, Professor Palma is Scientist Researcher of National Scientific and Technical Research Council (CONICET). His research activity has resulted in the publication of over 35 original refereed scientific articles and more than 95 contributions at conferences. Currently, his interest is directed towards the conception of new pharmaceutical platforms for drug delivery.

Gabriela Ullio Gamboa (26 years old) graduated as a pharmacist in 2009 and is currently studying for a Ph.D. at Chemistry School (National University of Cordoba-Argentina). She has fellowships for research activities from the Scientist Researcher of National Scientific and the Technical Research Council (CONICET). Her teaching activities involve lecturing, as well as practical classes, on the subject of Pharmacotechnia II at Pharmacy school. She is a member of a research team, directed by Professor Daniel Allemandi, which aims to develop new pharmaceutical platforms for drug delivery. Her subjects of research are nanotechonology and nanomedicines, covering both the development of encapsulation methods for active ingredients and drug targeting.

Daniel Allemandi (50 years old) graduated as a pharmacist in 1985 and Ph.D. in Pharmacy in 1993, at the National University of Cordoba-Argentina. He is Full Professor and researcher of the National Research Council (CONICET). His teaching activities at graduate and postgraduate level involve lecturing in topics related to Pharmaceutics and Biopharmaceutics. He is director of a team whose subjects of research are novel modified drug delivery systems with emphasis in nanoparticles and localized drug release. His work is supported by several grants provided by national scientific agencies. He is author and co-author or numerous papers and scientific articles concerning his expertise area.

birefringence (see next section). The semisolid consistency of such gels is an interesting property in order to formulate pharmaceutical dosage forms able to solubilize and stabilize different drugs for dermatological use. Therefore, these amphiphiles combine the powerful antioxidant properties of ascorbic acid with the capability of producing supramolecular aggregates.

The synthesis of ASCn was reported elsewhere.[16] The variation in side chain length has a direct influence on their physico-chemical properties, for example the melting point increases with the side chain length.[10] The pKa values for OH(3) and OH(2) are 4.2 and 11.6, respectively. Therefore, ascorbyl-6-O-alkanoates behave as anionic surfactants in pure water.[17]

Their amphiphilic nature allows these vitamin C derivatives to form aggregates (coagel) that provide an ideal environment for the solubilization of hydrophobic and sensitive drugs that might be otherwise easily degraded and oxidized when exposed to light, heat, dissolved oxygen, and other radical-producing species.

2.2. Phase Behavior and Aggregation Properties

At room temperature the water solubility of alkanoyl-6-o-ascorbic acids (ASCn) is poor, except for the short chained ASC8.[18–20] Their solubility increases with temperature, so that depending on the side chain length, the surfactants form clear micellar dispersions above a critical micellar concentration (CMC) and at temperatures higher than their critical micellar temperature (CMT).[21] On cooling, a solution of ascorbyl alkanoate produces an opaque curd.[22] Such a phase is typically termed semicrystalline mesophase, poorly hydrated crystal, or more usually *coagel.*

These supramolecular assemblies, in which the surfactant molecules are arranged in closely packed lamellar structures, undergo either a coagel-to-micelle or a coagel-to-gel phase transition depending on the aliphatic side chain length, as depicted in Figure 2.

The water in the system is in three different states.[23] The first hydration layer is composed of about 11 water molecules per surfactant molecule which are strongly attached to the oxygen and the hydrogens of the –OH groups of the polar headgroups by hydrogen bonds and are not detectable by DSC, included in a 3 Å thick layer. The second hydration layer is formed by about 50–60 water molecules per surfactant one, which are associated with that of the first hydration layer and are then affected enough by the presence of the polar headgroups to have a different melting peak from that of bulk water. The second hydration layer is extended up to 9 Å from the polar group. Water not included in these two categories behaves as bulk water.

2.3. Rheology of Nanostructured Aggregates

The semisolid system formed from ASCn self-assembly shows very particular rheological behavior.[24] ASC8, ASC12, ASC14, and ASC16 coagels show a complex rheology, with the appearance of spur rheograms, while coagels of ASC10 and ASC11 exhibit pseudoplastic flow. ASC11 also shows thixotropy.

