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Design of Original Bioactive Formulations Based on Sugar–Surfactant/Non-steroidal Anti-inflammatory Catanionic Self-Assemblies: A New Way of Dermal Drug Delivery**

Sabrina Consola,[a] Muriel Blanzat,[a] Emile Perez,[a] Jean-Christophe Garrigues,[a] Pascal Bordat,[b] and Isabelle Rico-Lattes*[a]

Abstract: A new kind of catanionic assembly was developed that associates a sugar-based surfactant with a non-steroidal anti-inflammatory drug (NSAID). Three different assemblies using indomethacin, ibuprofen and ketoprofen as NSAIDs were easily obtained in water by an acid–base reaction. These assemblies formed new amphiphilic entities because of electrostatic and hydrophobic effects in water and led to the spontaneous formation of vesicles. These catanionic vesicles were then tested as potential NSAID delivery systems for dermatological application. The anti-inflammatory activity was evaluated in vivo, and this study clearly showed an improved therapeutic effect for NSAIDs that were formulated as catanionic vesicles. These vesicles ensured a slower diffusion of the NSAID through the skin. This release probably increased the time of retention of the NSAID in the targeted strata of the skin. Thus, the present study suggests that this catanionic bioactive formulation could be a promising dermal delivery system for NSAIDs in the course of skin inflammation treatment.

Keywords: anti-inflammatory agents · drug delivery · NSAIDs (non-steroidal anti-inflammatory drug) · surfactants · vesicles

Introduction

Inflammatory skin diseases represent an important proportion of all skin disorders and are the subject of much research in the dermatological industry. Atopic dermatitis, contact dermatitis and psoriasis are the most representative examples of inflammatory skin disorders. Inflammation, which is essentially a protective response that initiates the process of tissue repair can be deleterious when it becomes uncontrolled, and can lead to chronic diseases.

In the course of inflammation treatment, dermal drug delivery is a promising approach for targeting skin diseases or rheumatism pathologies more specifically. Previous studies have clearly shown that the dermal route is particularly suitable and effective for delivering drugs into the skin and into areas under it, like muscles and articulations, while avoiding a large systemic distribution.[1]

Despite the potential of dermal drug delivery, this administration route is used for few drugs because of the remarkable properties of the skin. The barrier function of the skin, which is essentially ensured by the stratum corneum, the outermost layer, makes this epithelium impermeable to exogenous substances.[2,3]

Therefore, the major challenge for dermatological research is the design of new drug delivery systems that are able to provide a sufficient increase in drug concentration in the skin to have a therapeutic effect. An additional requirement is to achieve suitable skin penetration without inducing significant irreversible alterations in the skin's barrier function.

[a] Dr. S. Consola, Dr. M. Blanzat, Dr. E. Perez, J.-C. Garrigues, Dr. I. Rico-Lattes
Laboratoire des Interactions Moléculaires et Réactivité Chimique et Photochimique
UMR 5623, 31062 Toulouse cedex 4 (France)
Fax: (+33) 5-6155-8155
E-mail: rico@chimie.ups-tlse.fr

[b] P. Bordat
Directeur Recherche et Développement, Pascal Bordat
Pierre Fabre Dermo-Cosmétique, 17 allée Camille Soula BP 74
31322 Castanet Tolosan Cedex (France)

[**] Abbreviations used in this manuscript: NSAID: non-steroidal anti-inflammatory drug; Lhyd12: *N*-dodecylamino-1-deoxylactitol; IndoNa: sodium salt of indomethacin; Indo12: catanionic association between indomethacin and Lhyd12; Ibu12: catanionic association between ibuprofen and Lhyd12; Keto12: catanionic association between ketoprofen and Lhyd12; DLS: dynamic light scattering; TEM: transmission electron microscopy.

In the treatment of inflammatory conditions, non-steroidal anti-inflammatory drugs (NSAIDs) form an essential therapeutic class. These drugs are widely used as analgesics, and for the treatment of local and chronic inflammatory pathologies like arthritis and arthrosis. For instance, indomethacin is an effective NSAID used in the treatment of rheumatoid arthritis and ankylosing spondylitis, and has also been used as a model drug for many anti-inflammatory investigations. These NSAIDs are non-selective cyclooxygenase (COX) inhibitors and play an important part in the synthesis of prostaglandins.

Comparative studies to evaluate the suitability of NSAIDs for dermal delivery have shown the need for enhancers for effective penetration through the skin.[4–6] It is generally recognized that NSAIDs are characterized by a low water solubility, which is associated with their hydrophobicity, and these features are responsible for their low intrinsic dermal bioavailability.

Numerous approaches have been developed in the aim of finding a safe, effective formulation for NSAID dermal delivery. They include the use of chemical permeation enhancers,[7–9] microemulsions,[10–13] cyclodextrins[14–17] and prodrugs.[18]

In this context, vesicular formulations are receiving increasing attention. These carriers are particularly interesting because they can be used as solvents and enhancers, and they can also increase the time of residence of the drug in the stratum corneum and epidermis, while reducing the systemic absorption of the drug.[19–21]

In the field of vesicle preparation, catanionic surfactants have attracted considerable attention in the last two decades. Indeed, this surfactant family, obtained by mixing two oppositely charged surfactants, exhibits a unique ability to form stable vesicles spontaneously in an aqueous solution.[22–24] It is worth noting that these surfactant mixtures are usually prepared with an excess of one of the two surfactants to ensure water solubility.

