

Liposomes: Topical and Oral Bioavailability
Julio Cortijo, PhD, Patricia Almudéver Folch, PhD, Juan Manuel Serrano Núñez
 Sesderma Laboratories, Polígono Industrial Rafelbuñol
 C/Massamagrell 3, Rafelbuñol
 46138 (Valencia) Spain
Julio.Cortijo@uv.es, Patricia.Almudever@uv.es, j.serrano@sesderma.com

Liposomes are small vesicles composed of one or more lipid bilayers. The size can go from 30nm up to several microns. Liposomes can encapsulate hydrophilic solutes in the aqueous core and lipophilic solutes in the membrane. These vesicles can be classified according to their size and number of bilayers: Multilamellar (100-10.000nm), Small Unilamellar (less than 100nm), Large Unilamellar (100-500nm). Sesderma manufactures very uniform, unilamellar liposome populations of between 50-150nm.

The advantages of liposomes are that, the structure is very similar to biological membranes and thus, are biodegradable and non toxic, they can reach the deepest layers of the skin, they provide a sustained release of the active ingredients, they prevent the oxidation and degradation of the ingredients and they show higher efficiencies at lower concentrations.

We have carried out three different experiments on topical bioavailability: liposome penetration through skin, hair follicles and nails.

All the ingredients used to prepare the liposomes are classified as GRAS (generally recognized as safe).

In the first one, we compared the permeation capacity through human skin, using a Franz Diffusion Cell, of two different substances encapsulated and not encapsulated in liposomes: fluorescein and sodium ascorbate. Aliquots were taken from the receptor chamber at different times. The concentration of sodium ascorbate was determined by high performance liquid chromatography with ultraviolet detection (HPLC-UV) and that of fluorescein by spectrofluorimetry. The results were as follows:

| PERMEABILITY CONSTANT | EPIDERMIS | DERMIS | COMPLETE SKIN |
|----------------------------|---------------------|---------------------|---------------------|
| Liposomal Fluorescein | 3.80E-03 ± 1.01E-03 | 4.97E-05 ± 6.47E-06 | 7.70E-05 ± 4.47E-06 |
| Fluorescein Solution | 2.55E-05 ± 8.55E-06 | 1.08E-04 ± 2E-05 | 1.76E-06 ± 3.26E-07 |
| Liposomal Sodium Ascorbate | 2.43E-04 ± 4.55E-05 | 7.55E-04 ± 8E-05 | 8.94E-05 ± 3.90E-06 |
| Sodium Ascorbate Solution | 3.60E-06 ± 2.50E-07 | 4.81E-03 ± 8.91E-04 | 5.39E-06 ± 5.52E-07 |

These results might be due to the nature and size of the active ingredients, and the characteristics of the layers of the skin. The epidermis is a stratified layer with plenty of cells, this is why liposomes can get through it easier than the ingredients in solution. Fluorescein can diffuse faster through the epidermis than sodium ascorbate because fluorescein is more lipophilic than sodium ascorbate. The dermis has less cells and more fibres, and has a greater aqueous volume, so the preparation that permeates faster is that of sodium ascorbate solution due to its hydrophilic nature and small size. Finally, we can confirm that liposomes help substances pass through the skin.

In the second case, liposome ability to go across the follicular canal was assayed with liposomal fluorescein. The skin samples were extracted from human scalp and the equipment used was the same as in the prior experiment: Franz Diffusion Cell. Pictures were taken at different times with a fluorescence microscope. We concluded that the follicular canal is an excellent penetration enhancer; a liposome reservoir is formed, facilitating its pass through the hair follicle and into the dermis.

In the third experiment, we assessed the penetration capacity of liposomal fluorescein on one hand and a solution of fluorescein on the other hand, through human nails. The equipment utilized was a Franz Diffusion Cell with a coupling device for nails. Aliquots were taken from the receptor chamber at different times and the concentrations of fluorescein were determined by

spectrofluorimetry. Pictures were also taken with a fluorescence microscope. The results showed that the maximum quantity of absorption for both formulations was obtained after 2 days in contact with the products. The concentration of fluorescein ($2.96 \pm 1.02 \mu\text{g}/\text{cm}^2$) for the liposomal formulation was 2.5 times higher than the solution ($1.22 \pm 0.2 \mu\text{g}/\text{cm}^2$). However, the permeability constant is very similar for both preparations: fluorescein solution ($0.006 \pm 0.002 \text{ cm}^2/\text{s}$) and liposomal fluorescein ($0.008 \pm 0.001 \text{ cm}^2/\text{s}$). We could also observe that there was an increase in the thickness of the nail treated with liposomal fluorescein whilst there were no changes observed in the nail treated with the solution of fluorescein.

Ascorbic Acid Oral Pharmacokinetics in Rats

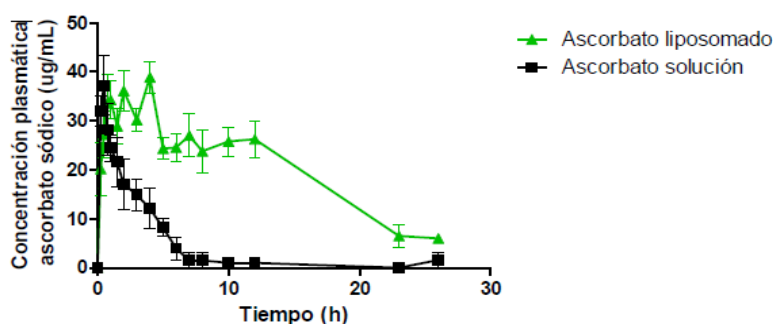
Aim: Compare the pharmacokinetics of two sodium ascorbate formulations:

- Sodium ascorbate solution (extemporaneously prepared).
- Sodium ascorbate encapsulated in liposomes.

Method:

The day prior to the administration of the formulations, 12 Wistar rats (280-310 g) were cannulated in the jugular vein to allow blood sampling at preset times. A volume of 2.5 mL of each fresh formulation was administered orally as single dose with intraoesophageal cannula. Six replicates were performed for each formulation. 200 μL blood samples were taken at the following times: 0, 15, 30, 45, 60, 90, 120 minutes and 3, 4, 5, 6, 7, 8, 10, 12, 23, 26 hours. The samples were centrifuged at 2000g for 5 min to obtain plasma which was immediately deproteinized with ice-cold MPA 10% (metaphosphoric acid). The samples were filtered through a pore diameter of $0.45 \mu\text{m}$. The analytical method used to measure vitamin C (sodium ascorbate) was HPLC-UV with UV detection at 254nm. The mobile phase used consisted of a KH_2PO_4 (0.1M) solution: ACN (95:5) at a pH of 2. As the stationary phase, the column Sherisorb ODS1 5 μm 25x0.4mm was used and the selected flow rate was 1 ml / min. The injection volume used was 60 μL .

Results:



Plasma concentration versus time after oral administration of 250 mg of sodium ascorbate formulated in an extemporaneous solution (black line) or in liposomes (green line). Mean \pm SEM, n = 6.

Conclusions: liposomes enable a better control of the release of the drug in plasma and maintains it for a longer period of time. A liposomal formulation of sodium ascorbate requires a smaller dose to reach the desired plasma concentration and, therefore, the desired therapeutic effect.

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