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Physicochemical characterization and effect of additives of membrane vesicles from *Brassica oleracea* L. to be used in nanofertilization

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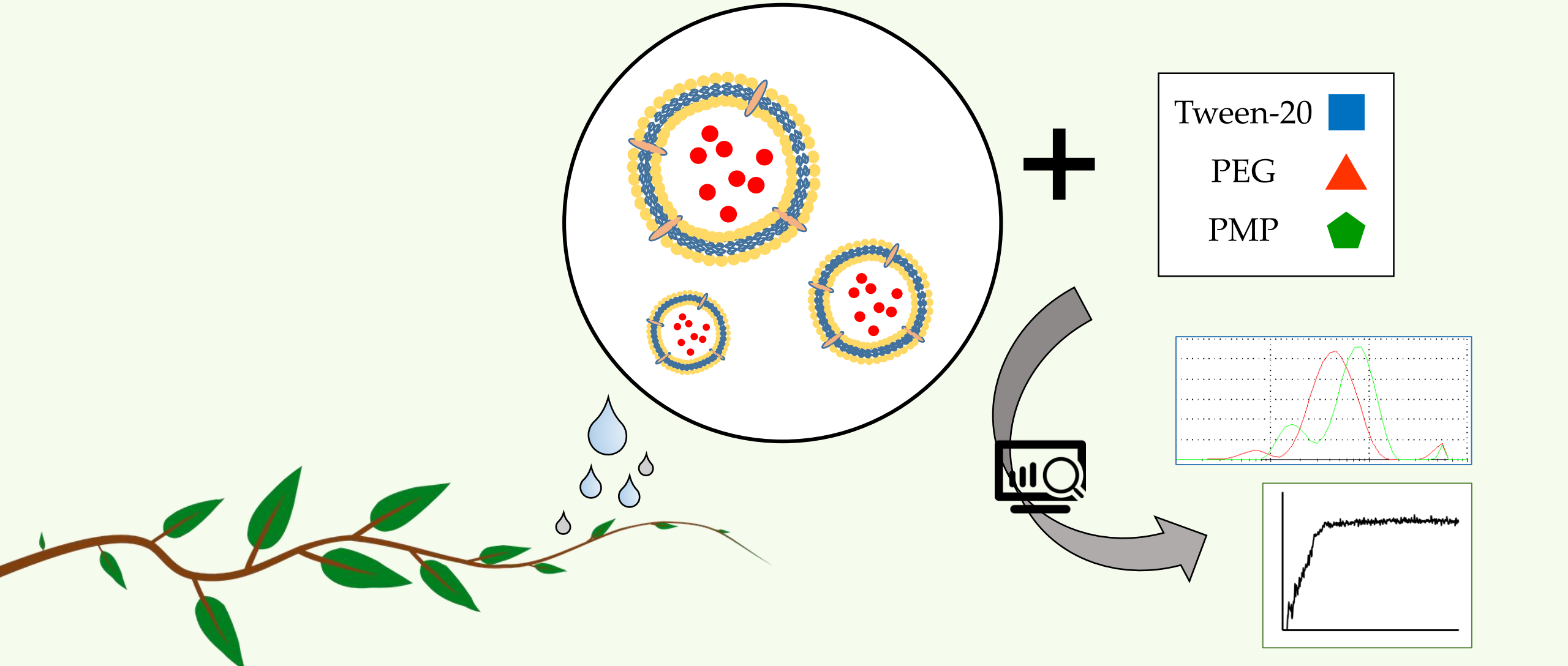
Physicochemical characterization and effect of additives of membrane vesicles from *Brassica oleracea* L. to be used in nanofertilization

Graphical Abstract



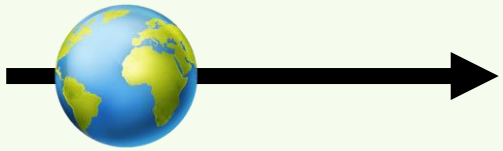
Physicochemical characterization and effect of additives of membrane vesicles from *Brassica oleracea* L. to be used in nanofertilization

