Lithuanian Journal of Physics
Vol. **66**, No. 1, pp. 40–47 (2026)

ISSN 1648-8504 eISSN 2424-3647
DOI: https://doi.org/10.3952/physics.2026.66.1.5

RELAXATION DYNAMICS OF GUANOSINE 5'-MONOPHOSPHATE WITH Na$^+$ AND K$^+$ IN AQUEOUS SOLUTIONS INVESTIGATED BY BROADBAND DIELECTRIC SPECTROSCOPY

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Received 14 November 2025; revised 9 December 2025; accepted 29 December 2025

To gain insights into nucleotide–monovalent ions interaction, the dynamics of counterions (sodium and potassium) with guanosine 5'-monophosphate (5'-GMP) were studied in an aqueous solution via broadband dielectric spectroscopy measurements. Complex reflection coefficients of aqueous Na-GMP and K-GMP solutions are measured in the frequency domain at room temperature. Complex specific resistance spectra are analyzed at various mononucleotide concentrations. One dispersion region located at 100 MHz was observed. It is interpreted as being caused by DNA counterion fluctuations. It largely depends on the GMP concentration in the solution.

Keywords: dielectric spectroscopy, Na$^+$ and K$^+$ counterions, relaxation dynamics, biological matter, polyelectrolytes

1. Introduction

Water and dissolved ions play a central role within the human body, even affecting the DNA in our cells. DNA is surrounded by several hydration layers, the organization of which strongly influences its conformational preferences and flexibility [1–5]. Electrostatic screening by counterions is equally essential: without monovalent cations, the canonical B-form double helix becomes destabilized and its biological functionality can be compromised [6]. The ionic atmosphere around DNA is therefore a critical determinant of nucleic acid behaviour, and both the valence and chemical nature of the ions govern binding modes and structural outcomes. Divalent and trivalent cations often bind in a site-specific manner to phosphate groups or nucleobases, giving rise to significant conformational changes, including condensation and base mismatches [7–9].

While the role of multivalent ions has been extensively studied, ion-specific effects among monovalent cations have only recently gained attention. Guanine-rich sequences, in particular, form stable G-quadruplexes through the Hoogsteen hydrogen bonding, and these structures exhibit strong preferences for either Na$^+$ or K$^+$ ions. Traditionally, Na$^+$ and K$^+$ were assumed to generate similar ionic atmospheres due to their identical charge. However, molecular dynamics simulations have demonstrated substantial differences in their spatial distributions near DNA, hydration properties, and groove-binding propensities [10]. These findings challenge the widespread assumption that Na$^+$ and K$^+$ ions are interchangeable, especially considering that intracellular environments are dominated by K$^+$, whereas most *in vitro* experiments rely on Na$^+$-based buffers. Despite this, experimental studies directly comparing Na$^+$ and K$^+$ interactions with nucleic acid building blocks remain limited.

Numerous dielectric spectroscopy investigations of DNA have been performed [8, 11–22]. However, the samples investigated were usually derived from animals, so their properties were not well defined, that is, their sequence and length were not exactly known. For this reason, we have acquired the dielectric spectra of the nucleotide guanosine, one of the main building blocks of DNA, in its form as a monophosphate (GMP, Fig. 1(a)). However,

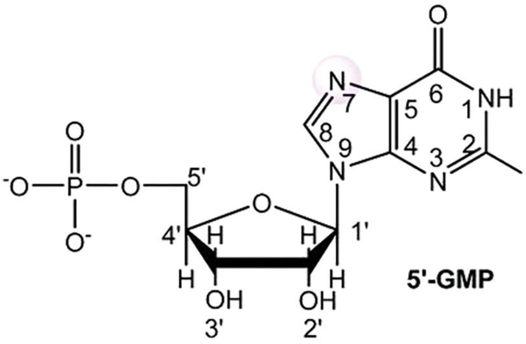

Fig. 1. Schematic representation of the structure of the used guanosine 5'-monophosphate (5'-GMP) [34].

the significance of 5'-guanosine monophosphate (GMP) lies in its important role in the synthesis of nucleic acids. GMP is a molecule known to form quartets and quadruplexes, the occurrence of which depends on the temperature and concentration. Under conditions of low temperatures and high concentrations, the G4 conformation is favoured, whereas at high temperatures and low concentrations the equilibrium tends to shift towards the formation of stacks. In contrast, solutions of other DNA bases, such as adenosine, thymine, and cytosine monophosphate (AMP, TMP and CMP), typically form stacks as they cannot establish Hoogsteen bonds [23].

