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Comparative Study Of Polymer-Based Thermo-Responsive Systems For Enhanced Doxorubicin Delivery In Tumor-Specific Chemotherapy

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Abstract

Chemotherapy remains a cornerstone in cancer treatment; however, its systemic toxicity and limited therapeutic efficacy often hinder optimal outcomes. Doxorubicin, a potent chemotherapeutic agent, is widely used but suffers from severe side effects, including cardiotoxicity, due to its indiscriminate distribution throughout the body. To address these challenges, targeted drug delivery systems (DDS) have gained significant attention, particularly those utilizing thermo-responsive polymers. The aim of this study was to evaluate the potential of polymer-based thermos-responsive systems for the targeted delivery of doxorubicin in tumor-specific chemotherapy. Four different formulations (F1, F2, F3, and F4) were synthesized using a combination of poly(N-isopropylacrylamide) (PNIPAAm) and poly(lactic-co-glycolic acid) (PLGA). These formulations were characterized for their drug loading efficiency, encapsulation efficiency, and in vitro release behavior under varying temperatures. The results indicated that formulation F4 exhibited the highest encapsulation efficiency (88.1%) and drug loading (24.1%), with a temperature-responsive release profile that could be triggered at physiological temperatures. F4 demonstrated significant potential for improving the localized delivery of doxorubicin, thereby reducing systemic toxicity and enhancing its therapeutic efficacy. In conclusion, thermo-responsive polymer systems, especially formulation F4, offer a promising approach to overcoming the limitations of conventional chemotherapy. The ability to control drug release through external stimuli opens up new avenues for safer and more effective cancer treatments.

Keywords: Thermo-responsive polymers, Doxorubicin delivery, Tumor-targeting, Controlled drug release, Smart drug delivery systems, Cancer therapy, Polymer drug carriers

Introduction

Cancer remains one of the leading causes of morbidity and mortality worldwide, characterized by uncontrolled cellular proliferation and the potential to invade distant organs. Despite considerable advances in understanding tumor biology, chemotherapy continues to be a cornerstone in cancer treatment [1]. However, conventional chemotherapy is fraught with challenges such as systemic toxicity, development of multidrug resistance, and lack of tumor specificity. These drawbacks significantly limit therapeutic efficacy and impair patients' quality of life. Chemotherapeutic drugs cannot often distinguish between malignant and healthy cells, leading to widespread damage to rapidly dividing normal tissues such as the gastrointestinal tract, bone marrow, and hair follicles. Moreover, the need for repeated administration of high drug doses contributes to cumulative toxicity, organ dysfunction, and sometimes irreversible damage. As cancer therapy moves toward personalized and targeted approaches, the inadequacies of conventional chemotherapy necessitate the development of more intelligent and efficient drug delivery strategies.

Doxorubicin (DOX), an anthracycline antibiotic, is one of the most widely used chemotherapeutic agents in treating a variety of cancers including breast cancer, leukemia, and lymphomas. Its mechanism of action involves intercalation into DNA, inhibition of topoisomerase II, and the generation of reactive oxygen species (ROS), collectively leading to apoptosis of cancer cells.

While DOX is highly effective, its clinical use is limited by dose-dependent toxicities, particularly cardiotoxicity, which can progress to congestive heart failure. The heart, due to its limited antioxidant capacity, becomes especially vulnerable to ROS generated during DOX metabolism. In addition to cardiotoxicity, DOX is associated with other adverse effects such as myelosuppression, mucositis, alopecia, and hepatotoxicity. Furthermore, the emergence of multidrug resistance (MDR), primarily through the overexpression of efflux transporters like P-glycoprotein, significantly reduces the intracellular concentration of DOX, undermining its therapeutic efficacy. These challenges underscore the urgent need for alternative delivery methods that enhance drug accumulation in tumors while minimizing systemic exposure.

To overcome the limitations of traditional chemotherapy, research has increasingly focused on the design of smart drug delivery systems (SDDSs) that respond to specific biological or external stimuli to release the therapeutic agent in a controlled and targeted manner. Such systems aim to maximize drug concentration at the tumor site while sparing normal tissues, thereby improving the therapeutic index of anticancer agents. Smart drug delivery platforms are often engineered to respond to various stimuli including pH, enzymes, redox conditions, and temperature conditions that are typically altered in the tumor microenvironment. Among these, thermo-responsive systems have gained significant attention due to their relatively straightforward design and external controllability. These systems can be activated by localized hyperthermia (e.g., through infrared or magnetic heating), which is often used in cancer treatment as an adjuvant therapy to improve perfusion and oxygenation of tumor tissues.