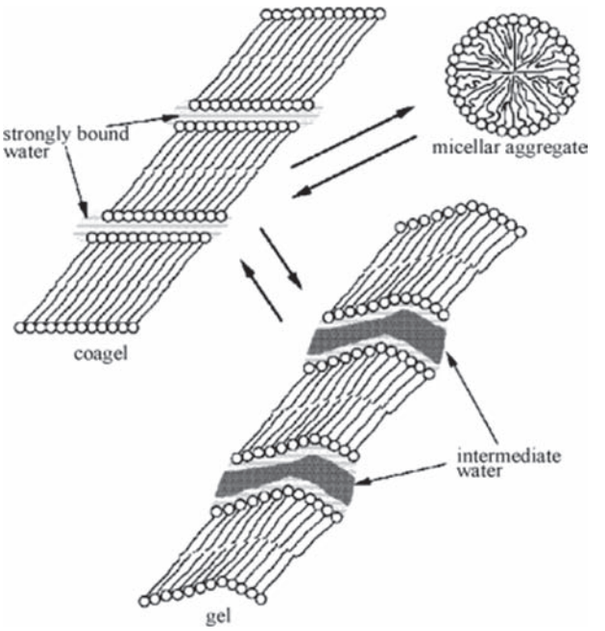

Fig. 2. Schematic picture of a coagel in equilibrium with a micelle or with a gel phase. Reproduced with permission from [10], S. Palma, et al., *Langmuir* 18, 9219 **(2002)**. © 2002.

Coagels of ASC12, ASC14 and ASC16 form a 'house of cards' structure, where swelling and strengthening of the semisolid network occurs, due to the presence of water pools between the amphiphilic bilayers. On the other hand, for ASC10 and ASC11 coagels, this kind of arrangement apparently is not permitted and flexible bimolecular sheets arrange parallel to each other.

When a spur value is reached, ASC12, ASC14 and ASC16 coagels acquire pseudoplastic flow. In this way, according to the handling of the semisolids, the rheological properties of the system can change.

3. OCULAR DRUG DELIVERY SYSTEMS

Topical application on the eye's surface is the common method of drug administration to treat ocular diseases. However, a pharmaceutical carrier used for this objective has to be formulated according to the disease nature, as different pathologies may affect the external (conjunctivitis, blefaritis, dry eye syndrome, etc.) or internal (uveitis, endophthalmitis, glaucoma, etc.) segments of the eye.

It has been reported that the intraocular bioavailability is very low ($< 5\%$) after topical application, mainly due to the particular characteristics of the corneal epithelium. Since precorneal area receives most of the administered drug, it is expectable that high systemic absorption through

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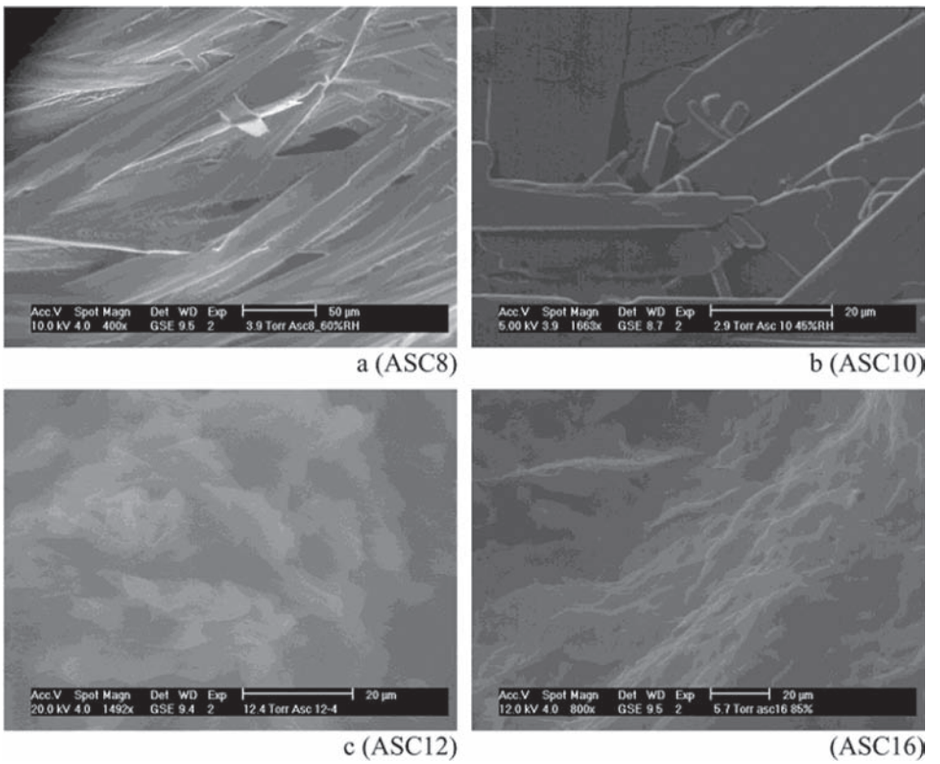

Fig. 3. ESEM pictures of ASCn. (a) ASC8, 400×, RH 60%, 3.9 Torr. (b) ASC10, 1663×, RH 45%, 2.9 Torr. (c) ASC12, RH 70%, 12.4 Torr. (d) ASC16, 800×, RH 85%, 5.7 Torr. Reproduced with permission from [10], S. Palma, et al., *Langmuir* 18, 9219 **(2002)**. © 2002.

conjunctiva and nasolacrimal duct may occur.[25] The cornea is practically impermeable and, therefore, behaves as a very efficient barrier against chemical compounds.

