

Systematic Characterization of Doxorubicin Hydrochloride Solutions: Viscosity, Density, and Refractive Index at Different Concentration and Temperatures

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Abstract

This study presents a systematic investigation of the thermophysical properties of doxorubicin hydrochloride (DOX·HCl) solutions at clinically relevant temperatures (5-50°C) and concentration (1-10 mg/mL) ranges. Using precision instrumentation including DMA 4500 M densitometer, Lovis 2000 M/ME viscometer, and Abbemat 500 refractometer, density, dynamic viscosity, and refractive index with high reproducibility (variation coefficients < 0.2%) were measured. The results demonstrate consistent and predictable variations: density decreases with temperature but increases linearly with concentration. Dynamic viscosity shows strong temperature dependence (64% reduction from 5 to 50 °C) but modest concentration sensitivity (≤ 8% increase). Refractive index exhibits linear concentration dependence with high sensitivity (~2.8 × 10⁻⁴ RIU per mg/mL). These findings provide crucial insights for clinical practice, enabling optimization of infusion accuracy through temperature management, rapid quality control via refractive index measurements, and evidence-based storage protocols. The established property correlations form a robust physicochemical foundation for enhancing doxorubicin formulation stability, administration safety, and therapeutic reliability in cancer chemotherapy.

Keywords: Doxorubicin Hydrochloride; Viscosity; Density; Refractive Index ; Physicochemical Properties

Introduction :

Doxorubicin is one of the most important and potent chemotherapeutic agents in the history of oncology. Its significance stems from its role as a cornerstone in the treatment of numerous cancers including sarcomas, breast cancer, leukemia, and lymphoma covering a broad spectrum of malignancies. This drug is a highly potent cytotoxic agent that acts through three primary mechanisms: (1) intercalation between DNA base pairs, (2) inhibition of topoisomerase II leading to double-strand DNA breaks, and (3) generation of reactive oxygen species (ROS) that cause oxidative damage to cellular components. Although doxorubicin is associated with significant adverse effects particularly cumulative cardiotoxicity its role in saving the lives of millions of cancer patients worldwide is undeniable [1].

Nevertheless, the stability, bioavailability, and clinical safety of this drug are directly influenced by the physicochemical properties of its solution including density, dynamic viscosity, and refractive index [2].

Density is essential for accurate drug concentration calculation and the design of infusion systems [3]. Dynamic viscosity not only affects injectability and drug flow rate in delivery systems [4], but in fluid biology, viscosity is also recognized as a key indicator of fluid behavior in microcirculation and mass transfer as demonstrated by epidemiological studies on blood and plasma, which have shown that elevated viscosity is associated with a higher risk of stroke and cardiovascular disease [5,6].

Refractive index serves as a sensitive parameter for quality control, homogeneity assessment, and detection of photodegradation in drug formulations [7].

Given doxorubicin's sensitivity to temperature and light, systematic measurement of these three parameters over the temperature range of 5–50 °C and concentration range of 1–10 mg/mL provides valuable data for optimizing formulation, storage conditions, and clinical transport and administration.

Materials and method

2.1. Materials

Doxorubicin hydrochloride powder (DOX·HCl) of pharmaceutical-grade purity was obtained from the manufacturer. Deionized water was produced in the laboratory using an ion-exchange system. All materials were used at ambient temperature without any further purification.

2.2. Solution Preparation

Aqueous solutions of doxorubicin hydrochloride were prepared in the concentration range of 1 to 10 mg/mL by dissolving precise quantities of the drug powder in deionized water. The uncertainty of the balance used for weighing was ±0.0003 g. All preparation steps were carried out under ambient conditions (25 °C and atmospheric pressure). To prevent photodegradation, all solution containers were wrapped in aluminum foil and stored in the dark at 25 °C.

2.3. Measurement of Physicochemical Properties

2.3.1. Density Measurement

Density was measured using the DMA 4500 M densitometer (Anton Paar GmbH, Austria) over the temperature range of 278.15 to 323.15 K (5 to 50 °C). The instrument employs U-tube oscillation technology with pulsed excitation, enabling automatic viscosity correction and density measurements with a precision of up to 1 × 10⁻⁶ g/cm³.

2.3.2. Viscosity Measurement

Dynamic viscosity was determined using the Lovis 2000 M/ME rolling-ball viscometer (Anton Paar). This instrument operates on the rolling-ball principle (a modification of the Hoeppler method) and is suitable for measuring the viscosity of low-viscosity.