Among catanionic mixtures, it is important to consider the sugar-based ones. Sugar-based surfactants are advantageous because they are produced from renewable raw materials, they are highly biodegradable and non-toxic.[25,26] Moreover, sugar-based surfactants enhance the water solubility of catanionic mixtures and therefore lead to soluble equimolar systems with no excess of cationic or anionic surfactant.[27,28] Finally, catanionic sugar-based surfactants can be easily obtained by an acid–base reaction between equimolar amounts of an amino sugar and a fatty acid. According to this method, which was described for the first time by our laboratory, the equimolar mixture is free of salt.[28] This feature is very interesting for biological applications, for which salt concentrations are of particular importance. Catanionic analogues of galactosylceramide have been synthesized according this protocol and are under investigation for HIV therapy.[29,30]

In this context, we designed a new kind of catanionic assembly in order to obtain a NSAID delivery system for dermatological applications. The originality of this catanionic mixture lies in the nature of one of the two components, which is a bioactive compound with anti-inflammatory properties. This strategy was developed to take advantage of the spontaneous vesicle formation of the catanionic surfactant family. In this way, the amphiphilic ion-pair obtained would confer self-assembling properties to the drug, and the drug could then participate in its self-formulation. Furthermore, this kind of association would overcome the low water solubility of the NSAID. On the one hand, the NSAID is administered in its ionic carboxylate form which is more water soluble. On the other hand, the sugar-based surfactant increases the hydrophilicity, improving the water solubility of the association. Moreover, the use of a sugar-based surfactant could lead to safe, biocompatible formulations.

To set up this concept, we studied three NSAIDs: indomethacin, ibuprofen and ketoprofen. This choice was made because these three drugs possess a carboxylic acid function and different steric demands associated with similar hydrophobicity. In this way, we could evaluate the influence of the structure of the NSAID on the physicochemical behaviour of the corresponding catanionic assemblies.

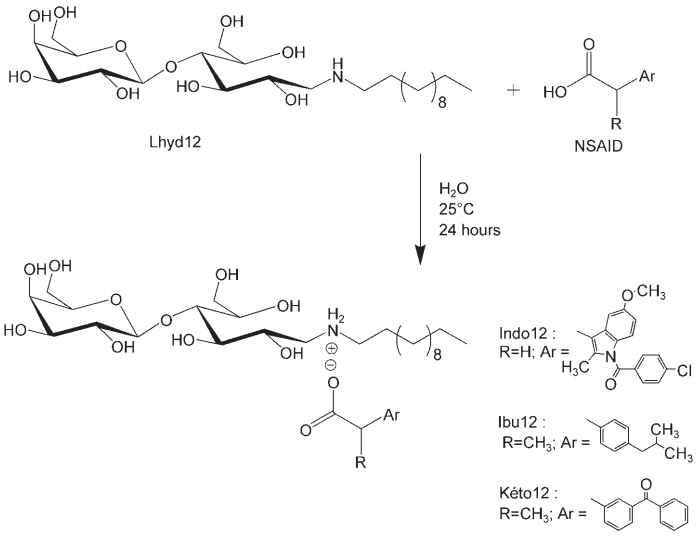
In this paper, we first describe the synthesis of a new kind of catanionic assemblies that are prepared with NSAIDs. Then, we analyse the self-assembling properties of these compounds in aqueous solution. Finally, we evaluate the potential of the bioactive formulation based on catanionic vesicles as a dermal drug delivery system. With this in mind, vesicular formulations were tested in vivo on the anti-inflammatory experimental model of the arachidonic acid induced mouse ear oedema. To further investigate the pharmacological activity, the cutaneous bioavailability was studied for the most active compound, the catanionic formulation prepared with indomethacin. This formulation was tested ex vivo in the skin penetration experimental model of pig ear skin.

Results and Discussion

Synthesis: All syntheses performed are presented on Scheme 1. The synthesis of the catanionic assemblies was performed in water by an acid–base reaction between equimolar amounts of the previously described amino sugar Lhyd12[31] and the NSAID carboxylic acids at room temperature. This method, first used for the synthesis of analogues of galactosylceramide,[28] led easily and quantitatively to water soluble ion-pairs, the so-called catanionic assemblies, which are free from salt.

All the associations were fully characterized by ^{1}H NMR, ^{13}C NMR, IR spectra and high-resolution electrospray mass spectrometry (HRMS).

NMR (^{1}H and ^{13}C) and IR spectra showed the proton transfer reaction, that is, the replacement of the carboxylic acid group by an anionic carboxylate, between the NSAID and the basic aminosugar, which was also confirmed by the pH decrease from 10 to 8 during NSAID dissolution in the aqueous solution.



Scheme 1. Preparation of the catanionic assemblies between Lhyd12 and indomethacin, ibuprofen and ketoprofen, named Indo12, Ibu12 and Keto12, respectively.

