IL NUOVO CIMENTO **48 C** (2025) 235
DOI 10.1393/ncc/i2025-25235-6

COMMUNICATIONS: SIF Congress 2024

Unveiling guanosine hydrogel properties: Mid and Far infrared spectroscopic insights

V. NOTARSTEFANO([1])([*]), A. PEPE([2]), F. RIPANTI([2]), P. MORETTI([2]) and P. MARIANI([2])

([1]) *Department of Bioscience and Technology for Agriculture, Food and Environment, University of Teramo - Teramo, Italy*
([2]) *Department of Life and Environmental Sciences, Polytechnic University of Marche Ancona, Italy*

received 24 February 2025

Summary. — Guanosine possesses the intrinsic ability to self-assemble in aqueous environments, giving rise to supramolecular architectures ranging from G-quartets to G-quadruplexes. These, in turn, can undergo cross-linking or entanglement, forming a molecular hydrogel with promising biotechnological properties. Infrared spectroscopy can successfully unravel the structural and dynamic properties of these supramolecular assemblies, revealing spectral features indicative of quartets, octamers, and quadruplex formation, and is used to monitor the formation of G-hydrogels.

Self-assembling G-hydrogels have gained increasing attention due to their unique and tunable mechanical properties, including softness, deformability, and quasi-liquid behavior, making them highly promising for biotechnological applications [1]. Hence, there is a growing need to develop reliable analytical tools for monitoring the formation of these structures and fine-tuning their characteristics.

1. – From guanosine quartets to hydrogels

Due to their unique ability to interact between themselves, guanosine nucleosides and nucleotides can self-organize into complex supramolecular structures, such as G-quartets, in aqueous environments [1, 2]. These G-quartets stack to form chiral columnar structures known as G-quadruplexes, with guanosine (Gua) and guanosine 5′-monophosphate (GMP) being key participants in this process. The G-quartet structure is stabilized by Hoogsteen hydrogen bonds [2] and undergoes ordered arrangements in the presence of alkali metal cations, leading to the formation of the higher-order structures of G-octamers, G-dodecamers, and G-quadruplexes.

([*]) E-mail: vnotarstefano@unite.it

In water, G-quadruplexes form columnar liquid crystalline lyotropic phases, and their rigidity and stability is given by the lateral repulsive forces due to phosphates, and the stabilization of the cation coordinating the oxygen atoms at the center of the G-quartet [3]. Replacing a portion of GMP with Gua at an appropriate molar ratio maintains the G-quadruplex structure, but increases its flexibility and van der Waals interactions, leading to the formation of a 3D hydrogel network [1, 4].

2. – Mid-infrared spectroscopy for monitoring G-hydrogel formation

Mid-IR spectroscopy can efficiently characterize the molecular structure of guanosine-based architectures, from the organization of guanosine quartets and quadruplexes, to the formation of hydrogels [5, 6]. IR techniques are valuable tools to obtain critical molecular and chemical insights that are not normally accessible through conventional experimental techniques used for the characterization of such systems.

Since the supramolecular architectures formed by Gua and GMP in water do not rely on the formation of covalent bonds, their spectral signatures arise from slight shifts and alterations of their fundamental building blocks. Hence, a precise point-by-point evaluation of all these spectral features is the most efficient way to point out the structural and dynamic characteristics of these complexes (fig. 1).

Several works played a role in the identification of valuable markers for the study of G-hydrogels. Among all, there is a general consensus on the pivotal importance of the peak assigned to the C6 = O6 ($\nu_{C6=O6}$) of guanine, centered at ∼1685 cm^{-1} [7-9]. The position of this peak is highly informative, since it depends on the formation of Hoogsteen-type hydrogen bonds, the cation coordination, and the stacking interaction between contiguous G-quartet planes [5, 7]. It has been reported that the center of this band shifts linearly with the increasing Gua:GMP ratio, likely influencing base stacking. This peak shift can be thus used to monitor the formation of thicker and denser hydrogels [9]. These findings suggest that this peak could serve as a useful tool in G-hydrogel production studies and applications.

