**From molecular modelling to Raman microspectroscopy: unprecedented demonstration of the hygroscopic properties of apiogalacturonans**

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**Abstract (Maximum of 250 words)**

To meet the needs of dehydrated skin, molecules with a high hygroscopic potential are necessary to hydrate it effectively and durably. In this context, SILAB was interested in pectins, and more precisely in APGs (APG), a singular one that is currently only found in a few species of aquatic plants. As key structures in water regulation of aquatic plants, we hypothesized that it could have beneficial role for skin hydration. *Spirodela polyrhiza* is a duckweed used in traditional Chinese medicine for its effects on water metabolism and known to be naturally rich in APG. SILAB thus supplied in this natural raw material. The aim of this study was to investigate the hygroscopic potential of APG and their moisturizing potential on skin suffering from dehydration. Firstly, an APG model was built based on structural information obtained from the literature. Molecular dynamics (MD) simulations were performed, and the hygroscopic potential was predicted *in silico* by analyzing the frequency of interaction of water molecules with each APG residue. Secondly, the hygroscopic properties were investigated directly *in vivo.* The water capture in the skin was tracked *in vivo* by Raman microspectroscopy. Measurements were also conducted on Caucasian and Asian volunteers with a dehydrated skin to investigate respectively the *stratum corneum (SC)* and epidermis to superficial dermis hydration levels. Quantification of interactions identified the presence of 23 water molecules on average in contact with each residue of APG. Thanks to the *in vivo* deuterated water ($D_20$) tracking by Raman microspectroscopy, investigations revealed that APG significantly capture and retain more water in the epidermis and deeper than a placebo control and hydrate the skin from the *SC*. Not only do these original natural molecules interact with water molecules, but they capture and retain them efficiently and durably in the skin. Hence, APG are of great interest for the care of dehydrated skin.

**Keywords:** Molecular Dynamics; Solvation; hygroscopic potential; hydration.

**Introduction**.

In the field of cosmetics, the issue of hydration is one of constant attention, recently boosted by the emergence of new interest focusing on dehydrated skin. This state of skin is characterized by a lack of water, a state that can be affected by a variety of factors (environmental, emotional, medicinal, etc.). It is very frequent and can involve all skin types, whether dry, mixed or oily. Tightness, lack of suppleness, loss of volume, lines and wrinkles that appear are all features that characterize dehydrated skin. To specifically meet their needs, the use of molecules with elevated hygroscopic potential is therefore necessary for effective, immediate and long-lasting hydration.

SILAB has extensive expertise in glycobiology and has been studying the natural properties of pectins for more than 25 years. Pectins are a family of macromolecules with highly varied compositions and structures. They are present in plant cell walls and in addition to their structural role, they can bind water, a capacity closely linked to the type of pectin [1, 2]. SILAB thus interested in pectins, and more precisely in apiogalacturonans (APG), a singular one that is currently only found in a few species of aquatic plants. As key structures in water regulation of aquatic plants, we hypothesized that it could have beneficial role for skin hydration. A special attention was paid to pectins found in the giant duckweed, *Spirodela polyrhiza*. Used for over 2,000 years in traditional Chinese medicine for its capacities to promote water metabolism, the cell wall of giant duckweed is one of the species containing the highest amount of APG [3, 4].

The aim of this study was to evaluate and thus to predict the hygroscopic potential of APG through the prediction of 3D structure and evaluate their capacity to capture and to retain water into the skin for a high skin hydration.

**Materials and Methods**.

***In silico* prediction of 3D structure and hygroscopic potential of APG**

First, an APG model was built based on structural information obtained from the literature [4, 5]. In this study, an APG polymer fragment was defined as a chain of 12 residues of α-D-galacturonic acid, with 3 ramifications, each containing two α-D-apiose (Figure 1). The α-D-galacturonic acid chain was constructed using the carbohydrate builder program glycam.org [6]. The α-D-apiose structure was obtained from an experimental X-ray crystallographic structure (PDB: 5IBQ) in which the APG is co-crystallized with the ABC solute Binding Protein. The APG polymer fragment was modelled by fusing the apiose molecules with homogalacturonan polymer fragment, using UCSF Chimera program. The glycosidic linkages between galacturonic acid with the first apiose molecule and between the first apiose and terminal apiose molecules were set to β1-2 and β1-3 respectively.