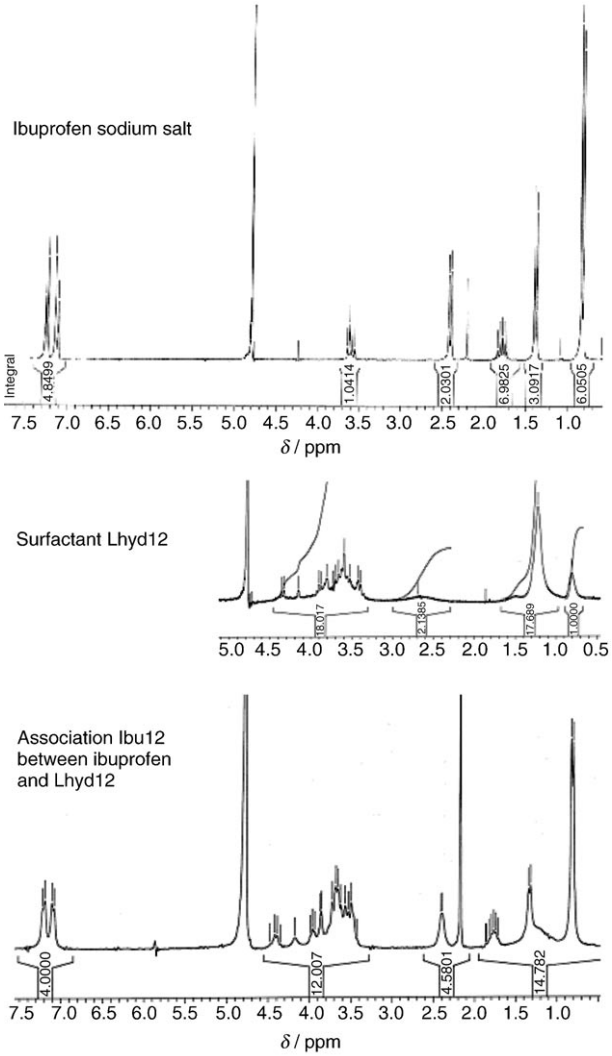


Figure 1. ^{1}H NMR spectra (250 MHz, D_2O) of ibuprofen sodium salt, surfactant Lyhd12 and catanionic assembly Ibu12.

It is worth noting that ^{1}H NMR spectra of the ion-pairs, which were perfectly soluble in aqueous solutions, were characterized by a considerable loss of resolution compared to the isolated species under the same conditions of analysis. This modification in the relaxation time of the protons was attributed to a self-assembling phenomenon in which protons could be enclosed in a less dynamic and more structured environment. This point is illustrated by Figure 1 for the catanionic assembly Ibu12, and was confirmed by additional analyses (DLS and TEM).

Furthermore, the ion-pair entity was detected and confirmed by electrospray HRMS. In catanionic equimolar mixtures, the ion-pair is globally neutral because the carboxylate anionic charge is neutralised by the ammonium cationic one. Consequently, their electrospray characterisation requires an additional cationisation, which can be achieved by adding small amounts of sodium iodide, because of the strong affinity of sodium ion for some sugars.

Figure 2 illustrates this point and shows the electrospray mass spectrum that was obtained for the catanionic assembly Indo12. The spectrum is enlarged in the area of molecular ion peaks between 889 and 894. The peak observed at $m/z = 891.5278$ represents the sodium adduct of the ion-pair Indo12 after a calibration on the molecular ion peak of the protonated Lhyd12 at 512.4245 (see Experimental Section).

All of the analyses provided complementary evidence for the formation of a new entity: the association between the sugar-based surfactant and the NSAID. The formation of this ion-pair was based on an ionic bond reinforced by the hydrophobic effect in aqueous solution. Consequently, these interactions resulted in the solubilisation of the hydrophobic drug, and in an environmental modification for both entities; this led to new physicochemical properties which differed from those of the starting materials.

Self-assembly properties

Surface tension measurements: To investigate the ion-pair behaviour in aqueous solution, the surface activity of the catanionic assemblies was studied by surface tension measurements using the Wilhelmy method.

Figure 3 shows the variation of the water surface tension as a function of the logarithm of the concentration for compounds Indo12, Ibu12 and Keto12. This graph indicates that all the compounds exhibited surface-active properties, shown by the lowering of the water surface tension. Moreover, the values obtained for the surface tension of water were lower than those obtained with conventional surfactants (30–40 mN/m), indicating a high surface activity for these catanionic assemblies.[32,33] From this graph, a critical aggregation concentration (CAC) was also determined for all of the compounds (Table 1). The break in the curves indicates the minimum concentration for the spontaneous formation of catanionic assemblies.

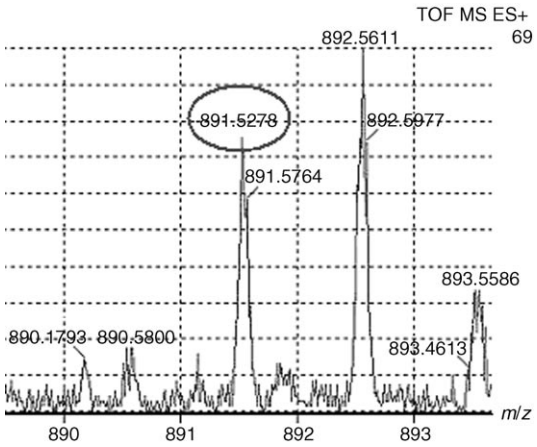


Figure 2. Electrospray mass spectrum of Indo12.

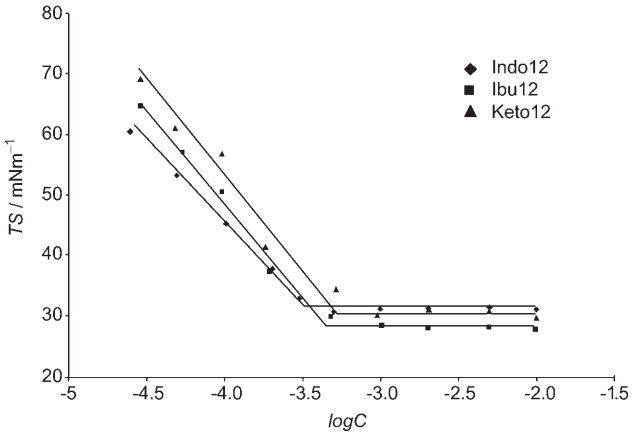


Figure 3. Plot of surface tension versus logarithm of the concentration for the catanionic assemblies Indo12, Ibu12 and Keto12 at 20 °C.

