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Contribution of Nanotechnology In the Improvement of the Anti-Inflammatory activity of Shea butter.

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ABSTRACT

Unrefined shea butter is a vegetable fat that can be used for its anti-inflammatory properties related to its unsaponifiable fraction but also as excipient for various forms. In this study, the aim was to see how to take advantage of this dual status in order to improve the anti-inflammatory activity of shea butter through nanotechnology. In other words, it was to test the hypothesis of an improvement of the anti-inflammatory activity of unrefined shea butter when used in the form of nanoparticles and gel / nanoparticle system. The nanoparticles were prepared by a phase inversion method and had an average size of 400 nm with a poly dispersion index of 0.416, a zeta potential between -5 and -7mV and a pH around 5.3-5.4. The gel / nanoparticle complex was obtained by mixing the nanoparticles with a gel based on polyethylene glycol, water and HydroxyPropyl Methylcellulose. The mouse ear edema test was implemented for the evaluation of the anti-inflammatory activity and the results showed a clear improvement of the latter. Indeed, the ratios (R) between the percentage inhibition of the anti-inflammatory activity (I) and the real percentage of shea butter used in the various preparations are respectively: crude shea butter (I = 62.52%; R = 0.62), nanoparticles (I = 60.80%, R = 1.05) and gel / nanoparticle complex system (I = 52.41, R = 6.38) (for the latter, the proportion of nanoparticles in the complex system was 4/24).

Keywords: Shea butter, phase inversion, lipid, nanoparticles, nanoparticle/gel complex, anti-inflammatory activity

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INTRODUCTION

Shea butter is a fat produced from the seeds of Vitallaria paradoxa (sapotaceae). Unrefined, it has anti-inflammatory activity revealed by traditional medicine (1, 2) and confirmed by various scientific works (3-7). In addition, it can be used as an excipient for various conventional dosage forms such as ointments and creams (8-13) but also as a base material for the preparation of drug carriers (14).

The aim of this study was to show if it was possible to improve the anti-inflammatory activity of shea butter by putting it in the form of nanoparticles. Indeed, it has been shown that the effectiveness of the active molecules can be greatly increased if they were associated with nanoparticulate vectors. Thanks to these, the total dose to be administered would be reduced compared to the conventional form with, in addition, limited potential side effects (15-19). Compared to conventional nanoparticles in which active molecules are incorporated, nanoparticles of shea butter will have the distinction of being manufactured with a material that already has in its structure the active principles to convey.

The mouse ear edema test was used to evaluate the anti-inflammatory activity of nanoparticles of shea butter obtained by a phase inversion method (20) and a complex system consisting of these nanoparticles incorporated into a gel.

MATERIALS AND METHOD

The raw materials used for the formulation of nanoparticles were: unrefined shea butter, purchased from women producers of Kedougou (South-East of Senegal), a non-ionic surfactant, Cremophore (Kolliphor ELP (BASF)), distilled water. For the preparation of the gel, polyethylene glycol, distilled water and HydroxyPropylMethylCellulose were used.

Preparation of nanoparticles

The lipid nanoparticles were obtained by a phase inversion method described in the literature (20). After various tests using a three-dimensional diagram (Figure 1), a stable dispersion was obtained with: 57.14% of crude shea butter, 14.26% of cremophor and 28.57% of distilled water.