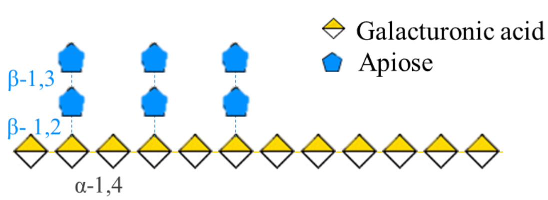


**Figure 1.** Schematic representation of the 2D structure of an APG from *Spirodela polyrhiza.*

Since the parameters for the apiose residue are not characterized in any of the conventional force fields, the parameters were derived from *ab initio* calculations [7,8,9,10]. To characterize the three-dimensional structures, bond lengths, bond angles and dihedral parameters were derived from GLYCAM_06j-1 force field for the apiose atoms that are connected to the atoms of galacturonic acids. The same parameters involving the rest of the apiose atoms were derived from General Atom Force Field (GAFF) [11].

The generated APG structures were solvated in a cubic box with edge distance of 8Å. Water molecules from TIP3P model were added with also 12 neutralizing $Na^+$ counterions [12]. The solvated systems were energy-minimized, and heating equilibrated. A 50 ns simulation was performed. All the above-mentioned preparatory steps as well as simulations were performed using GROMACS 2020.4 software [13, 14]. Then, the hygroscopic potential was predicted *in silico* by analyzing the frequency of interaction of water molecules with each APG residue along the Molecular Dynamics (MD) trajectory.

**Obtention of a natural active ingredient enriched in APG**

A specific extraction process was implemented on *Spirodela polyrhiza* to develop a natural active ingredient enriched in APG. The challenge was thus to define extraction conditions to obtain APG molecules of optimal size, while preserving the di-apiose ramifications. Indeed, preserving the native structure is unavoidable to maintain the intrinsic properties of molecules. Using mild acid hydrolysis coupled with optimized reaction temperature and pH, SILAB has developed an active ingredient enriched to more than 50% in APG.

***In vivo* investigation of the APG hygroscopic potential**

All the volunteers of this study were selected with a dehydrated skin and display daily sensation of tightness and low levels of hydration (MoistureMeter D < 40 for Caucasian and Corneometer < 55 for Asian).

The water capture in the skin was investigated directly *in vivo* by Raman microspectroscopy by using $D_20$ on the arm of 8 volunteers. The $D_2O$ is a molecule displaying the same behavior than $H_20$ but with a characteristic Raman spectrum allowing it to be tracked specifically. For this purpose, an emulsion containing APG was applied at the surface of the skin for 3 hours and then, an occlusive patch containing $D_20$ was applied for 30 minutes. Acquisitions were conducted on different skin depths for 15 minutes.

Moreover, to determine and validate the cosmetic interest, *in vivo* measurements were conducted on Caucasian and Asian volunteers with a dehydrated skin by using a Corneometer and a MoistureMeterD to investigate respectively the *Stratum corneum* (SC) and epidermis to superficial dermis hydration levels.

**Results**.

***In silico* prediction of 3D structure and hygroscopic potential of APG**

For the first time, molecular modeling investigations and MD allowed us to characterize the 3D structure and dynamics of APG. Figure 2A corresponds to a conformation of the APG molecule obtained during MD simulation. Molecular dynamic simulations revealed that these molecules have characteristic shell of water in their solvation process (Figure 2B). Moreover, the investigation of water molecules in interaction with this APG model involve computation of frequency of water molecules present at less than 0.3 nm from an atom of APG during the simulation. The contact frequency was then normalized with respect to each residue of APG, including apiose as well as galacturonic acid. Quantification of interactions identified the presence of 23 water molecules on average in contact with each residue of APG. From a theoretical point of view, this building fragments have an interesting hygroscopic potential that seems twice as high as the one of hyaluronic acid [15].