In response, different strategies have been developed to improve drug permeation through this membrane. Among these, the use of absorption enhancers is perhaps one of the most effective.[26]

Regarding usual ocular pathologies, glaucoma involves progressive optic nerve damage associated with the loss of visual function and is frequently related to elevated intraocular pressure (IOP).[27]

Such pathology is usually treated with the administration of different classes of topical medications such as prostaglandin analogs (latanoprost, travoprost, and bimatoprost), selective β-adrenergic agonists (apraclonidine, brimonidine, and clonidine) and carbonic anhydrase inhibitors (CAIs; brinzolamide).

Nowadays, acetazolamide (AZM), a CAI, can be orally used for the reduction of IOP in patients suffering from glaucoma, in the preoperative management of closed-angle glaucoma, or as an adjuvant therapy in the treatment of open-angle glaucoma.[28]

However, in order to obtain the desired lowering in IOP, large oral doses of AZM have to be administered, and can lead to a wide range of side effects that usually appear due to the extensive distribution of carbonic anhydrase in the body's organs.

These deleterious systemic side effects of AZM could be avoided if AZM is topically administered to the eyes. Nevertheless, the poor aqueous solubility (0.7 mg/mL) and low corneal permeability (4.1×10^{-6} g · cm/s) limit the ocular bioavailability of AZM.[29]

In a recent work[30] we reported the results concerning the potential utility of ASCn coagels for AZM ophthalmic administration. These systems were evaluated in terms of their *in vitro* permeability (isolated cornea), pharmacological effectiveness (IOP reduction in normotensive rabbits) and potential irritant effects.

Besides low permeability, the poor solubility of AZM in water is also a limiting factor for drug absorption. Regarding this, the lamellar structure of coagels may increase the amount of solubilized drug in the system. The highest AZM solubility (> 0.5% W/V) was observed for 2% ASC12 coagels, and in comparison to ringer's solutions, the apparent solubility of AZM was increased 4-fold.

Based on these results, for permeation studies, we used 2% ASC12 coagel containing AZM at 2 different concentrations (0.1% and 0.4%) aiming to evaluate the influence of drug concentration on the therapeutical response. Then, bearing in mind the pharmaceutical requirements for ophthalmic formulations such as tonicity and pH, the coagels were formulated using glucose isotonic solutions as vehicle.

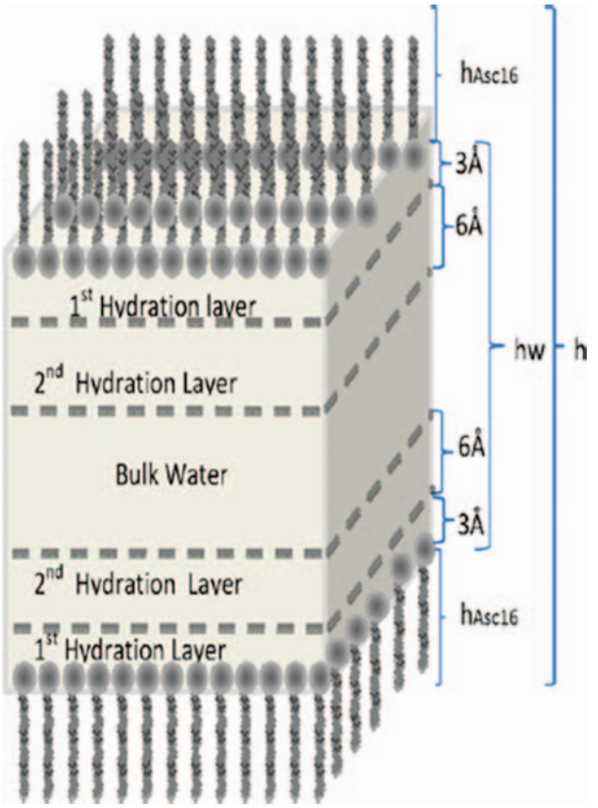

Fig. 4. Structural model of the hydrated aggregates (crystals and lamellar mesophases). Reproduced with permission from [23], L. Benedini, et al., *Colloids Surf., A: Physicochemical and Engineering Aspect.* 375, 178 **(2011)**. © 2011.

The formulations obtained were slightly hypo-osmotic, although in compliance studies on animals, we detected neither discomfort nor histological evidence of mucosa damage. The low pH observed may be aggressive for ocular mucosa. However, the normal pH value of lacrimal fluid was rapidly restored (about 30 s) after administration of AZM coagel in rabbits, without any detectable side effects (unpublished data).

3.1. *In Vitro* Drug Release

This assay was performed in order to evaluate the effect of coagels on AZM release. Taking into account that a drug permeable membrane was used, the observed release kinetic should only be attributed to the influence of coagels on drug diffusion.