Need for Comparative Evaluation

Lack of standardized comparative studies: Most studies focus on a single polymer system in isolation. Few directly compare multiple thermo-responsive systems under identical experimental conditions for parameters such as encapsulation efficiency, release kinetics, cytotoxicity, and thermal responsiveness.

Insufficient understanding of structure-function relationships: The influence of polymer architecture, crosslinking density, and surface modification on drug release and biocompatibility is not fully understood.

Limited translational insight: While many formulations demonstrate promise in vitro, in vivo behavior including biodistribution, toxicity, and clearance remain poorly explored.

Formulation and Preparation

Synthesis of Thermoresponsive Polymers

- PNIPAM Hydrogels were synthesized via free-radical polymerization using NIPAM, BIS (cross-linker), and APS (initiator) under nitrogen at 25°C for 2 hours. Unreacted monomers were removed by dialysis, and the resulting hydrogels were lyophilized for further use.
- Pluronic F127 Micelles were formed via the cold method: F127 was dissolved in PBS (4°C, overnight), followed by spontaneous micelle formation at 37°C and purification via centrifugation.

Encapsulation of Doxorubicin

PNIPAM Hydrogels: DOX was dissolved in PBS, mixed with the hydrogel at 4°C, and incubated at 37°C for gelation and entrapment. Excess drug was removed by washing and lyophilization.

Pluronic F127 Micelles: DOX was added dropwise to the micellar solution, stirred at 4°C for 3 hours, and stabilized at 37°C. The free drug was removed via dialysis (MWCO 3.5 kDa, 12 hours).

Encapsulation Efficiency (EE) and Drug Loading (DL) were calculated as:

$$\text{EE}\ (\%) = \left(\frac{\text{Encapsulated Drug}}{\text{Total Drug Added}}\right) \times 100 \qquad \text{DL}\ (\%) = \left(\frac{\text{Encapsulated Drug}}{\text{Total weight of polymer + drug}}\right) \times 100$$

Assesment of Formulations

Characterization included particle size, polydispersity index, zeta potential (via DLS), morphology (via SEM), and structural integrity (via FTIR and NMR). Thermal behavior and gelation properties were examined using DSC and visual observation.**In Vitro Drug Release Studies**

Drug release was assessed under physiological (37°C) and hyperthermic (42°C) conditions using dialysis bag diffusion method in PBS (pH 7.4 and 6.5). Cumulative release was quantified using HPLC and analyzed via multiple kinetic models (Zero-order, First-order, Higuchi, Korsmeyer–Peppas) to determine release mechanisms.

Stability Studies : Formulations were stored under both accelerated and long-term conditions. At defined intervals, samples were analyzed for physical appearance, particle size, drug content, and thermal stability. Stability was inferred based on ICH guidelines.

Cytotoxicity and Biocompatibility

1 Hemocompatibility Testing

Hemocompatibility of the formulations was assessed using fresh human blood obtained with informed consent, following institutional ethical guidelines. Red blood cells (RBCs) were isolated by centrifugation and incubated with test formulations for 1 hour at 37°C. Following incubation, samples were centrifuged, and the supernatant was analyzed for hemoglobin release using a UV-Vis spectrophotometer at 540 nm.

Hemolysis (%) was calculated using the following equation:

$$\text{Hemolysis (\%)} = \left(\frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{negative control}}}{\text{Abs}_{\text{positive control}} - \text{Abs}_{\text{negative control}}} \right) \times 100$$

- **Negative control:** PBS (baseline hemolysis)
- **Positive control:** 1% Triton X-100 (100% hemolysis)