Structurally, DNA, proteins and liquids differ in many ways, but their dielectric relaxation dynamical properties are expected to be similar, as the former can be interpreted in terms of polyelectrolytes [2, 24–29]. Namely, from a physical perspective, nucleic acids and other charged biopolymers can be regarded as polyelectrolytes, the dielectric response of which is dominated by counterion dynamics, hydration-shell fluctuations, and dipolar relaxations. However, polyelectrolyte solutions are challenging to model because their effective charge, shape, and dynamic behaviour are strongly influenced by the surrounding ions. Polyelectrolytes are long linear or branched polymers onto which charges are appended. When these polymers are dispersed in water or a polar solvent, the counterions responsible for balancing their charge undergo partial dissociation. Consequently, the properties of polyelectrolytes in the solution are significantly influenced by the nature of their counterions. Because they are sensitive to changes in their environment, polyelectrolytes hold promise for use in biomedical applications [30]. Two principal theoretical frameworks – counterion condensation (CC) theory and Poisson–Boltzmann (PB) theory – are commonly employed to describe such systems [31].

In this study, we investigate the relaxation dynamics of GMP in aqueous solutions containing Na^+ or K^+ ions using broadband dielectric spectroscopy over a wide frequency range. Our aim is to determine whether Na^+ and K^+ produce measurably distinct dielectric signatures in GMP solutions.

2. Experiment

2.1. Materials

Guanosine-5'-monophosphate disodium salt hydrate (5'-GMP) was obtained from *Sigma-Aldrich* (Germany) and used without further purification. Its molecular structure is shown in Fig. 1. Sodium and potassium phosphate salts (analytical grade, *Sigma-Aldrich*) were used to prepare 20 mM Na-phosphate and K-phosphate buffer solutions at pH 7.0. All solutions were prepared using deionized water (resistivity ≥ 18.2 MΩ cm).

2.2. Sample preparation

GMP stock solutions were prepared by dissolving the appropriate amount of 5'-GMP in the Na- or K-phosphate buffer and mixing until complete dissolution. Working solutions covering the concentration range from 1 to 20 $mg{\cdot}mL^{-1}$ were obtained by serial dilution. All samples were allowed to equilibrate at 298 K for at least 1 h prior to measurements to ensure temperature and structural stabilization. The pH of each solution was determined after GMP addition.

2.2. Dielectric measurements

The dielectric permittivity of Na-GMP and K-GMP was measured in a frequency range of 10 MHz to 40 GHz at a temperature of 298 K (room temperature). The sample cell consisted of a circular waveguide with a transition to an open-ended coaxial probe. Reflectance measurements were performed using a vector network analyzer *Agilent* E8363B. The dielectric permittivity was extracted from the reflectance data using a full-wave model corresponding to the employed waveguide configuration;

a detailed description of this measurement methodology can be found in Ref. [32]. Temperature control was ensured using a T-type thermocouple connected to a *Keithley* Integra 2700 multimeter.

3. Results and discussion

Deoxyribonucleic acid (DNA) is a biological polymer comprised of nucleotide monomers [33]. Each nucleotide monomer consists of three fundamental components: a planar aromatic derivative derived from either a pyrimidine or purine base, deoxyribose sugar, and a phosphate group. DNA structure involves four distinct nitrogenous bases: adenine (A) and guanine (G) belonging to the purine group, and thymine (T) and cytosine (C) belonging to the pyrimidine group (see Fig. 1).