Table 1. CAC values determined by surface tension measurements for catanionic assemblies Indo12, Ibu12 and Keto12 in aqueous solutions at 20 °C and calculated logP for non-associated NSAIDs.

	CAC [mM]	TS [mN m^{-1}][a]	NSAID	log$P$[b]
Indo12	0.40	31	indomethacin	4.68
Ibu12	0.50	28	ibuprofen	4.59
Keto12	0.60	30	ketoprofen	4.15

[a] TS for the CAC. [b] Calculated log$P$[a] (by replacing the carboxylic acidic moiety by a proton) of the hydrophobic part of the NSAID.

Table 1 lists the CAC values for the three catanionic assemblies. These CAC values were close to 0.5 mM for the three compounds, which is in agreement with the NSAIDs' hydrophobicity, and is particularly close for the three NSAIDs (log P around 4). Moreover, these CAC values indicated that the catanionic assemblies were able to form aggregates spontaneously in aqueous solution. It is noteworthy that the CAC values differed from the critical micelle concentration (CMC) value determined for the cationic sugar-based surfactant alone ($CMC_{(Lhyd12H+)} = 1.50$ mM) and from the CAC previously obtained for the sodium salts of the NSAID ($CAC_{(indomethacin)} = 30$ mM,[34] $CAC_{(ibuprofen)} = 180$ mM,[35] $CAC_{(ketoprofen)} = 2.20$ mM[36]).

DLS and TEM: To investigate the aggregation behaviour of the ion-pairs and to achieve a better understanding of the part played by both entities, the nature of the aggregates was studied.

To determine the hydrodynamic diameters and the size distribution of the aggregates, dynamic light scattering (DLS) studies were carried out on the three assemblies at 20 °C in 10^{-2} M aqueous solution. The light-scattering intensity measurements were performed at an angle of 90°, and the automatic Contin method was used for the data analysis.

Table 2 summarizes the hydrodynamic diameters measured and the polydispersity index that was evaluated for the aggregate dispersions in aqueous solution. DLS studies showed the formation of aggregates with diameters ranging from 20 to 280 nm. In all cases, these diameters were larger than those obtained for a micelle, which generally ranged between 5 and 10 nm, and suggest the formation of vesicles. The broad distribution of vesicle sizes that were observed for these catanionic assemblies was in agreement with those obtained in previous works for catanionic analogues of galactosylceramide (10–500 nm).[28]

Table 2. Mean hydrodynamic diameters and polydispersity index (determination according the scattered light intensity) for objects spontaneously formed in aqueous solutions of Indo12, Ibu12 and Keto12 at 20 °C.

	Concentration	Mean hydrodynamic diameter [nm]	Polydispersity index
Indo12	10^{-2} M	40	0.33
Ibu12	10^{-2} M	90	0.53
Keto12	10^{-2} M	20 (32%) 285 (68%)	1.00

TEM analyses (Figure 4) clearly indicated the formation of unilamellar vesicles. The micrographs show spherical structures with a closed, stained periphery, corresponding to a single-layered membrane in agreement with the vesicular structures currently observed with catanionic mixtures. For the three catanionic assemblies, the aggregate sizes observed by TEM were in agreement with those evaluated by DLS with diameters between 30–300 nm. All the results indicated the spontaneous formation of vesicles in aqueous solution for the three catanionic assemblies. This aggregation behaviour differed from that of the isolated entities, which formed micelles in aqueous solution.

To explain the new aggregation behaviour, the thermodynamic model of Israelachvili[37] can be considered. In this model, the aggregate morphology is related to the surfactant structure through a molecular packing parameter P given in Equation (1), in which v is the volume of the hydrophobic part, a_0 is the optimal head group cross-sectional area and l_C the critical alkyl chain length.

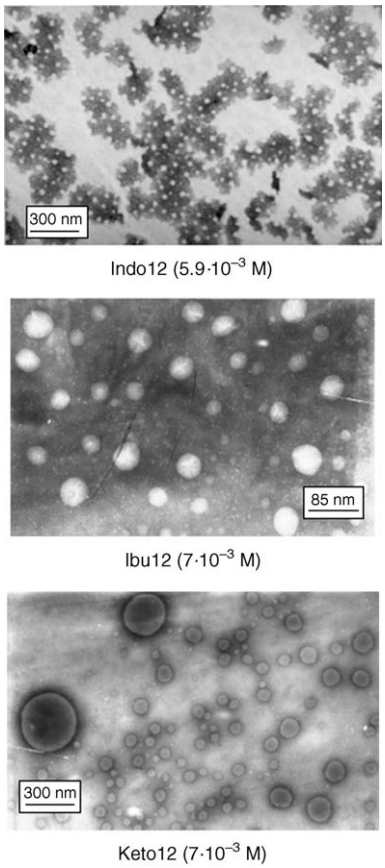


Figure 4. Negatively stained electron micrographs of NSAID vesicles that spontaneously formed in aqueous solutions.

$$P = \frac{v}{a_0 l_c} \quad (1)$$

Table 3 indicates the relationship between some surfactant shapes and the corresponding aggregate morphologies. For instance, micelles are formed by surfactants characterized by a molecular packing parameter of 1/3, while vesicles are associated with a packing parameter of 1/2.