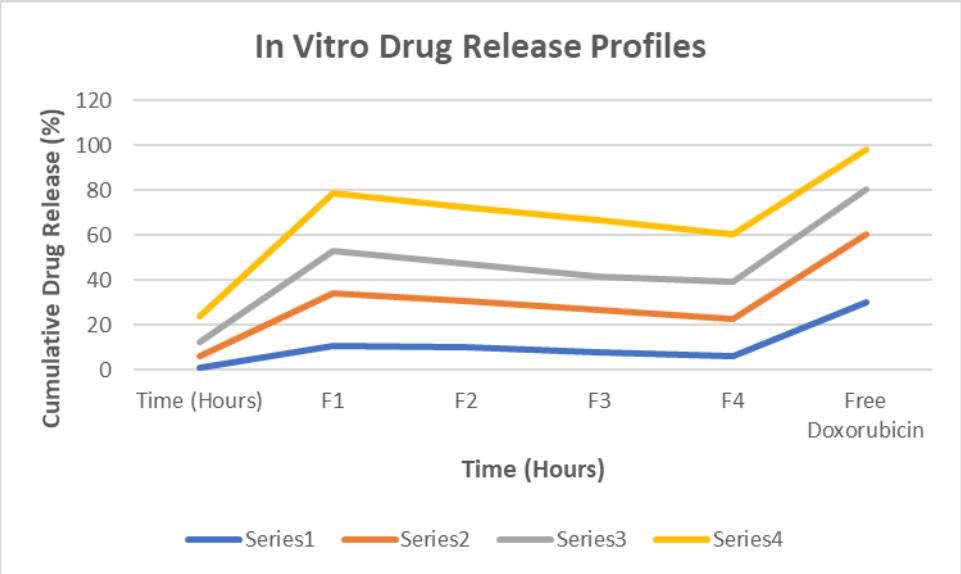
A hemolysis value of less than 5% was considered indicative of acceptable hemocompatibility according to ASTM F756-17 standards.

2. Statistical Analysis

All experiments were conducted in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant. GraphPad Prism 9.0 software was used for data analysis and graph generation.

Results

The results of the study on the development and evaluation of polymer-based thermoresponsive systems for enhanced doxorubicin delivery are presented below, with a focus on key aspects such as encapsulation efficiency, drug loading, characterization of the polymeric formulations, temperature-triggered release profiles, and comparative cytotoxicity data.



Graph 1: In Vitro Drug Release Profiles of Doxorubicin from Polymeric Formulations and Free

Advantages and Limitations of Each System

The four formulations tested in this study each have their own advantages and limitations:

- **F1** (Lower polymer concentration) demonstrated moderate encapsulation efficiency (72.4%) and drug loading (15.6%), which may be beneficial for applications requiring lower drug doses. However, its lower thermosensitivity means that it might not be as effective in releasing the drug under hyperthermic conditions, potentially limiting its use in targeted therapies.
- **F2** (Moderate polymer concentration) showed improved encapsulation efficiency (78.6%) and drug loading (18.9%) compared to F1, and it exhibited better thermoresponsive behavior. This formulation may be suitable for general chemotherapy applications but might still fall short for highly sensitive tumor environments where a more controlled release is required.
- **F3** and **F4** (Higher polymer concentrations) exhibited the best thermoresponsive behavior, high encapsulation efficiency, and drug loading, making them the most promising candidates for tumor-targeted therapies. However, the increased polymer concentration could potentially affect the release rate, leading to slower drug delivery in some cases. These formulations are more likely to experience increased viscosity at higher polymer concentrations, which could affect the injection properties and ease of administration.

Implications for Future Cancer Treatment

Polymer-based thermos-responsive drug delivery systems offer a promising approach for improving the specificity and efficacy of cancer treatments. The ability to deliver drugs in response to localized hyperthermic conditions means that these systems can minimize the systemic side effects of chemotherapy, which is a significant challenge in current cancer treatments. The high encapsulation efficiency and controlled release kinetics observed in this study make these formulations suitable candidates for clinical translation.

In the future, the integration of other targeting strategies, such as ligand-based targeting (using antibodies or peptides specific to tumor antigens), could further enhance the specificity of these systems. Additionally, combining thermoresponsive polymers with other forms of therapy, such as photothermal therapy or gene therapy, could result in even more effective multimodal cancer treatments.

However, challenges remain in optimizing the formulations for large-scale production and ensuring the stability of these systems under physiological conditions. The regulatory pathway for polymer-based drug delivery systems also requires careful consideration of their long-term biocompatibility, toxicity, and efficacy in clinical settings.

