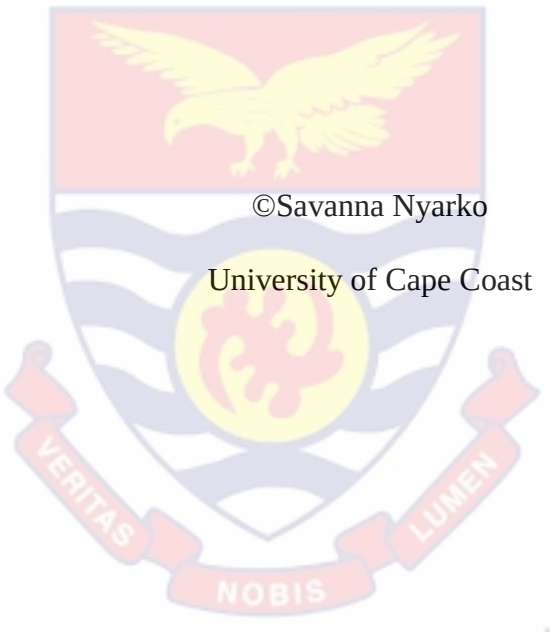
UNIVERSITY OF CAPE COAST

DRUG DELIVERY BY ZEOLITE NANOMATERIALS IN TREATMENT OF BREAST CANCER: *IN VITRO*

SAVANNA NYARKO

2024



UNIVERSITY OF CAPE COAST

DRUG DELIVERY BY ZEOLITE NANOMATERIALS IN TREATMENT OF BREAST CANCER: *IN VITRO*

BY

SAVANNA NYARKO

Thesis submitted to the Department of Physics of the School of Physical Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Doctor of Philosophy degree in Physics

JANUARY, 2024

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature…….. Date……

Name: Savanna Nyarko

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature…… Date……...

Name: Prof. Moses Jojo Eghan

Co-Supervisor's Signature…… Date……

Name: Prof. Elvis Kwason Tiburu

ABSTRACT

In this work, synthetic Linde type A (LTA) zeolites were examined to find out how well they could encapsulate and release doxorubicin cancer drug. Synthetic zeolites were used for this study because of their uniform pore distribution and crystal purity. The samples were characterized using X-ray diffraction spectroscopy (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). The XRD data on the control LTA zeolite showed average crystallite size of 40.89 nm, 28.40 nm and 29.76 nm at 60℃, 80 ℃ and 105 ℃ respectively. The percentage crystallinity also revealed values of 65.99, 71.39 and 76.37 at 60℃, 80 ℃ and 105 ℃ respectively. The XRD diffraction pattern on drug loaded LTA zeolite showed average crystallite size of 24.89 nm, 16.44 nm and 26.91 nm at 60℃, 80 ℃ and 105 ℃ respectively. The percentage crystallinity of the loaded drug on LTA zeolite also revealed values of 70.79, 83.78 and 68.82 at 60℃, 80 ℃ and 105 ℃ respectively. The FTIR data also showed the signature peaks characteristics of LTA zeolites at all the three temperatures (60℃, 80 ℃ and 105 ℃). The morphology of the control and loaded LTA zeolites were determined by Helium Ion Microscope (HIM) and Scanning Electron Microscope (SEM). Brunauer-Emmett- Teller (BET) surface area, pore size and pore volume were also determined. The drug release data from 60 ℃ had a correlation (R2) values of 0.9139, 0.8764 and 0.7844 with the first-order, Hixson-Crowell and zero-order models respectively. Drug release data for 80 ℃ and 105 ℃ also had a (R2) values of 0.7345 and 0.5160 respectively for the Korsmeyer-Peppas model. The Alamar blue assay cell viability results showed that 105 ℃ was cytotoxic to the cells with an IC50 of 92 µg/ml.

KEY WORDS

Breast Cancer

Drug Delivery

FTIR Characterization

Temperature

XRD Characterization

Zeolites Synthesis

ACKNOWLEDGEMENTS

Glory to God for being a pillar of support during my academic career. I am very grateful to Directorate of Research, Innovation and Consultancy and Training and Development section, University of Cape Coast for providing the funding for this doctoral study. I am also grateful to Erasmus + grant for the opportunity to undertake a three months training on drug delivery at the Norwegian University of Science and Technology. My heartfelt gratitude goes to my supervisors; Prof. Moses Jojo Eghan of the Department of Physics, University of Cape Coast and Prof. Elvis Kwason Tiburu of the Department of Biomedical Engineering, University of Ghana for their contributions, advise, encouragements, guidance and constructive suggestions on this study.

First and foremost, I want to sincerely appreciate Professor Catharina de Lange Davies, of the Department of Physics, Norwegian University of Science and Technology (NTNU) for training me on drug delivery during my stay at NTNU. Your time, technical knowledge, and insightful advice really enhanced both the overall tone of this thesis and my research experience. You have greatly inspired me with your expertise in the field of drug delivery. Very special thanks to Astrid Bjørkøy and Kristin Sæterbo at NTNU for their technical support at the lab in NTNU. I gratefully recognize Dr Anastasia Aikins, Department of Biochemistry, University of Ghana for providing the cell lines for this study. Thanks to Mr. Justum Nii Kotei Amon, Mr. Solomon Katu, Mr. Srinivasan Shankar, Mr. Richard Asiamah, Mr. Ernest Obeng, Mr. Edward Amenyaglo and finally Mr. Wisdom Amenyikor for the diverse support.

DEDICATION

To my parents; Nana Kobina Amoansah and Madam Sarah Sam.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AFI	Aluminophosphate-five
BET	Brunauer-Emmett-Teller
DDS	Drug Delivery System
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DOX	Doxorubicin
FAU	Faujasite
FBS	Fetal Bovine Serum
FER	Ferrierite
FTIR	Fourier Transform Infra-Red
HER 2	Human Epidermal Growth Factor Receptor 2
HIM	Helium Ion Microscope
LTA	Linde Type A
MDA-MB	Monroe Dunaway Anderson-Metastasis Breast
MOR	Mordenite
NP	Nanoparticles
QD	Quantum Dot
SEM	Scanning Electron Microscope
UV-Vis	Ultraviolet-Visible
XRD	X-ray Diffractometer
1D	One-Dimensional
2D	Two-Dimensional

CHAPTER ONE

INTRODUCTION

The most prevalent ailment that affects women and the primary cause of cancer deaths in Ghana is breast cancer (Thomas *et al*., 2017), which is distinguished by a proliferation of aberrant cells in the breast. These cells can gradually infect neighboring healthy breast tissue as well as the lymphatic system in the armpit when unnoticed and over time. Invasive cancer can also spread (metastasis) to other organs such the lungs, bone, brain, and liver when left undiagnosed and untreated for a long time. With changing lifestyles and more instances being reported, the number of women developing breast cancer in Ghana has increased over time and is predicted to continue to climb (WHO, 2008). This highlights the urgent need to advance breast cancer treatment. The context of the study, the study's problem statement, the study's aim and objectives, its importance, its constraints, and its organization are all covered in this chapter.

1.1 Background to the Study

Despite several technical advancements, even the most effective breast cancer treatments available today are not always 100% successful. Surgery, radiation and chemotherapy used in conventional cancer treatment do not target tumor tissues specifically. As a result, the course of treatment also causes harm to healthy tissues. Despite advancements in chemotherapy, many medications still fail to reach the tumor site at therapeutic levels and are frequently linked to severe system toxicity and subpar pharmacokinetics (Rocha *et al*., 2017).

The considerable adverse consequences of radiotherapy and chemotherapy are widely documented, and the majority of treatments target fast dividing cells in general, whether or not they are malignant. Additionally, the ineffectiveness, toxicity, and restricted biodistribution of cancer medications caused by their poor solubility, stability, and metabolism present additional difficulties (Navya *et al*., 2019).

There has been an unprecedented growth when it comes to the study of nanotechnology pertaining to medication administration for the treatment of illnesses. This is observed as researchers investigate various approaches for diagnosis and treatment for cancer (Malik *et al*., 2023). The study of matter at the nanoscale and its manipulation is known as nanotechnology, or around 1 to 100 nanometers, by the United States' National Nanotechnology Initiative (Heath, 2015). Nanoparticles (NPs) are used as drug carriers, because they can deliver active drug to cancerous cells or tissues without endangering nearby healthy cells or tissues and still have the intended pharmacological effect. NPs that act as drug carriers should be biodegradable, should not trigger any immunological response, and be able to deliver the medication to the intended spot (Portioli *et al*., 2017). In relation to having high levels of stability, specificity, possibilities for regulated drug release, drug loading, and transport of both hydrophilic and hydrophobic compounds, NPs provide a number of benefits more than traditional delivery techniques. NPs as drug delivery systems have many benefits, but they still have some drawbacks, such as burst or rapid release at specific places, which necessitates a sustained or protracted release and significantly reduces therapeutic efficacy. There is a growing demand for drug carriers that provide long-term drug release. Nanomedicine is

a potential alternative for breast cancer therapy. The application of nanotechnology in cancer diagnosis and treatment is still mostly in its infancy (Yan *et al*., 2020).

A new drug delivery method called drug targeting aims to only release the drug at the targeted location while inhibiting its release at all other places (Prabahar *et al*., 2021). A fascinating development in medicine is the use of NPs in drug delivery systems, which allows for focused drug distribution to achieve therapeutic efficacy. Additionally, due to off-target activity and extremely harmful health effects, conventional drug delivery systems have some disadvantages (Punia *et al*., 2020). Most anticancer drugs are injected or infused intravenously, resulting in an initial burst release and a drop in blood drug levels below therapeutic values (Manocha *et al*., 2010). Successful sustained release of medicine formulations have demonstrated that sick tissues benefit more from prolonged exposure to low drug concentrations than from a greater concentration of the medicine in a pulsed supply (Feng *et al*., 2003; Moghimi *et al*., 2001). Doxorubicin is among the widely prescribed anticancer drugs (Mishra *et al*., 2021). Resistance to the traditional chemotherapeutic agent, which is regarded as a major obstacle and poses significant hurdles in cancer therapy is one of the primary components causing the high mortality rate among cancer patients (Housman *et al*., 2014; Wambang *et al*., 2016; Wang *et al*., 2019).

Nanoparticles have displayed different hues and characteristics with a range in shape and size, which is applicable in biomedical imaging (Dreaden *et al*., 2012). One of the three layers that make up a nanoparticle is a range of small molecules, metal ions, surfactants, and polymers which can be used to

functionalize the surface layer (b) the core, which essentially refers to the nanoparticle's central area and usually refers to the nanoparticles themselves; (c) the shell layer, which in every way differs chemically from the core's composition (Shin *et al*., 2016). Nanoparticles are classified by different approaches and basis of dimensions as one, two and three dimensions (Tyagi, 2016). Because they are dispersed, nanoparticles have more surface area and more particles per unit mass than other types of particles (Khan *et al*., 2019).

Furthermore, a larger ratio of atoms are located on the exterior of nanomaterials, and these atoms once more have fewer close neighbours (Nadeem *et al*., 2022). One dimensional (1D) NPs have so far attracted more interest because of their intriguing characteristics and an extensive array of possible uses (Yun *et al*., 2013). Examples of ID nanomaterials include nanotubes, nanofiber, nanowire, nanorod and nanofilament. Amorphous or crystalline, single or polycrystalline, metallic, and ceramic 1D nanomaterials (Bashir *et al*., 2015). Two dimensional (2D) NPs are often classified to be the thinnest layer of nanomaterials due to their thickness and dimensions on nanoscale (Zahra, 2019). With three dimensional (3D) NPs, their components are smaller than 100 nm, yet all three dimensions are larger than 100 nm. (Verma *et al*., 2023).

These nanomaterials are generally nonporous and have many different uses. Nanocomposites, bundles of nanofibers, and multinanolayer-type structures are the most prevalent examples of three-dimensional nanomaterials (Jeevanandam *et al*., 2018).

The synthesis of nanoparticles as drug delivery methods that use nanotechnology has the potential to improve medicine bioavailability,

solubility, change the biodistribution of chemotherapy drugs, get rid of treatment-related drug resistance, and lessen nonspecific toxicity.

When utilized as a medication delivery mechanism, nanoparticles can lessen the negative side effects and adverse events that chemotherapy causes while also enhancing patient quality of life and extending patient survival (Yan *et al*., 2020). However, the use of several nanocarrier-based drugs has significant issues in the treatment and management of cancer which have been exposed by latest developments in the drug delivery system with desirable qualities. The design of effective cancer nano – based therapeutics, controlling drug release and preventing opsonization still remain a great challenge (Chenthamara *et al*., 2019) and just a few nano-formulations have enrolled in clinical studies. Moreover, another difficulty with using nanoparticles for drug delivery is ensuring the safety of human health. Nanocarriers may cause unintended toxicity as a result of adverse connections to biological systems when used in the treatment of cancer, and there have been numerous investigations that have demonstrated the negative effects of nanocarriers due to their toxicity (Navya *et al*., 2019). Despite all the shortcomings of nanomedicine, medication delivery systems based on nanoparticles for the treatment of cancer are still an effective choice (Yanru *et al*., 2017).

1.2 Statement of the Problem

Surgery, radiation, and chemotherapy used in conventional cancer treatment do not target tumor tissues specifically. As a result, the course of treatment also causes damage to healthy tissues. For instance, cytotoxic drugs used in chemotherapy frequently have a restricted therapeutic index because they are very harmful to healthy tissues (Drummond *et al*., 1999). A one

cancerous cell has the potential to trigger the growth of a tumor again, effective cancer therapy necessitates that cytotoxic medicines arrive at the targeted place in the right concentrations to kill every lethal cell. To boost the therapeutic dosage at the desired spot and achieve localized delivery is through the use of nanoparticles as a medication delivery system, chemotherapy drug side effects and adverse events can be reduced, again patients' quality of life can be improved and their survival time can be extended (Afadzi *et al*., 2012 ; Yan *et al*; 2020).

1.3 Purpose of Study

This study's goal was to explore local source nanomaterials for intelligent drug delivery in treatment of breast cancer.

1.3.1 *Research Objectives*

The specific objectives of this research work included:

1. Characterization of zeolite nanoparticle
2. Characterization of loaded doxorubicin on zeolite
3. Sustained release kinetics
4. Delivery of loaded drug for breast cancer treatment

1.4 Significance of the Study

Breast cancer, which has the highest mortality and incidence rates among women in Ghana, is a serious public health issue (Thomas *et al*., 2023). Breast cancer is the most second prevalent cancer in Ghana after cervical cancer, according to data from the 2012 Korle Bu Teaching Hospital Cancer Register (Ghartey *et al*., 2016). This indicates that Ghana is seeing a rise in the prevalence of breast cancer (Agyemang *et al*., 2020). Malignant tumors account for 12.8% of admissions to the Korle Bu Teaching Hospital in 1996

were for breast cancer (Ghartey *et al*., 2016). Breast cancer became the most common cancer in women in Ghana in 2009, accounting for about 16% of all cancers (Thomas *et al*., 2023). Additionally, Ghana lacks a thorough strategy and treatment recommendations for preventing breast cancer. Most Ghanaian women are unable to get treatment since most hospitals only offer radiation therapy, chemotherapy and surgery, which are all concentrated in the city areas (Zelle *et al*., 2012). Breast cancer has racial and social variations and is a complicated and diverse disease (Carey *et al*., 2006). Allied health professionals will gain knowledge on the use of the novel technology through training workshops. Hospitals, Ghana Health Service, Ghana Cancer Registry and the University of Cape Coast will benefit from the new knowledge technique in drug delivery by nanotechnology. Zeolite nanomaterial drug delivery will prove to be an effective method for improving breast cancer treatment.