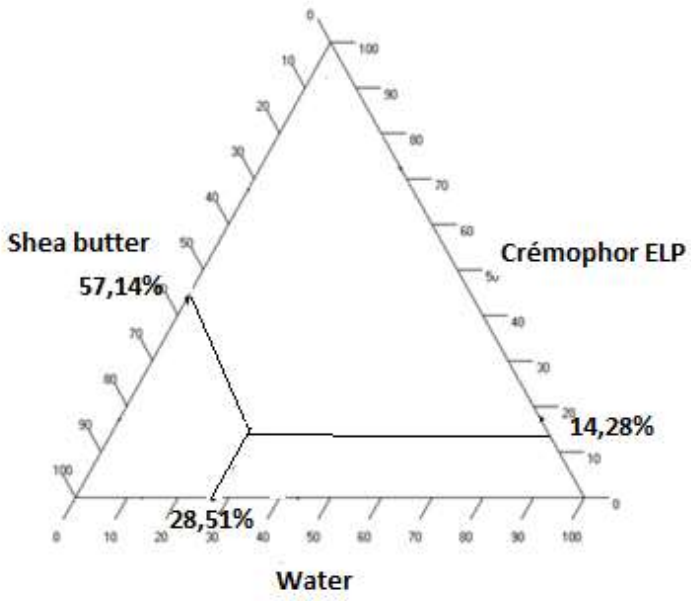

Figure 1: Representation of the three-dimensional diagram for the preparation of shea butter nanoparticles

Preparation of the gel

The surfactant (Cremophor) was mixed under stirring in with the shea butter which has been melted at a temperature below 60 ° C. When the mixture became completely homogeneous, the temperature was brought to 80 ° C. This oily phase was then added abruptly in the aqueous phase at 0 ° C. Stirring was maintained at 350 rpm 60 ° C for 10 minutes. This resulted in the formation of lipid nanoparticles which were then distributed in test tubes and left out of light and at room temperature.

Physico-chemical characterization of lipid nanoparticles

Macroscopic examination: the purpose of this examination was to look for possible instability phenomena such as deposition formation, phase separation, aggregate formation, etc

Microscopic examination: it was carried out with a fluorescence optical microscope (Zeiss Primo Star) at X 40 magnification. Size, PDI and Zeta potential: these parameters were determined by a Zetasizer Nano ZS 90 (Malvern Instruments, England). All experiments were performed at 20°C, in triplicate. pH measurement: it was carried out with a pH meter CG 820 (SCHOTT GERATE).

Preparation of the gel-nanoparticle system

The preparation of the system consists of a mixture of the nanoparticles with the already prepared gel. Different ratios have been used and the most stable was that with 14.28% nanoparticles and 85.72% gel.

Anti-inflammatory test

The croton oil induced ear swelling test has been implemented using the method described by Sosa (21). The mice were divided into 4 lots of 5 each: a control group and three groups (L1, L2, L3) treated respectively by unrefined shea butter (3mg/ear), the shea butter nanoparticles (1.71 mg/ear) and the gel / nanoparticles complex (0.24 mg/ear).

Statistical analysis

The results were expressed as mean ± standard error at mean. An analysis of variance (ANOVA) was performed to verify the homogeneity of the groups. A Student's test was used to highlight the existence of a significant difference between the different groups with a threshold of significance $p < 0.05$.

RESULTS AND DISCUSSION

Physico-chemical characterization of lipid nanoparticles

Macroscopic examination

After formulation, all the preparations were stable. They were whitish in color and viscous in appearance (figure 2).

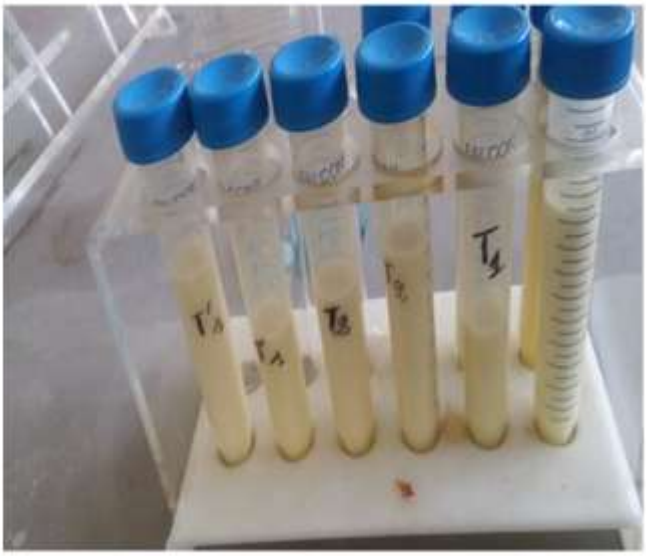

Figure 2: Macroscopic aspect of shea butter nanoparticles suspension

Microscopic examination

Figure 3 shows the microscopic aspect of the suspension of lipid nanoparticles.