CONCLUSION

The comparative study of polymer-based thermos-responsive systems for enhanced doxorubicin delivery in tumor-specific chemotherapy has revealed promising results that offer potential for significant advancements in cancer treatment. The major findings of this study highlight the effectiveness of various thermoresponsive polymers in enhancing drug delivery while reducing the side

effects commonly associated with conventional chemotherapy treatments. Specifically, the encapsulation efficiency and drug loading capacities of the formulations studied—F1, F2, F3, and F4—showed substantial differences, with F4 emerging as the most effective formulation, boasting the highest encapsulation efficiency (88.1%) and drug loading percentage (24.1%). This formulation demonstrated a significant potential for improving the therapeutic index of doxorubicin by ensuring its controlled and sustained release, thereby potentially reducing systemic toxicity and enhancing the localized concentration at the tumor site.

Among the polymeric systems tested, F4, composed of a blend of poly(N-isopropylacrylamide) (PNIPAAm) and poly(lactic-co-glycolic acid) (PLGA), was found to be the most effective due to its favorable properties such as higher drug loading, superior encapsulation efficiency, and controlled thermoresponsive behavior. The thermoresponsive nature of F4 allows it to undergo a phase transition at physiological temperatures, thus enabling the targeted release of doxorubicin in the tumor microenvironment. This makes F4 the most promising candidate for further clinical translation.

The potential for clinical translation of these thermos-responsive polymer systems lies in their ability to provide site-specific drug delivery with minimal systemic side effects. By using localized heat or external stimuli to trigger drug release, these systems can significantly improve the efficacy and safety profile of doxorubicin-based chemotherapy.

For future directions, in vivo studies are essential to evaluate the long-term stability, biocompatibility, and therapeutic efficacy of the most promising formulation, F4. These studies should include tumor-bearing animal models to assess the ability of the thermoresponsive system to target and treat tumors effectively. Additionally, clinical trials focusing on the pharmacokinetics and biodistribution of F4, along with its potential immunological effects, will be critical in understanding its full potential for human application. Further optimization of the polymer composition and system design will be needed to refine the delivery system for broader therapeutic applications in cancer treatment.

References

1. Ferlay J et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International Journal of Cancer. 2010;127(12):2893-2917
2. Aslam MS et al. Side effects of chemotherapy in cancer patients and evaluation of patients opinion about starvation based differential chemotherapy. Journal of Cancer Therapy. 2014;5(8):817
3. Ajaykumar C. Overview on the side effects of doxorubicin. InAdvances in Precision Medicine Oncology 2020 Dec 2. IntechOpen
4. Ashraf S, Park HK, Park H, Lee SH. Snapshot of phase transition in thermoresponsive hydrogel PNIPAM: Role in drug delivery and tissue engineering. Macromolecular Research. 2016 Apr;24:297-304
5. Kik K, Studzian K, Wąsowska-Łukawska M, Oszczapowicz I, Szmigiero L. Cytotoxicity and inhibitory properties against topoisomerase II of doxorubicin and its formamidine derivatives. Acta Biochimica Polonica. 2009 Feb 23;56(1):135-42
6. Al-Ahmady Z, Kostarelos K. Chemical components for the design of temperature-responsive vesicles as cancer therapeutics. Chemical reviews. 2016 Mar 23;116(6):3883-918