Abstract: Traditional fertilizers and their intensive use cause different environmental problems and new strategies are necessary to deal with these aspects. In these sense, foliar nanofertilization is a new technology postulate as one of the most promising to use in the near future. This type of fertilization has many environmental advantages but there are different factors that its necessary to solve, as they need to be compatible with other additives. Membrane vesicles derived from plant material have been showed in preliminary studies by their great potential as nanocarriers of different micronutrients such as iron (Fe) or boron (B). A complete optimization of fertilizer system based on nanocarriers to encapsulate different elements from different approach is key to obtaining a suitable and profitable from an economic point of view. In this work, different physicochemical parameters such as size, potential Z or osmotic water permeability were measured in membrane vesicles obtained from *Brassica oleracea* L. to check the integrity of vesicles for further biotechnological application. Besides, different additives (polyether-modified-polysiloxane [PMP], Tween-20 and polyethylene glycol [PEG]) were added to vesicles at concentration of application to determine an effect in the integrity and functionality of the membranes. The results show that functionality of membrane vesicles was only reduced with polyether-modified-polysiloxane [PMP], but not altered by the rest of the additives. These analyses serve to support subsequent research to advance the implementation of this nanotechnology.

Keywords: agriculture; nanocarriers; nanofertilization; surfactants.

INTRODUCTION

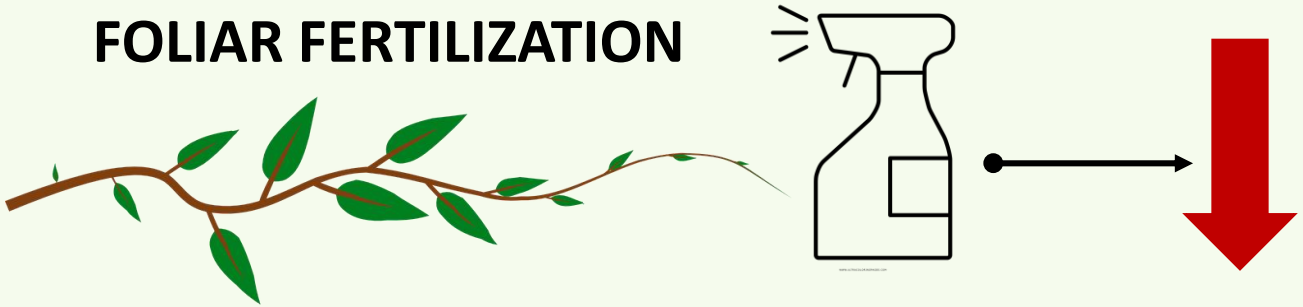
Glogal objective



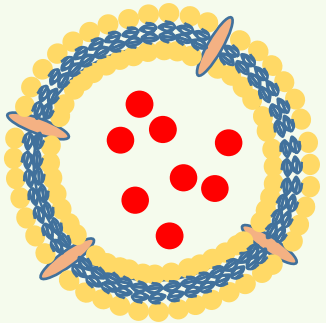
Efficient agricultural technologies

Improvement of nutrients uptake

FOLIAR FERTILIZATION



- The use of nanocarriers provide an enhancement of efficiency of foliar applications.