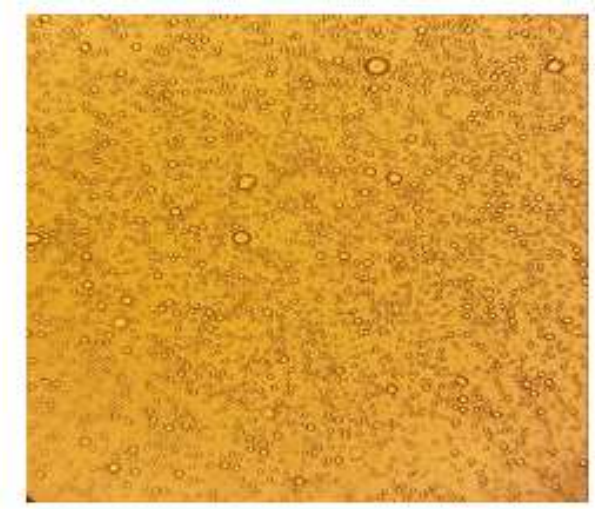

Figure 3: Image of shea butter nanoparticles observed with a fluorescence optical microscope (Zeiss Primo Star) at X 40 magnification.

The results of the measurement of the size and the poly dispersion index of the nanoparticles were respectively 403.1 ± 1.266 nm and 0.416

Zeta potential

Measurement of the zeta potential of our different preparations yielded values between -5 and -7 millivolts.

pH

The table I gives the average pH values measured for each sample for 28 days.

Table I: pH values over time

	J1	**J7**	**J14**	**J28**
pH	5.34	5 .48	5.35	5.33

Anti-inflammatory activity

The percentages of increase (% AUG) of the weight of the ears and the inhibition of the edema were respectively calculated according to the formulas F1 and F2.

$$\%AUG = \frac{\text{Weight OG} - \text{Weight OD X 100}}{\text{Weight OG}} \text{ (F1)}$$

$$\%INH = \frac{\text{Avg (\%) Aug T} - \text{Avg (\%) Aug Batch Tr}}{\text{Avg (\%) Aug T}} \text{ (F2)}$$

The results are shown in table II and figure 4.

Table II : Percentage of edema weight as percentage of the increase in ear weight and percentage of edema inhibition

	percentage of the increase in ear weigh (%)	Inhibition (%)
Control	122.78 ± 12.44	
Shea butter	45.05 ± 8.72	62.52 ± 6.55
Nanoparticles	46.35 ± 7.98	60.80 ± 7,42
Gel/nanoparticles system	56.59 ± 6.74	52.41 ± 7.32

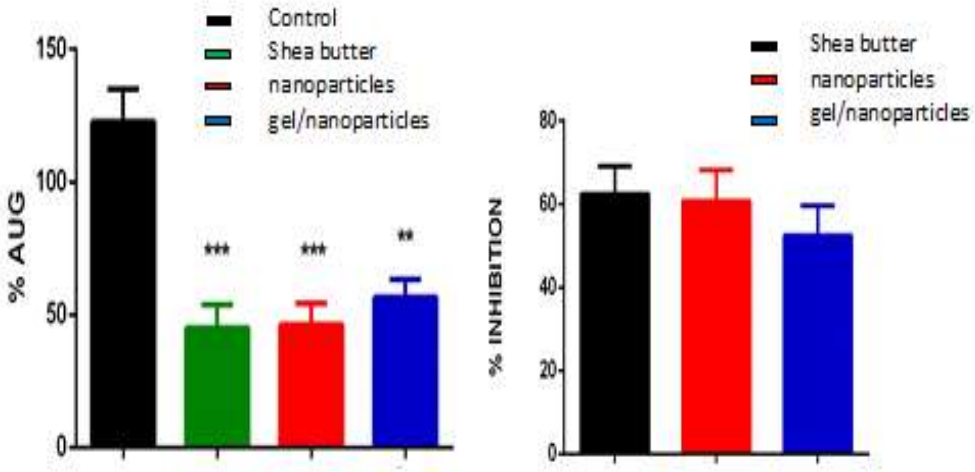

Figure4.1 **Figure 4.2**

Figure 4: Percentages of edema (4.1) and inhibition of the ear (4.2).