3. – A preliminary Far-IR spectroscopy approach

Compared to Mid-IR, Far-IR spectroscopy has not been equally investigated for the study of guanosine supramolecular complexes. Messinger and colleagues proposed for the

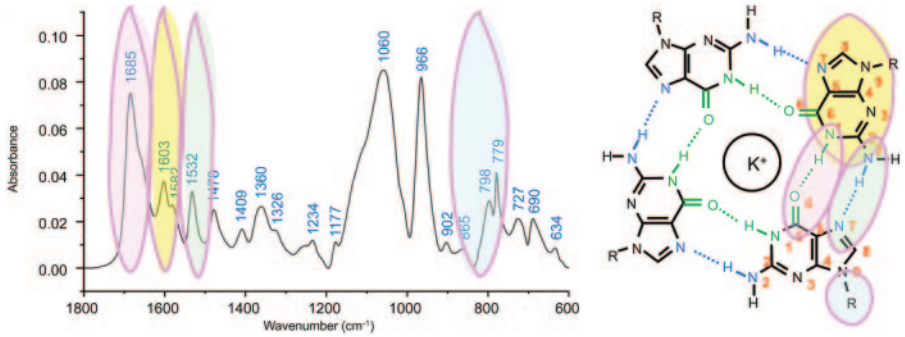

Fig. 1. – Spectral profile of GMP/K quadruplexes and color-guided correspondence with the G-quartet structure. Modified from [9].

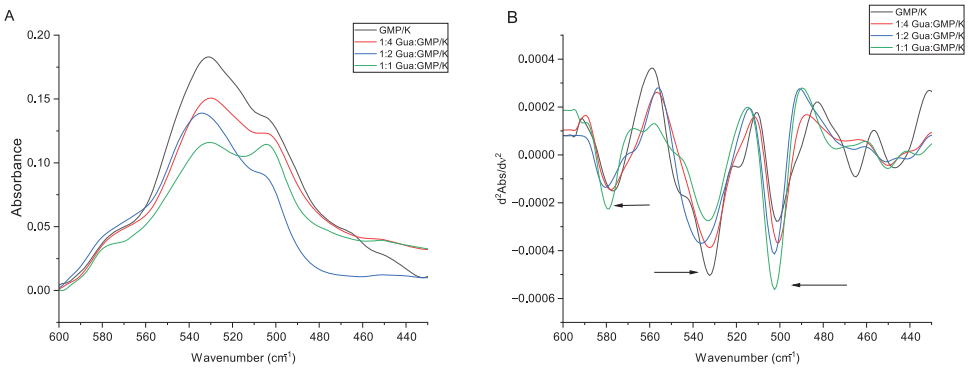

Fig. 2. – Comparison of the spectral profiles ((A) absorbance; (B) second derivative) of hydrogels of GMP/K (black), 1:4 Gua:GMP/K (red), 1:2 Gua:GMP/K (blue), and 1:1 Gua:GMP/K (green) in the 600–430 cm^{-1} spectral region.

first time Far-IR spectra (600–50 cm^{-1}) of GMP/K and GMP/Na salts, both in crystal and gel conformation [10]. Here, Far-IR spectroscopy is used to propose, in a preliminary way, other spectral markers useful for monitoring the formation of G-hydrogels upon the addition of Gua to GMP, to be combined with those successfully assessed from Mid-IR spectroscopy.

Figure 2 displays the 600–430 cm^{-1} spectral range of GMP/K and 1:4, 1:2, 1:1 Gua:GMP/ K mixtures. The most prominent band is centered at ∼530 cm^{-1}, attributable to guanosine ring deformation, in particular to the bending of N9-H [11]. This band, together with those centered at ∼580 and ∼500 cm^{-1}, displays a blue shift linearly correlated with the increasing Gua:GMP ratio (see second derivative spectra). These bands are all assigned to deformations of N1-H and N9-H moieties and to the bending modes of guanine rings: their behavior may be attributable to a modified freedom of movement of Gua and GMP subunits as a consequence of the the lack of phosphate groups following the inclusion of Gua molecules into the quartets.