1.5 Delimitations

This research focuses on the use of Linde Type A zeolite synthesized at three different temperatures, evaluating the doxorubicin drug loading capabilities and study the release rate of doxorubicin from the zeolite as a method of delivering drugs for effective breast cancer treatment.

1.6 Limitations

The following studies were not covered in this work

1. In - vivo imaging; that is, to know the circulation time of the drug loaded zeolite
2. Transmission Electron Microscope (TEM) of the synthesized zeolite

1.7 Organization of the Study

There are five chapters in this thesis. The study is introduced in Chapter 1. It focuses on the study's historical context, which describes the major challenge with regards to treatment of breast cancer and the types of treatment modalities. Additionally, it talks about the study's goal and its goals. In this chapter, it is stated how important the study is and how it was limited. The review of the literature is covered in Chapter 2. This chapter examines current literary works related to the research field. It goes over drug delivery systems, several kinds of nanoparticles used in medication delivery and Linde Type A zeolite nanomaterials. Literature on doxorubicin drug for cancer treatment is also presented.

The research approach is covered in Chapter 3. The components and procedures of this research investigation are clearly described in this chapter. The data collecting tool, the data collection processes, and the processing and analyses are all presented in detail. Additionally, it will describe the study's design and the manner in which the experiment was conducted.

The findings and discussion are presented in chapter four. In this chapter, the findings from the experimental procedures are reported. The outcomes of characterization of raw zeolite and drug loaded zeolite, SEM images, HIM images, BET pore volume, pore size, and surface area, doxorubicin drug loading on zeolite, drug release kinetics and cell viability studies (Alamar blue assay) are presented.

The summary, conclusion, and recommendations are found in the final chapter. This chapter presents an overview of the complete body of research and discusses the conclusions and suggested courses of action.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

This chapter reviews the underlining theoretical concepts used in this work. It focuses on overview of drug delivery systems, implication for therapeutics, drug delivery by doxorubicin drug, MDA MB 468 breast cancer cell line, zeolitic materials with various applications including drug delivery in literature.

2.1 Overview of Drug Delivery Systems: Implication for Therapeutics

2.1.1 Drug Delivery

Drug delivery is a fast-evolving multidisciplinary field within the health sciences. The area of drug delivery is vast which describes the creation of innovative carrier systems for safe and effective distribution of medicinal substances to their target locations to achieve therapeutic effect (Tiwari *et al*., 2012). Pharmacodynamics and pharmacokinetics are essential for drug delivery. Pharmacodynamics describes the physiologic and biochemical effects of the drug on the body (Rimmington, 2020). Pharmacokinetics refers to the duration of the concentration of a drug in the body, from absorption, bioavailability, distribution, metabolism through to excretion (Thürmann, 2020). For a disease to be properly managed, pharmacokinetic profiles of therapeutic drugs must be consistent. This is contingent on the patient's continued compliance in taking the medication as prescribed. Patients on prolonged release therapies have been shown in the literature to have lower

rates of relapse due to constant dosing of the drug (Senior *et al*., 2000). This enables the achievement of a consistent and stable pharmacodynamics profile.

Conventional method of drug delivery is effective when a quick response of the drug is required. However, drugs with narrow therapeutic window and low solubility are ineffective when delivered by the conventional method.

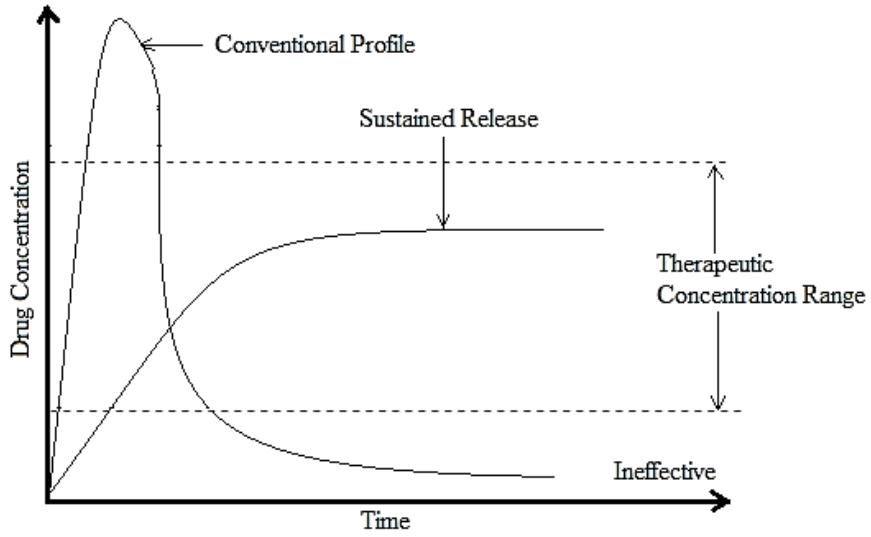


Figure 2.1: Pharmacokinetic profiles of sustained release vs. conventional release of drugs (Versypt, 2015).

In Figure 2.1, sustained release is achieved when a drug concentration increases steadily and plateaus in the therapeutic range in the blood plasma of a specific target and releases using the medication for a long time and that is where maximum therapeutic efficiency is reached. Once the drug concentration goes above within the therapeutic range, it is thought to be toxic and when the concentration goes below the therapeutic range, it is rendered ineffective.

During the 1950s, the first controlled release formulation received approval (Lee *et al*., 2010). In contrast to previous years, there has been a

continuous increase in the development of innovative drug delivery development of new drugs (Park, 2014). Among the key factors for this drug delivery development is due to the high drug delivery other than going through the long process of drug discovery, clinical testing and drug development through to regulatory approval cost and time involved in creating new medications. Research in 2013 found that the cost involved in developing a drug carrier was 10% of a new drug's development costs (He *et al*., 2013). As such research scientists prefer to develop drug carriers to increase the effectiveness of drug targeting.

2.2 Drug Delivery Methods

Drugs are administered by swallowing, absorption, inhalation, intravenous injection, or through the skin to create an overall pharmacological result. The oral method which is the common method experiences the following problems; low bioavailability, poor absorption of protein and this results in low permeability of drugs (Gopinath *et al*., 2020). For systemic treatment by pulmonary delivery, inhalation is used. Intravenous infusion is often used to deliver sedatives, analgesics, providing patients with fluids, anaesthetics, and chemotherapeutic agents (Ferrari *et al*., 2018). For drug distribution via the transdermal route, the skin's barrier defences allows compounds with appropriate physiochemical the ability to penetrate skin (Alkilani *et al*., 2015). Some advantages of delivery of drugs through the skin are less frequent dosing, large area for absorption, noninvasive among others (Saravanakumar *et al*., 2015).

2.3 Drug Delivery Systems

Drug delivery systems (DDSs) are pharmaceutical formulations or engineering tools that help with the targeted distribution and/or controlled release of pharmaceutical compounds throughout the body. By regulating the speed, timing, and location of medicine release in the body, DDSs increase the effectiveness and safety of medications (Jain, 2020). DDSs release the active pharmaceutical compounds when administered. When these compounds are encapsulated within a protective structure, potential disruptions among the active substances are reduced (Wang *et al*., 2021). Aside the increment in bioavailability of the pharmaceutical compound, unwanted adverse consequences brought on via systemic dispersion and frequent dosing are reduced (Kok-Yong *et al*., 2015). The bioactive molecules reach the site of action as they pass through many biological barriers. Research has revealed that these barriers play different roles in the transport of drugs in the circulatory systems and through cells and tissues (Yang *et al*., 2017). Research scientists have capitalized on the roles of these biological barriers to develop new modes of drug delivery (Agrahari & Kumar, 2022). Current novel DDS trigger the drug's discharge only at the target site with sustained or controlled release.

Other DDS deliver based on stimuli such as pH, light, and temperature among others. One important advantage of the medication delivery systems is controlled drug released in a time-dependent manner through both inactive and active targeting (Kumar *et al*., 2012). Passive targeting works based on the pathophysiological traits of tumor vessels which allow DDS to assemble

within the tumor at much higher concentrations than in normal tissues since tumor vessels become more permeable than healthy state (Attia *et al*., 2019).

This kind of targeting is based on the delivery system's size and circulation time. Ligand-receptor binding, which enhances the accumulation of DDS at target locations, is necessary for active targeting (Yoo *et al*., 2019). This makes it easier for the tumour cells to absorb DDS. DDS could be activated by a trigger that is peculiar to the desired location, including a pH responsive material. For example, most parts of the body have a consistent neutral pH. Some other parts may become more acidic than others especially in tumor environments. DDSs can however, use the change in pH to trigger the release of drugs. Another mechanism that can be used to start the release of a medication is the redox potential (Mollazadeh *et al*., 2021). Tumors change the redox potential in its environment. Based on this, a payload of medication can be made available based on a change in redox potential in the tumors.

2.4 Classes of Therapeutic and Delivery Paradigm

By moving and delivering the medication either inactively or actively towards the tissue and avoiding unintended accumulation of the drug, drug delivery seeks to optimize the effectiveness of treatment. (Vargason *et al*., 2021). Techniques for delivering drugs have greatly benefited in the transformation of effective pharmaceuticals from possible therapies (Vargason *et al*., 2021). Classes of therapeutic and numerous terms can be used to describe drug delivery technologies. The first drug delivery systems were built on four drug release mechanisms that were based on formulations for use with the mouth and skin, namely; diffusion, dissolution, osmosis, and ion

exchange-controlled drug release. Out of these four drug release mechanisms, processes of diffusion and dissolution are widely used (Park *et al*., 2022). Nanomedicine technology's effects on tumor-targeted medication delivery have not been as anticipated, and there have only been a limited number of newly authorized anticancer medication formulations (Bae & Park, 2011; Park, 2017; Bae & Park; 2020).

The development of formulations with predetermined release kinetics and duration is a medication delivery strategy. Almost solely oral formulations for 12 or 24 hour delivery were used for controlled medication release formulations at the beginning of the technological advancement (Park *et al*., 2022).

A small molecule like a peptide drug which is the most practical drug administration method is a type of oral drug delivery. Oral obstacles to medication distribution include biological obstacles, limited water solubility, low stability, low permeability or absorption, and pre-systemic clearance (Amidon *et al*., 1995). Some techniques and tools have been used to improve peptide and protein medication absorption which is still at the research and development stages (Ahiadan *et al*., 2020; Brayden *et al*., 2020; Brown *et al*., 2020; Bryne *et al*., 2021). The most popular method of administration has been an oral medication delivery (Alqahtani *et al*., 2021). Nevertheless, additional mechanism of critical processes of understanding the need for oral absorption is achieved, such as inter-individual variations, gastric retention, medication absorption disparities between genders and ages, formulation restrictions for poorly water-soluble and weakly permeable medicines, and others (Vinaroz *et al*., 2021). Antibodies also are the most common type of

therapeutic, which has increased clinical clearances in the United States (Anselmo *et al*., 2019; Lu *et al*., 2020). Therapeutic targets and the immune system can interact in particular ways according to the slightly different structure of antibodies compared to other groups of biological systems. These antibodies bind to cellular targets and send signals to the immune system (Chames *et al*., 2009). Antibodies binding can neutralize a target antigen and again prevent signalling molecule adsorption and initiate unfavorable cell behaviors (Shih *et al*., 2006).

Live cells therapy is the most recent therapeutic generation. They utilize particular cell types' inherent therapeutic abilities to control or facilitate important biological processes (Vargason *et al*., 2021). Drug delivery systems include three different types of drug delivery paradigms, drug changes, and microenvironmental modifications. Drug modifications mostly alter the qualities that affect the concentration and solubility of the chemical, the duration of the drug action also affects the compound's concentration solubility, the length of the movement and the ability of the molecules to be permeable in membranes (Saltzman, 2001). Microenvironment modifications have several techniques, ranging from a very specific adjustments when used with adjuvants at the point of action, when drugs are delivered systemically and affect the environment of the host. Again, a variety of medication delivery strategies that can assist in navigating biological barriers include microenvironment changes (Vargason *et al*., 2021). Drug delivery systems describe how medications are packaged, such as in the shape of micelles or nanoparticles, which shields them from degradation and enables them to move wherever they are needed in the body. In recent years, medication delivery

technology has evolved significantly, and future developments are anticipated to be much more significant (NIH, 2022).

2.5 Requirements for Drug Delivery Systems

Release of drugs at the intended location acts in accordance with patient requirement is guaranteed by ideal drug delivery systems. The drug focus should be between the minimum level of focus and the minimum harmful concentration at the targeted site (Hedaya, 2012). The drug delivery systems must also regulate the rate of release of the medication under strict control.

Patient adherence increases and the frequency of drug administration decreases when the drug's duration of action is prolonged and its plasma level fluctuation is reduced. The components utilized for DDSs should not elicit any negative reactions, the matrix material and its degraded products should be biocompatible and the material's mechanical characteristics should be able to provide prolonged protection of the therapeutic cargo; thus, ensuring chemical stability of the medication before it gets to the intended location (Parhiz *et al*., 2018). For cell-specific moieties, the DDS surface should be functionally changed. A triggerable or regulated drug release is also necessary.

2.6 Types of Drug Delivery Systems

It is essential to preserve the product's and the delivery's stability when dealing with different physiological factors. DDS should be easy to administer to patients as well as be trustworthy and safe. Examples of DDSs developed over the years include liposomes, micelles, polymeric particles, hydrogels, dendrimers, inorganic particles, quantum dots, and nanospheres.

2.6.1 Liposomes

One or more concentric phospholipid bilayers make up liposomes (He *et al*., 2019). Liposomes are very lipid-rich multilayered spherical structures with a wide range of classification criteria, including as structural characteristics and parameters, size, production techniques, processing, and drug loading (Nakhaei *et al*., 2021). Hydrophilic drugs are loaded in their core because of its polar nature. They are able to entrap both amphiphilic and lipophilic compounds' affinity towards the phospholipids. Because liposomes are more biocompatible than polymeric nanoparticles, they are a more preferable option for biomedical applications (Muller *et al*., 2000). Liposomes were originally utilized as a delivery system in the early 1990s. Natural and artificial liposomes are both considered as drug carrier systems because of their distinct structural characteristics (Nakhaei *et al*., 2021).