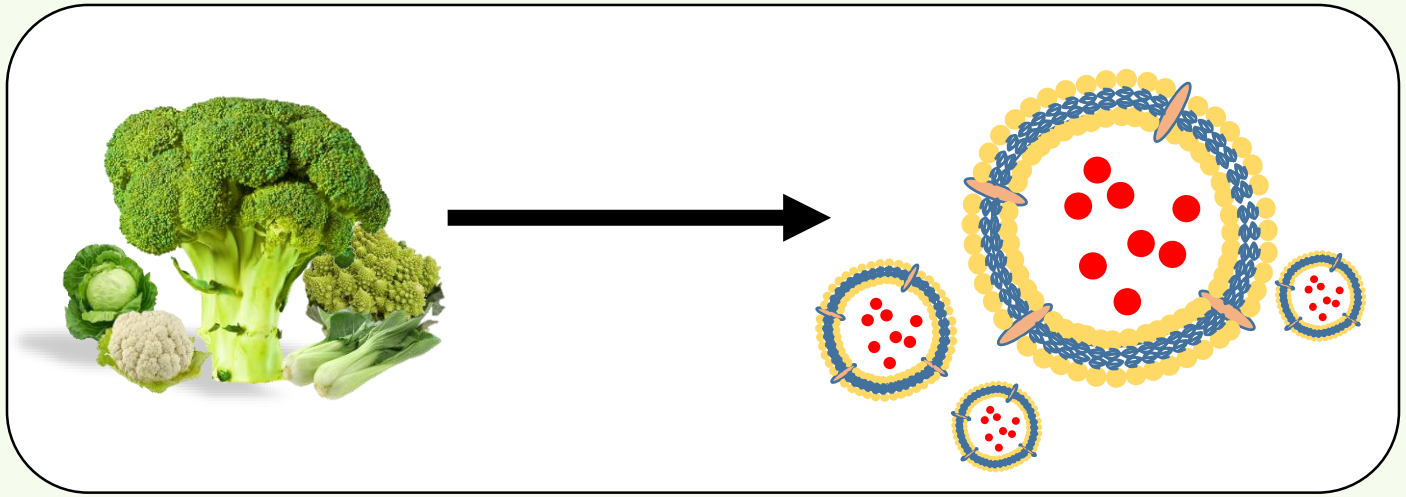


Nanocarriers

+

Nutrients

INTRODUCTION

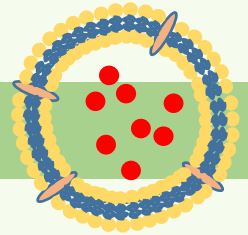


Proteoliposomes from *brassicas* have been tested as carrier of micronutrients.

- Zinc (Zn) was encapsulated with high efficiency in this type of membrane vesicles and the delivery of Zn into protoplast was reported (*Rios et al. 2019*).
- An increase in the penetrability through stomatal pores for B and Fe was showed when micronutrients were encapsulated in membrane vesicles (Ríos et al. 2020).

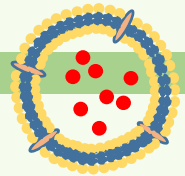
Modify the vesicle surface with surfactants to enhance the efficiency and stability.

In medical areas, many advances have been reached in the modification of liposomes surface for example with polyethylene-glycol (**PEG**), which conjugated to the surface, increase the targeting to specific tissues. Nevertheless, **in agriculture there are limited results in this aspect**.



OBJECTIVE

Characterize physico-chemically membrane vesicles, measure the stability of the vesicles over time and determine the effect of different surfactants (Tween-20, PEG and PMP) in osmotic water permeability (*Pf*) as parameter that reflect the functionality of the membrane vesicles.



RESULTS AND DISCUSSION

Different population of membrane vesicles with different average size were obtained by filtration.

Size between 400 and 200 nm were obtained without modify the homogeneity (PdI) and the charge (Zeta potential) of the samples.

These sizes are suitable to be use in different applications, including foliar application, in which an entry through the stomatal pore is possible, since this has a range of 500-100 nm (Fanourakis et al., 2015).

