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NMR SPECTRAL CHARACTERISTICS OF PHASES IN A PEG-DEXTRAN-WATER AQUEOUS TWO-PHASE SYSTEM

Jala TEYMUROVA
Baku State University, Baku, Azerbaijan
jalateymurova98@gmail.com

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ABSTRACT

In this study, the properties of the upper and lower phases obtained after phase separation in a PEG (polyethylene glycol)–dextran–water aqueous two-phase system were comparatively investigated using ^1H NMR spectroscopy. For comparison, a bi-distilled water sample was used as a reference, and the spectra of the phase samples were recorded under identical measurement conditions. The position and shape of the water signal in the NMR spectra, as well as the appearance of polymer-derived proton signals, enabled the assessment of compositional and structural differences between the phases. The results showed that, although the phases share the same aqueous medium, the spectral patterns differ due to interactions of the polymers with hydrophobic and hydrophilic groups. Thus, ^1H NMR is presented as an informative approach for characterization and interpretation of interphase differences in aqueous two-phase systems.

1. INTRODUCTION

As is well known, to study each component of biological mixtures (cells, viruses, proteins, etc.) individually, it is first necessary to separate them from one another. Although numerous chemical methods exist for the separation and purification of biological particle mixtures, these approaches are generally unsuitable for the separation of biological materials [1]. This is because, during the application of such methods, biological objects may undergo denaturation under the influence of various factors, thereby losing their biological activity and original properties. Other potentially applicable methods are complex, labor-intensive, and economically inefficient.

In recent years, the Swedish scientist P. A. Albertsson [2] developed a new method for the separation and purification of biological materials that is simple, cost-effective, does not require large-scale equipment, and is convenient to implement. This method is based on the unequal partitioning of substances in aqueous polymer two-phase systems. The essence of the method is as follows: when two polymers of different chemical nature are dissolved together in water, at polymer concentrations exceeding certain threshold values, the homogeneous solution separates into two immiscible liquid phases that are in equilibrium and possess different physicochemical properties (primarily due to hydrophobic and hydrophilic interactions); that is, a homogeneous-to-heterogeneous phase transition occurs. In such an equilibrium system, one phase becomes enriched with one component, while the other is enriched with the second component. Both phases consist predominantly of water (70–90%), which is the natural environment of living organisms [3]. The physicochemical properties of the phases differ, and consequently, the affinities of solutes distributed in an aqueous polymer system toward the phases are not the

same. Therefore, substances partition unevenly between the phases. Because water constitutes the major component of both phases, the structure of biomaterials is not damaged during partitioning, and thus their biological functions remain unchanged. One of the key advantages of this method is that the partitioning process can be readily transferred from laboratory conditions to biotechnological and industrial-scale applications.

It should be specifically noted that this modified method also has broad application potential in medical, biological, and pharmacological fields. In particular, it has helped to overcome the long-standing simplistic view of the key concepts of hydrophilicity, hydrophobicity, the hydrophobic effect, and hydrophobic interactions, and it enables the quantitative assessment of the relative hydrophobicity of substances, which cannot be determined by other methods [4]. Furthermore, this approach is widely used in primary medical diagnostics and facilitates understanding of the reasons underlying the existence of numerous energetic (histo-hematic) barriers in living organisms.

Thus, the search for, identification of, and recommendation of aqueous two-phase systems (ATPS) applicable to specific practical cases is highly relevant. To experimentally investigate phase separation in such systems, one of the simplest and most accessible model systems is an aqueous polymer system. In the present work, a PEG–dextran–water aqueous two-phase system was selected as the object of study. As noted above, phase separation in this system occurs when the concentrations of both polymers (PEG and dextran) exceed certain threshold values, leading to the formation of two distinct phases in water. One phase is dextran-rich, whereas the other is PEG-rich. Water constitutes the major fraction of both phases (approximately 70–90%). The structural temperature of each phase was determined by the viscometric method, and it was confirmed that the PEG-rich phase is more structured than the dextran-rich phase [5]. The obtained results indicate that the upper and lower phases differ in terms of the level of aqueous polymer structuring.