ASC12 coagels containing AZM showed to be able to modulate drug release (Fig. 6). In both cases (0.1% and 0.4%), a near-zero-order release was observed. As expected, the drug release was higher for AZM 0.4%.

3.2. Evaluation of AZM Transcorneal Permeation

The corneal epithelium is less permeable compared with other epithelial tissues (intestinal, nasal, bronchial, and tracheal), although it is more permeable than the stratum corneum.[31] The results concerning AZM permeation through the cornea are presented in Figure 7. In these assays, we also evaluated the permeation of AZM suspended in ringer's solution (AZM-R) and the marketed brinzolamide drug product AZOPT®. This marketed formulation containing 1% w/w of brinzolamide, according to the information provided by the producer (Alcon Laboratories), was used as a reference. Although this latest is a newer and lesser potent drug compared to AZM, nowadays is the selected drug for first instance treatment of glaucoma.

AZM-loaded coagels were able to promote AZM permeation, which was higher compared to ringer solution. This increase in permeation was proportional to AZM concentration according to the measured AZM steady-state flux (J) and apparent permeation coefficient (P_{app}) (J = 1.43 mg/min and $P_{app} = 3.04\ cm \cdot s^{-1}$).[31]

In this way, for AZM 0.1% the transcorneal permeation was thrice higher than AZM-R for the same drug concentration. This increase was even higher in the case of AZM 0.4% coagels, mainly due to the higher amount of AZM available for absorption. In the case of AZM-R, drug permeation was very low as consequence of the physicochemical properties of AZM. Similar results were observed with brizolamide (AZOPT®), although this drug is quite different to AZM. Therefore, based on these results, preliminary *in vitro* permeation studies have demonstrated that coagels could be an advantageous drug delivery system for the ocular administration of AZM.

3.3. *In Vivo* IOP Measurements

In order to evaluate the possible correlation of coagels behavior observed *in vitro* with the *in vivo* effect, the variation of IOP in rabbits after formulation administration was measured.

In Figure 8, we show the results concerning IOP diminution after the administration of the formulations under investigation. In the case of coagels containing AZM, the hipotensor effect (HE) was proportional to AZM concentration, whereas for AZM-R, this correlation was practically negligible. It was possible to appreciate the maximum difference in HE (24.84%–2.02%) 2 h post-administration for AZM 0.4% coagels in comparison to AZM-R. This implies that the effect of a higher concentration alone does not seem to be sufficient to explain this observation, as AZM-R is a solution where AZM is immediately available for absorption. Nevertheless, the corneal permeation and HE were both lower.

With regard to HE of AZOPT, this was similar to that observed for AZM 0.4% coagel during the first 2 h post-administration. However, this effect rapidly diminished for AZOPT after 2 h, whereas for AZM 0.4% coagel the HE reached a maximum at 2 h and remained at similar values during 3 h.

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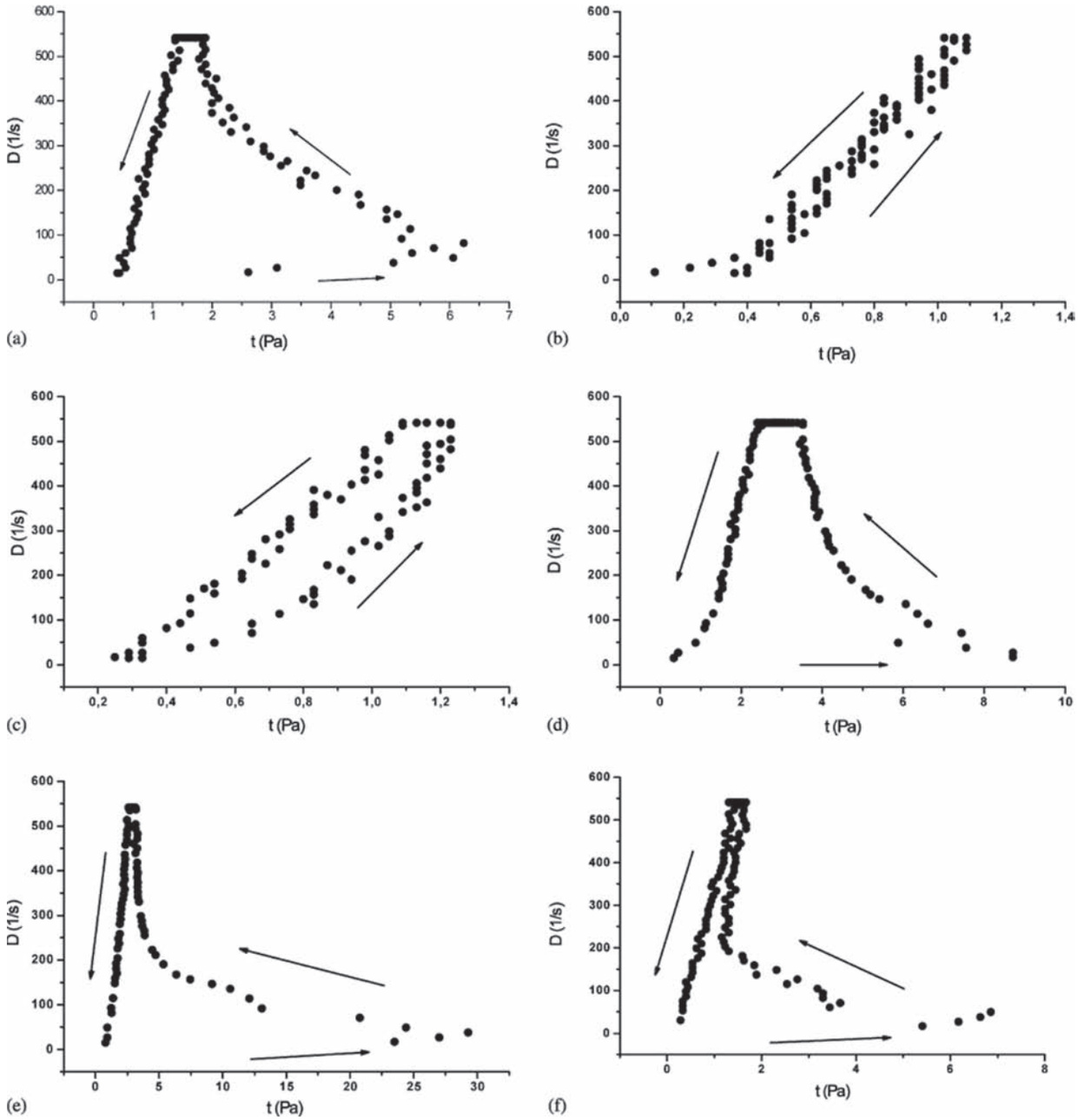