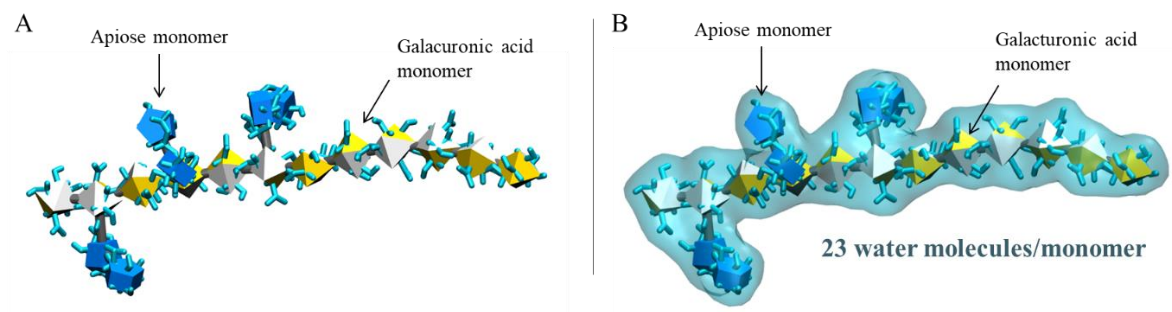


**Figure 2.** A. Tridimensional representation of an APG polymer fragment obtained by molecular modeling. B. Representation of the shell of water around the APG polymer fragment (derived from contacts during the MD simulation).

## *In vivo* investigation of the APG hygroscopic potential

The second part of this work involved validating the capacity of APG contained in an emulsion to take up water directly in volunteers with dehydrated skin. Firstly, Raman microspectroscopy was done to follow the penetration of $D_2O$, a labeled molecule of water, brought directly on the skin of volunteers having previously applied a placebo formula or one containing APG, for 3 hours. Thanks to the *in vivo* $D_20$ tracking by Raman microspectroscopy, investigations revealed that APG significantly capture and retain more water in the epidermis and deeper than a placebo control (Figure 3).

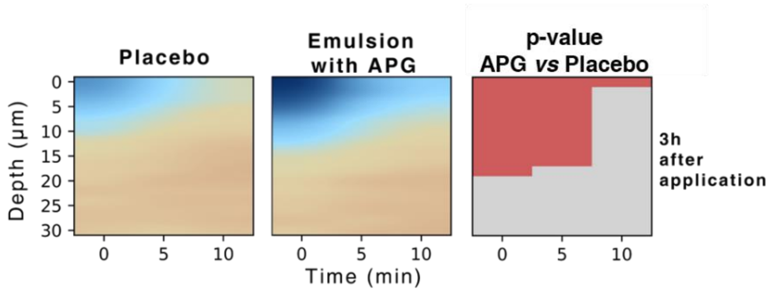


**Figure 3.** Effect of APG on the quantity and depth of $D_20$ taken up by the skin 3 hours after a single application.

An additional study was conducted with the same experimental approach in order to compare the hygroscopic capacity of APG to that of other glycocompounds whether of pectic origin (homogalacturonans) or not (fructosans). Results presented in Figure 4 revealed that APG can take up and retain more water, deeper and longer compared to other glycocompounds.