Phase separation can be qualitatively explained as follows. The polymers introduced into water differ in their chemical composition. This difference is associated with variations in the hydrophobic and hydrophilic groups present in each polymer. Naturally, such diversity should manifest itself in the interactions between the polymers and water. For this reason, when polymers are added to water, their distinct hydrophobic and hydrophilic groups affect the hydrogen-bond network in different ways—weakening or strengthening hydrogen bonding to varying extents. Consequently, as the distribution of electron density responsible for hydrogen-bond formation changes, water molecules become oriented differently, and characteristic microdomains are formed in the immediate vicinity of each polymer [6]. As a result, these processes occurring throughout the bulk lead to the formation of nuclei (incipient domains) of the emerging phases (Figure 1).

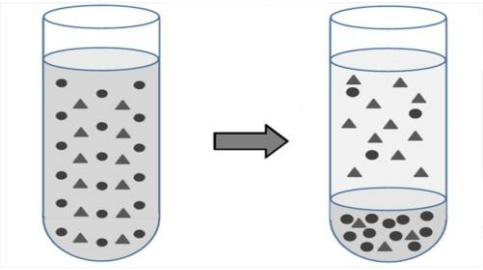

Fig. 1 *Schematic representation of the phase-separation process*

When the size (r) and concentration (C) of these newly formed structures are small, they are disrupted by thermal motion. This process is fluctuation-driven: over time, new structures continuously form and disappear, and they remain thermodynamically unstable. As the polymer concentrations are gradually increased, the microdomains grow through fluctuation-induced coalescence, and both their size and concentration increase. Once the size and concentration exceed certain critical values, i.e., when $r > r_{cr}$ and $C > C_{cr}$, thermodynamically stable, mutually immiscible phases are formed in accordance with the specific polymer–water interactions.

To describe aqueous polymer two-phase systems, unlike conventional phase diagrams, a phase diagram is used in which the coordinates represent the concentrations (in weight %) of the polymer components constituting the phases. The phase diagram is characterized by a number of parameters, including the binodal curve, tie-line, critical point, and the slope of the tie-line [7].

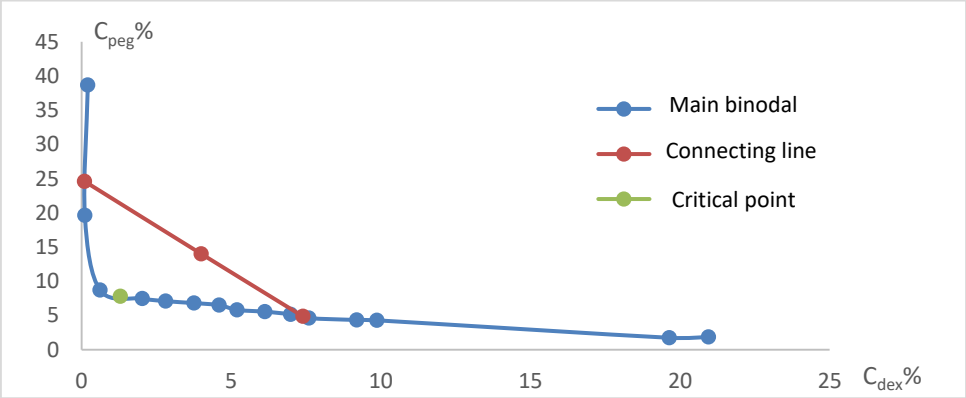

Fig. 2 *Phase diagram of the PEG-dextran-water biphasic system*

The concentrations of the components in aqueous solutions at which two-phase systems separate into two distinct phases are determined using binodal curves. The binodal curve represents the locus of points on the phase diagram that separates the single-phase (homogeneous) region from the two-phase (heterogeneous) region for a given system. Another characteristic parameter of two-phase systems is the tie-line. A tie-line indicates the compositions of the upper and lower phases: specifically, the intersections of the tie-line with the binodal, as well as any point along the line, correspond to the component concentrations in the coexisting upper and lower phases. The critical point is a hypothetical point at which the phase volumes and compositions are equal.