2.3.3. Refractive Index Measurement

The refractive index was measured using the Abbemat 500 automatic refractometer (Anton Paar).

2.4. Experimental Protocol

Prior to measurement, samples were sonicated for one hour in an ultrasonic bath to ensure complete degassing and homogeneity. The temperature range of 5-50 °C was selected to reflect realistic conditions for drug storage, transport, and clinical use, while remaining safely below the thermal degradation temperature of doxorubicin. The concentration range of 1-10 mg/mL covers all relevant pharmaceutical formulations and provides sufficient data points for robust mathematical modeling.

2.5. Calibration and Quality Control

All instruments were calibrated prior to use according to the manufacturer's protocols:

- Densitometer: with deionized water and air;
- Viscometer: with standard oil (product code APN26, Anton Paar);
- Refractometer: with deionized water at 20 °C.

Method validation was confirmed using reference data and control measurements. All measurements were performed in triplicate, and results are reported as mean standard deviation. These standardized methods, combined with advanced instrumentation, enable a systematic and accurate investigation of the effects of temperature and concentration on the physicochemical properties of doxorubicin hydrochloride solutions.

Results and Discussion

This study systematically investigates the effects of temperature (5-50 °C) and concentration (1-10 mg/mL) on the density, dynamic viscosity, and refractive index of aqueous doxorubicin hydrochloride (DOX·HCl) solutions. All measurements were performed in triplicate, demonstrating high reproducibility (variation coefficients < 0.2% in most cases). As shown in Fig. 1, doxorubicin HCl properties vary systematically with temperature and concentration.

Density

Density decreased with increasing temperature for both pure water and DOX solutions, consistent with thermal expansion. However, at any fixed temperature, density increased linearly with DOX concentration. For example, at 25 °C, density increases from 0.99709 g/cm³ (water) to 1.00055 g/cm³ at 10 mg/mL - a 0.35% increase. The slope of density vs. concentration was nearly constant across all temperatures (R² > 0.998), confirming additive solute contribution without significant solute-solute interactions in this concentration range.

Dynamic Viscosity

Dynamic viscosity exhibited a strong inverse relationship with temperature. For instance, the 5 mg/mL solution showed a viscosity drop from 1.5408 mPa·s at 5 °C to 0.5589 mPa·s at 50 °C - a 64% reduction. Surprisingly, dynamic viscosity showed only a modest increase (≤ 8%) across the 1-10 mg/mL range at fixed temperatures, despite the rising density and molecular load. This suggests minimal impact of DOX on the microviscous structure of water at clinically relevant concentrations.

Refractive Index (RI)

RI increased linearly with concentration and decreased with temperature, showing high sensitivity to both parameters. At 25 °C, RI increases from 1.33247 (water) to 1.33529 (10 mg/mL), yielding a sensitivity of ~2.8 × 10⁻⁴ RIU per mg/mL. The monotonic, non-scattering behavior of RI across all conditions confirms the absence of aggregation, precipitation, or photodegradation - supporting the efficacy of light-protective handling protocols.

Implications

These results have direct relevance to clinical practice:

Infusion accuracy: Temperature-dependent viscosity changes can affect flow rates in gravity-driven or pump-based systems.

Quality control: Refractive index and density offer rapid, non-invasive metrics for verifying concentration and stability.

Storage & transport: Solutions should ideally be equilibrated to room temperature before administration to ensure predictable rheological behavior.

Collectively, this dataset provides a robust physicochemical foundation for optimizing DOX formulation, handling, and delivery - ultimately contributing to therapeutic reliability and patient safety.