In literature, Ketoprofen drug was encapsulated in pharmacosomes and showed an improved dissolution of 93.3 % as compared to free ketoprofen which showed 49.77 % dissolution (Amandeep, 2013; Zylberberg & Matosevic, 2016). Furthermore, in order to create delivery mechanisms that can trap volatile substances (such as antimicrobials, antioxidants, flavours, and bioactive components) and safeguard their functionality, the food and agricultural industries have intensively investigated using liposomes as a medication delivery system (Akbarzadeh *et al*., 2013). The uses of liposomes in biomedical application can be separated into diagnostics and therapeutics.

The use of liposomes as a model, tool, or substance in the fundamental research of cell contacts, recognition mechanisms, and the method of action for specific compounds encompasses a number of indicators or medications

(Banerjee *et al*., 2004). When compared to free pharmaceuticals, the pharmacokinetics of liposomal medications can increase drug bioavailability at a specific target tumour location (Akbarzadeh *et al*., 2013). Liposomes currently is commercially used drug delivery system because it can trap hydrophilic medicines and is biocompatible, biodegradable, low-toxic (Johnson *et al*., 2007; Akbarzadeh *et al*., 2013) and also a localized medication delivery to tumors (Hofheinz *et al*., 2005). Despite the advantages of liposomes, they have some disadvantages as well. Their physical and chemical stabilities are liposomes' most important drawback (Bakker-Woudenberg, 2002). Liposomes are not highly water soluble (Li *et al*., 2019), they only last for a brief period of time inside the body (Kshirsagar *et al*., 2005). Liposome synthesis is expensive (Noble *et al*., 2014). They may result in the drug-loaded container leaking and fusing (Joly *et al*., 2011). There can be allergic reaction to some of the liposome compounds (Mansoori *et al*., 2012). Applications in medicine and the pharmaceutical industry frequently use liposomes.

2.6.2 Polymer Micelles

Nano-drug delivery devices called micelles made of polymer have a shell-like core which develops when block copolymers that are amphiphilic self-assemble in aqueous solution (Ghezzi *et al*., 2021). Amphiphilic di-block copolymers (like polystyrene and poly (ethylene glycol)) and tri-block copolymers (like poloxamers) are the most often used polymeric micelles. Again, G chitosan and ionic (e.g. poly (ethylene glycol)-poly(ε-caprolactone)-g polyethyleneimine) copolymers are also used (Yadav *et al*., 2019; Kulthe *et al*; 2012; Jiang *et al*., 2006; Li *et al*., 2019). PEG (polyethylenglycole) makes up the hydrophilic component, although other polymers including poly (vinyl

pyrrolidone), poly (acryl oylmorpholine), and poly (trimethylene carbonate) are also utilized. Poly (propylene oxide), polyesters like poly(-caprolactone), and co-polymers of glycolic and organic acids make up the hydrophobic component (Kulthe *et al*., 2012). Drugs may be produced with polymeric micelles, in regard to the manufacture process and the medication's physicochemical properties (Makhamalzade *et al*.,2018; Gaucher *et al*., 2005). Considering the qualities of the medication, different places, such as those near the outside or the inside core, can host the drug since the polarity and degree of hydration of micelles within the carrier are not uniform (Makhamalzade *et al*.,2018). Drugs that are hydrophobic are frequently housed and loaded in the inner core. The medicine may also be covalently bonded to the polymer in some circumstances (Ghezzi *et al*., 2021). Polymeric micelles are interesting conduits for a variety of drug administration because of their characteristics including small size, simplicity in synthesis, and efficient dissolution. To lessen side effects, polymeric micelles increase drug bioavailability and generate a prolonged drug release (Ambade *et al*., 2005; Mikhail *et al*., 2009). The intravenous (i.v.) injection or infusion is the micelles delivery method that has received the most research (Zhang *et al*., 2014). Additionally, polymeric micelles have been demonstrated to have increased medication bioavailability after oral administration within the literature (Lu *et al*., 2013; Gaucher *et al*., 2005) and topical (ocular, nasal, buccal) (Khan *et al*., 2017; Grimuado *et al*., 2019) administration. Micelles are frequently described as having spherical shapes, though occasionally rod-, worm-, or disk-shaped structures can be seen (Owen *et al*., 2012; Zhong *et al*., 2010; Troung *et al*., 2015). The polymers' composition utilized is mostly

responsible for the variations in micelle form (Owen *et al*., 2012; Kuntsche *et al*., 2011) and to the environmental features of the area in terms of its temperature, pH, and composition (Kuntsche *et al*., 2011).

The two basic ways that drugs are released from polymeric micelles are medication absorption from whole micelles or micelles breakdown. Micelles should have strong kinetic and thermodynamic stability to prevent an uncontrolled delivery when given drugs are released (Trivedi *et al*., 2010; Imran *et al*., 2018; Ahmad *et al*., 2014). The literature provides a clear explanation of how a nanocarrier's size affects how widely it is distributed in the body (Duan & Li, 2013; Hoshyar *et al*., 2016) and in the case of polymeric micelles, this has also been validated (Yue *et al*., 2022; Wang *et al*., 2015). The body of literature has shown that micelles with sizes between 30.00 nm and 100.00 nm can easily collect in highly permeability tumors, but micelles smaller than 30.00 nm can only penetrate poorly permeable tumors, highlighting the critical significance of size (Cabral *et al*., 2011).

Both natural and artificial polymers can be utilized for medication delivery. Considering the features of the polymer utilized in the formulation, polymeric system formulations are able to distribute medications sustainably longer than necessary. Depending on the use, polymers' characteristics can be modified (Sharma *et al*., 2022). This increases interest among research scientists in using polymers for applications involving drug delivery. The environment of the released media, diffusion from the polymer, and chemical composition of the delivery system all have an impact on the drug released from polymers.

2.6.3 Dendrimers

Highly branching and specialized dendrimers monodispersed with symmetrical structure. They have branching units, a centre core, and terminal groupings that are functional (Dias *et al*., 2020). Glues are affixed to the terminal ends of dendrimers to improve drug targeting. Dendrimers are several functional groups combined with a tight molecular structure typically characterize well defined artificial macromolecules (Tomalia *et al*., 2002). Dendrimer macromolecules tend to linearly become larger and take on a globular form as dendrimitic production increases (Abbasi *et al*., 2014). The dendrimers are functionalized by polyethylene glycol chains to keep it stable and protect them from Mononuclear Phagocyte System. It was observed from research that the solubility of aceclofenac, a nonsteroidal anti-inflammatory drug, was improved by using G0 PAMAM dendrimers. The study showed that the enhancement of aceclofenac was depending on the amount of the dendrimer (Patel *et al*., 2011). Inorganic materials such as silica, graphene oxide, and hydroxyapatite and gold nanoparticles have been abused and used to deliver drugs. These nano-carriers which serve as skeletons in these systems are able to launch and load drugs but not diminishing its framework in the blood (Zhaoqing *et al*., 2020). They are biocompatible and possess good pharmaceutical characteristics. Dendrimers can be employed as a drug carrier for several medicinal treatments, lowering drug toxicity and increasing drug efficacy (Sherje *et al*., 2018). Dendrimers' structural components and surface capabilities have a notable effect in their therapeutic potential, consequently, understanding the architectural elements of the various dendrimer types is vital for exploring their use in drug delivery (Jain *et al*., 2020). Dendrimers of

various sorts, such as tecto dendrimers, chiral dendrimers, amphiphilic, hybrid, poly(amidoamine-organosilicon) (PAMAMOS), and liquid crystalline dendrimers, have been reported in the literature. In tecto dendrimers, the dendrimer itself serves as the core and is surrounded by additional dendrimers.

For example, the building blocks of liquid crystalline dendrimers are mesogen-functionalized carbosilane dendrimers, whereas PAMAMOS are hydrophilic, nucleophilic PAMAM inside and exteriors of hydrophobic organosilicon inverted unimolecular micelles. Dendrimers that contain both linear and dendritic polymers are known as hybrid dendrimers (Gupta *et al*., 2014). Due to its hydrophilic qualities coming from the numerous surface functional groups, the PAMAM dendrimer is among the most researched using dendrimers to release antibacterial medications. This interaction with water soluble antibiotics can boost antibacterial capabilities (Adriana *et al*., 2020). Mesoporous silica nanoparticles were coated with polydopamine to develop an approach to drug delivery that could make the medication available in reaction to low pH or high glutathione levels (Lei *et al*., 2021). The degree and kind of interactions determine how quickly the medicine is released from the dendrimer drug complex (Chauhan, 2018). Due to dendrimers' benefits, such as their pharmacokinetic and pharmacodynamic features, toxicological reports have been made surrounding dendrimers throughout the literature (Pandita *et al*., 2014). Dendrimer size and surface charge are typically correlated with toxicity. PAMAM dendrimers from the sixth generation and later are more poisonous and expensive (Wang *et al*., 2022). While cationic dendrimers usually demonstrate neutral, and anionic dendrimers are less hazardous than high toxicity. (Janaszewska *et al*., 2019).

2.6.4 Quantum Dots

Quantum dots (QDs) are semiconductor nano-crystals materials that are available in less than 10 nm and have developed into intriguing nanoparticles with a wide range of applications (Mohamed *et al*., 2021). Transistors, light-emitting diodes (LEDs), diode lasers, solar cells, and medicinal applications are some of the potential uses for QDs (Coe- Sullivan *et al*., 2005; Ramirez *et al*., 2015), inkjet printing, waste water treatment (Coe-Sullivan *et al*., 2005), spin-coating (Ramirez *et al*., 2015) and quantum computing (Mohamed *et al*., 2021). QDs in biomedical applications are injected into specific living cell tissues as tracers. QDs applications for cutting-edge cancer therapies can be created since they can focus on a particular organ rather than using conventional chemotherapy (Bhardwaj *et al*., 2016). According to published research, the toxicity of QDs both in the short and long term affects their use in biological systems, including both cellular and animal models (Gao *et al*., 2007; Gao *et al*., 2005; Li *et al*., 2008). Because the quantum confinement zone of these QDs spans the whole optical spectrum, they are the most widely utilized and researched QDs (Gerion *et al*., 2006). Due to their distinct physical and optical characteristics and ability to attach a variety of biomolecules on their surface, QDs are attractive candidates for biosensing (Sapsford *et al*., 2006). QDs created for biological systems are typically used in solution (colloidal form), although literature has also noted a desire for QDs placed on diverse applications of solid surfaces in biomedicine (Bodas *et al*., 2007; Ma *et al*., 2008). QD encapsulation techniques have been successful using solid lipid nanoparticles made of highly biocompatible lipids with long-term chemical and physical stability (Liu *et al*., 2008). These

capping ligand's hydrolysis or oxidation can readily degrade stable solid lipid nanoparticles, making them more practical than tiny compounds (such as mercaptopropionic acid), which are typically employed to modify QD's surfaces (Jana *et al*., 2009). Although quantum dots have many promising uses in the area of medication delivery, biomedical imaging, and sensors (Matea *et al*., 2017).

They also have drawbacks including toxicity, body clearance, synthesis technique, environmental effect, and high manufacturing costs (Matea *et al*., 2017). Figure 2.2 shows the various nano carriers used as drug delivery.

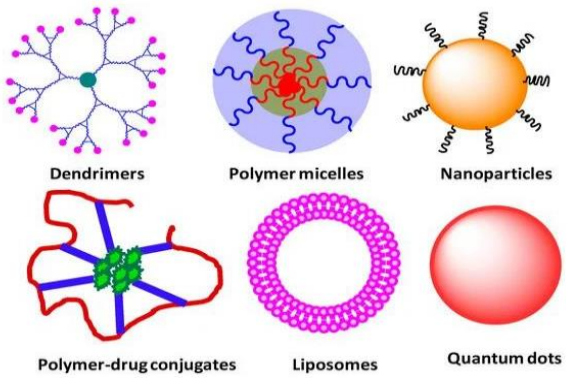


Figure 2.2: Different types of carriers used for drug delivery (Sharma *et al*., 2015).

2.7 Drug delivery by Doxorubicin

Doxorubicin is a widely prescribed chemotherapeutic drug (Mishra *et al*., 2021). Resistance to the traditional chemotherapeutic agent, which is regarded as a major obstacle and poses significant hurdles in the treatment of cancer, and is among the main components causing high mortality rate among cancer patients (Housman *et al*., 2014; Wambang *et al*., 2016; Wang *et al*., 2019). Unfortunately, doxorubicin has a variety of harmful adverse consequences that aim to limit its usefulness in chemotherapy, including

cardiotoxicity, alopecia, vomiting, leucopenia, and stomatitis (Chudoba *et al*., 2021). Doxorubicin (DOX) have been revealed that because of its cytotoxic properties, new drug delivery formulations are required for cancer treatment (Vyas *et al*., 2020). To administer DOX to particular cells or tissues in order to achieve targeted therapy, various medication delivery methods have been created (Xu *et al*., 2017). These methods for delivering drugs lessen the negative consequences of DOX and increase the efficacy of chemotherapy (Xu *et al*., 2017). DOX is a type of anthracycline antibiotic prototype molecule. While DOX is effective in treating the majority of solid tumors, such as sarcomas, neuroblastoma, and malignancies of the breast, thyroid, ovary, bladder, and lung, it is mostly used to treat acute leukemias and lymphomas (Wambang *et al*., 2016). DOX is a crystalline powder that is orange-red in color, has a faint ethanolic odor, and is hygroscopic, water-soluble, and just marginally methanol-soluble (Molavi *et al*., 2013; Kolhatkar *et al*., 2008). Doxorubicin can be encapsulated into liposomes to reduce the cardiotoxicity associated with the drug's free form while preserving its anticancer efficacy (Torchilin, 2005; Tardi *et al*., 1996; Petersen *et al*., 2016). Dox has been designed and tested for systemic distribution and sustained release using various types of nanoparticles as drug carriers (Zhao *et al*., 2018).

2.8 MDA MB 468 Breast Cancer Cell Line

In medical research facilities, the cell line for human breast cancer MDA-MB-468 is frequently employed (Wagner, 2022). MDA-MB-468 is a triple negative breast cancer (Huang *et al*., 2020) and is prone to be metastasis and relapse (Zou *et al*., 2021). Because chemotherapy is now only available with standard chemotherapy drugs, triple negative breast cancer (TNBC) is

typically linked with a low survival rate (Jung *et al*., 2018). Invasive MDA-MB-468 cells can form xenografts that spontaneously metastasis to lymph nodes when transplanted orthotopically (Welsh, 2013). The MDA-MB-468 cell line is described as aggressive, poorly differentiated triple negative breast cancer cells that lack HER2 (human epidermal growth factor receptor 2) amplification and oestrogen receptor (ER), progesterone receptor (PR) expression (Liu *et al*., 2003; Chavez *et al*., 2010).

Cell line for breast cancer MDA-MB-468 normally grows randomly in a spindle shape (Tsai *et al*., 2019) and these cells can survive without glucose, however, they cannot survive in the absence of glutamine (Ocana *et al*., 2020). Figure 2.3 shows the image of MDA MB 468 breast cancer cells.

