Hydroxypropyl methylcellulose hydrogel of berberine chloride-loaded escinosomes: dermal absorption and biocompatibility

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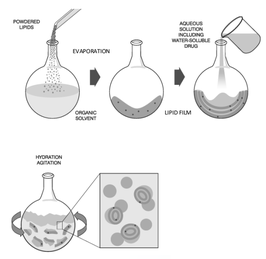
Thin Layer Evaporation method



Transmission Electron Microscopy

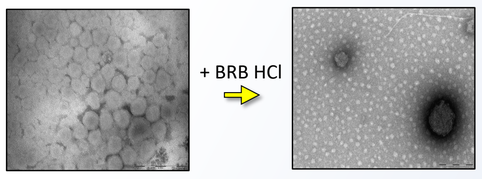


Figure 3. TEM images of escinosomes (EL) on left and BRB-loaded escinosomes (B-EL) on right.

Dynamic/Electrophoretic Light Scattering

Formulation	Composition (ratio w/w)	AHD (nm)	PdI	ζ-potential (mV)	Deformability
EL	ESN-P90G (5:33)	137.4 ± 6.9	0.26 ± 0.01	-40.5 ± 3.1	0.89 ± 0.01
B-EL	BRB + ESN-P90G (1.3:5:33)	150.1 ± 5.2	0.17 ± 0.02	-34.8 ± 5.1	1.09 ± 0.01

Table 1. Physical characterization of escinosomes (EL) and BRB-loaded escinosomes (B-EL). Mean ± SD (*n*=3). AHD = Average Hydrodynamic Diameter; PdI = Polydispersity Index; * = not deformable

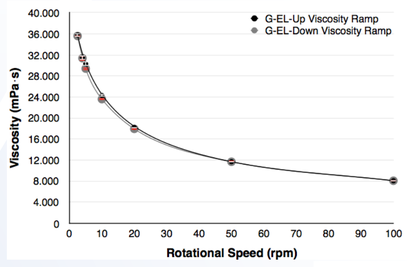
HPLC-DAD

Formulation	ESN			BRB		
	ESN %w/v	R%	EE%	BRB %w/v	R%	EE%
EL	0.5%	97.34 ± 3.40	95.46 ± 0.93	/	/	/
B-EL	0.5%	96.50 ± 3.90	93.16 ± 9.68	0.13%	97.06 ± 7.10	66.70 ± 5.33

Table 2. Chemical characterization of escinosomes (EL) and BRB-loaded escinosomes (B-EL). Mean ± SD (*n*=3). R = Recovery; EE = Encapsulation Efficiency.

Vesicle gelation by manual stirring

Brookfield DVE-RV Digital Viscometer



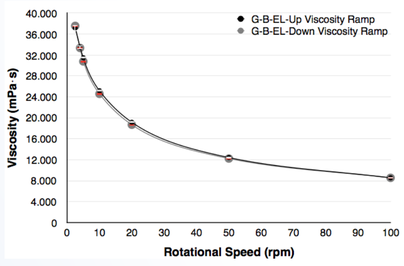


Figure 4. Up-down ramp viscosity of G-EL (hydrogel of escinosomes) and G-B-EL (hydrogel of BRB-loaded escinosomes). Mean±SD (*n*=3).

The hydrogels are:
- ✓ Non-Newtonian
- ✓ Time-indipendent
- ✓ Shear thinning

SUMMARY

Aim of this work was to prepare and characterize new nanocarrier-loaded hydrogel formulations for topical application, using hydroxypropyl methylcellulose (HMPC) and special nanovesicles, the escinosomes [1]. The combination of the two technological strategies, nanocarriers and hydrogels, was selected to circumvent some drawbacks of nanovesicles and develop stable and efficient skin-delivery systems [2]. HPMC is a derivative of cellulose with a wide range of physicochemical properties, forming suitable hydrogel for dermatological applications. Escinosomes are a new vesicular carrier made of escin (ESN), a natural bioactive saponin clinically used for the anti-edematous and anti-inflammatory effects [3], plus phosphatidylcholine, where ESN also represented a vesicle bilayer forming material. Escinosomes are able to maintain the hyaluronidase inhibition activity of ESN, and load other active molecules such as berberine chloride (BRB), a natural quaternary isoquinoline alkaloid traditionally used for various therapeutic effects [4]. BRB-loaded escinosomes were then entrapped in the polymeric matrix of HPMC and they were studied for viscosity, drug release, dermal permeation and biocompatibility properties. BRB did not affect ESN release and the low ESN release from the escinosome hydrogels confirmed the strong ESN interaction with the vesicular bilayer. Permeation profiles of aqueous ESN/BRB dispersions and escinosomes were compared with the corresponding hydrogels, showing a higher residence time of the HPMC-hydrogel. Moreover, the viscosity measurements and the biocompatibility studies evidenced their suitability for topical applications.