Figure 3 compares the four samples in the 280–200 cm^{-1} spectral range. In general, it is possible to notice a narrower and more defined shape of the IR bands of the pure GMP/K sample: this may be ascribable to the known more ordered structure of

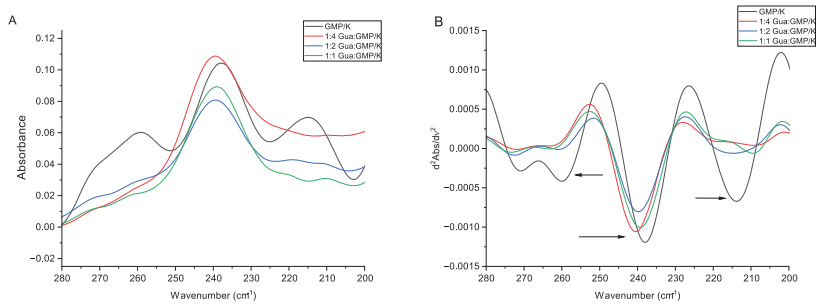

Fig. 3. – Comparison of the spectral profiles ((A) absorbance; (B) second derivative) of GMP/K (black), 1:4 Gua:GMP/K (red), 1:2 Gua:GMP/K (blue), and 1:1 Gua:GMP/K (green) in the 280–200 cm^{-1} spectral region.

GMP-quadruplexes respect to the more disordered 3D-network arising from the mixture of Gua and GMP. The three peaks detected in this spectral range, ∼260, ∼240, and ∼216 cm^{-1}, have not been thoroughly investigated in the literature: they may be assigned to C-O-P bending modes [10]. As expected, the increasing amount of Gua in the mixture determines a decrease in the relative quantity of phosphate groups. Moreover, the broader and less defined shape of these bands in Gua:GMP/K samples could be indicative of the loss of rigid and homogeneous structures in favor of a more flexible architecture.

4. – Conclusions

The unique biotechnological properties of self-assembling G-hydrogels made necessary to develop reliable analytical tools for monitoring the formation of these structures and fine-tuning their characteristics. In this regard, certain Mid-IR bands have proven to serve as reliable markers for this purpose. In this context, we present a preliminary exploration of the potential of Far-IR spectra, identifying promising spectral markers.

This brief review, along with new preliminary findings, highlights the significant potential of IR spectroscopy for studying G-hydrogels. It offers a cost-effective, objective, and rapid method to gather valuable information for the development of these systems for biotechnological and biomedical applications.

REFERENCES

[1] Carducci F., Yoneda J. S., Itri R. and Mariani P., *Soft Matter*, **15** (2018) 2938.
[2] Davis J. T., *Angew. Chem. Int. Ed.*, **43** (2004) 668.
[3] Baldassarri E. J., Ortore M. G., Spinozzi F., Round A., Ferrero C. and Mariani P., *Nanomaterials*, **10** (2020) 629.
[4] Pepe A., Moretti P., Yoneda J. S., Carducci F., Itri R. and Mariani P., *Nanoscale*, **15** (2023) 15196.
[5] Andrushchenko V. and Bouř P., *J. Phys. Chem. B*, **113** (2009) 283.
[6] Mathlouthi M., Seuvre A. M. and Koenig J. L., *Carbohydr. Res.*, **146** (1986) 15.
[7] Guzmán M. R., Liquier J., Brahmachari S. K. and Taillandier E., *Spectrochim. Acta A*, **64** (2006) 495.
[8] Gao M., Harish B., Berghaus M., Seymen R., Arns L., McCallum S. A., Royer C. A. and Winter R., *Sci. Rep.*, **7** (2017) 9864.
[9] Notarstefano V., Pepe A., Ripanti F., Piccirilli F., Vaccari L. and Mariani P., *Spectrochim. Acta A*, **324** (2025) 124939.
[10] Messinger A., Powell J. W., Weidlich T. and Genzel L., *J. Biomol. Struct. Dyn.*, **10** (1993) 841.
[11] Seki T., Ohki Y. and Mizuno M., *2020 IEEE Conference on Electrical Insulation and Dielectric Phenomena (CEIDP)* (IEEE) 2020, pp. 539–542.