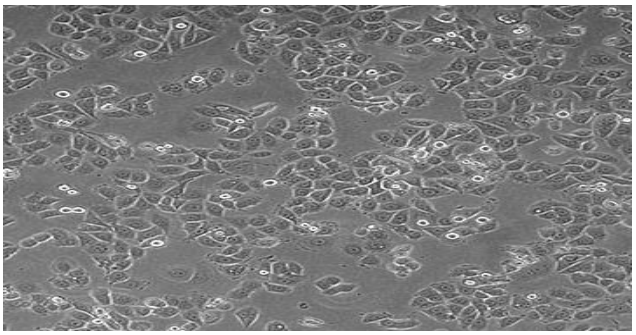


Figure 2.3: MDA-MB-468 breast cancer cells (Cell Avalanche online product detail website, 2014).

2.9 Zeolitic Materials with Various Applications Including Drug Delivery

Zeolites are naturally occurring crystalline alumino-silicate minerals. Zeolites have three dimensional structures which arises from a framework of $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ coordination polyhedral. Zeolites have recently become a subject of focus due to their capacity for controlled drug delivery (Servatan *et al*., 2020). The biomedical sector has paid close attention to their pores due to

their consistent and regular shape, their long biological durability, and their capacity to modify immune system performance (Safari *et al*., 2019). Given their unique structural characteristics, biocompatibility, high particular surface area that ranges from 200-1000 m^2 g^{-1} (Larysa *et al*., 2022), and ability to elicit control of physical characteristics like pH, porosity, size, bond length, and electronic structure, zeolites have demonstrated considerable promise as a potential drug delivery system. Zeolites of many sorts have been created and documented in the literature, such as LTA, FAU, MOR, FER, MEL, and AFI (Caro *et al*., 2008) and the zeolite's distinctive shapes and pore diameters are caused by variations in the aluminium to silica ratios (Julbe *et al*., 2016).

Zeolites have widespread application in the industry such as catalysis, separation and gas adsorption (Lehman *et al*., 2014). According to pore size, zeolites are classified as macroporous, mesoporous, and microporous; these distinctions affect each type of zeolite's capacity for absorption and catalysis (Chandak *et al*., 1997). Zeolite has a wide surface area and a porous structure that helps prevent the aggregation of active ingredients and increase a material's mechanical strength. Additionally, the abundance of acidic regions on zeolite's surface can lead to specific catalytic activity (Xue *et al*., 2021).

Zeolites have been utilized extensively as catalysts in petrochemistry and oil refining (Perez- Botella *et al*., 2022). Due to the properties of zeolite structure, they have been successfully applied to act as sieves and adsorbents for molecules in some separation processes (Yue *et al*., 2022). For numerous fluid mixes, such as air separation, hydrocarbon separation, drying, hydrogen purification, and water treatment, adsorption is one of the potential alternative separation processes. The first zeolite materials' commercialization is

inextricably linked to the creation of industrial adsorption processes (Perez-Botella *et al*., 2022).

Up to 60% of the weight of the water molecules can be retained because of the high porosity of the zeolite crystalline structure (Khaleque *et al*., 2020). Without harming the crystal structures, water in zeolite pores might be slowly evaporated or reabsorb (Cataldo *et al*., 2021).

Numerous agricultural uses have benefited from zeolites' physical and chemical characteristics as well as their profusion in rocks and sedimentary deposits that contain volcanic material (Hosokawa *et al*., 2003; Mintosa *et al*., 1998; Vilasseca *et al*., 2004; Zhu *et al*., 2001). Clinoptilolite which is a natural zeolite is the most prevalent zeolite used in agricultural applications, being the most plentiful in sediments and soils processes (Cataldo *et al*., 2021). Zeolites improve the ability of soils with delayed element releases to hold onto nutrients, such as potassium and ammonium for uptake by crops (Li, 2003).

Additionally, the zeolite addition had an impact on the nutrients measured in the maize tissues and the utilization of zeolites combined with inorganic fertilizers rose nitrogen and potassium ingestion and effectiveness of usage in roots, leaves, and stem (Ahmed *et al*., 2010). Zeolites have various possible applications in agriculture especially, the management of soil. For instance, zeolites can be employed as nutrient transporters to increase the effectiveness of nutrient utilization (Cataldo *et al*., 2021).

2.10 Linde Type A Zeolite Nanomaterials

Linde Type A (LTA) belongs to the aluminosilicate molecular sieve family (Julbe *et al*., 2016). The three-dimensional pores of Linde type A (LTA) zeolite have a high degree of symmetry (Lucena *et al*., 2022) and it is

typically distinguished by the formular $[(Na^{+}_{12}(H_2O)_{27}|_8[Al_{12}Si_{12}O_{48}]_8$ (Julbe *et al*., 2016). Lithium (Li-LTA), potassium (K-LTA), or calcium (Ca-LTA), as well as other cations, can be substituted for the sodium ions in LTA zeolite (Townsend *et al*., 2001). Sodalite cages, which are the main structural components of LTA zeolite, are joined by four-membered rings to form a network that is three- dimensional (Julbe *et al*., 2016) as shown in figure 2.4.

These cages have 11.4-mm-diameter core cavities that are connected by eight-ring openings with 4.1-mm-diameter apertures. This creates an incredibly open zeolite structure having a high 47% void volume fraction (McCusker *et al*., 2001).

As a result of their distinctive structural makeup and interactions with water, LTA zeolite membranes have showed good pervaporation separation (Wenten *et al*., 2017). Figure 2.4 shows the structure of LTA zeolite.

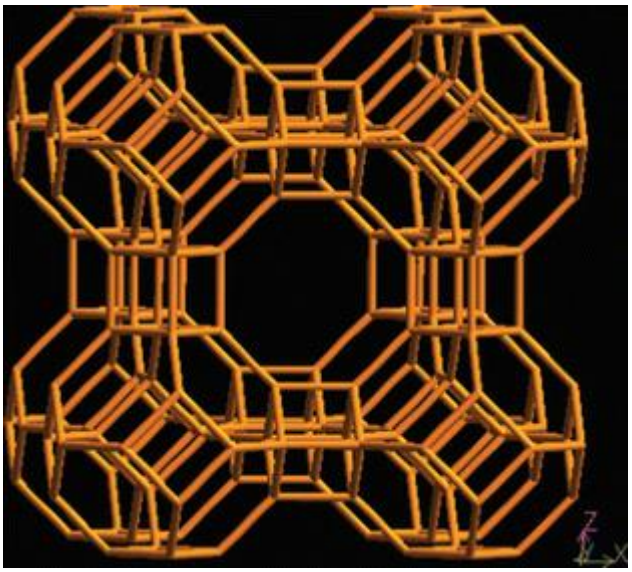


Figure 2.4: Structure of LTA zeolite (Rios-Reyes *et al*., 2021).

2.11 Drug Delivery by Synthetic LTA Zeolites

In order to create synthetic zeolites, a silica-alumina gel is typically slowly crystallized in the presence of organic templates and alkalis. This method can produce a wide variety of shapes (IIT JEE study material, 2023).

Zeolites have porous structures that can be microporous, mesoporous, or macroporous, allowing for the regulated and consistent administration of several chemotherapeutic medications at the intended location **(**Servatan *et al*., 2020). The ability of synthetic zeolites to encapsulate and release medications has been researched in literature (Rimoli *et al*., 2008). The methods that are most frequently employed for medication distribution can be steady, regulated, or targeted (Nayak *et al*., 2018). Because it is expensive to make synthetic LTA zeolites from chemical sources of silica and alumina, there is a need to lower the cost of making these materials from natural sources, including clays (Youcef *et al*., 2020).

In the biomedical area, synthetic zeolites are favored over natural zeolites primarily because of their excellent purity, crystallinity, and uniform particle size (Breck, 1974; Szostak, 1998). Once more, synthetic LTA zeolite offers greater purity, composition control, and most importantly, the chance to enhance key biological characteristics of drug delivery (Cavallaro *et al*., 2004). To adjust crystal size for particular purposes, LTA zeolite can be produced in the lab using organic templates and a variety of synthetic processes, including hydrothermal reactions (Zhou *et al*., 2013; Kyotani *et al*., 2006). LTA zeolites have received numerous scientific attentions because of their porous structure and potential as drug delivery methods. They can increase loading capacity, regulate drug release rate, provide a range of drugs

and biological materials to particular organs and tissues, and do so in a safe and effective manner (Servatan *et al*., 2020). Synthetic zeolites have been researched in the literature to find out if they can encapsulate and release medications. For instance, due of its pore structure, LTA zeolite was explored for the encapsulation of ketoprofen (Rimoli *et al*., 2008).

2.12 Chapter Summary

This chapter examined literature pertinent to the topic area**.** Drug delivery methods, different drug delivery methods, drug delivery by doxorubicin and LTA synthetic zeolite nanomaterials and their application in drug delivery were discussed. Drug delivered at a regulated pace, slowly, and precisely are all extremely appealing approaches that have been actively sought for.

Different drug delivery methods affect how quickly and effectively a drug reaches a target site. Drug delivery systems based on nanotechnology may be a better way to overcome side effects of drugs and provide numerous advantages over free drugs, including improved blood circulation, fewer drug interactions, increased solubility, controlled release properties, and the potential to promote drug accumulation in diseased sites. One of the commonly prescribed cancer treatments is doxorubicin. Doxorubicin has a number of harmful side effects that are intended to limit how well it can be used in chemotherapy, including cardiotoxicity, alopecia, vomiting, leucopenia, and stomatitis. One of the objectives of the assemblage is releasing drug to MDA-MB-468 breast cancer cells.

CHAPTER THREE

METHODOLOGY

3.0 Introduction

Effective research procedures are crucial for gathering the most accurate and useful data possible to address research topics. The precise methods and resources used to produce the results for this work are covered in depth in this chapter. It includes the research design, the field of investigation, the sampling techniques, the data collection tools, the data gathering techniques, the data processing and analysis, as well as the chapter summary.

3.1 Research Design

A plan or conceptual structure for study is known as a research design. It includes an approach for gathering, measuring, and analyzing data. Numerous research designs are available, and they can be chosen based on the sort of evidence required to address the study issue. Descriptive, explanatory, correlational, and experimental research designs are all possible (Creswell, 2003). To accomplish the stated goals, a quantitative experimental research methodology was chosen for this study. The choice of a certain research design typically depends on the research's strengths and limits as well as the information sought. The advantage of this experimental study is that it enables researchers to test hypotheses in a safe setting before moving forward with clinical trials.

3.2 Laboratory Centre

This study was done at the Oncology laboratory of the Department of Biomedical Engineering, School of Engineering Sciences and Virology

laboratory of West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana.

One of the World Bank's African centers of excellence, WACCBIP is run by academics from the University of Ghana's Department of Biochemistry, Cell and Molecular Biology and the Noguchi Memorial Institute for Medical Research.

3.3 Data Collection Instruments

To acquire the necessary data, six data collection instruments were created. These data collection instruments were created due to the process involved in the study.

The subsequent types of data gathering tools were created:

1. Data collection instrument for synthesized LTA zeolite
2. Data collection instrument for characterization of LTA zeolite
3. Data collection instrument for drug loading unto the LTA zeolite
4. Data collection instrument for drug loading characterization
5. Data collection instrument for drug release kinetics
6. Data collection instrument for cell culture and Alamar blue assay

3.3.1 Data Collection Instrument for Synthesized LTA Zeolite

This data collection instrument was developed to synthesize LTA zeolite obtained from kaolin deposits. It provides the methods of synthesis and details how LTA zeolite used for this study was obtained in the laboratory.

Zeolite synthesis from natural kaolin sources have shown many advantages economically which has been reported in several literatures. In order to control the crystal size, LTA zeolite was created in the laboratory using the hydrothermal conditions protocol.

3.3.2 Data Collection Instrument for Characterization of LTA Zeolite

To confirm that the kaolin source synthesized at the laboratory is LTA zeolite, a data collection instrument was developed which has been described in the synthesis method. According to the results of the characterization, zeolite exhibits a variety of distinctive qualities, including consistent pore size, acidic characteristics, thermal stability, ion exchange capacity, hydrophilicity, and hydrophobicity.

3.3.3 Data Collection Instrument for Drug Loading unto the LTA Zeolite

Data collection instrument was developed to confirm the immobilization of the drug unto the LTA zeolite. The instruments used were UV-Vis spectroscopy, X-ray Diffractometer (XRD), Fourier Transform Infra-Red (FTIR) Spectroscopy, Scanning Electron Microscope (SEM) and Helium-Ion Microscope (HIM).

3.4 Data Collection Procedure

This section provides comprehensive details about the data collection process. It includes methods of synthesized LTA zeolite, drug loading methods, characterization of LTA zeolite and drug release kinetics and methods of cell culturing and Alamar blue assay.

3.4.1 Synthesis of Linde Type A Zeolite

Kaolin was collected from deposits at Teleku Bokazo (Figure 3.1) in the Western Region, Ghana, West Africa, coordinates (4° 58' 19" N 2° 19' 13" W). The deposits were picked with gloves on and put in a container in a zip-lock bag and transported to the laboratory of the Department of Biomedical Engineering, School of Engineering Sciences and Virology laboratory of WACCBIP, University of Ghana. The collected kaolin deposits were

grounded into fine powder, and all the debris was removed by sieving it through a 0.25 mm mesh size. An amount of 200 g was weighed with analytical balance (Denver Instrument Analytical Balance, PI-214.3, USA) for the initial preparation. The zeolite preparation was done using hydrothermal synthesis protocol. The kaolin was heated at 100 °C to acquire the zeolite. The zeolites were then combined with NaOH (Sigma-Aldrich, USA) at a mass ratio of 2:3 after being calcined at 650°C for two hours. Following the calcination process, the mixture was then crushed and pulverized and combined with distilled water in a 1:5 mass ratio. The suspension was then mechanically mixed with sterile mortar and pestle in the laboratory for an hour. Subsequently, the mixture was subjected to an aging process where it was left for 24 hours at room temperature.

The mixture was separated into three different mixtures then subjected to crystallization at temperatures of 60 ℃, 80 ℃ and 105 ℃ for an additional 24 hours. Each of the resultant mixture was carefully rinsed and filtered until the filtrate's pH fell within the 7.5–8.0 range. The filtrate materials were then dried in a vacuum dryer (VWR Scientific Vacuum Oven) and powdery samples (LTA 60 ℃, LTA 80 ℃ and LTA 105 ℃) were obtained from the drying.