NANOVESICLE PHYSICAL AND CHEMICAL CHARACTERIZATION

NANOVESICLE PREPARATION

HPMC-HYDROGEL PREPARATION AND VISCOSITY MEASUREMENTS

ESCIN

Escin (ESN) is a mixture of triterpenic saponins isolated from seeds of *A. hippocastanum.* It exhibits anti-inflammatory, anti-oedematous and venotonic properties. It is clinically used to increase venous well tone in the treatment of chronic venous insufficiency and it also has a place in the treatment of post-operative oedema [1]. In the present work, ESN has been used as constituent of the liposome bilayer thanks to its chemical structure and edge activator function, in order to increase the cutaneous absorption of other poorly permeable molecules, as well as to get a synergic therapeutic effect.

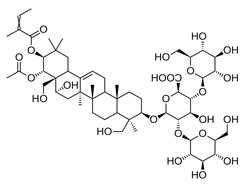


Figure 1. ESN chemical structure.

BERBERINE

Berberine (BRB) is a natural isoquinoline alkaloid, isolated from various medicinal plants such as *Berberis vulgaris* L. and *Hydrastis canadensis* L. It was widely used by the Traditional Chinese Medicine since the ancient times, because of its several therapeutic activities, such as anti-inflammatory, anti-microbial, and gastrointestinal effects, however it is recently largely studied also for its cardiovascular and anti-tumour activities [2]. In the present work it has been examined in combination with ESN inside vesicular systems relative to dermal administration.

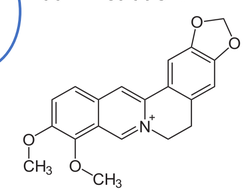


Figure 2. BRB chemical structure.

DRUG RELEASE FROM HPMC-HYDROGELS

IN VITRO ABSORPTION STUDIES ON NUDE MOUSE SKIN

IN VIVO TEST: ACUTE DERMAL IRRITATION/CORROSION

Vertical diffusion Franz cells: cellulose nitrate membranes

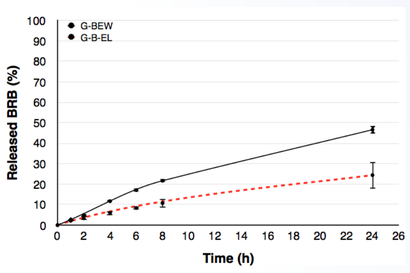


Figure 5. BRB release from G-B-EL (hydrogel of BRB-loaded escinosomes) and G-BE_W (hydrogel of ESN plus BRB water dispersion). Mean ± SD (*n*=3).

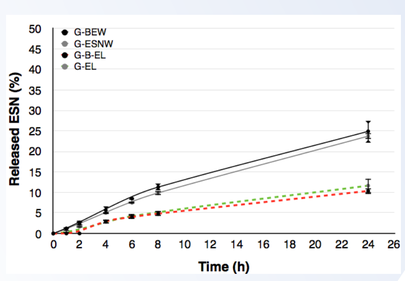


Figure 6. ESN release from escinosome HPMC-hydrogels (G-B-EL and G-EL), and from aqueous dispersion hydrogels (G-BE_W and G-ESN_W). Mean ± SD (*n*=3).