In this study, Lhyd12 formed micelles, while catanionic assemblies with NSAIDs led to the spontaneous formation of vesicles. The NSAIDs thus helped to change the molecular packing parameter from 1/3 to 1/2. Consequently, the behaviour of the NSAIDs was not that of a usual organic counterion for the amino sugar, but rather the NSAID participated actively in the self-association process and modified the self-assembly of Lhyd12. The ion-pair acted as a double-tailed zwitterionic surfactant, which is known to be able to form spontaneous vesicles in an aqueous medium.[22] This point is illustrated by Figure 5, which shows the participation of the NSAID in the vesicle formation. Indeed, the NSAID was not only dissolved in the hydrophobic bilayer, but was a component of this bilayer.

The NSAID and the sugar-based surfactant participated in a new amphiphilic entity by electrostatic and hydrophobic effects and this association led to the spontaneous formation of vesicles. Furthermore, this ion-pair showed an aggregation behaviour that can be compared to that of classic catanionic surfactant mixtures made of two oppositely charged surfactants. The ion-pairs prepared in this work constituted a new kind of catanionic assembly, which included a bioactive entity.

Table 3. Relationship between surfactant shape and aggregation behaviour based on the molecular packing parameter P of Israelachvili.

Packing parameter	Surfactant shape	Aggregation behaviour
$P < 1/2$	Cone	- Micelles - Hexagonal I
$1/2 < P < 1$	Truncated cone	Flexible lamellar = vesicle
$P \approx 1$	Cylinder	- Lamellar - Cubic
$P = 1$	Inverted truncated cone	- Reversed micelles - Hexagonal II

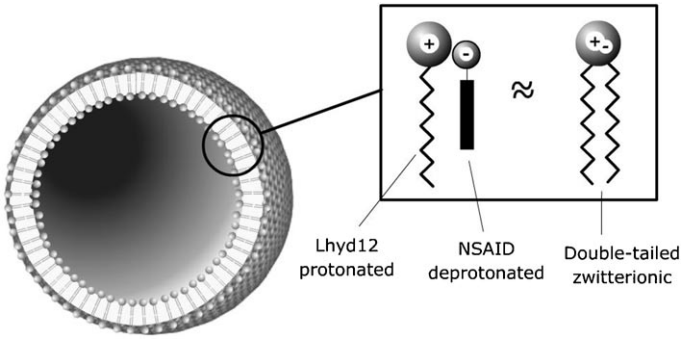


Figure 5. Schematic representation of the participation of the NSAID in the catanionic vesicle formation.

To conclude on the physicochemical modifications, this new catanionic association 1) improved the water solubility of the NSAID and 2) provided a spontaneous formulation of the drug, which was self-included within its own vesicle. These supramolecular structures constitute a promising dermal drug delivery system.

Anti-inflammatory activities of the catanionic assemblies: To evaluate the potential of these catanionic vesicles as a dermal drug delivery system, the bioactive formulations were tested in vivo on the arachidonic acid induced mouse ear oedema model.[38,39] This test consists of inducing an ear oedema with arachidonic acid application, and then measuring the anti-inflammatory activity of the NSAID administered through the skin.

The effects of the topical application of the NSAID formulations on arachidonic acid induced mouse ear oedema are summarized in Figure 6.

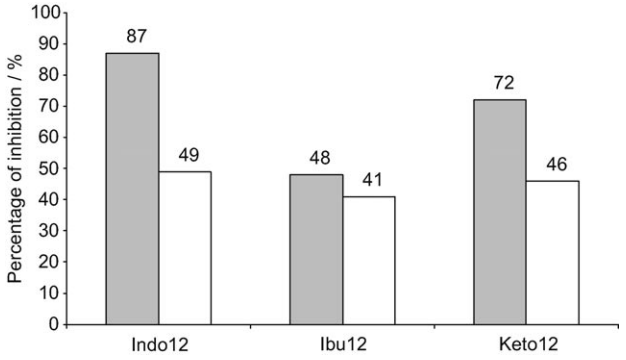


Figure 6. Comparative anti-inflammatory activities of the catanionic assemblies (■) and the non-associated NSAIDs (□) (expressed as a percentage of inhibition of the inflammation).

All the compounds tested were active against the arachidonic acid induced ear mouse oedema. NSAIDs showed percentages of inhibition close to 45%, while the anti-inflammatory activity of the catanionic assemblies ranged from 48 to 87%. Indo12 showed the highest activity, while Ibu12 was the least active compound. These results indicate first that the NSAIDs maintain their therapeutic properties when they are associated with a biocompatible sugar-based surfactant through electrostatic and hydrophobic effects. Moreover, the topical administration of catanionic assemblies of indomethacin and ketoprofen decreased the oedema more efficiently than the non-associated NSAIDs of the control groups (increase of 77 and 56% for indomethacin and ketoprofen, respectively), and these results were obtained for lower administered doses.

Thus, these new associations between a sugar-based surfactant and a NSAID improved the therapeutic effect of the anti-inflammatory drugs in skin treatment. The catanionic formulations appeared to be as efficient drug delivery systems for dermal administration.

Differences in oedema inhibition were observed between the three catanionic assemblies, while no difference appeared for the three non-associated NSAIDs. These distinctions indicate that the nature of the interaction between the sugar-based surfactant and the NSAID has an important influence on the anti-inflammatory activity of the catanionic assemblies.