Fig. 5. Rheograms for ASCn coagels: (a) ASC8; (b) ASC10; (c) ASC11; (d) ASC12; (e) ASC14; and (f) ASC16. Reproduced with permission from [24], S. Palma, et al., *Il Farmaco.* 58, 1271 **(2003)**. © 2003.

The behavior of AZM 0.1% coagel was quite different compared with earlier formulations. Its maximum HE was lower and was reached 3 h postadministration. After 4 h, the HE value was similar to the values observed for COA-AZM 0.4% and AZOPT at the same time. Evidently, according to Figure 8, lower AZM concentrations did not facilitate rapid drug absorption due to the low concentration gradient that impelled the diffusion process. In this context, it is important to analyze the results taking into account the relative drug concentration in each formulation.

The AZM permeation from AZM 0.4% coagel and brinzolamide were initially similar. Although the AZM concentration from AZM 0.4% coagel was 2.5-fold lower than brinzolamide concentration (AZOPT), the former was able to permeate the drug more efficiently. Besides, brinzolamide is known to be intrinsically more permeable than AZM, implying that the promoter effect of coagels is really remarkable.

In order to evaluate the potential irritant effects of the formulations, we performed a histological examination by

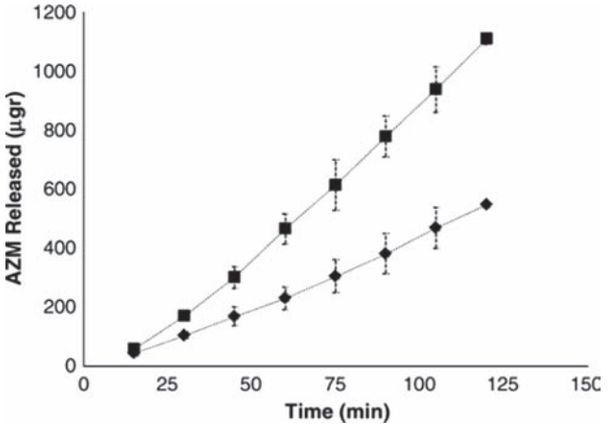

Fig. 6. *In vitro* release profile of acetazolamide (AZM) from ASC12 coagels. ■ AZM 0.4%; ♦ AZM 0.1%. Reproduced with permission from [30], L. I. Tártara, et al., *Journal of Ocular Pharmacology and Therapeutics* 28, 102 (**2012**). © 2012.