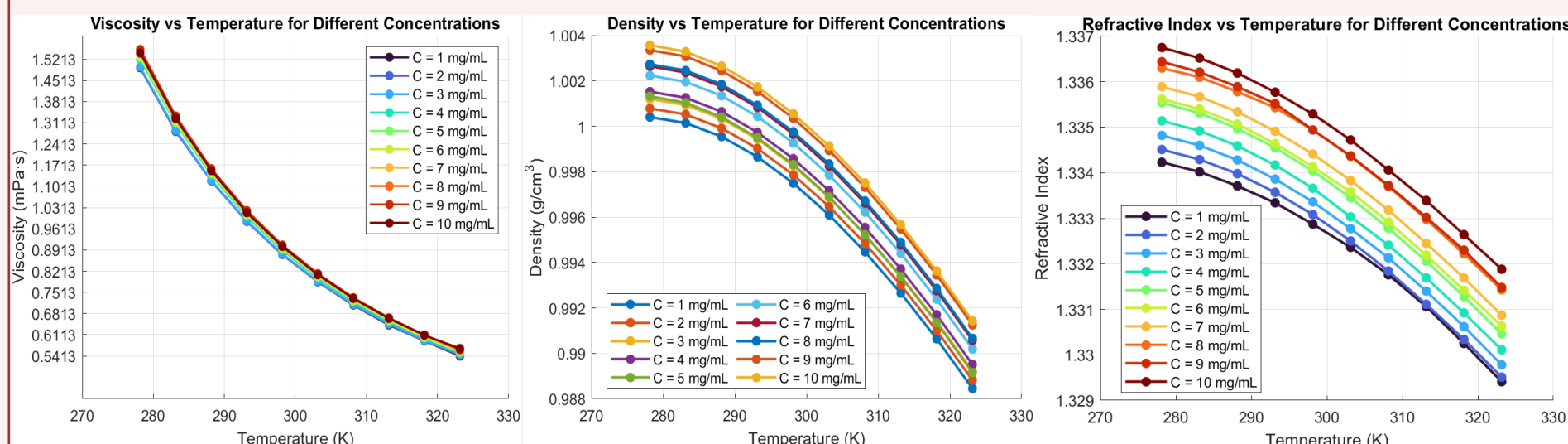


Fig. 1 Thermophysical Properties vs Temperature for Different Concentrations

Conclusions

This comprehensive study provides systematic experimental data on the thermophysical properties of doxorubicin hydrochloride solutions across clinically relevant temperature (5-50°C) and concentration (1-10 mg/mL) ranges. The results demonstrate that density, dynamic viscosity, and refractive index exhibit consistent and predictable variations with both temperature and concentration, following well-defined physicochemical relationships.

The linear concentration dependence of density confirms ideal solution behavior, while the strong temperature sensitivity of viscosity (up to 64% reduction) highlights the critical importance of thermal management during drug administration. The refractive index shows remarkable sensitivity to concentration changes (~2.8 × 10⁻⁴ RIU per mg/mL), establishing it as a valuable quality control parameter.

These findings have direct practical implications for clinical practice:

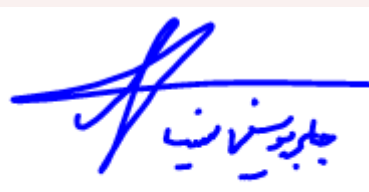
Formulation optimization: Enables precise concentration verification and stability assessment

Administration safety: Guides proper temperature equilibration to ensure accurate infusion rates

References

- [1] Minotti, G., et al. (2004). Anthracyclines: Molecular Advances and Pharmacologic Developments in Antitumor Activity and Cardiotoxicity. *Pharmacological Reviews*, 56(2), 185–229. <https://doi.org/10.1124/pr.56.2.6>
- [2] Gupta, P. K., & Rai, R. K. (2020). Refractive index as a tool for quality control and stability assessment of pharmaceutical solutions: A case study of doxorubicin hydrochloride. *Journal of Pharmaceutical Analysis*, 10(5), 487–493. <https://doi.org/10.1016/j.jpba.2020.01.005>
- [3] Allen, T. M., & Cullis, P. R. (2013). Liposomal Drug Delivery Systems: From Concept to Clinical Applications. *Advanced Drug Delivery Reviews*, 65(1), 36–48. <https://doi.org/10.1016/j.addr.2012.09.037>
- [4] Sharma, A., et al. (2021). Rheological characterization of injectable drug formulations: Implications for syringeability, injectability, and patient comfort. *European Journal of Pharmaceutics and Biopharmaceutics*, 162, 146–158. <https://doi.org/10.1016/j.ejpb.2021.02.014>
- [5] Lowe, G. D. O., et al. (1997). Blood viscosity and risk of cardiovascular events: the Edinburgh Artery Study. *British Journal of Haematology*, 96(1), 168–173. <https://doi.org/10.1046/j.1365-2141.1997.8532481.x>
- [6] Koenig, W., et al. (1998). Plasma Viscosity and the Risk of Coronary Heart Disease: Results From the MONICA-Augsburg Cohort Study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 18(5), 768–772. <https://doi.org/10.1161/01.ATV.18.5.768>
- [7] United States Pharmacopeia (USP). (2023). General Chapter <831> Refractive Index. <https://www.usp.org/compounding/general-chapter-refractive-index>

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