Skin penetration of the catanionic assemblies: To explain the anti-inflammatory activity of the catanionic assemblies, we also evaluated the cutaneous bioavailability of these compounds. This study was carried out with the compound Indo12, because it exhibited the highest anti-inflammatory activity in vivo in the arachidonic acid induced ear mouse oedema model.

To study the influence of self-assembly on skin permeation, we compared the catanionic vesicular formulation of Indo12 with an aqueous solution of the sodium salt of indomethacin used as reference. The same concentration was used for both solutions, and was chosen above the CAC of Indo12 in order to have spontaneous vesicle formation. The study was carried out ex vivo with an infinite dose of 200 mg, and under occlusive conditions to favour the drug penetration. The porcine ear skin model was used because it is predictive of human skin penetration.

Figure 7 shows the penetration profile of both formulations over 24 h. The dermal administration of the sodium salt of indomethacin resulted in two-fold higher cumulative

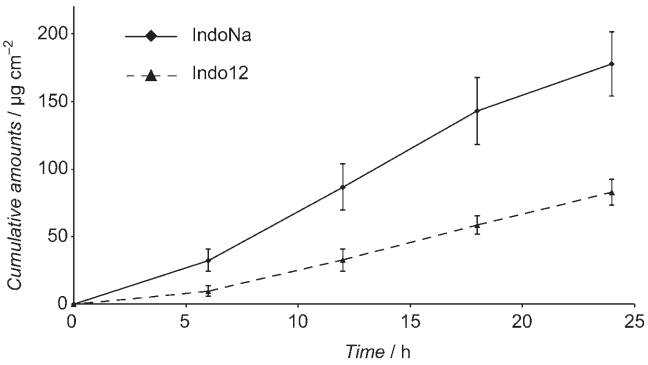


Figure 7. In vitro percutaneous penetration of indomethacin after dermal application of aqueous solutions of the sodium salt and the catanionic vesicular formulation of Indo12.

amounts than those obtained with the catanionic vesicular formulation. Thus, the comparison between these two formulations clearly showed a slower diffusion of indomethacin from the catanionic assembly.

The results indicate that the percutaneous penetration of indomethacin was modified by the sugar-based surfactant and by the aggregation behaviour of the amphiphilic ion-pair. The diffusion of the drug was slower when it was incorporated within the catanionic vesicles.

The drug penetration through the skin involved a process with two different steps. The drug first had to diffuse through the formulation before diffusing through the stratum corneum. The physicochemical properties of the drug and the formulation both influence the skin permeation. In this context, the colloidal structure of the formulation is of importance to determine the limiting step in the drug penetration.

Indomethacin incorporated within the aggregates was not just dissolved within the hydrophobic bilayer of the catanionic vesicle, but was a constitutive entity that participated in the spontaneous self-assembly process (Figure 5).

As in the micellisation process, in the formation of catanionic vesicles an equilibrium between monomers, the ion-pairs and vesicular structures can be considered. The concentration of free monomers was then lower than the concentration of sodium salt. Because the driving force of diffu-

sion through the skin is the concentration gradient, one can understand that the penetration of indomethacin through the skin was slower than in the case of sodium salt. The diffusion of catanionic formulations was thus governed by the aggregation behaviour.

The slower release of indomethacin could also be a consequence of the formation of a drug reservoir within the stratum corneum. The amphiphilic nature of the ion-pair could modify the lamellar structures that were formed by endogenous lipids of the stratum corneum, thus creating new areas for drug accumulation. This phenomenon has already been reported by Puglia et al.,[21] who observed that liposomes of phospholipids provided a sustained and prolonged release of indomethacin through the skin. It would be interesting to study the biodistribution of indomethacin through the epidermis by stripping the different layers in order to confirm this hypothesis.

The catanionic vesicles ensured a slower diffusion of the indomethacin through the skin that could increase the retention of the NSAID in specific skin strata. In this way, the NSAID could more efficiently target the COX enzyme and exhibit its therapeutic effect.

Conclusion

This work presents a new concept for the efficient delivery of NSAIDs through the skin. An original catanionic assembly based on a sugar-derived surfactant and a NSAID was designed to provide a bioactive formulation. The catanionic assemblies were easily obtained by an acid–base reaction thanks to electrostatic and hydrophobic effects in an aqueous solution. These mixtures were advantageously water soluble in an equimolar ratio and were free from salt. As in conventional catanionic mixtures, this new kind of catanionic assembly, which involved a bioactive compound led to the spontaneous formation of vesicles in aqueous solution.

These catanionic vesicles provided a bioactive formulation for the dermal administration of NSAIDs. This original formulation showed very interesting benefits. First, it improved the anti-inflammatory activity of the NSAID in vivo. Second, it ensured a slower diffusion of the NSAID through the skin, probably leading to a prolonged time of residence in the targeted sites. Finally, the formulation was obtained spontaneously and should be safer, thanks to the use of biocompatible surfactants and an aqueous vehicle.

Experimental Section

Materials: All starting materials were purchased from commercial sources and were used without further purification.

General methods: ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AC 250 spectrometer at nominal frequencies of 250 MHz for ^{1}H, and 62 MHz for ^{13}C. Chemical shifts are reported in ppm with respect to tetramethylsilane.

IR spectra were recorded on a Perkin Elmer FT-IR 1760-X spectrometer (0.5% in KBr) and the wavelengths are expressed in cm^{-1}.

