Phytosomes-Based Nanocarriers Enhanced with Seaweed Extracts: Overcoming the Blood-Brain Barrier

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INTRODUCTION

Neurodegenerative diseases result from the gradual decline in nerve cell function and are exacerbated by population aging. Macroalgae, particularly *Bifurcaria bifurcata* (BB), *Fucus spiralis* (FS), and *Ascophyllum nodosum* (AN), offer neuroprotective potential. This is attributed to their bioactive compounds, which possess antioxidant and anti-inflammatory properties [1,2]. However, in order to reach their target, these compounds have to cross the blood-brain barrier (BBB) (Fig. 1).

Phytosomes (Fig. 2) are nanocarriers made of phospholipid bilayers that enhance bioactive compound delivery by forming amphipathic complexes, improving their ability to cross the BBB through lipid-mediated or carrier-assisted transport [3].

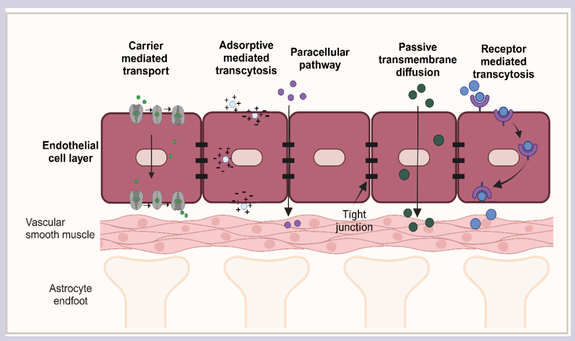

Fig. 1-Schematic representation of the BBB.

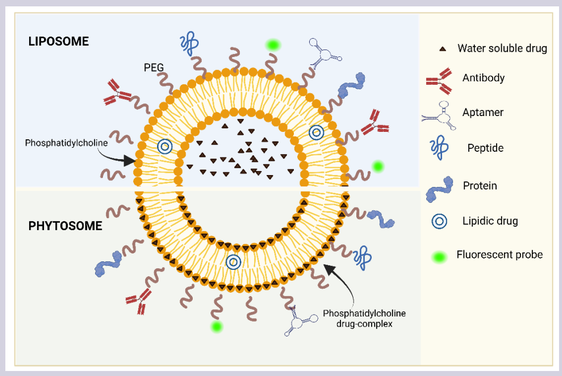

Fig. 2–Liposomes, phytosomes and possible ligands.

These nanocarriers offer biocompatibility, stability, and liposolubility, with surface functionalization (e.g., polyethylene glycol or apolipoprotein E (ApoE)) enhancing BBB penetration and reducing immune recognition.

METHODOLOGY

Macroalgae extraction: Extracts were obtained using microwave-assisted extraction (MAE) with ethanol-water as the solvent, lyophilized and kept at -80ºC until further use [4,5].

Phytosomes production and stability : Phosphatidylcholine (PC) and extract were combined in a 1:1 w:w and kept at 60ºC for 1h under agitation. The entrapment rate and stability were determined based on the absorbance at 280 nm. Phytosomes were further functionalized with DSPE-PEG(2000) and ApoE. To assess their stability, the phytosomes were dried under a N_2 flow, resuspended in CH_3Cl and the absorbance was read at 250nm for 4 weeks.

Phytosomes characterization: Phytosomes were analyzed by UV spectrophotometry, FTIR and the size distribution and zeta potential were characterized by dynamic light scattering (DLS).

hCMEC/D3 cells model of the BBB: To analyze if the phytosomes were able to cross the BBB a transwell hCMEC/D3 (Fig. 3) cell model was applied. The phytosomes under studied were marked with coumarin 6 1% (w:w) and the quantity that crossed the simulated BBB was read by fluorescence, emission at 501 nm after excitation at 457 nm, after 3h and 24 hours.

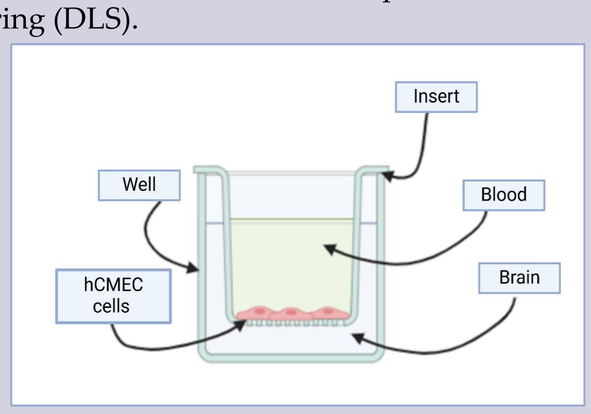

Fig. 3–Scheme of a transwell mimicking the BBB.

RESULTS

The encapsulation rates (Fig. 4 A) varied between 75% (BB) and 80% (AN). Concerning the stability of the phytosomes complex (Fig. 4 B), the absorbance measured at the maximum absorbance wavelength (280 nm) was stable for 4 weeks with variations under 20%.