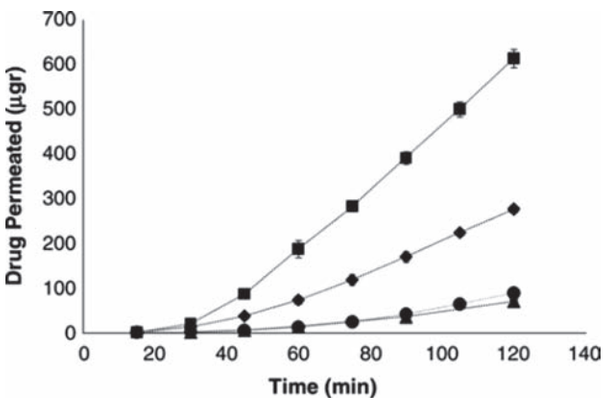

Fig. 7. *Ex vivo* permeation profile through isolated rabbit cornea. Comparison between formulations of ASC12 coagels containing AZM and AZOPT commercial formulation. ■ AZM 0.4%; ♦ AZM 0.1%; • AZM-R; ▲ AZOPT®. Reproduced with permission from [30], L. I. Tártara, et al., *Journal of Ocular Pharmacology and Therapeutics* 28, 102 (**2012**). © 2012.

light microscopy of the treated corneas.[31] In all cases, the intensity of irritation was time dependent.

The sodium dodecyl sulphate solution (SDS 2%) used as positive control produced a serious injury after 30 min post-administration. Although this irritation gradually decreased, at 180 min, it was still considerable. On the other hand, as expected, the irritant effect of AZM-R was practically negligible along the time of examination (180 min). Finally, in the case of AZM 0.1% and AZM 0.4% coagels, a mild-to-moderate effect was observed.

4. TRANSDERMAL DRUG DELIVERY SYSTEMS

Several factors may influence the skin permeation, and consequently, the improvement in the therapeutic efficacy of topically delivered drugs. Among these, the release of drug from the formulation, drug penetration into the stratum corneum and drug diffusion into the deeper skin layers may be included.

The permeation of ASCn as well as its effect on "*in vitro*" and "*in vivo*" drug diffusion through rat and mice skin was evaluated.[32] ASCn permeation through rat skin epidermis was very fast and quantitatively significant. ASC12 appears to be the compound that possesses the highest capacity to enhance the penetration of the drug as well as for self-penetration through the epidermis.

The ability of these compounds to permeate the rat skin is related to their chemical composition, since the flux of ASCn decreases as alkyl chain length increases. Furthermore, a burst effect was observed with ASC12. Also, the permeation of anthralin from ASCn coagels applied on rat skin was very increased compared to other pharmaceutical systems such as liposomal and niosomal carriers,[33] being ASC12 the most effective enhancer.

In line with this, a subsequent study was conducted with the goal of characterizing the transdermal permeation of ibuprofen (IBU).[34,35] In the case of ASC12 and ASC16 coagels, IBU release was sustained as indicated by the diffusion coefficient (n). IBU release from ASC16 was slightly lower than that of ASC12. This difference could be attributed to the higher viscosity of the former.[25]

Regarding IBU permeation through hairless mouse skin, we observed a noticeable increment in permeation in the case of ASCn coagels, especially for ASC12. The permeation of IBU from Arfen® (marketed formulation containing IBU 0.1%) was lower than ASC12 and ASC16. Considering lag time, Arfen® needed 4.5 h to saturate the skin, while ASC16 and ASC12 could cross the skin in 45 min and 60 min, respectively. We have previously corroborated the enhancing activity of ASC12 without noticeable injurious effects on the skin.[32] The influence of a co-solvent (PEG 400) was also evaluated.

ASC12 and ASC16 coagels increased IBU transdermal permeation about 21 and 12-fold compared to Arfen® ($p < 0.05$), respectively; demonstrating the ability of coagels to improve IBU skin permeation (Fig. 9). The addition of PEG to ASCn coagels produced a remarkable increase in IBU permeation compared to ASCn coagels.[35] This observation can be explained by the known enhancer properties of PEG. This polymer shows biocompatibility more suitable to the cutaneous administration with respect to short-chain alkanols also used as enhancers.

According to the attained results, both the *in vitro* test to characterize the drug partition from the vehicles and the *ex vivo* drug rat skin diffusion test to analyze the complexes interactions that occur between skin and excipients proved the efficacy of this new series of derivatives of 6-Oascorbic acid alkanoate.

The studied ASC16 and ASC12 coagels, defined as stable supramolecular assemblies in water or in water/PEG mixtures, allowed the solubilization of IBU (0.85%) and

