PREFACE

We successfully synthesized methotrexate-loaded chitosan nanoparticles in this study using the ionic gelation method. Taguchi design was employed to optimize formulation parameters such as particle size, entrapment efficiency, and drug loading capacity. The nanoparticles were designed to improve therapeutic efficacy and minimize the side effects of methotrexate in breast cancer treatment. Additionally, we developed a self-targeted formulation by modifying reaction conditions. Also, we synthesized fluorescent calcium carbonate nanoparticles functionalized with chitosan and loaded them with methotrexate for controlled drug delivery. The nanoparticles were extensively characterized using various techniques, including UV-vis spectrophotometry, HPLC, DLS, FTIR, XRD, SAED, fluorescence spectrophotometry, IVIS, and biochemical analysis. The nanoparticles exhibited spherical morphology and demonstrated good biocompatibility and anticancer effects. They effectively reduced cytokine levels and showed high potential for triple-negative breast cancer treatment. The optimized nanoparticles had desirable physicochemical properties, controlled drug release, and improved pharmacokinetic profiles compared to free methotrexate. They exhibited biocompatibility, minimal hemolysis, and higher cytotoxicity against breast cancer cells. In a chemically-induced breast cancer rat model, the nanoparticles significantly inhibited tumor growth compared to free methotrexate. These findings highlight the potential of the developed nanoparticles for breast cancer treatment, offering enhanced drug delivery, improved pharmacokinetics, and reduced toxicity. The functionalized fluorescent nanoparticles also demonstrated biocompatibility, bioimaging capabilities, and anticancer activity. This study provides a promising therapeutic approach with the potential for clinical translation in the future. The thesis work has been conceptualized considering the above-discussed aspects of biocompatible nanocarrier research for breast

cancer treatment. The thesis consists of seven chapters, with the first two devoted to the introduction, literature review & the objective of the work section. Four out of the remaining five chapters extensively discussed different aspects of prepared nanoparticles and their application in breast cancer treatment. The last chapter belongs to the summary of the overall research.

The present study is divided into the following chapters.

Chapter 1: This chapter provides an in-depth exploration of cancer, including its various types, prevalence, and the urgent need for effective treatment strategies. It highlights the wide-ranging prospects of nanoparticles in the context of breast cancer treatment, emphasizing their potential to revolutionize current therapeutic approaches. The chapter delves into the crucial considerations involved in material selection for nanoparticle synthesis, considering the desired properties and characteristics necessary for effective drug delivery. Furthermore, it discusses the adopted technologies employed during the synthesis of nanoparticles, focusing on the ionic gelation method and its advantages. Special emphasis is given to the superiority of polymeric nanoparticles and inorganic nanomaterials, discussing the unique properties that make them ideal candidates for targeted drug delivery and enhanced therapeutic outcomes in breast cancer treatment.

Chapter 2: In this chapter, exploring the comprehensive review critically examines the potential of Methotrexate-loaded or encapsulated chitosan-based nanoparticles for breast cancer treatment. It covers various aspects, including synthesis techniques, physicochemical characterization, drug release kinetics, cellular uptake mechanisms, and strategies to enhance therapeutic efficacy. This comprehensive analysis serves as a foundation for further research and development in the field of chitosan-based nanoparticle formulations for breast cancer therapy.

Chapter 3: This chapter focuses on synthesizing a 1-hour-methotrexate-encapsulated polymeric nanocarrier using the ionic gelation method. The formulation is optimized using Taguchi design, a Design of Experiment (DOE) approach, to enhance its effectiveness in improving therapeutic efficacy while minimizing the side effects of the drug. The chapter delves into the details of the synthesis process, discussing the specific parameters and conditions employed to achieve the desired nanocarrier formulation. To ensure the reliability and quality of the nanocarrier, a comprehensive characterization is conducted using various microscopy and spectroscopy techniques. Furthermore, the chapter addresses the crucial aspect of assessing the toxicity of the nanocarrier. To evaluate its safety, a toxicity study is conducted in normal rats, with the evaluation focusing on tissue histopathology analysis.

Chapter 4: This chapter focuses on formulating a single-step self-assembly 3-hour-methotrexate-encapsulated polymeric nanocarrier that exhibits pH-sensitive drug release specifically designed for the breast cancer microenvironment. To evaluate the anticancer efficacy of the nanoparticles, a chemically induced N-methyl-N-nitrosourea (NMU) breast tumor rat model is developed. Furthermore, the assessment includes evaluating tumor volume, tumor weight, and other relevant parameters to determine the therapeutic impact of the nanoparticles on breast cancer.

Chapter 5: This chapter described the histopathological and biochemical assessment using a developed nano-formulation chemically N-methyl-N-nitrosourea (NMU) induced breast tumor rat model. Histopathology was done for tumor tissue, Kidney, and liver to check any toxicity of nanoparticles. Nano-formulation intravenous administrated in tumor-bearing rats, liver and kidney biomarker analysis was done. And different pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 were also checked for tumor generation confirmation and treatment efficacy.

Chapter 6: This chapter focuses on synthesizing chitosan-functionalized luminescent calcium carbonate nanoparticles (@Cal-CS-MTX-Nps) as a carrier for methotrexate and uses it for bioimage and treatment in breast tumor-bearing rats. The nanoparticles were characterized by various spectroscopy and microscopy techniques. The anticancer efficacy of nanoparticles was assessed in DMBA (dimethylbenz (a) anthracene) induced breast tumor rat model.

Chapter 7: The final chapter summarizes the overall research findings and provides conclusions regarding the development and application of methotrexate-loaded chitosan nanoparticles for breast cancer treatment.