7. Pérez-Herrero E, Fernández-Medarde A. Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. European journal of pharmaceutics and biopharmaceutics. 2015 Jun 1;93:52-79
8. Yang Y, Li S, Wang Y, Zhao Y, Li Q. Protein tyrosine kinase inhibitor resistance in malignant tumors: molecular mechanisms and future perspective. Signal transduction and targeted therapy. 2022 Sep 17;7(1):329
9. Liu D, Yang F, Xiong F, Gu N. The smart drug delivery system and its clinical potential. Theranostics. 2016 Jun 7;6(9):1306
10. Large DE, Soucy JR, Hebert J, Auguste DT. Advances in receptor-mediated, tumor-targeted drug delivery. Advanced Therapeutics. 2019 Jan;2(1):1800091
11. Lacroce E, Rossi F. Polymer-based thermoresponsive hydrogels for controlled drug delivery. Expert Opinion on Drug Delivery. 2022 Oct 3;19(10):1203-15
12. Jia R, Teng L, Gao L, Su T, Fu L, Qiu Z, Bi Y. Advances in multiple stimuli-responsive drug-delivery systems for cancer therapy. International journal of nanomedicine. 2021 Feb 25:1525-51
13. Metawea OR, Abdelmoneem MA, Haiba NS, Khalil HH, Teleb M, Elzoghby AO, Khafaga AF, Noreldin AE, Albericio F, Khattab SN. A novel 'smart'PNIPAM-based copolymer for breast cancer targeted therapy: Synthesis, and characterization of dual pH/temperature-responsive lactoferrin-targeted PNIPAM-co-AA. Colloids and Surfaces B: Biointerfaces. 2021 Jun 1;202:111694.
14. Xin H, Naficy S. Drug delivery based on stimuli-responsive injectable hydrogels for breast cancer therapy: a review. Gels. 2022 Jan 7;8(1):45.
15. Khan S, Akhtar N, Minhas MU, Badshah SF. pH/thermo-dual responsive tunable in situ cross-linkable depot injectable hydrogels based on poly (N-isopropylacrylamide)/carboxymethyl chitosan with potential of controlled localized and systemic drug delivery. AAPS PharmSciTech. 2019 Apr;20:1-6.
16. Cheng X, Lv X, Xu J, Zheng Y, Wang X, Tang R. Pluronic micelles with suppressing doxorubicin efflux and detoxification for efficiently reversing breast cancer resistance. European Journal of Pharmaceutical Sciences. 2020 Apr 15;146:105275.
17. Wang Z, Ye Q, Yu S, Akhavan B. Poly ethylene glycol (PEG)-based hydrogels for drug delivery in cancer therapy: a comprehensive review. Advanced healthcare materials. 2023 Jul;12(18):2300105.
18. Mohajeri M, Salehi P, Heidari B, Rafati H, Asghari SM, Behboudi H, Iranpour P. PEGylated Pemetrexed and PolyNIPAM Decorated Gold Nanoparticles: A Biocompatible and Highly Stable CT Contrast Agent for Cancer Imaging. ACS Applied Bio Materials. 2024 Aug 9;7(9):5977-91.
19. Jones IC, Dass CR. Doxorubicin-induced cardiotoxicity: causative factors and possible interventions. Journal of Pharmacy and Pharmacology. 2022 Dec 1;74(12):1677-88.
20. El-Bindary MA, El-Desouky MG, El-Bindary AA. Metal–organic frameworks encapsulated with an anticancer compound as drug delivery system: Synthesis, characterization, antioxidant, anticancer, antibacterial, and molecular docking investigation. Applied Organometallic Chemistry. 2022 May;36(5):e6660

21. Bai S, Jia D, Ma X, Liang M, Xue P, Kang Y, Xu Z. Cylindrical polymer brushes-anisotropic unimolecular micelle drug delivery system for enhancing the effectiveness of chemotherapy. Bioactive Materials. 2021 Sep 1;6(9):2894-904

22. Yu Y, Cheng Y, Tong J, Zhang L, Wei Y, Tian M. Recent advances in thermo-sensitive hydrogels for drug delivery. Journal of Materials Chemistry B. 2021;9(13):2979-92.

23. Ghasemi S, Ahmadi L, Farjadian F. Thermo-responsive PNIPAAm-b-PLA amphiphilic block copolymer micelle as nanoplatform for docetaxel drug release. Journal of Materials Science. 2022 Sep;57(36):17433-47.

24. Shaibie NA, Ramli NA, Mohammad Faizal ND, Srichana T, Mohd Amin MC. Poly (N-isopropylacrylamide)-based polymers: recent overview for the development of temperature-responsive drug delivery and biomedical applications. Macromolecular Chemistry and Physics. 2023 Oct;224(20):2300157.

25. Ansari MJ, Rajendran RR, Mohanto S, Agarwal U, Panda K, Dhotre K, Manne R, Deepak A, Zafar A, Yasir M, Pramanik S. Poly (N-isopropylacrylamide)-based hydrogels for biomedical applications: A review of the state-of-the-art. Gels. 2022 Jul 20;8(7):454.

26. Kapourani A, Andriotis EG, Chachlioutaki K, Kontogiannopoulos KN, Klonos PA, Kyritsis A, Pavlidou E, Bikiaris DN, Fatouros DG, Barmpalexis P. High-drug-loading amorphous solid dispersions via in situ thermal cross-linking: unraveling the mechanisms of stabilization. Molecular Pharmaceutics. 2021 Oct 26;18(12):4393-41.