Electrospray mass spectra were recorded on a Waters Qtof Ultima API. An aliquot of a 1 mg mL^{-1} aqueous solution of catanionic assembly (50 μL) was mixed with an aqueous solution of NaI (50 μL; 1 mg mL^{-1}) and distilled water (900 μL). The injection was performed with a flow of 10 μL min^{-1}. The capillarity voltage was 3 kV, the cone-to-skimmer voltage was set at 100 V and a collision energy of 10 eV was used. Spectrum calibration was performed with the molecular-ion peak of the protonated Lhyd12. Despite the relatively soft conditions, a partial dissociation occurred, and the catanionic entity of the ion-pair was systematically observed.

Surface tensions were measured at 20 °C by using the Wilhelmy method with a VWR TD1 Lauda tensiometer. The aqueous solutions were prepared in a concentration range between 10^{-6} M and 10^{-2} M.

Vesicles were characterised by dynamic light scattering (DLS) to determine the mean hydrodynamic diameter and the size distribution. DLS was performed with a Malvern Instruments Zetasizer 3000 HR that could be used for samples containing particles from 1 nm to 10 μm. Analyses were carried out at 20 °C on aqueous solutions of 10^{-2} M. All solvents were previously filtered using a filter with a pore size of 1.20 μm. The wavelength was set at 633 nm and scattered light was analysed at an angle of 90°. Data were fitted by the automatic Contin method.

Vesicle formation was observed by transmission electron microscopy (TEM). Aliquots of vesicle solutions were applied on carbon-coated Formvar grids, negatively stained with a 2% solution (w/v) of sodium phosphotungstate (pH 7.5). The formation of aggregates was examined and photographed with a JEOL JEM 200 CX electron microscope operating at an 200 kV accelerating voltage.

Preparation of the catanionic assemblies between the sugar-based surfactant and the NSAID—General procedure: NSAID [indomethacin (238.5 mg, 0.66 mmol), ibuprofen (136.1 mg, 0.66 mmol), ketoprofen, (167.8 mg, 0.66 mmol)] was added to a micellar solution (30 mL) of Lhyd12 (456.3 mg, 0.66 mmol). After 24 h of stirring at room temperature, a homogeneous solution was obtained and was freeze-dried to give the catanionic assembly in a quantitative yield.

Indo12—catanionic assembly between Lhyd12 and indomethacin: Yield: 100%; ^{1}H NMR (250 MHz, D_2O, 25 °C, TMS): $\delta = 7.31$ (AB syst, ^{3}J(A,B) = 8.1 Hz, 4H; Ar*H*), 6.89 (brs, 1H; CH), 6.51 (d, ^{3}J(H,H) = 8.3 Hz, 1H; Ar*H*), 6.31 (d, ^{3}J(H,H) = 8.3 Hz, 1H; Ar*H*), 4.39 (d, ^{3}J(H,H) = 7.5 Hz, 1H; C*H*OCH), 4.18–3.39 (m, 12H; CH and CH_2), 3.61 (s, 3H; OCH_3), 3.47 (m, 2H; CH_2COO^-), 2.90 (brs, 1H; CHOHC$H_2NH_2^+$), 2.62 (brs, 1H; $CH_2CH_2NH_2^+$), 2.11 (s, 3H; CH_3), 1.22 (brs, 12H; CH_2), 0.90 ppm (brs, 3H; CH_3); ^{13}C-(^{1}H) NMR (75 MHz, D_2O): $\delta = 181.6$ (COO^-), 171.1 (NCO), 157.9 (Ar*C*), 141.1 (Ar*C*), 137.8 (Ar*C*), 136.4 (Ar*C*), 134.2 (Ar*C*), 133.8 (Ar*C*), 133.1 (Ar*C*), 131.3 (Ar*C*), 118.8 (Ar*C*), 114.1 (Ar*C*), 112.4 (Ar*C*), 103.1 (*C*HOCH), 78.6–71.2 (CHOH), 66.1–63.5 (CH_2OH), 58.1 (OCH_3), 50.6 ($CH_2NH_2^+$), 35.2–34.7 (CH_2), 32.7 (CH_2COO^-), 29.2–28.4 (CH_2), 25.5 (Ar*C*), 16.7 (CH_3), 15.5 ppm (CH_3); IR (KBr): $\tilde{\nu} = 2470$ (s, N–H), 1572 (s, C=O_{asym}), 1384 cm^{-1} (s, C=O_{sym}); MS (10 eV, EI): m/z: 891.4 $[M+Na]^+$.

Ibu12—catanionic assembly of Lhyd12 and ibuprofen: Yield: 100%; ^{1}H NMR (250 MHz, D_2O, 25 °C, TMS): $\delta = 7.15$ (AB syst, ^{3}J(A,B) = 7.0 Hz, 4H; Ar*H*), 4.40 (d, ^{3}J(H,H) = 8.3 Hz, 1H; C*H*OCH), 4.18–3.42 (m, 13H; CH_2OH, C*H*OH and $CHCOO^-$), 2.39 (brs, 2H; CH_2), 1.77 (m, 1H; C$H(CH_3)_2$), 1.33 (brd, ^{3}J(H,H) = 5.9 Hz ,3H; CH_3CHCOO$^-$), 0.82 ppm (brd, ^{3}J(H,H) = 5.7 Hz, 9H; (CH_3)$_2$CH and CH_3); ^{13}C-(^{1}H) NMR (62 MHz, D_2O): $\delta = 186.4$ (COO^-), 143.7 (Ar*C*), 142.8 (Ar*C*), 132.1 (Ar*C*), 130.0 (Ar*C*), 77.8–71.4 (CHOH), 66.3–63.8 (CH_2OH), 51.1 (*C*HCOO$^-$), 47.3 (Ar*C*), 32.7 (Ar*C*), 24.7 (CH(CH_3)$_2$), 21.6 (CH_3CHCOO$^-$), 16.7 ppm (CH_3); IR (KBr): $\tilde{\nu} = 2555$ (s, N–H), 1579 (s, C=O_{asym}), 1397 cm^{-1} (s, C=O_{sym}); MS (10 eV, EI): m/z: 740.4 $[M+Na]^+$.