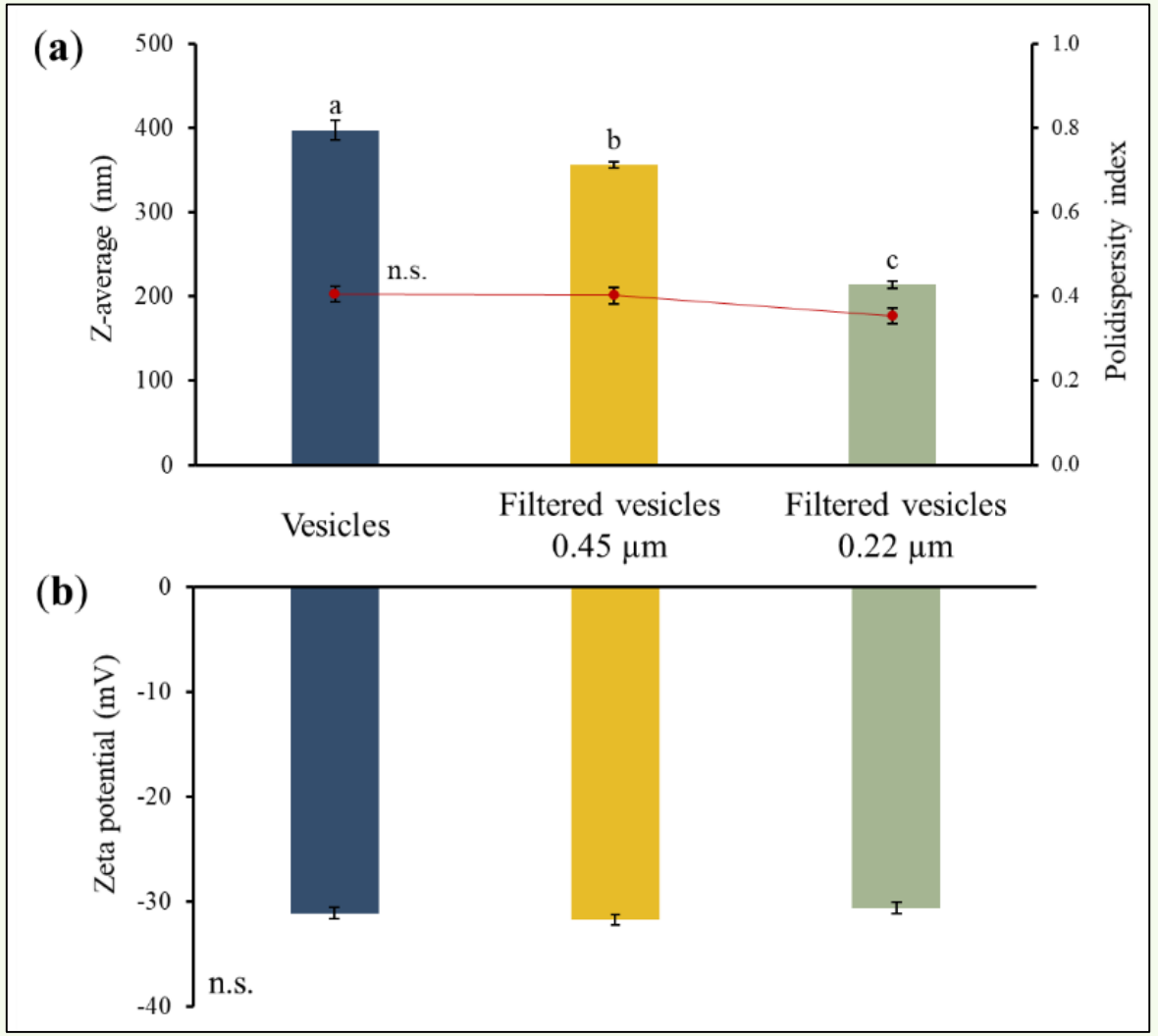


Figure 1. Physicochemical parameters membrane vesicles. (**a**) Z-average (nm) and polydispersity index and (**b**) Zeta potential (mV) of vesicles, vesicles filtered by 0.45 µm and 0.22 µm. Data are means ± SE (n = 3).