2. RESULTS AND DISCUSSION

In the present work, to more clearly observe differences in the structure of the aqueous media of the phases in an aqueous polymer two-phase system (ATPS), and considering that both phases are water-rich (70–90%), the nuclear magnetic resonance spectra of pure water and of the aqueous media of the phases were investigated. The experiments were performed on a Spinsolve instrument operating at a working frequency of 60 MHz ($\omega_0 = \gamma H$). Based on the obtained results, the origins of the differences observed between the phases were interpreted, and the applicability of the NMR method for the characterization of two-phase systems was demonstrated.

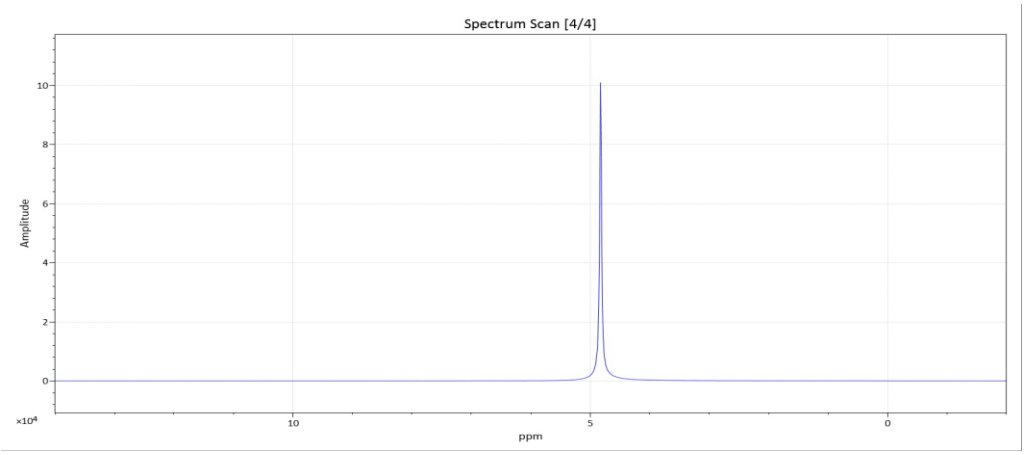

Fig. 3 *^{1}H NMR spectrum of distilled water*

Figure 3 shows the NMR spectrum of bi-distilled pure water. As can be seen, the spectrum obtained for the bi-distilled water sample exhibits a single, high-intensity, narrow peak in the range of 4.7–5.0 ppm. This peak corresponds to the protons of free water molecules. The narrow and symmetric peak shape indicates high mobility of water molecules. The absence of additional proton signals confirms that no impurities are present and that the water is pure. This spectrum was taken as the reference for comparison with the other samples (the phase spectra).