The complex dielectric function ε^* of polyelectrolyte solutions can be written as

$$\varepsilon^*(\omega) = \varepsilon'(\omega) - \mathrm{i}\varepsilon''(\omega) = \varepsilon'(\omega) - \mathrm{i}\left[\varepsilon''(\omega) + \frac{\sigma_0}{\varepsilon_0\omega}\right], \quad (1)$$

where $\varepsilon'(\omega)$ is the real part that reflects the ability of the material to be polarized under the influence of the external electric field. The dielectric loss consists of two components: one is due to the dielectric process $\varepsilon''(\omega)$ and the other is due to the DC electrical conductivity, which is the low-frequency limit of $\sigma(\omega)$. The high DC conductance in the solutions is a result of the charged phosphate groups and of the Na^+ and K^+ ions present. The frequency dependence of the complex dielectric permittivity $\varepsilon^*(\omega)$ of guanosine 5'-monophosphate (GMP) at room temperature is shown in Fig. 2. Although various polarization mechanisms govern the dielectric response of aqueous polymer solutions, some general features emerge that are common to all polyelectrolyte solutions. Dielectric spectra of aqueous polyelectrolyte solutions typically exhibit at least three dispersion regions [24]. First, the dispersion at approximately 18 GHz stems from the orientational polarization of water molecules like that in pure water. Water is the main component of most aqueous solutions and has a large orientational dielectric polarization that contributes to the dielectric spectra. However, bound water within these solutions displays distinct properties from bulk water which forms hydration layers. The relaxation of the bound water spans from kHz to hundreds of MHz. Additionally, in the intermediate-frequency range, typically between 1 and 100 MHz, another relaxation process occurs.

Generally, a dependence of the dielectric spectra on the mononucleotide concentration should be expected as the presence of the charged biomolecule contributes to the dielectric spectra. In our case, only a moderate difference can be seen in the frequency dependence of the complex dielectric permittivity even varying the mononucleotide concentration from 1 up to 20 mg mL^{-1}. The DC conductivity loss $\sigma_0/\varepsilon_0\omega$ increases with decreasing frequency and even obscures $\varepsilon''(\omega)$ at the lowest frequencies. This complicates the detection and

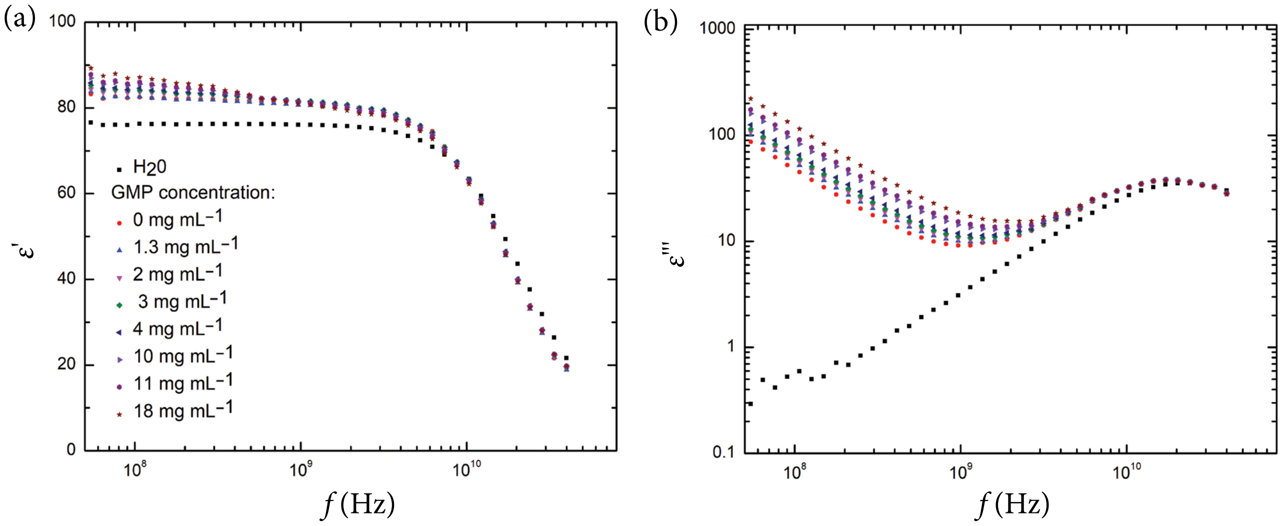

Fig. 2. Frequency dependences of the ε' and ε'' of the Na-phosphate buffer solution 20 mM mixed with a different concentration of guanosine 5'-monophosphate.