Discussion

It must be remembered that the development of nanoparticles as drug vectors involves a basic material (polymer, lipid, etc.), a preparation method and a molecule of interest. Therefore, the issues lie around questions related to the toxicity and the material used but also to the implementation of the method of preparation (complexity, yield, rate of encapsulation of active ingredients, etc.).

In general, these nanoparticles may be nanocapsules (Fig 5. a.1) (polymeric membrane surrounding a cavity containing active ingredients or nanospheres that are matrix-type nanoparticles (Fig 5. a.2) (the active ingredients are dispersed or dissolved in the polymeric matrix) (22).

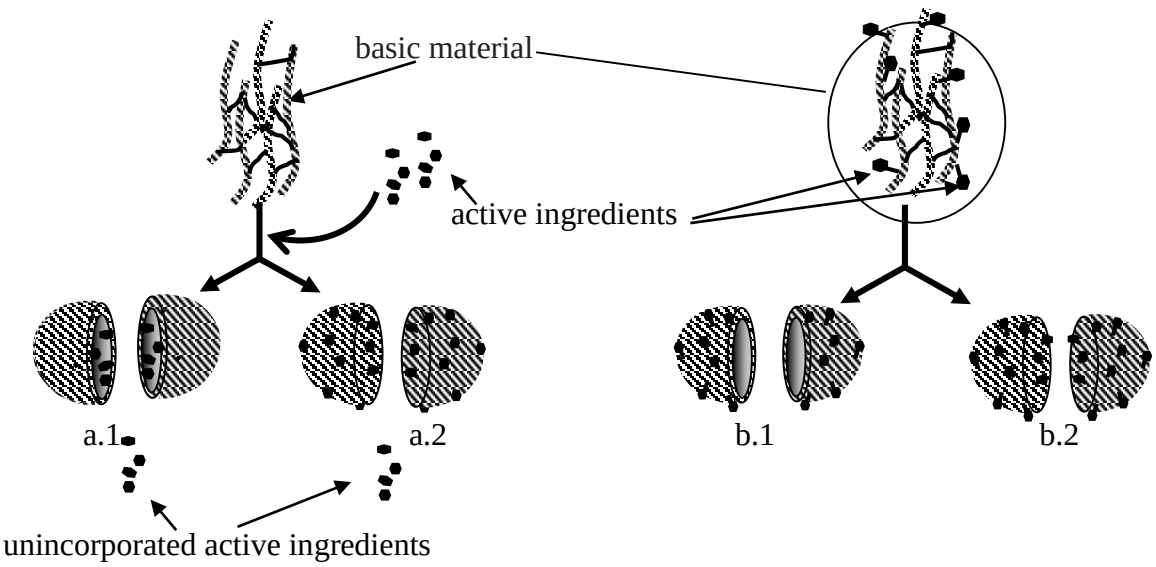

Figure 5: Nanoparticles and vectorized active ingredients

a.1. nanocapsule: active ingredients are inside the capsule; a.2. nanosphere: the active ingredients are incustrated in the mass of the basic material; b.1 and b.2 . shea butter nanocapsule and nanosphere: the active ingredients (anti inflammatory) are part of the structure of the material

When implementing the drug nanovector preparation , some of the active ingredients added to the base material are not encapsulated or incorporated into the nanospheres (Fig 5. a.1 and 5. a.2). Therefore the choice of a method of preparation that can ensure a good rate of incorporation of the active ingredients has become, as shown several authors (23-25), a major challenge in the field of vectorization.