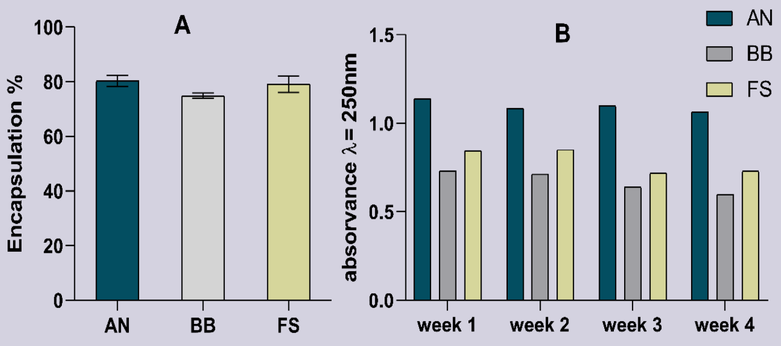

Fig. 4- A) Phytosomes encapsulation rate; and B) stability studies.

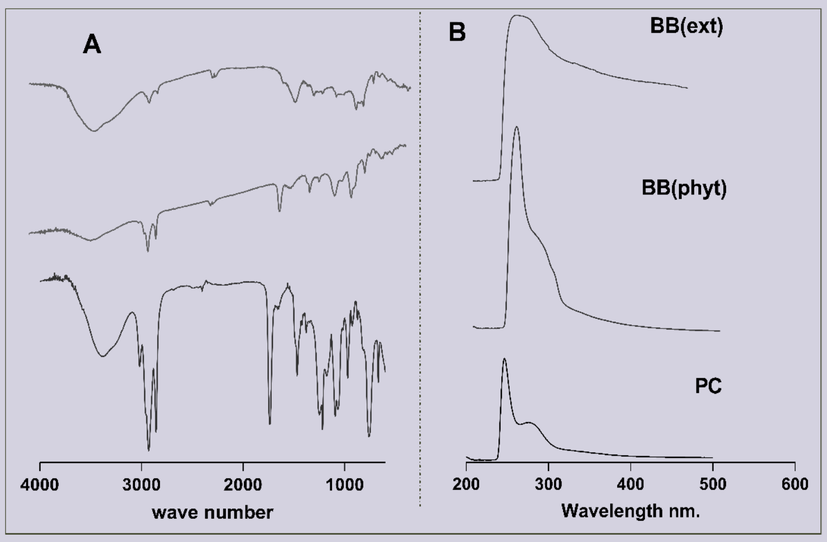

Fig. 5-Spectral profile of the BB extract, the phytosomes and the phosphatidylcholine molecule. A) FTIR B) UV-VIS

As an example, the FTIR and UV-Vis spectra of the BB extract, the respective phytosomes and PC is presented in Fig. 5.

Analyzing the FTIR spectra the hydroxyl group (OH) is confirmed by a characteristic peak at 3400 nm in the extract and the phytosomes.

Common groups:

- C-H (peak around 3000 nm)
- C=O (peak around 1700 nm)
- P=O (peak at 1200 nm) - unique to the complex
- C-O (peak at 1100 nm) - significantly intensified in the complex compared to the extract.
- C-O-C (peak near 1000 nm) - exclusive to the extract.

The UV-VIS spectrum of the phytosomes reveals a noticeable narrowing of the absorption band around 250 nm.

Table 1-Results obtained by DLS analysis for the different phytosomes.

Phytosomes	Average size (nm)	Polydispersity	Zeta Potential (mV)
AN (phyt)	167.73 ± 18.01	0.286 ± 0.019	2.22 ± 0.52
BB (phyt)	150.79 ± 60.30	0.395 ± 0.086	2.15 ± 0.12
FS (phyt)	117.71 ± 26.10	0.411 ± 0.178	1.91 ± 0.10
AN (PEG)	365.6± 34.16	0.587 ± 0.138	0.63 ± 0.47
BB (PEG)	297 ± 58.43	0.566 ± 0.032	0.87 ± 0.58
FS (PEG)	254.25 ± 88.35	0.685 ± 0.171	-7.27 ± 1.81
AN (ApoE/PEG)	277.07 ± 38.31	0.389 ± 0.013	-0.68 ± 0.26
BB (ApoE/PEG)	309.68± 85.56	0.539 ± 0.088	-3.31 ± 1.05
FS (ApoE/PEG)	361.44 ± 43.97	0.419 ± 0.100	-2.30 ± 0.64

Table 1 highlights differences between simple and functionalized phytosomes across three extracts, confirming an additional functional layer. Functionalized phytosomes show higher polydispersity, especially with PEG alone, compared to ApoE + PEG combinations. Functionalization also lowers the zeta potential, often yielding negative surface charges. Table 2 suggests that particle transport may occur independently of ApoE receptors. At 24 hours, permeability increased, but the impact of functionalization remained unclear.

Table 2-Percentage permeability of functionalized and non-functionalized phytosomes across the simulated BBB after 3 and 24 hours.

Phytosomes	Permeability (%) 3h	Permeability (%)24 h
AN (phyt)	0.079 ± 0.026	27.411 ± 0.936
BB (phyt)	3.763 ± 0.042	22.865 ± 2.017
FS (phyt)	5.152 ± 0.039	23.276 ± 0.612
AN (ApoE3/PEG)	4.672 ± 0.028	13.144 ± 0.393
BB (ApoE3/PEG)	4.603 ± 0.032	12.480 ± 0.280
FS (ApoE3/PEG)	4.788 ± 0.032	20.598 ± 2.099

CONCLUSIONS

- **Development of Phytosomes**: Natural macroalgae extracts were successfully incorporated into a lipid nanocarrier through the development of phytosomes.
- **Stability**: The phytosomes demonstrated stability for at least four weeks.
- **BBB Permeability**: Functionalization with ApoE was not essential for phytosomes to cross the BBB, as they passed through hCMEC/D3 cell monolayers regardless of formulation.

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