- ✓ Escinosomes make the release of ESN and BRB slower
- ✓ BRB do not affect ESN release

Vertical diffusion Franz cells: nude mouse skin

	BRB		
	A_{24} (%)	S_{24} (%)	TA_{24} (%)
B-EL	69.16±11.77	4.30±0.59	73.41±12.59
BE_W	53.75±2.69	5.11±1.89	59.27±5.16
	ESN		
	A_{24} (%)	S_{24} (%)	TA_{24} (%)
EL	76.59±1.82	24.37±1.44	100.96±0.38
B-EL	26.10±5.00	26.97±10.13	36.90±12.74
ESN_W	24.51±4.04	3.53±0.46	26.69±2.61
BE_W	0.90±0.25	6.19±3.41	7.09±3.47

Table 3. BRB and ESN skin absorption parameters related to escinosomes and water dispersions, obtained from the *in vitro* test with nude mouse skin, by vertical diffusion Franz cells. Mean ± SD (*n*=3).

	ESN		
	A_{24} (%)	S_{24} (%)	TA_{24} (%)
G-EL	4.67±2.62	0.56±0.35	5.23±2.29
G-B-EL	2.52±0.90	0.39±0.08	2.90±0.79
G-ESN_W	-	0.99±0.16	0.99±0.16
G-BE_W	3.67±0.28	0.82±0.12	4.48±0.41
	BRB		
	A_{24} (%)	S_{24} (%)	TA_{24} (%)
G-B-EL	46.09±13.83	4.13±0.27	50.22±13.67
G-BE_W	41.45±3.24	2.98±0.27	44.43±3.36

Table 4. BRB and ESN skin absorption parameters related to hydrogels of escinosomes and hydrogels of water dispersions, obtained from the *in vitro* test with nude mouse skin, by vertical diffusion Franz cells. Mean ± SD (*n*=3).

- ✓ Hydrogels increase the residence time of the formulation on the skin
- ✓ Hydrogel of BRB-loaded escinosomes increases BRB skin absorption
- ✓ BRB sligthly decreases ESN skin absorption

Sprague-Dawley rats

Table 5. Appearance of rats belonging to the control group (C) and to the groups treated with: G-L (hydrogel of blank liposome), G-EL (hydrogel of escinosomes) and G-B-EL (hydrogel of BRB-loaded escinosomes). Experiments were completed on 6 animals for each group.

Group	Erythema (normal for"√")	Oedema (normal for"√")	Death/Total animals
C	√	√	0/3
G-L	√	√	0/6
G-EL	√	√	0/6
G-B-EL	√	√	0/6

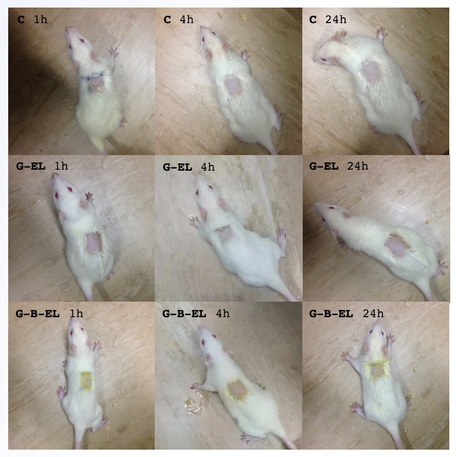


Figure 7. Acute dermal irritation/corrosion test. Rat skin appearance after 1 h, 4 h and 24 h of observation (from left to right, respectively). First line: rats not treated with escinosome HPMC- hydrogel, C (control group). Second line: rats treated with G-EL (hydrogel of escinosomes). Third line: rats treated with G-B-EL (hydrogel of escinosomes loaded with BRB). Experiments were done on 6 animals for each group.

- ✓ No irritant effects: hydrogels are safe for human dermal use

ABBREVIATIONS

EL: escinosomes
B-EL: berberine chloride-loaded escinosomes
ESN_W: escin water dispersion
BE_W: berberine chloride plus escin water dispersion
G-EL: hydrogel of escinosomes
G-B-EL: hydrogel of berberine chloride-loaded escinosomes
G-ESN_W: hydrogel of escin water dispersion
G-BE_W: hydrogel of berberine chloride plus escin water dispersion
A_{24}= absorbed dose
S_{24}= absorbable dose retained inside the skin
TA_{24}= total absorbed dose

CONCLUSIONS

The new escinosome HPMC-hydrogel formulations combine the advantages of a modified release and increased transdermal permeability (escinosome components), with better viscosity properties (polysaccharide matrix). In addition, the developed escinosome HPMC-hydrogels were very stable with a very good safety profile, since biocompatibility studies showed no potentially hazardous skin irritation.

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Acknowledgments

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