RESULTS AND DISCUSSION

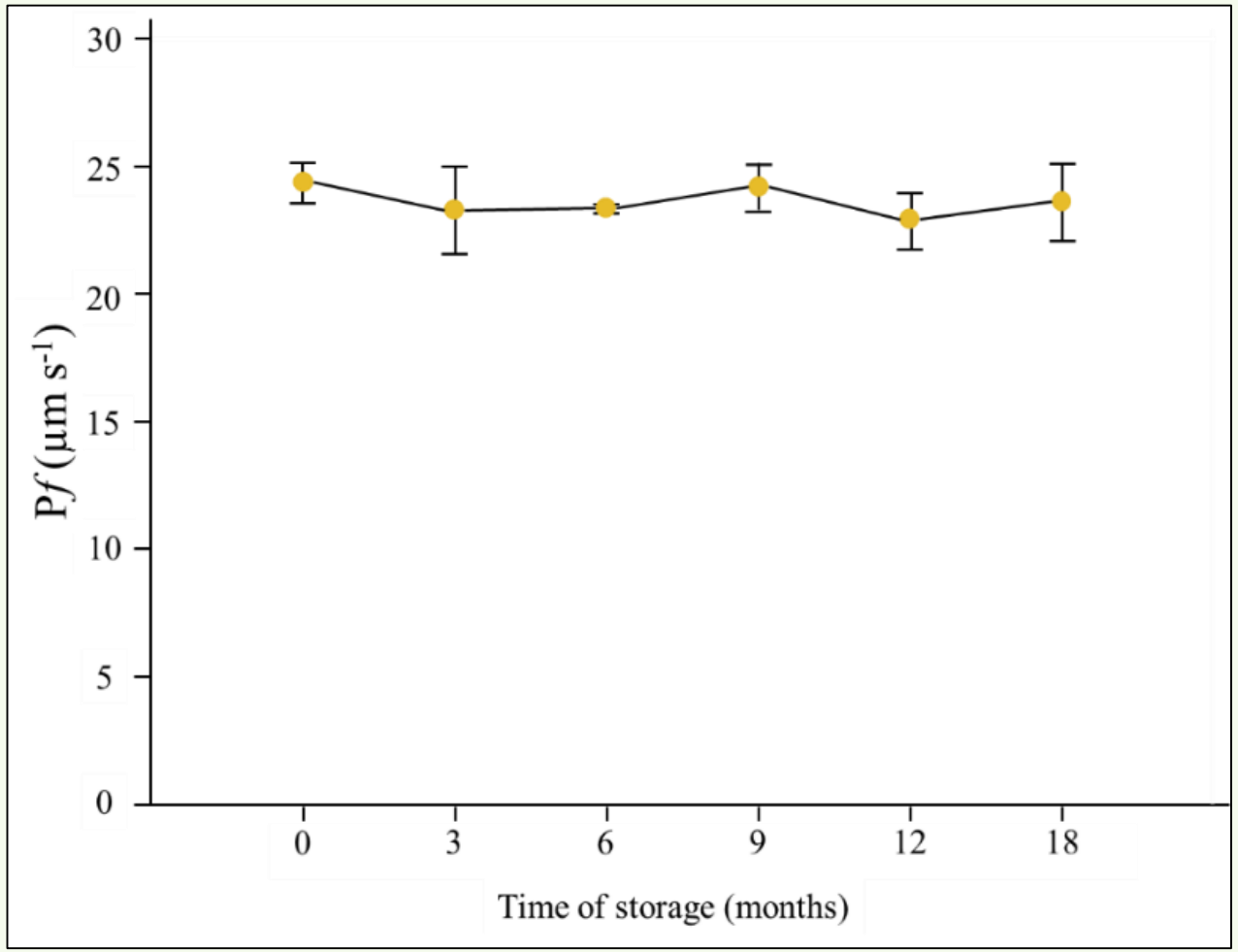


Figure 2. Membrane vesicles stability. Time course (months) of the osmotic water permeability (Pf) of membrane vesicles at 20ºC resuspended in potassium phosphate buffer and stabilized with polyalcohol. Data are means ± SE (n = 30).

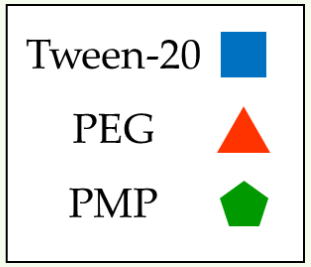
Water permeability value (*Pf*) could be a parameter to determine the **functionality and integrity** of the membrane vesicles.

An initial value about **25 µm s^{-1}** was kept for 18 months when vesicles were stabilized with polyalcohol.

A long shelf-life is essential to use vesicles in a final aaplication, for example in a fertilizer.

RESULTS AND DISCUSSION

Different surfactants were added to the vesicles to determine if the osmotic water permeability was altered.



Only PMP modified Pf of membrane vesicles, which was reduced by about 30%.

Table 1. Osmotic water permeability values of membrane vesicles with surfactants at different concentrations. Data are means ± SE (n = 30).

Applied surfactant in membrane vesicles	*Pf* (μm s^{-1})
Control	22.5 ± 2.8
1% Tween-20	21.8 ± 1.3
2% Tween-20	23.6 ± 2.4
1% PEG	25.4 ± 5.0
2% PEG	22.8 ± 3.1
0.1% PMP	15.0 ± 1.2 *

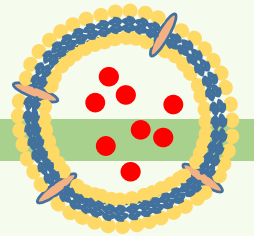
PEG, Polyethylene Glycol; *Pf*, osmotic water permeability; PMP, polyether-modified-polysiloxane.

- *Modification of vesicle surface is key to improve the characteristic of de nano-systems.*
- *Addition of surfactants could be interesting to increase the shelf-life or the capacity*

CONCLUSION

- Membrane vesicles from *Brassica* plants have suitable physicochemical characteristics and stability over time to be used in biotechnological applications.

- Results obtained from surfactant screening opens a new area of research, the modification of the surface of membrane vesicles by surfactants with the aim of improving shelf life and cargo targeting, and efficiency since some of surfactant tested did not altered the functionality of vesicles.



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