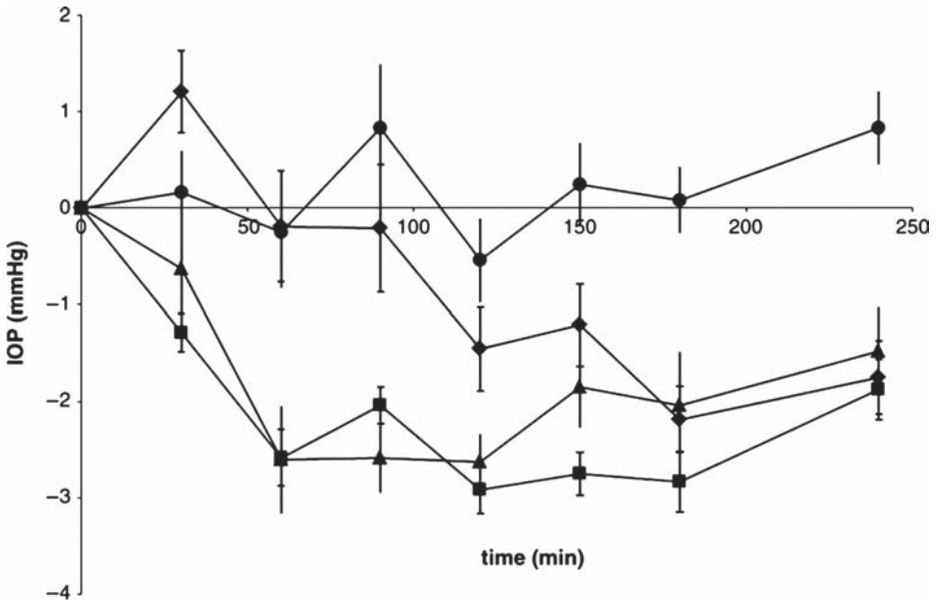

Fig. 8. *In vivo* profile variation of intraocular pressure in rabbits. Comparison between formulations of coagels containing acetazolamide and marketed formulation. • AZM-R; ♦ COA-AZM 0.1%; ■ COA-AZM 0.4%; ▲ AZOPT®. Reproduced with permission from [30], L. I. Tártara, et al., *Journal of Ocular Pharmacology and Therapeutics* 28, 102 **(2012)**. © 2012.

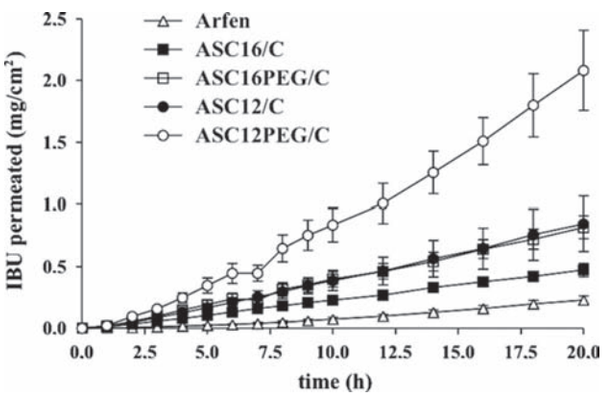

Fig. 9. Permeation profile through rat skin of IBU from ACS12PEG/C (s), ACS12/C (d), CS16PEG/C (h), ACS16/C (j) coagels and reference commercial formulation (4) (mean SE, $n = 6$). Reproduced with permission from [35], V. Saino, et al., *European Journal of Pharmaceutics and Biopharmaceutics* 76, 443 **(2010)**. © 2010.

controlled the drug release rate. Our results demonstrated that ASC16 and ASC12 coagels and in particular those vehicles containing PEG, producing a higher amount of permeated drug through the skin compared to commercial Arfen®, are a promising tool as carriers for cutaneous IBU delivery.

5. SUMMARY AND PERSPECTIVE

The nanostructured systems derived from the self-assembly properties of ASCn showed appropriated characteristics as pharmaceutical platform for drug delivery, especially through administration routes where permeation enhancement is necessary. In this way, coagels of ASC16 and ASC12 were able to increase drug permeation in formulation intended for ocular and transdermal administration.

According to our studies carried out so far, ocular hypotensive drugs such as AZM were more effective when vehiculized in ASC12 coagels comparatively to other pharmaceutical systems or marketed formulations. Similar conclusions were obtained in the case of NASDs such as ibuprofen incorporated in ASC12 coagels and administrated topically on to the skin. Besides, other properties such as drug release modulation, antioxidant activity and adequate rheology make this kind of systems very promissory as pharmaceutical dosage form.

Based on these observations, very interesting perspectives are derived for the potential utility of ASCn coagels, since there is a wide range of relevant pharmaceutical drugs whose therapeutic effectiveness is dependent on its penetration capacity across mucoses.

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References and Notes

1. D. Myers, Surfactant Science and Technology, Second edn., VCH, New York **(1994)**.
2. J. Clint (ed.), Surfactant Aggregation, Blackie, London **(1992)**.