Keto12--catanionic assembly of Lhyd12 and ibuprofen: Yield: 100%; ^{1}H NMR (250 MHz, D_2O, 25 °C, TMS): $\delta = 7.73$–7.49 (m, 9H; Ar*H*), 4.41 (brs, 2H; C*H*OCH), 4.20–3.45 (m, 13H; CH_2, CH and $CHCOO^-$), 2.90 (brs, 1H; C$H_2NH_2^+CH_2$), 1.38 (brs, 3H; CH_3CHCOO$^-$), 1.04 (brs, 6H; CH_2), 0.70 ppm (brs, 1H; CH_3); ^{13}C-(^{1}H) NMR (62 MHz, D_2O): $\delta = 202.6$ (C=O), 185.5 (COO^-), 146.8 (Ar*C*), 139.8 (Ar*C*), 136.0 (Ar*C*), 135.3 (Ar*C*), 133.1 (Ar*C*), 132.0 (Ar*C*), 131.3 (Ar*C*), 77.8–71.4 (CHOH), 66.3–63.0 (CH_2OH), 51.3 (*C*HCOO$^-$), 34.8–25.5 (CH_2), 21.4 (CH_3CHCOO$^-$),

16.7 ppm (CH_3); IR (KBr): $\tilde{\nu}$ = 2430 (s, N–H), 1580 (s, C=O_{asym}), 1393 cm^{-1} (s, C=O_{sym}); MS (10 eV, EI): m/z: 788.4 $[M+Na]^+$.

Anti-inflammatory activity: Anti-inflammatory activity of the bioactive formulations was evaluated in vivo by the arachidonic acid induced mouse ear oedema experimental model. The animals used were male mice (Swiss, crl CD-1) weighing 14–16 g, in groups of ten, supplied by the Charles River Laboratories (France). Before ear oedema was induced, 25 μL of aqueous vehicle was applied to the inner and outer surfaces of the both ears of the mice, 30 minutes before the inducer application. Arachidonic acid induced ear oedema was formed by applying arachidonic acid (2 mg) dissolved in acetone (25 μL) to both surfaces of the right ear of the mice. After 2 minutes, the anti-inflammatory formulations (4 mg) dissolved in the vehicle (25 μL) were topically applied to both surfaces of both ears. For the control animals, only the vehicle was applied and for the references, NSAID (2 mg) dissolved in the vehicle (25 μL) were applied on both surfaces of both ears. 30 Minutes after the beginning of the experiment, the animals were sacrificed by cervical dislocation, and a plug (6 mm diameter) was removed with a biopsy-punch from each ear. The oedematous response was measured as the weight difference between the two plugs of the same mouse. The anti-inflammatory activity was expressed as the percentage of inhibition of oedema in treated mice compared to the control animals.

Evaluation of the skin penetration of bioactive formulations of NSAID: In vitro skin permeation studies were performed by using Franz diffusion cells with dynamic flow and an effective diffusion area of 1 cm^2. The experiments were carried out using porcine ear skin. The pigs were obtained from a local slaughterhouse (Montauban, France). After cleaning, hair was removed with a depilatory and the skin was excised from the outer surface of the ears. A 500 μm thick section was removed using an Electro-dermatome (Aescilap). This section included the stratum corneum and a part of the dermis. The skin, which was frozen at −18 °C, was placed in a refrigerator at 4 °C overnight until the experiment. Square pieces of skin 1.5 cm long were prepared. They were placed on the Franz cells with the stratum corneum side facing the donor compartment. The transepidermal water loss was measured before running the experiment in order to guarantee skin integrity.

The experiment was carried out by applying an infinite dose (200 mg) of formulation under occlusive conditions for 24 h. The tested 0.3 % (w/w) vesicular formulations of indomethacin were applied on the skin surface by using a micropipette. One piece of skin was kept untreated to serve as a blank for the experiment.

The receiver compartment was filled with a hydroalcoholic solution (ethanol/phosphate buffer 0.01 M pH 7, 50:50) with a continuous flow of 1.5 $mL\,h^{-1}$ and thermostated at 37 °C throughout the experiment. Receiver medium was collected every 6 h for 24 h in different collectors and the collected volumes were carefully measured. Indomethacin in the collected samples was quantified by HPLC.

HPLC analysis of samples from receiver compartment: Indomethacin samples from the receiver compartment were prepared by adding ethanol (100 μL) to the collected medium (1 mL). Aliquots of 20 μL from each sample were injected into an HPLC system, equipped with a C18 column (Symmetry®, 5 μm, 150 mm × 4.6 mm, Waters). The HPLC system (Hewlett–Packard series) consisted of an autosampler and a multiwavelength UV detector. The quantification of indomethacin was carried out at 260 nm. The samples were analysed using an isocratic mobile-phase consisting of 0.05 % methanol/acetic acid (80:20) at a flow rate of 1 $mL\,min^{-1}$. A calibration curve (peak area versus drug concentration) was plotted by running standard indomethacin solutions in a receiver medium of ethanol/phosphate buffer 0.01 M, pH 7 (50:50).

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