analysis of the occurring processes. Therefore, any attempt to try to analyze results by approximating the dielectric permittivity spectra with a Cole–Cole or any similar equation, which is usual to use in the dielectric spectroscopy experiment, would not give reliable results. To overcome this difficulty, an alternative approach to calculate dielectric parameters should be adopted. The complex specific resistivity function $\rho^*(\omega) = \rho'(\omega) - \mathrm{i}\rho''(\omega)$ was used to represent the frequency- and concentration-dependent data:

$$\rho'(\omega) = \frac{1}{\varepsilon_0\omega} \cdot \frac{\varepsilon''}{\varepsilon'^2 + \varepsilon''^2}, \tag{2}$$

$$\rho'(\omega) = \frac{1}{\varepsilon_0\omega} \cdot \frac{\varepsilon'}{\varepsilon'^2 + \varepsilon''^2}. \tag{3}$$

This formalism offers notable benefits, especially in cases where the system demonstrates conductivity that varies with frequency. The frequency dependence of complex specific resistance ($\rho^*(f)$) of Na-GMP covering the frequency range from 10 MHz to 40 GHz for the several selected concentrations of GMP in the aqueous sodium phosphate buffer solutions is shown in Fig. 3. In a similar manner, the $\rho^*(f)$ of K-GMP is shown in Fig. 4. The result shows that GMP with Na-phosphate and K-phosphate buffers exhibit relaxation from 70 to 100 MHz and from 100 to 130 MHz, respectively (see Figs. 3–4). This indicates that guanosine monophosphate (GMP), a derivative of one of DNA's primary building blocks, exhibits characteristics of a polar molecule in various ionic environments [33]. Within the biophysics community, the dispersion observed at such frequencies is commonly referred to as β-relaxations. This relaxation can be attributed to the rotation of polyelectrolyte, counterion fluctuations or even the dynamics of the hydration shell. β-relaxation is particularly intriguing as it can be influenced by concentration, flexibility, and the contour length of the polyelectrolyte, as well as its interactions with small ions (both counterions and added salt). From Figs. 3–4, it is clearly seen that the dielectric relaxations tend to shift towards higher frequencies with increasing concentrations of GMP in the solutions.

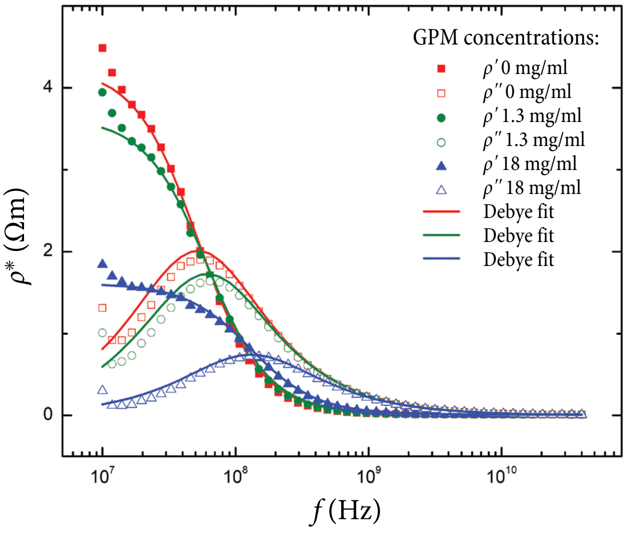

Fig. 3. Frequency dependence of the ρ' and ρ'' of the Na-phosphate buffer solution 20 mM mixed with a different concentration of guanosine 5'-monophosphate. The line shows the Debye fit curve.