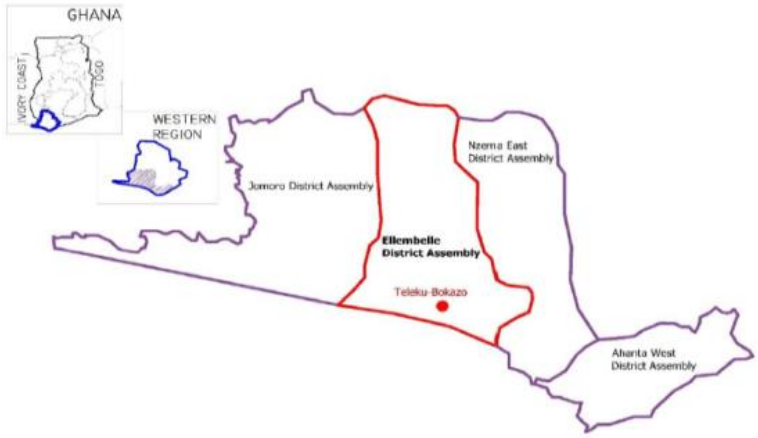


Figure 3.1: A map showing the location of Teleku Bokazo (Abankwah *et al*., 2021)

3.4.2 Drug Loading

100 mg of synthesized LTA zeolites at 60, 80 and 105 ℃ were each weighed and transferred into two separate sets of clean Eppendorf tubes. From a commercial doxorubicin stock solution of total concentration, 50 mg/25 ml (w/v), 1mg/ml and 2 mg/ml aliquots were respectively measured and transferred into each of the two separate sets of 60, 80 and 105 ℃ synthesized LTA zeolites contained in the respective Eppendorf tubes. The mixtures were then agitated gently using the rotating equipment (LA620 TNK00012189) (Figure 3.2) at 18 rpm to ensure the adsorption of the drug doxorubicin onto the composites. At predetermined times, each of the two sets of mixtures (60, 80 and 105 ℃ synthesized LTA zeolites, 1mg/ml and 2 mg/ml doxorubicin aliquots) were centrifuged at 3000 rpm for 1 minute and 50 µl of the supernatant for the individual preparation mixtures of the two sets were pipetted and diluted with 950µl of 50% Dimethyl sulfoxide (DMSO) solution for UV-VIS spectroscopy. The absorbance of the resulting solution for all individual sample mixtures were measured at an optical density (wavelength)

of 486 nm using the JENWAY 6705 UV-Vis Spectrophotometer (Antylia Scientific, USA). 50 µl of fresh 50% DMSO solution was replaced in the solution to maintain sink conditions. The drug loading was done for 24 hours. The respective prepared zeolite (60, 80 and 105 ℃ synthesized LTA zeolites) doxorubicin was centrifuged at 3600 rpm for 10 minutes to obtain the 60, 80 and 105 ℃ synthesized LTA zeolites) /doxorubicin nanocomposites. The sample products were washed with distilled water for three times. Final dry sample powders of the zeolite/drug nanocomposites were obtained by drying the product in a vacuum oven (VWR Scientific Vacuum Oven).

Equation 3.1 was used to determine the amount of doxorubicin (thus, aliquots of 1mg/ml and 2mg/ml) entrapped onto the 60, 80 and 105 ℃ synthesized LTA zeolites.

$$\text{Entrapment Efficiency (\% EE)} = \frac{x-y}{x} \times 100 \qquad (3.1)$$

Where: x = total concentration of drug added

y = concentration of drug in the supernatant.

Entrapment efficiency estimates the percentage of the active ingredient that is adsorbed on the 60, 80 and 105 ℃ synthesized LTA zeolites compared to the total amount of drug added.

Figure 3.2: Agitation of 100 mg each of 60, 80 and 105 ℃ synthesized LTA zeolites in 1mg/ml and 2 mg/ml doxorubicin aliquots drug solution to ensure adsorption of drug onto the zeolites.

3.4.3 Characterization of LTA Zeolite

3.4.31 Fourier Transform Infra-Red Spectroscopy

In Fourier transform infrared (FTIR) spectroscopy the raw data of the interferogram is transformed into the actual spectrum using the mathematical method (Fourier transform).

In this study, the Nicolet MAGNA-IR 750 Spectrometer (Nicolet Instrument Co., USA), shown in Figure 3.3 was used to obtain the FTIR spectra. Before readings were made, the sample holder was properly cleaned with an organic solvent to make sure there were no contaminants that could affect readings. A 5 mg of the LTA 60 ℃ sample was placed in the sample holder and placed in the FTIR machine and scanned from lower wavelength to higher wavelength. Using 16 scans with a 4 cm^{-1}spectral resolution, all spectra were captured between 4000 cm^{-1} and 500 cm^{-1} and data stored on the computer for further analysis. This was repeated for the other samples (LTA

80 ℃ and LTA 105 ℃). After data processing, absorbance was converted from transmittance and plotted against wavelength.

Figure 3.3: A photograph of Nicolet MAGNA-IR 750 FTIR Spectrometer used for the spectra acquisition of LTA- 60, 80 and 105 ℃.

3.4.32 Xray Diffraction

X-ray diffraction (XRD) is a non-invasive technique that provides details about the physical characteristics and crystalline structure, and chemical composition of materials (Jurásek *et al*., 2019). The control LTA zeolites powdered samples crystallized at 60 ℃, and was loaded into sample holder wells on a back loader and pressed firmly to obtain a smooth levelled surface. The sample was examined by X-ray diffraction using the Empyrean series 2, X-ray diffractometer shown in Figure 3.4, which the image was obtained from University of Ghana, Department of Physics, XRD laboratory.

The instrument was operated at 45Kv, 40mA with Cu-Kα radiation (λ=1.54060 Å) in the angular region, 2Θ =5.053° to 99.87°, step size of 0.1050 (°2Θ) and a scan speed of 47.6850(s). The K- beta xray line was removed with a nickel filter on the receiving optics. The detector used was a Pixel line detector. The same procedure was repeated for the LTA zeolites crystallized at

80 ℃ and 105 ℃ temperatures for both LTA zeolites and drug loaded zeolites samples. The resultant data were stored for further analysis with OriginPro 8.5 software.

Figure 3.4: A photograph of Empyrean series 2 XRD system used for scanning both LTA zeolites and drug loaded zeolites samples.

3.4.33 Ultraviolet-visible spectroscopy

A compound's solution-based absorbance spectra are found via Ultraviolet-visible spectroscopy. The concentration of the solution and absorbance have a linear relationship, according the Beer-Lambert law.

$$A = \varepsilon \times b \times c \qquad (3.2)$$

where, A is the absorbance, ε is the molar absorptivity of the solution, b represents the cuvette's route length which is usually 1 cm, and c is the solution's concentration.

In this study, the upload and release rates were studied using the UV-vis spectrophotometer. It helps to determine the amount of drugs that was loaded and the amount of drugs released from the loaded sample by measuring the absorbance within 24 hours with 1 hour time interval.

The JENWAY 6705 UV-vis spectrophotometer was used for UV-vis characterization. Initially, an amount of 3000 µl of doxorubicin drug was measured and poured in a cuvette, and placed in the UV-vis machine to determine the absorbance. A wavelength of 486 nm was selected and the UV-vis machine was pressed on for the absorbance measurement. The absorbance measurement was then recorded and stored for further analysis. A 2950 µl supernatant of the drug loaded on LTA 60 °C was taken and added with 50 µl of 25 % Dimethyl sulfoxide (DMSO) to obtain 3000 µl of the supernatant. The supernatant was then placed in the UV-vis machine to measure the absorbance. A wavelength of 486 nm was selected and the UV-vis machine was pressed on for the absorbance measurement. The absorbance measurement was recorded and stored for further analysis. The same procedure was repeated for drug loaded of LTA 80 °C and 105 °C. OriginPro 8.5 software was used to analyze the data obtained from the absorbance measurements.

3.4.34 Brunauer–Emmett–Teller Specific Surface Area

The Brunauer-Emmett-Teller (BET) hypothesis is used to calculate the surface area of solid or porous materials.

In this study, a Micromeritics 3 Flex Surface area and Pore size analyzer and the Brunauer-Emmett-Teller method were used. With the size and volume of the pores distribution of the control zeolites and drug loaded

zeolites measurements, the gas pressure of the pore size analyzer was increased until all the pores were filled with nitrogen molecules. Then the pore size analyzer's nitrogen gas was gradually dissipated when the gas pressure was lowered. The LTA 60 ℃ sample's weight was ranged from 0.10 to 0.20 g which was degassed under vacuum for 24 hours at 110 ℃ to flush out any moisture and other gases that had been adsorbed. The surface area of the LTA 60 ℃ sample was calculated using nitrogen sorption and the relative pressure (p/p0) range. The identical process was carried out again for the LTA zeolites samples crystallized at 80 ℃ and 105 ℃ temperatures.

3.4.35 Scanning Electron Microscopy

A popular imaging technique for determining the morphology of materials is scanning electron microscopy (SEM) (Omidi *et al*., 2017) and also imaging the soft and hard materials' sub-micron surface ultrastructures (Joens *et al*., 2013).

A JEOL JSM-7100F Field Emission Scanning Electron Microscope with an accelerating voltage of 10 kV was used to obtain the SEM micrographs for this study. Conductive carbon tabs were carefully positioned on an aluminum stub to prepare the samples (Ted Pella, Inc.). Surplus particles inside the LTA 60 ℃ sample were then eliminated by tapping the stub sideways. A low- electron beam of energy was directed to the LTA 60 ℃ sample inside the SEM machine and after the interaction, signals were detected and recorded with the SEM machine. The SEM images acquired were stored and further analysis was done with ImageJ NIH 2.1.4.7 software.

The same procedure was repeated for LTA 80 ℃ and 105 ℃ samples.

3.4.36 Helium Ion Microscope

Instead of using an electron beam, Helium Ion Microscope (HIM) uses an ionized helium beam. The HIM has improvements in charge reduction, reduced sample damage, enhanced depth of field, 5 angstrom imaging resolution, and high surface contrast without the requirement for metal coating (Joens *et al*., 2013). The LTA 60 ℃ sample was prepared by being evenly distributed on a tape that enables the sample to firmly adhere to the surface and spread out on a double-sided tape that is fastened to a sample container and image under elevated vacuum pressure about 2 x 10^{-7} Torr. The imaging on the LTA 60 ℃ sample was done by using an accelerating voltage of 30 kV. The beam current ranged from 0.7 to 1 pA, and the imaging aperture was 10 µm. The LTA 60 ℃ sample surface was then charged using flood gun. The resultant data from the scanning was recorded and afterwards ImageJ (1.53p version) was used for the analysis to obtain the images. The same procedure was repeated for LTA 80 ℃ and LTA 105 ℃ samples.

3.5 In-Vitro Drug Release

The unloaded doxorubicin was discarded, leaving the loaded doxorubin-LTA zeolites with three different temperatures in three separated eppendorf tubes. 2 ml of the release medium made up of the working solution of 5 ml of 25 % Dimethyl sulfoxide (DMSO) at pH of 6.8 diluted with 10 ml of distilled water was added to each of sample in the three separate eppendorf tubes. The mixtures were then centrifuged at 500 rpm for 1 minute and 50 µl of the supernatant of the mixtures were carefully pipetted and diluted with 2950 µl of 25% DMSO solution. Absorbances of the resulting solutions were measured at wavelength of 486 nm using the UV-Vis Spectrophotometer. 50

µl of fresh 25% DMSO solution was replaced in each of the solution to maintain sink conditions. The drug release was done for 24 hours at 37 °C.

The concentration of the released doxorubicin was established utilising a standard curve of doxorubicin in 25% DMSO.

3.6 Calibration Curve of Model Drug (Doxorubicin)

The calibration curve developed in this study was utilized to assess the amounts of doxorubicin in the release medium. From the doxorubicin stock concentration of 50 mg/25 ml w/v per vial, serial concentrations of 0.02, 0.04, 0.08.0.10 and 0.12, 0.14, 0.16, 0.18 and 0.20 mg/ml were prepared respectively using 25% DMSO solution with the dilution formular (Concentration 1 Volume 1) C1V1 = C2V2 (Concentration 2 Volume 2).

A graph of absorbance against concentration was plotted. A calibration curve (R^2 = 0.979) was plotted through the measurement of the absorbance of the diluted solutions at a wavelength of 486 nm. The graph was later fitted in order for it to obey Beer-Lambert law (Delgado, 2021). The equation and R^2 value of the fitted graph were obtained.

3.7 Culturing of MDA MB 468 Breast Cancer Cell Line

The Sigma Aldrich-purchased MDA-MB 468 breast cancer cells were cultured in 10% foetal bovine serum (FBS) in Dulbecco's Modified Eagle Medium (DMEM) at 37 °C, 1 mM L-glutamine, 100 units/mL penicillin, and 100 g/mL streptomycin. When MDA-MB 468 cells were 70 to 80% confluent, they were sub-cultured after being seeded at a density of one to three 104 cells/cm^2.

3.8 Alamar Blue Cytotoxicity Assay

Alamar blue is among the most widely utilized substances for cytotoxicity and viability tests, which has primarily been used in cytotoxicity and cell viability research over the years in the cell laboratory (Sephra, 2012).

MDA MB 468 breast cancer cells used in this study was harvested at the growth phase and the cell counts determined. A 90 μl of media (DMEM, Sigma -Aldrich) was suspended to cell density of 1×10^4 cells seeded in 96 well plate. Cells were allowed to attach for 24 hours, which was confirmed under microscope in a 5 % CO_2 environment at 37°C. After that, the cells were then treated with 10 μl of the doxorubicin loaded on the three different synthesized LTA zeolites in the concentrations of 0, 0.5,1,5,10,20,40,80 and 100 ng/ml. After incubating the treated cells for 48 hours, 10 μl of the alamar solution was added into each of the well and incubated again for 6 hours. The fluorescence was then measured with microplate reader with absorbance wavelengths of 600 and 570 nm following the necessary incubation.

3.9 Data Analysis

Data were obtained as described in 3.4.2, 3.5 and 3.8 from the drug loading, drug release and Alamar blue assay experiments respectively. The XRD and FTIR tests generated data in the form of spectra. Origin Pro 8.5 software was employed for the analysis of the XRD and FTIR spectra. The data obtained for the calibration curve for the drug release obeyed the Beer-Lambert law.

3.10 Chapter Summary

The techniques and supplies needed to get the outcomes are covered in this chapter. To accomplish the stated goals, a quantitative experimental

research methodology was chosen for this study. University of Ghana's WACCBIP and the Department of Biomedical Engineering's Oncology and Virology laboratories served as the study sites. The drug loading and release studies were done at the Oncology laboratory at the Department of Biomedical Engineering and the cells work was done at the Virology laboratory of WACCBIP, University of Ghana.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Introduction

This chapter presents the results and discussion on the characterization of LTA zeolites synthesized at three different temperatures. The chapter also analyzes the properties of the zeolites when doxorubicin drug is loaded and released. Brunauer-Emmett-Teller was also used to characterize pore size, surface area of the drug loaded zeolites to estimate the amount of drug uptake by the materials. Cytotoxicity studies were conducted in vitro on MDA MB 468 breast cancer cell lines and cell death were studied using the Alamar blue cytotoxicity assay.