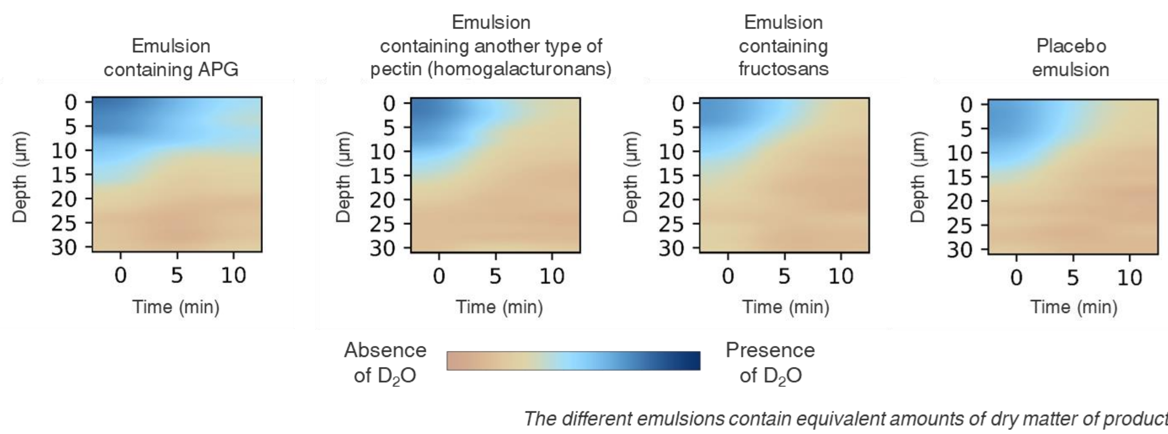


**Figure 4.** Effect of APG and other glycocompounds on the quantity and depth of $D_20$ taken up by the skin 3 hours after a single application.

If the capacity of APG to capture and retain water into the skin was demonstrated, it was then of interest to investigate the moisturizing effect of these molecules on dehydrated skin. Indeed, in Caucasian skin, as of 3 hours after a single application, APG significantly increase hydration of the SC by 17.8% compared to placebo. This action continues and intensifies 6 hours after treatment with a significant 25.1% improvement of superficial hydration compared to placebo (Figure 5A).

Figure 5B displays the results obtained by MoistureMeterD from the epidermis up to the superficial dermis. As of 3 hours after a single application and in comparison to the placebo, APG significantly increase hydration by 11.9%. This effect continues 6 hours after application, with a 15.8% increase of hydration (Figure 5B).

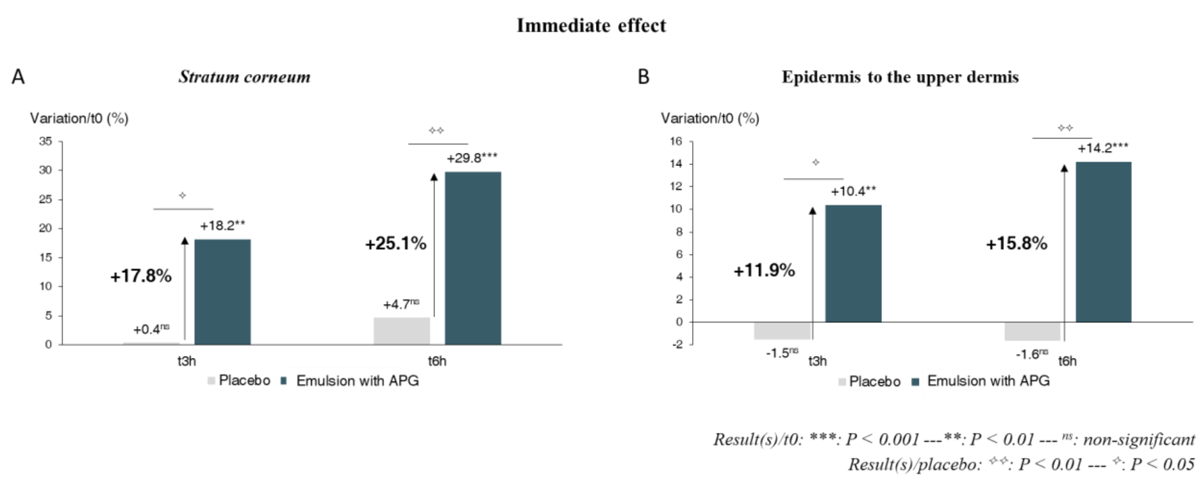


**Figure 5.** Effect of an emulsion containing APG on the hydration of Caucasian volunteers on the SC measured by Corneometer (A.) and from the epidermis to the upper dermis by MoistureMeterD (B).

Moreover, APG improve long-term superficial hydration of the face of Caucasian volunteers. Figure 6 corresponds to a representative map of 23 measurements obtained with a Corneometer after 21 and 42 days of twice daily treatment. Results revealed a significant 5.9% improvement of hydration of the skin surface. This effect continues and intensifies after 42 days, with a 10.0% increase of hydration.