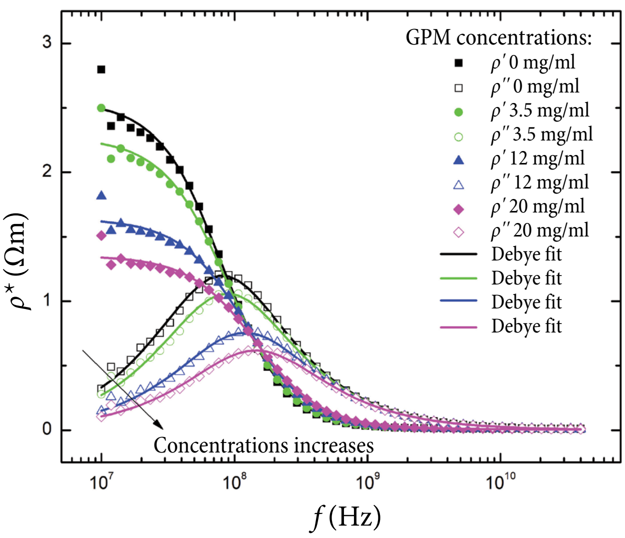

Fig. 4. Frequency dependence of the ρ' and ρ'' of the K-phosphate buffer solution 20 mM mixed with a different concentration of guanosine 5'-monophosphate. The line shows the Debye fit curve.

For further quantitative analysis of relaxational processes, a suitable model function, simultaneously fitted to both the real and imaginary part of the complex specific resistance, is essential. In the simplest case, the contributions of orientation polarization may be described using the Debye formula,

$$\rho^*(\omega) = \rho(\infty) + \frac{\Delta\rho}{1 + \mathrm{i}\omega\tau}, \tag{4}$$

where $\Delta\rho = \rho(0) - \rho(\infty)$, $\rho(\infty)$ is the specific resistance at frequency $f \to \infty$ (residual specific resistance); $\rho(0)$ is the specific resistance at frequency

$f = 0$ Hz (static specific resistance); the alternating-current angular frequency ω is given by $\omega = 2\pi f$; τ is the specific relaxation time in which polar molecules can respond to the electrical field.

The β-relaxations are frequently observed to obey the Debye equation or exhibit the behaviour closely resembling a monodispersed process [4, 22, 24, 37]. Not many researchers utilize the Cole–Cole function for the description of the β-process. In the case of the β-relaxation, it is reasonable to anticipate the Debye behaviour since interactions among GMP are not highly expected, and each molecule should experience a consistent environment primarily governed by rapidly fluctuating water molecules over the time scale of the β-relaxation.

As a model function, we used the Debye formula corresponding to the relaxations centred at 100 MHz. Reasonable fits of the experimental spectra were achieved. The concentration dependence of the relaxation time, associated with the movement of DNA counterions, is shown in Fig. 5. With the GMP concentration increasing up to 20 mg mL^{-1}, the relaxation time of GMP notably decreases. However, at higher concentrations, the relaxation time decreases to a lesser extent. Our results show that the movement of counterions in the presence of GMP remains diffusive, the movement of K$^+$ being faster than that of Na$^+$. Takashima et al. [6, 33, 35, 36] carried out dielectric measurements on double stranded helical DNA. They have observed three dielectric dispersions. Figure 6 shows the variation of specific resistance strength with concentration. The strength of the low-frequency dispersion, which is centred at around 100 MHz, increases with nucleotide concentration. This is an indication that the screening by the hydration shell of molecules inhibits any mutual interactions. The stronger relaxation of GMP can be explained by its higher dipole moment and supports the view that this dispersion results from the dipole relaxation of nucleotides.