4.1 Characterization of LTA Zeolites Synthesized at Three Different Temperatures

4.1.1 Fourier-transform infrared spectra and X-ray Diffraction analysis of the control

The control is the LTA zeolites without the drug. Fourier Transform Infra-Red Spectroscopy gives the functional groups of the material under study. Figure 4.1(A) shows the FTIR spectra of LTA zeolites synthesized at three different temperatures (60℃, 80 ℃ and 105 ℃). Figure 4.1 (B) shows the XRD spectra of LTA zeolites synthesized at three different temperatures (60℃, 80 ℃ and 105 ℃). The functional groups present in the LTA zeolite were disclosed by the FTIR characteristic peak intensities of the LTA zeolite. The peak position was the same for the zeolite material at three different temperatures. There was reduction in intensity as a function of temperature of the zeolite nanomaterial.

There was a constant decrease of the broad peak which was due to water as a function of temperature. The wavenumbers (561 cm^{-1}, 670 cm^{-1} , 751 cm^{-1} , 1000 cm^{-1} and 1639 cm^{-1}) corresponded to vibration Si-O-Si, symmetrical stretching vibration of the Si-O-Al, symmetrical stretching vibration, the uneven stretching vibrations of the Si-O-Si bond across all temperatures and the water deformation respectively.

X-ray diffraction was employed to determine the crystal structure of the crystalline material. With the sharp peaks indicating high material crystallinity. LTA zeolite miller indices from XRD pattern (200), (220), (222), (420), (440), (442), (622), (642), (840), (842), (664) and (666) clearly corresponded to LTA. The intensity of the peak reduced as a function of the temperature. As the temperature of the LTA zeolite material increased, the intensity of the peaks reduced and hence the crystallite size reduced due to the presence of the drug.

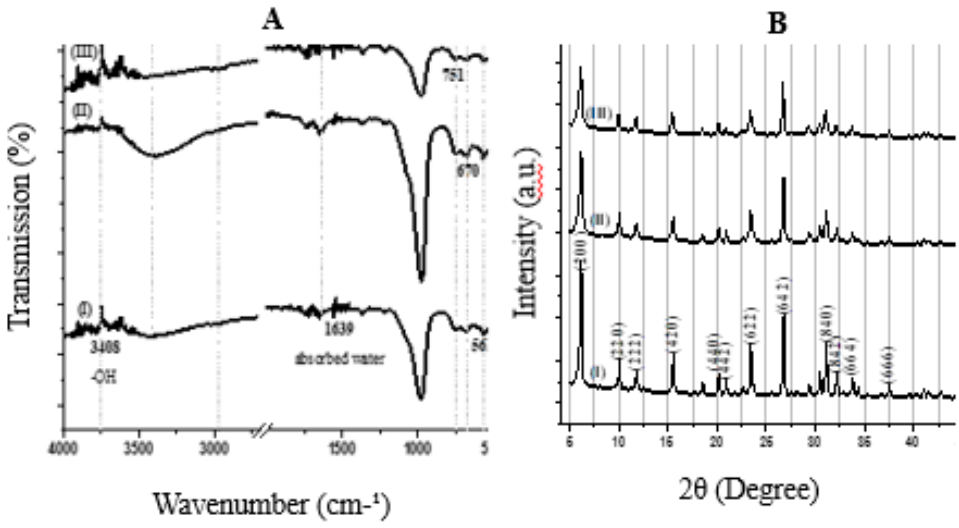


Figure 4.1: (A) FTIR and (B) XRD spectra of LTA zeolites at three different temperatures (I) 60℃, (II)80 ℃ and (III) 105 ℃

4.1.2 Fourier-transform infrared spectra and Xray Diffraction analysis of the drug loaded zeolite materials

Figure 4.2 (A) shows FTIR spectra of loaded doxorubicin drug on (I) control zeolites materials, (II) drug loaded at LTA 60 ℃ at drug concentration of 2 mg and (III) drug loaded at LTA 60 ℃ at drug concentration of 4 mg. Figure 4.2 (B) shows XRD spectra of loaded doxorubicin drug on (I) control zeolite material, (II) drug loaded at LTA 60 ℃ at drug concentration of 2 mg and (III) drug loaded at LTA 60 ℃ at 4 mg. There was unique peaks appearing on (II) and (III) due to the presence of the drug and that shows clearly that the drug was loaded on the zeolite material.

Figure 4.3 (A) shows FTIR spectra of loaded doxorubicin drug on (I) control zeolite material, (II) drug loaded at LTA 80 ℃ at drug concentration of 2 mg and (III) drug loaded at LTA 80℃ at 4 mg. Figure 4.3 (B) shows XRD spectra of loaded doxorubicin drug on (I) control zeolite material, (II) drug loaded at LTA 80 ℃ at drug concentration of 2 mg and (III) drug loaded at LTA 80 ℃ at 4 mg. Similarly, there were new peaks appearing on (II) and (III) when the drug was loaded on the zeolite material. Figure 4.4 (A) shows FTIR spectra of loaded doxorubicin drug on (I) control zeolite material, (II) drug loaded at LTA 105 ℃ at drug concentration of 2 mg and (III) drug loaded at LTA 105 ℃ at 4 mg. Figure 4.4 (B) shows XRD spectra of loaded doxorubicin drug on (I) control zeolite material, (II) drug loaded at LTA 105 ℃ at drug concentration of 2 mg and (III) drug loaded at LTA 105 ℃ at 4 mg. There were new peaks also appearing due to the presence of the drugs.

It is observed that, with the presence of the drug, there was unique peaks appearing on (II) and (III) and these peaks was not corresponding to the peaks on (I) which clearly showed that the drug had effect on the crystallinity of the zeolite. Also, the sharp peak on (I) was reduced in (II) and (III) which also confirm that the drug had effect on the crystallinity of the zeolite. There was new peaks appearing in (II) and (III) and this is due to the reforming of the zeolites due to the presence of the drug.

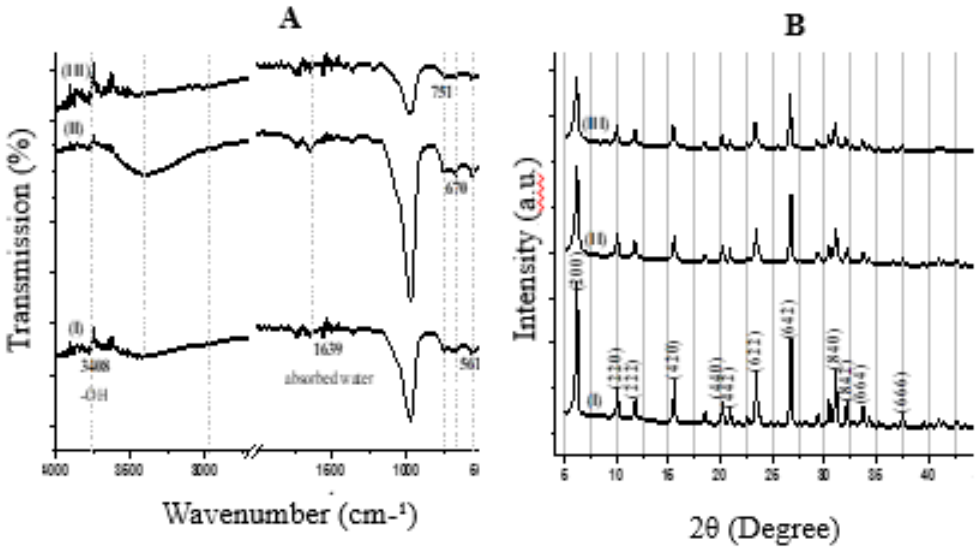


Figure 4.2: (A) FTIR and (B) XRD spectra of loaded doxorubicin drug on (I) control (raw zeolites), (II) drug loaded at LTA 60℃ at drug concentration of 2 mg and (III) drug loaded at LTA 60℃ at drug concentration of 4 mg

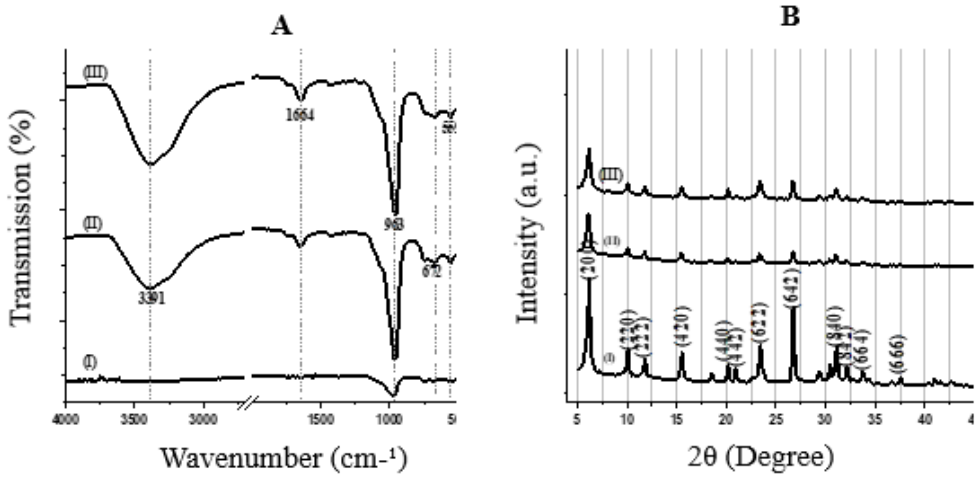


Figure 4.3: (A) FTIR and (B) XRD spectra of loaded doxorubicin drug on (I) control (raw zeolites), (II) drug loaded at LTA 80℃ at drug concentration of 2 mg and (III) drug loaded at LTA 80℃ at drug concentration of 4 mg

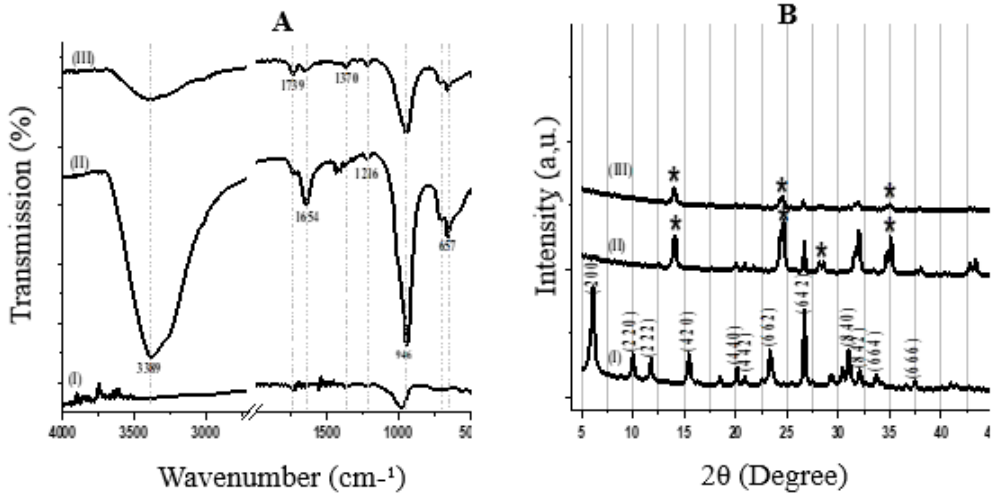


Figure 4.4: (A) FTIR and (B) XRD spectra of loaded doxorubicin drug on (I) control (raw zeolites), (II) drug loaded at LTA 105 ℃ at drug concentration of 2 mg and (III) drug loaded at LTA 105 ℃ at drug concentration of 4 mg

To confirm the peak features, the crystal sizes at different temperatures were extracted as shown in Table 4. 1. The Scherrer and modified Scherrer equations were used to determine the average crystallite size.

Table 4.1: Average crystallite size and percentage crystallinity of LTA zeolites and drug loaded LTA zeolites

	Average crystallite size/nm			Crystallinity/%		
Drug loading	0 mg	2 mg	4 mg	0 mg	2 mg	4 mg
Sample						
LTA 60 °C	40.89 ±1	20.37	19.41	65.99 ±1	66.47	70.20
LTA 80 °C	28.40 ±1	21.93	18.88	71.39 ±1	71.64	78.12
LTA 105 °C	29.76 ±1	20.79	17.01	76.37 ±1	78.02	57.58

Table 4.1, the LTA 60 °C of the control zeolite had an average crystallite size higher than LTA 80 °C and LTA 105 °C, which shows that LTA 60 °C has a bigger crystal size than LTA 80 °C and LTA 105 °C. However, the concentrations of the drug to the zeolites at these temperatures reduced the average crystallite sizes and therefore indicate that the drug has been loaded. Using HIM and SEM, the zeolites' morphology was examined as shown in Figures 4.5 to 4.7.

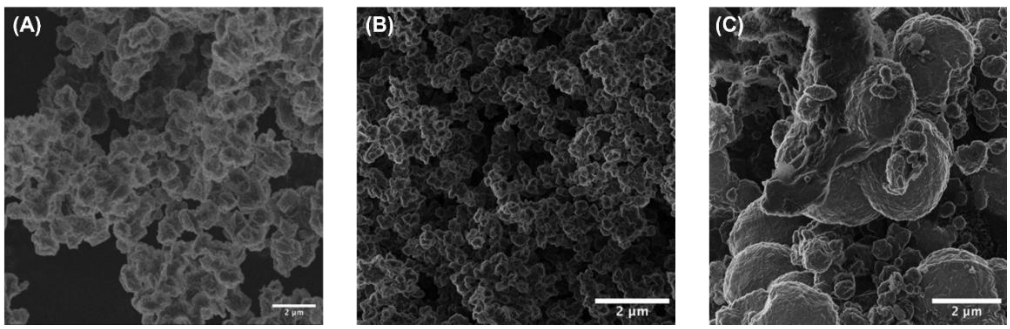


Figure 4.5: HIM images of LTA zeolites at resolution of 2 µm at various temperatures (**A**) LTA 60 °C, (B) LTA 80 °C, and (**C**) LTA 105 °C.

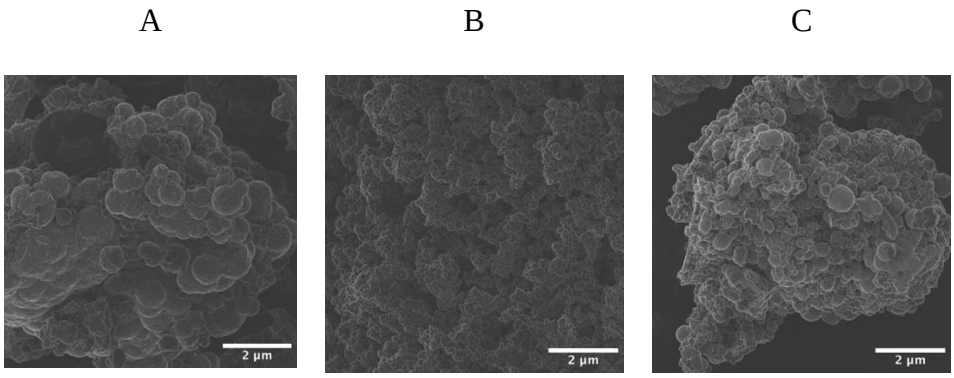


Figure 4.6: HIM images of loaded LTA zeolites at resolution of 2 µm at various temperatures (**A**) LTA 60 °C, (B) LTA 80 °C, and (**C**) LTA 105 °C.