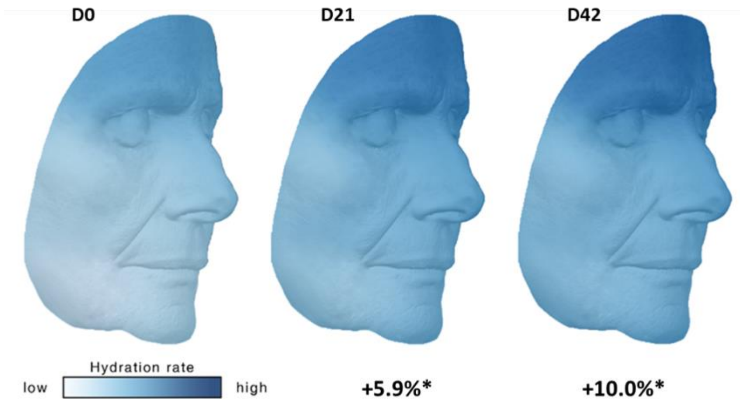


**Figure 6.** Representative map of hydration done using 23 measurements by corneometry on the face of Caucasian volunteers after 42 days of twice daily application of APG (Results / placebo: * < 0.05).

In Asian volunteers, APG also improve long-term superficial hydration. Indeed, after 21 days of treatment, there is a significant 12.9% increase in hydration of the SC that intensifies after 42 days of treatment with a significant 22.5% augmentation (Figure 7).

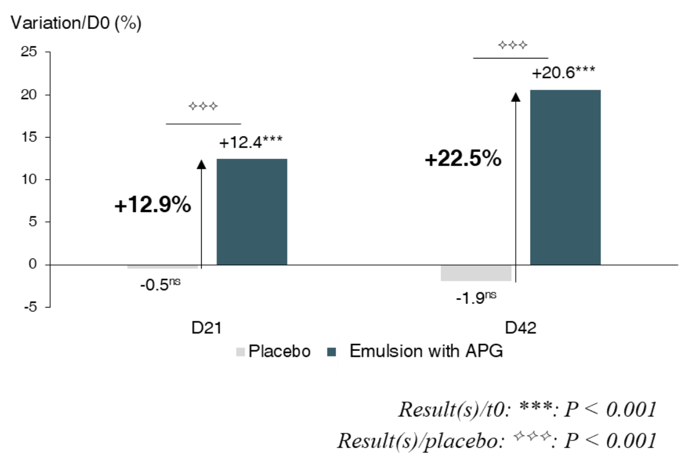


**Figure 7.** Effect of an emulsion containing APG on the hydration of Asian volunteers on the SC measured by using a Corneometer.

**Discussion.**

By combining both theoretical molecular modeling and experimental Raman microspectroscopy, SILAB has for the first time shown the outstanding hygroscopic properties of APG. *In silico* approach allowed the development of a theoretical model of APG with a 3D representation of its structure and molecular simulation revealed a specific shell of water. Quantification of interaction between water molecules and APG revealed for the first time the outstanding properties of this type of pectin. SILAB then develop an eco-designed process from the giant duckweed (*Spirodela polyrhiza*), resulting in a natural active ingredient composed of pectic glycocompounds purified in APG. To demonstrate the remarkable hygroscopic properties predicted *in silico*, *in vivo* investigations were conducted. The tracking of $D_2O$ by Raman microspectroscopy revealed that APG capture and retain more water in the skin in comparison to other glycocompounds and pectin. This result thus confirm that the structure impacts the hygroscopic potential of these molecules and highlight the importance of apiose in the hygroscopic potential thanks to the result obtained with homogalacturonans. *In vivo* measurements revealed that APG procure an immediate hydration of the skin. After a prolonged treatment of 21 and 42 days of twice daily application, this effect continues and is accompanied by smoothing, plumping and radiance boosting actions in Caucasian and Asian skins.

**Conclusion**.

In an unprecedented manner, SILAB has predicted and validated the hygroscopic potential of APG, by combining the power of molecular modeling numerical tools and Raman microspectroscopy. The resulting natural active ingredient, enriched in APG, provides flash and long-lasting hydration, superficially and in depth. Complexion radiance is revived, lines smoothed, and facial volumes redefined.

**Conflict of Interest Statement**.

NONE.

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