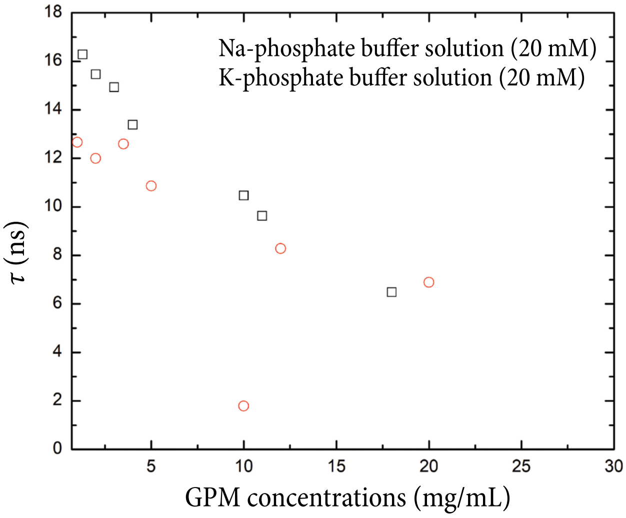

Fig. 5. Fitting parameters of the Debye fit. The solvent relaxation time of 5'-GMP solutions in different buffers as a function of 5'-GMP concentration.

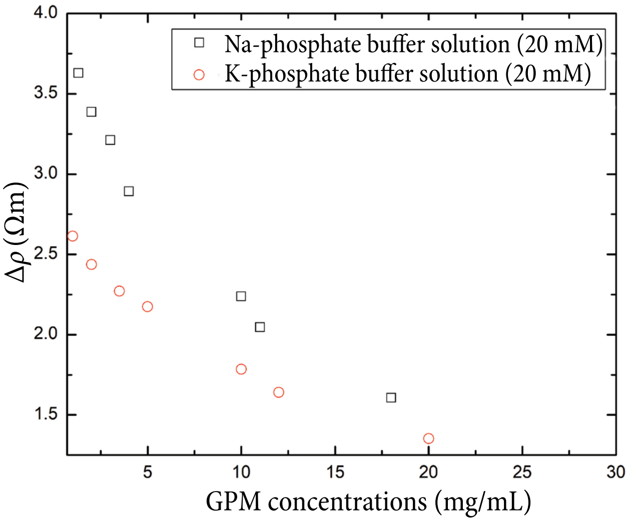

Fig. 6. Fitting parameters of the Debye fit. The solvent specific resistance strength of 5'-GMP mononucleotide solutions in different buffers as a function of 5'-GMP concentration.

4. Conclusions

Using broadband dielectric spectroscopy, we measured the complex specific resistance of aqueous GMP solutions containing either Na$^+$ or K$^+$ ions over the frequency range from 10 MHz to 40 GHz. It was possible to resolve intermediate-frequency (located at ~100 MHz) dispersion. This dispersion depends on the GMP concentration in the solution and added monovalent ions species. Increasing the GMP concentration results in a systematic shift of the relaxation peak toward higher frequencies and a broadening of the dispersion. These observations indicate concentration-dependent modifications of counterion dynamics and hydration-shell fluctuations around GMP molecules.

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GUANOZINO 5'-MONOFOSFATO RELAKSACIJOS DINAMIKA NATRIO IR KALIO JONŲ TIRPALUOSE, TIRTA PLAČIAJUOSTE DIELEKTRINE SPEKTROSKOPIJA

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Santrauka

Siekiant geriau suprasti nukleotidų ir monovalentinių jonų sąveiką, šiame darbe tirta guanozino 5'-monofosfato (5'-GMP) dinamika natrio ir kalio vandeniniuose tirpaluose, taikant plačiajuostę dielektrinę spektroskopiją. Gauti kompleksinės specifinės varžos spektrai plačiame dažnių diapazone (nuo 50 MHz iki 40 GHz) skirtingos GMP koncentracijos vandeniniams tirpalams. Dispersijos sritis ties ~100 MHz siejama su priešingo krūvio jonų bei prie mononukleotido paviršiaus sujungto vandens dinamika. Dielektrinės relaksacijos parametrai priklauso nuo GMP koncentracijos ir tirpale esančio monovalentinio jono tipo.