3. F. Caboi, G. S. Amico, P. Pitzalis, M. Monduzzi, T. Nylander, and K. Larsson, *Chem. Phys. Lipids* 109, 47 **(2001)**.
4. J. C. Shah, Y. Sadhale, and D. M. Chilukuri, *Advanced Drug Delivery Reviews* 47, 229 **(2001)**.
5. C. Guo, J. Wang, F. Cao, R. J. Lee, and G. Zhai, *Drug Discovery Today* 15, 1032 **(2010)**.
6. L. Gan, S. Han, J. Shen, J. Zhu, C. Zhu, X. Zhang, and Y. Gan, *Int. J. Pharm.* 396, 179 **(2010)**.
7. J. Lee and I. W. Kellaway, *Int. J. Pharm.* 195, 29 **(2000)**.
8. L. S. Nielsen, L. Schubert, and J. Hansen, *European Journal of Pharmaceutical Sciences* 6, 231 **(1998)**.
9. C. C. Muller-Goymann, *European Journal of Pharmaceutics and Biopharmaceutics* 58, 343 **(2004)**.
10. S. Palma, R. Manzo, D. Allemandi, L. Fratoni, and P. Lo Nostro, *Langmuir* 18, 9219 **(2002)**.
11. M. Ambrosi, P. Lo Nostro, L. Fratoni, L. Dei, B. W. Ninham, S. Palma, R. H. Manzo, D. Allemandi, and P. Baglioni, *Phys. Chem. Chem. Phys.* 6, 1401 **(2004)**.
12. S. D. Palma, B. Maletto, P. Lo Nostro, R. H. Manzo, M. C. Pistoresi-Palencia, and D. A. Allemandi, *Drug Dev. Ind. Pharm.* 32, 1 **(2006)**.
13. M. Rasia, M. I. Spengler, S. Palma, R. Manzo, P. Lo Nostro, and D. Allemandi.
14. H. S. Nalwa (ed.), Handbook of Nanostructured Biomaterials and Their Applications in Nanobiotechnology, American Scientific Publishers, Los Angeles **(2005)**, Vols. 1–2.
15. P. K. Martindale, The Complete Drug Reference, 34th edn., The Pharmaceutical Press, London **(2005)**.
16. G. Capuzzi, G. Kulkarni, J. Fernandez, J. Vincieri, and P. Lo Nostro, *J. Colloid Interface Sci.* 186, 271 **(1997)**.
17. P. Lo Nostro, G. Capuzzi, and N. Mulinacci, *Langmuir* 16, 1744 **(2000)**.
18. G. Capuzzi, P. Lo Nostro, K. Kulkarni, and J. E. Fernandez, *Langmuir* 12, 3957 **(1996)**.
19. G. Capuzzi, P. Lo Nostro, K. Kulkarni, J. E. Fernandez, and F. F. Vincieri, *Langmuir* 12, 5413 **(1996)**.
20. G. Capuzzi, K. Kulkarni, J. E. Fernandez, and F. F. Vincieri, P. Lo Nostro, *J. Colloid Interface Sci.* 186, 271 **(1997)**.
21. U. Kohler, P. W. Yang, S. Weng, and H. H. C. Mantsch, *J. Spectrosc.* 33, 122 **(1988)**.
22. H. Sapper, D. G. Cameron and H. H. C. Mantsch, *J. Chem.* 59, 2543 **(1981)**.
23. L. Benedini, E. P. Schulz, P. V. Messina, S. D. Palma, D. A. Allemandi, and P. C. Schulz, *Colloids and Surfaces A: Physicochemical and Engineering Aspect.* 375, 178 **(2011)**.
24. S. Palma, A. Jiménez-Kairuz, L. Fratoni, P. Lo Nostro, R. Manzo, and D. Allemandi, *Il Farmaco.* 58, 1271 **(2003)**.
25. A. Urtti, *Adv. Drug Deliv. Rev.* 58, 1131 **(2006)**.
26. D. Shulin, *PSSP* 1, 328 **(1998)**.
27. J. G. Flanagan, *Ophthal. Physiol. Opt.* 18, 126 **(1998)**.
28. I. P. Kaur, R. Smith, D. Aggarwal, and M. Kapil, *Int. J. Pharm.* 248, 1 **(2002)**.
29. M. W. Duffel, Y. S. Segarra, T. M. Ing, J. A. Dixson, C. F. Barfknecht, and R. D. Schoenwald, *J. Med. Chem.* 29, 1488 **(1986)**.
30. L. I. Tártara, D. A. Quinteros, V. Saino, D. A. Allemandi, and S. D. Palma, *Journal of Ocular Pharmacology and Therapeutics* 28, 102 **(2012)**.
31. Y. Rojanasakul, L. Y. Wang, M. Bhat, D. D. Glover, C. J. Malanga, and J. K. H. Ma, *Pharm. Res.* 9, 1029 **(1992)**.
32. S. D. Palma, B. Maletto, P. Lo Nostro, R. H. Manzo, M. C. Pistoresi-Palencia, and D. A. Allemandi, *Drug Dev. Ind. Pharm.* 32, 1 **(2006)**.
33. R. Agarwal, O. Katare, and S. Vyas, *Int. J. Pharm.* 228, 43 **(2001)**.
34. V. Saino, S. Palma, P. Chetoni, and D. Allemandi, *Lat. Am. J. Pharm.* 28, 3438 **(2009)**.
35. V. Saino, D. Monti, S. Burgalassi, S. Tampucci, S. Palma, D. Allemandi, and P. Chetoni, *European Journal of Pharmaceutics and Biopharmaceutics* 76, 443 **(2010)**.

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