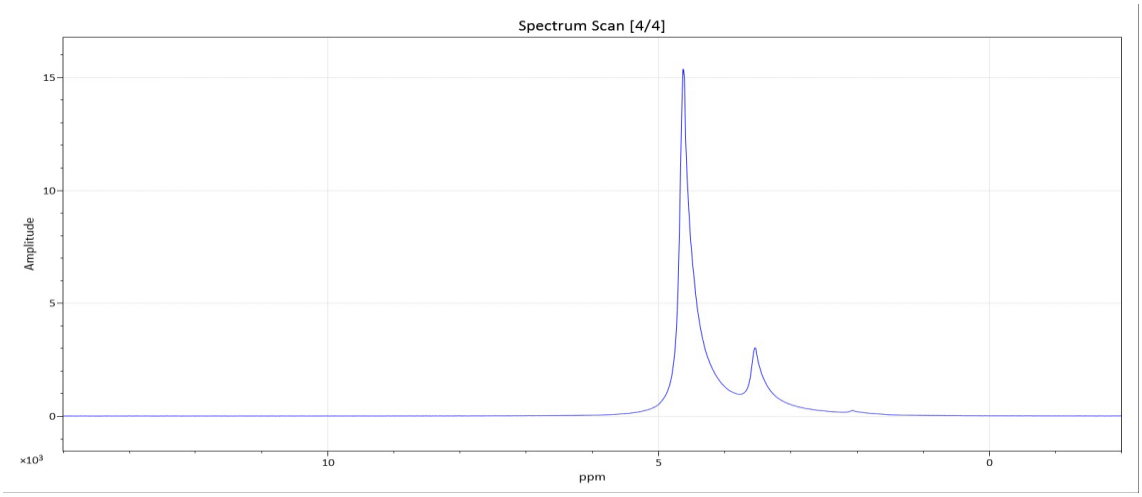

Fig. 4 *^{1}H NMR spectrum of the upper phase*

The spectrum of the upper phase is shown in the sample presented in Figure 4. In this spectrum, the main dominant signal (peak) again corresponds to water protons. However, in comparison with bi-distilled water, asymmetry and broadening of the water peak are observed. In addition, extra signals appear in the range of 3.5–3.8 ppm. The additional signal observed at 3.5–3.8 ppm is attributed to the $–CH_2–CH_2–O–$ groups of PEG, since it is generated by PEG protons. On the other hand, the oxygen atoms of PEG form hydrogen bonds with water molecules, rendering a fraction of the water "bound." This interaction alters the electronic environment of water protons and leads to changes in the chemical shift and line shape of the water peak. This indicates that the upper phase is PEG-rich.

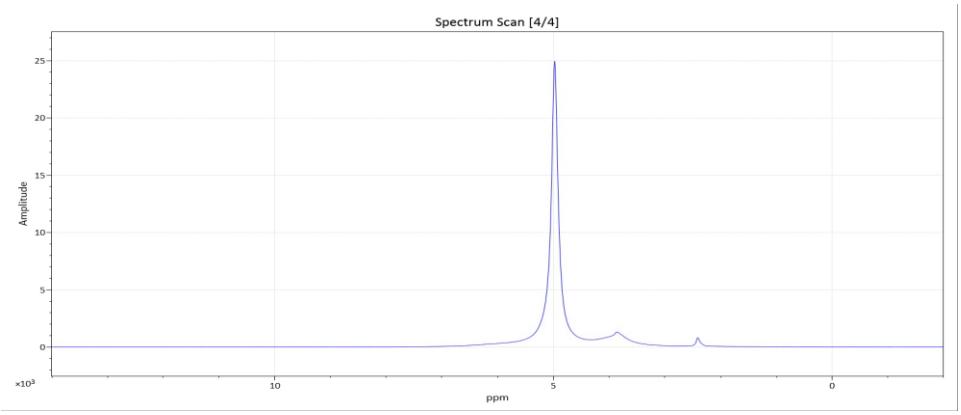

Fig. 5 *^{1}H NMR spectrum of the lower phase*

As can be seen from Figure 5, the water peak remains the dominant signal in the spectrum of the lower phase; however, the following additional features are observed:

1. A more complex structure is observed in the vicinity of the water peak

2. Several weak but discernible signals appear in the 3.0–4.5 ppm region

3. Additional weak signals are also observed in the 2.0–2.5 ppm region

The signals observed in the 2.0–2.5 ppm range are attributed to protons located in a modified magnetic environment resulting from the strong interaction of dextran molecules with water. The signals in the 3.0–4.5 ppm region are associated with ring protons of the carbohydrate rings of dextran. In addition, weak signals corresponding to the anomeric protons of dextran are likely present in the ~5.0–5.4 ppm interval (near the water peak). Because a fraction of the anomeric protons participates in bonding, their corresponding signal becomes weakened. Therefore, it is entirely expected that this signal appears not as a distinct, separate peak, but adjacent to and partially overlapping with the water peak. These findings indicate that the lower phase is dextran-rich.

3. CONCLUSION

Thus, the performed ^{1}H NMR study demonstrated that the upper and lower phases in the PEG–dextran–water aqueous two-phase system differ in their spectral features. Comparison with the bi-distilled water reference revealed changes in the line shape of the water signal and in the overall spectral background in the phase samples, indicating a reorganization of the water microstructure within the polymer environment. The intensity and spectral regions of polymer-derived proton signals in the upper and lower phases confirmed that the phases differ in composition. In particular, the more pronounced signals attributed to ring protons in environments more strongly associated with dextran highlight the role of polymer–water interactions. The results suggest that the differences between the phases are not limited to concentration variations, but also involve changes in hydration and in the state of the hydrogen-bond network. In this respect, ^{1}H NMR spectroscopy can be recommended as an effective method for the rapid assessment of phase composition and structural differences in the aqueous medium in ATPS.

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