With regard to shea butter nanoparticles, it has already been demonstrated that they can be obtained using high shear mixer (14), but the advantage of the method used is that it is simpler, less energetic and faster. As described in the literature (20), this method leads to the formation of nanocapsules.

What makes the interest of nanoparticles of shea butter is that their obtaining is not concerned with the incorporation rate of active ingredients. Indeed, the latter (anti inflammatory active ingredients) have the distinction of being an integral part of the basic material, in particular of its unsaponifiable fraction; no action was necessary to add them to the latter (Figures 4. b.1 and b.2). The encapsulation rate therefore depends on the level of unsaponifiable which is here of 16%.

If we consider the low zeta potential values, the electrostatic stability could not be mentioned here. It should be remembered, however, that steric hindrance due to surfactants is a factor of stability which may be sufficient to prevent aggregation of the particles (26- 28). This is probably why,

during the observation period, the dispersions remained stable. Indeed, no sign of destabilization (formation of deposits or phase separation) has occurred. Microscopic observation reveals spherical particles with some extensions on the surface of the particles.

With regard to pH, the results obtained indicated an acid character for all the samples, with average values between 5.2 and 5.3. These values are perfectly compatible with an incorporation of these lipid nanoparticles in forms such as creams, ointments and gels intended for cutaneous application, since the pH of the skin is generally between 5.4 and 5.9 (29). These data are compatible with the wide use of traditional shea butter for various skin conditions, particularly those with an inflammatory character.

According to several studies, the presence of compounds such as sterols, triterpenic alcohols, phenols and tocopherol in the unsaponifiable fraction of shea butter confer to the latter anti-inflammatory and antioxidant effects which would justify such use (4, 30-32). Further studies have shown that, more specifically, triterpen acetates and triterpen cinnamates were responsible for the anti-inflammatory activity (5).

The results of the anti-inflammatory tests show average inhibition percentages of 62.52%, 60.80% and 52.41% respectively for unrefined shea butter, nanoparticles and nanoparticles incorporated in gel. The ratio of the percent inhibition of ear edema to the real percentage of shea butter in the different preparations was 0.62, 1.05 and 6.38 respectively for unrefined shea butter, nanoparticles and the gel-nanoparticle system. Thus, there was a very great improvement in the anti-inflammatory activity for the last two forms. This may be due to the fact that they ensure better penetration via the different layers of the skin. The influence of the form of use appears clearly here.

The question of the penetration of lipid particles in the skin has been the subject of many studies that have shown that several mechanisms could be involved. Among these, intercellular penetration route could be mentioned. Such a mechanism concerns ultra-flexible liposomes (33). Indeed, despite their large size, the liposomes would be able to deform to slide through the intercellular canals. This mechanism does not seem to be involved in the case of the lipid nanoparticles studied here because the latter do not have the flexibility of liposomes.

However, for the lipid nanocapsules of a size between 100 nm and 400 nm, other authors explain the mechanism of their penetration into the skin by their solubilization in the stratum corneum (34, 35). It is more likely that it is such a mechanism that explains the improvement of the anti-inflammatory activity observed with the nanoparticles.

The best anti-inflammatory activity was obtained with the gel-nanoparticle system. This could be explained by the ability of the gel to allow better penetration of the skin barrier. This is in agreement with the work of several authors for whom this greater efficiency of nanoparticle gel systems compared to conventional forms can be explained on the one hand by a greater contact between the active ingredients and the skin because of the large specific surface area of the nanoparticles (36, 37) and on the other hand by the fact that the hydrophilic gels constitute an additional barrier favorable to a sustained release over time (38, 39).

CONCLUSION

This study showed the benefit of using solid solid lipid nanoparticles to contribute to the increase of the effectiveness of the molecules administered topically. Shea butter, which contains anti-inflammatory products in its composition, has been put into the form of nanoparticles by a phase inversion method to verify this hypothesis. Used alone or in combination with a gel, shea butter nanoparticles showed significantly greater anti-inflammatory activity than shea butter used as such.

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