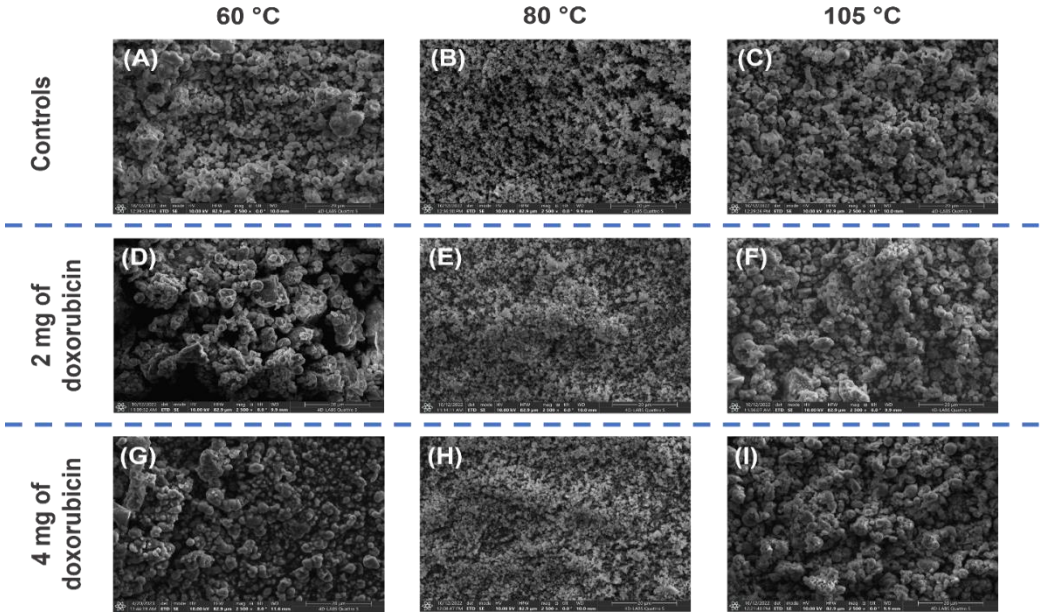


Figure 4.7: SEM images of control LTA zeolites loaded with 2 and 4 mg of doxorubicin drug at three different temperatures of the zeolites at 60 °C, 80 °C, and 105 °C.

The HIM images in Figure 4.6 confirms temperature effect on the morphology LTA zeolites with and without the drug. As the temperature of the LTA zeolite increases, the crystallinity reduces due to the presence of the drug. The SEM images also display the shape of the control zeolites and drug

loaded zeolites to confirm the XRD results. The drug is amorphous and the zeolites is crystalline, with the HIM images of the drug loaded represented in Figure 4.6 show a modification of the zeolites in the presence of the drug. It is observed that the higher drug concentration decreased the crystallinity.

However, with the SEM images shown in Figure 4.7, it is seen that at the drug concentration at 4 mg, there was decreased in crystallinity at LTA 105 °C. Due to the amorphous nature of the drug, it compromised the crystallinity at LTA 105°C, which also shows that most of the drug was entrapped because of the surface area of the LTA 105 °C.

4.2 Specific Surface area and pore distribution measured by Brunauer-Emmett- Teller

LTA zeolites have a three dimensional pore structure with high degree of symmetry but there are variations in the locations of the cations which lead to different energy levels in their cages (De Lucena *et al*., 2022). To calculate the size of a solid or porous material's surface, one uses the BET principle.

Given that a material's surface area determines how that solid will interact with its environment, it provides the zeolite‘s surface changes information about that material's physical structure.

Table 4.2: BET measurements of control zeolites and drug loaded zeolites

Sample	Surface area/m^2g^{-1}			Pore size/ Å			Pore Volume/cm^3g^{-1}		
	Control	2mg	4 mg	Control	2mg	4 mg	Control	2mg	4mg
LTA 60 °C	8.96	9.69	12.61	716.25	230.44	961.51	0.16	0.05	0.30
LTA 80 °C	650.65	166.54	322.62	24.25	53.77	35.11	0.39	0.22	0.28
LTA105 °C	10.02	171.84	5.08	356.60	97.74	418.19	0.09	0.42	0.15

(Researcher, 2024)

Table 4.2, the BET measurement on LTA 60 °C at the presence of 2 mg and 4 mg of the drug, which increased the surface area and affected the crystallinity. It is also observed in Table 4.1 that as the drug concentrations were loaded to the LTA 60°C and LTA 105 °C, the crystallite sizes reduced, which means that as crystallite size decreases, the surface area increases as stated in literature that surface area is mostly dependent on crystallite size (Hobday *et al*., 2021).

The BET measurement on LTA 80°C, the findings revealed a decrease in the surface area when the drug concentrations were loaded, this could possibly mean that there was different mechanism responsible for the uptake of the drug. The drug can be adhered to the surface or probably acting differently on the material.

4.3 Standard Calibration Curve of Model Drug (Doxorubicin)

The calibration curve as shown in Figure 4.8 is employed to ascertain the drug's concentration during the UV-Vis characterization. Known concentrations are utilized to plot the calibration curves and it helps to estimate the concentration of the absorbance values during the drug loading and release. R squared value close to 1 indicate the reliability of the curve is high and can be used to validate the concentration of the doxorubicin drug.

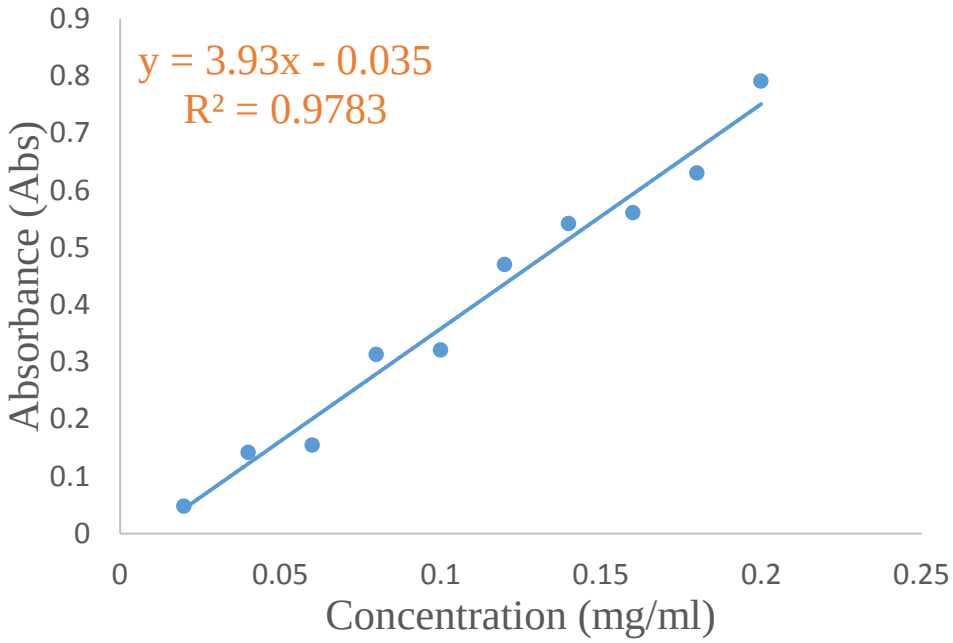


Figure 4.8: A standard calibration curve of doxorubicin

4.4 In-vitro Drug Release Kinetics

The data for the drug release was fitted to kinetic models, including zero-order, first-order, Korsmeyer-Peppas, and Hixson-Crowell models (Jahromi *et al*., 2020), in order to anticipate the drug's release behaviour from the nanomaterial. These kinetic models were used to explain the mechanisms of drug release from the samples using the correlation coefficients achieved by regression evaluation. Figures 4.9, 4.10, 4.11 and 4.12 show how the kinetic models fitted to the release data. Table 4.3 also give a summary of the R-squared values and constants from the fitted models. From the Table 4.3, it can be observed that LTA 60°C release shows a higher correlation with the first-order model (R^2 = 0.9139) and Hixson-Crowell model (R^2 = 0.8764) as compared to the Zero-order (R^2 = 0.7844) and Korsmeyer-Peppas (R^2 = 0.2457) models. With the LTA 80°C, it had a higher correlation with the Korsmeyer-Peppas model (0.7345) than with the Zero-order model (R^2 =

0.4412), first-order model (0.4017) and Hixson-Crowell (R^2 = 0.4151). But, LTA 80°C release shows higher correlation with the Hixson-Cromwell model (R^2 = 0.7345). A higher correlation was observed with Korsmeyer-Peppas (R^2 = 0.5160) and a contrast in Zero-order (R^2 = 0.0351), First-order (R^2 = 0.0712) and the Hixson-Crowell model (R^2 = 0.0575) for LTA 105°C.

The release profiles of the LTA 60°C best fitted with the Zero-order, First-order and Hixson-Crowell models as compared to LTA 80°C and LTA 105°C which best fitted to Korsmeyer-Peppas model. When a drug's rate of release from the drug carrier is constant, the zero-order model can be used to explain the release. The systems that exhibit this type of release behavior are suitable for prolonged drug release (Bruschi, 2015) and is independent of concentration (Lisik & Musial, 2019) which was fairly exhibited by the LTA 60°C sample as compared to the LTA 80°C and LTA 105°C samples. In the first-order model, the amount of drug released each time is fixed with respect to its concentration.

With the LTA 60°C sample showing a high correlation with both the zero-order and first-order models, it will be a suitable vehicle for prolonged, controlled and sustained delivery of drugs as compared to the LTA 80°C and LTA 105°C samples, which exhibited a low correlation in both kinetic models.

The Hixson-Crowell model focuses on the rate of dissolution with respect to the sample solution interaction with the surface. A greater surface area leads to quicker dissolution. The Hixson-Crowell model again fitted well with the LTA 60°C sample which confirms that with the increase in the surface area of the LTA 60°C, the rate of dissolution too increases as well. In contrast, LTA 80°C and LTA 105°C can best be described using the Korsmeyer-Peppas

model (Jahromi *et al*., 2020). In this model, the drug release is diffusion controlled and characterized using the release exponent (n) value in a function with time.

There is a direct correlation between the type of release from the vehicles and the rate constants. In Table 4.3, the rate at which a unit amount of the drug is made available in the release medium per unit time can be attributed to the kinetic constants. For a diffusion-controlled release, the kinetic constant should follow a sequential ascending or descending sequence. From Table 4.3, the values of n are 0.10 and 0.01 for LTA 80°C and LTA 105°C respectively. The calculated value of the release exponent (n) is less than 0.45, which confirms the release follows a Fickian diffusion behavior of drugs (Lavrentev *et al*., 2023).

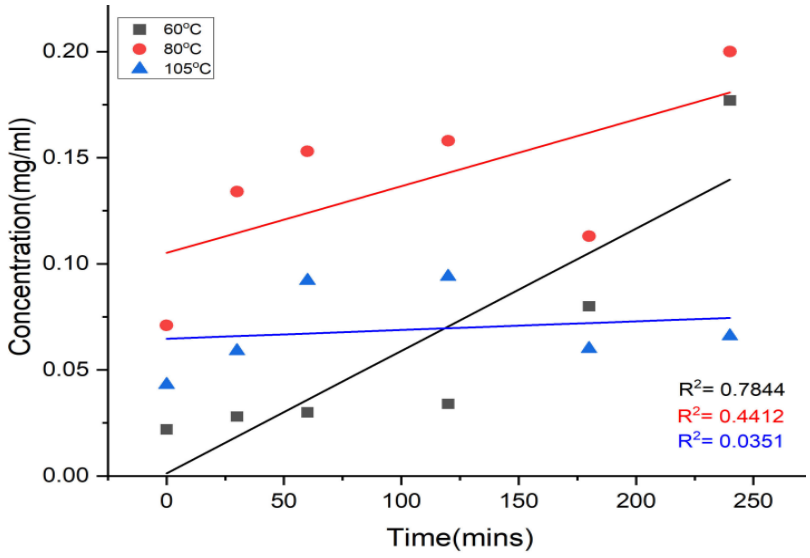


Figure 4.9: Zero-order release profile for LTA 60 °C, LTA 80 °C and LTA 105 °C.

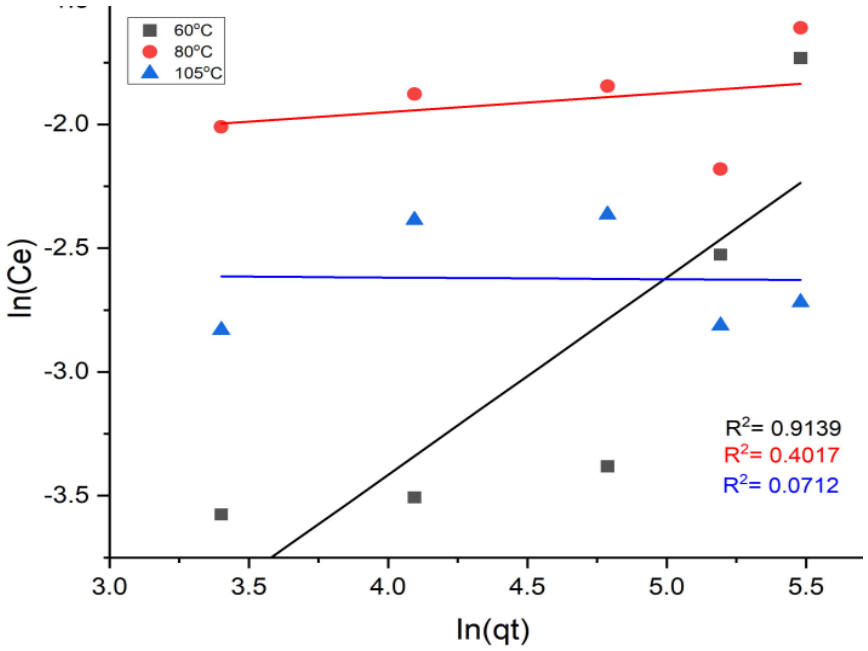


Figure 4.10: First-order release profile for LTA 60 °C, LTA 80 °C and LTA 105 °C.

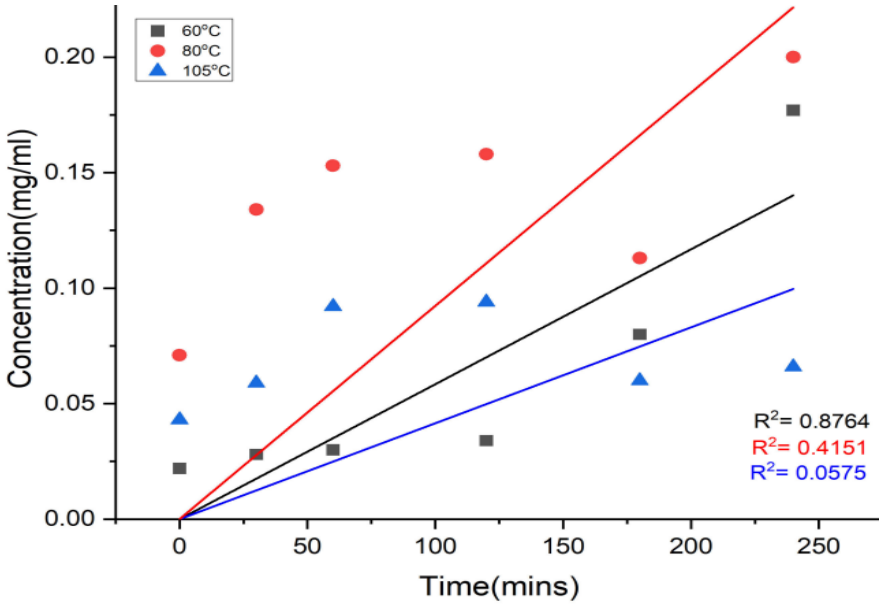


Figure 4.11: Hixson-Cromwell release profile for LTA 60 °C, LTA 80 °C and LTA 105 °C.

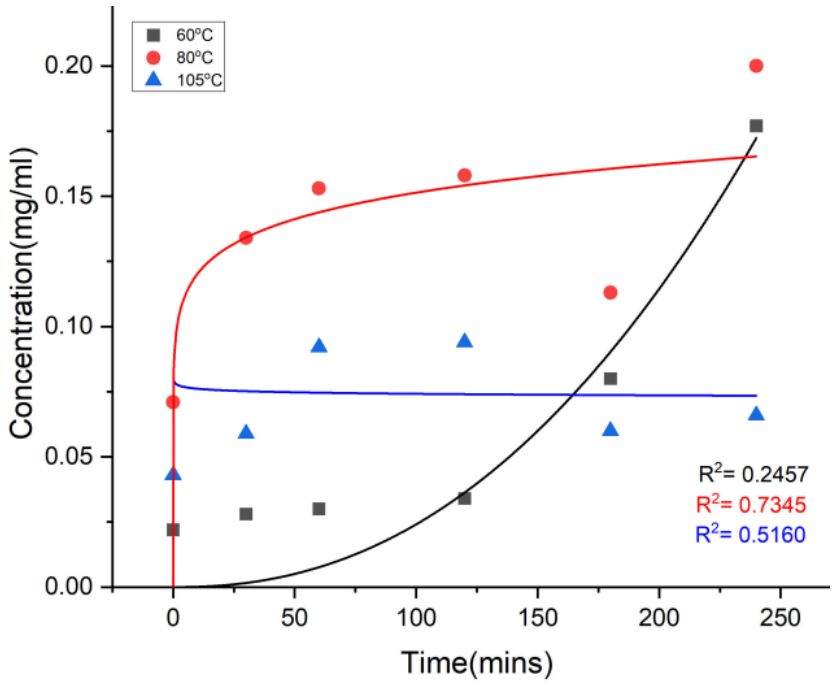


Figure 4.12: Korsmeyer-Peppas release profile for LTA 60 °C, LTA 80 °C and LTA 105 °C.

Table 4.3: Rate constants of different kinetic models

Model	Parameter	LTA 60°C	LTA 80°C	LTA 105°C
Zero-order	R^2	0.7844	0.4412	0.0351
	K_0	$5.76491x10^{4}$	$3.1473x10^{-4}$	$4.0701x10^{-5}$
First-order	R^2	0.9139	0.4017	0.0712
	K_1	0.7970	0.0771	- 0.0069
Korsmeyer-	R^2	0.2457	0.7345	0.5160
Peppas	K_{KP}	$7.698\ x10^{-7}$	0.0954	0.0781
	N	2.25	0.10	- 0.01
Hixson-Crowell	R^2	0.8764	0.4151	0.0575
	K_{HC}	$5.840x10^{-4}$	$9.2287x10^{-4}$	$4.1515x10^{-4}$

4.5 Alamar Blue Cytotoxicity Assay

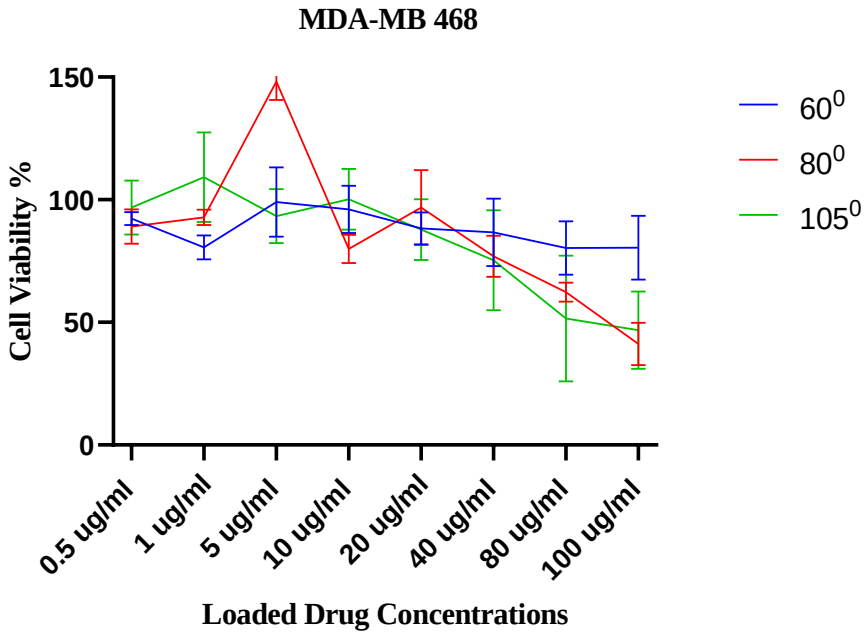
The cytotoxicity studies were carried out in a triple-negative breast cancer cell line, MDA-MB- 468. In this study, different concentrations of the different material loaded with doxorubicin on the MDA-MB-468 cell line were studied. The doxorubicin was released from the loaded materials unto the MDA- MB- 468 cell lines and it was observed that cell death was dependent on the particular material synthesized at different temperatures, indicating that the drug concentration was higher on LTA zeolite material synthesized at 105°C. After 48 hours of cell treatment, the drug demonstrated high levels of toxicity on cell line (MDA-MB-468), with IC_{50} of 92 µg/ml at LTA 105°C.

The findings correlate with literature that suggests that the synthesis of the nanoparticle at temperature of 105°C provides an optimum crystallization temperature for the effective and systematic release of doxorubicin (Iftitahiyah *et al*., 2018; Ji *et al*., 2020).

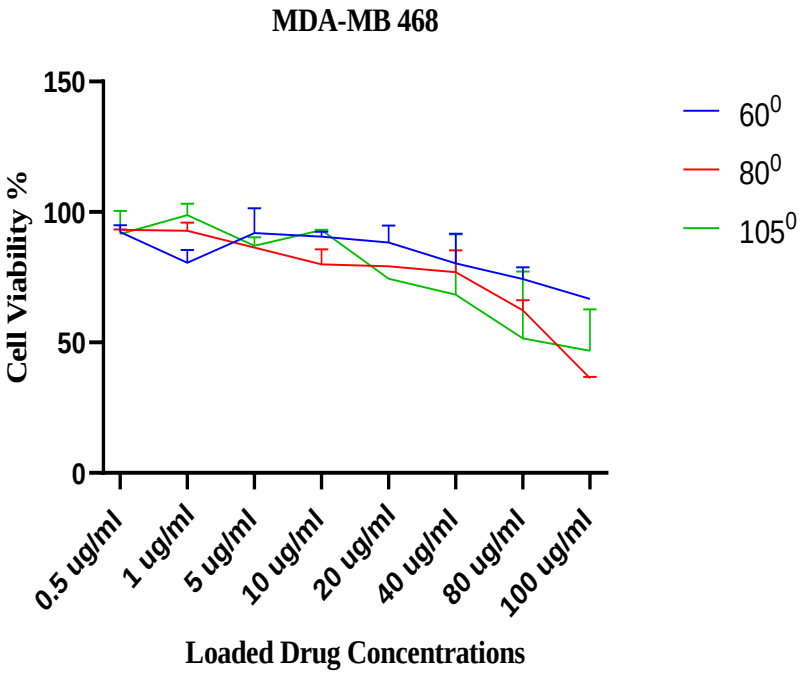
The doxorubicin loaded on LTA 60 °C exhibited a steady increase in MDA- MB 468 cell deaths with increase in the concentration of the doxorubicin as shown in Figures 4.13 and 4.14. These may probably mean that, LTA 60 °C had a lot of drugs in the pores that were causing the steady increase in the cell deaths. The doxorubicin loaded on LTA 80 °C also exhibited a shock at 10 µg/ml as shown in figure 4.14 which created an instantaneous growth, however, as the concentration increased, there was a cytotoxic effect. Hydrothermal crystallization temperature is one of the key determinants of drug release in biological systems. The crystallization temperature is not a fixed value but rather influenced by the properties of the nanoparticle. Studies using zeolites have identified the LTA 105°C to be ideal

for the development of microporous and mesoporous structures for effective drug release in systems (Iftitahiyah *et al*., 2018).

(A)



(B)



(C)

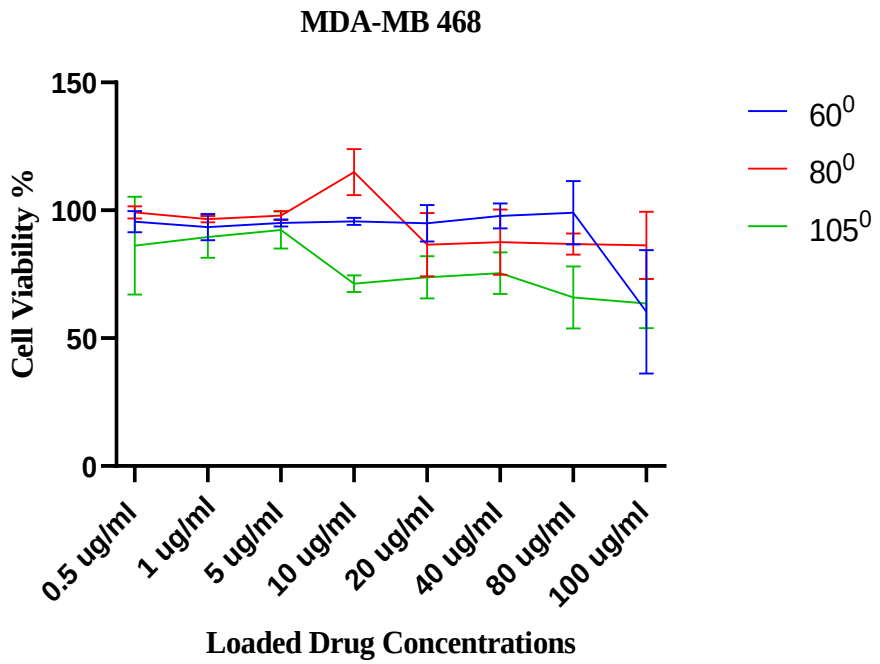


Figure 4.13: Effect of drug loaded doxorubicin on MDA-MB-468 for (A) Replicate 1 (B) Replicate 2 and (C) Replicate 3

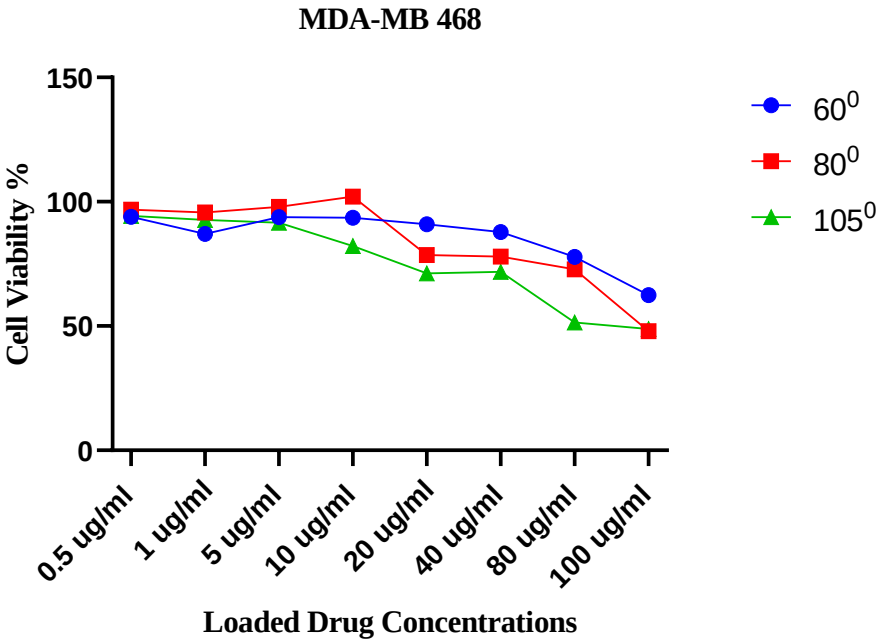


Figure 4.14: Percentage cell viability test against different concentrations of drug loaded doxorubicin on MDA -MB- 468

4.6 Chapter Summary

The results and discussions are summarized in this chapter as follows; The Fourier Transform Infrared spectroscopy (FTIR) and Xray diffraction spectroscopy (XRD) confirmed that the nanomaterial was Linde Type A (LTA) nanomaterial. Helium Ion microscope (HIM) and scanning electron microscope (SEM) visualization also confirmed the uptake of the drug by the nanomaterial. The pore volume, pore size, and surface area were determined for both the LTA zeolite and the drug loaded zeolite using the Brunauer-Emmett- Teller (BET) measurements. It was observed from the BET measurement that, both 60 °C and 105 °C LTA zeolite surface area increased as the drug of two different concentrations were loaded. This shows that the drug was trapped in the pores of the LTA zeolite. But with 80 °C LTA zeolite, the surface area decreased when the drug was loaded and this could possibly be that the drug was on the surface of the LTA zeolite. The release profiles of the sample prepared at 60 °C best fitted with the Zero-order, First-order and Hixson-Crowell models as compared to 80 °C and 105 °C which best fitted to Korsmeyer-Peppas model. After 48 hours of cell treatment, using the Alamar blue assay toxicity, the drug demonstrated high levels of toxicity on cell line (MDA-MB- 468), with IC_{50} of 92 µg/ml at the LTA zeolite of 105°C.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.0 Overview

The discovery of nanomedicine in the last century brings into perspective a new concept and dimension in the advancement of technology in the health sector. Our understanding of how medications are absorbed and released in living systems is improved by the use of nanoparticles as a drug delivery technology. Since 1995, the United States of America Food and Drug Administration has approved over 50 smart nanocarriers of different kinds to supply healing agents to diseased cells. There have been many attempts to explore the potential of a huge range of nanoparticles as drug delivery systems. In the current study, synthetic zeolite was employed as a drug delivery system in the delivery of drug to breast cancer cells. X-ray diffractometer and FTIR were used as the characterization techniques to confirm the synthetic zeolites as Linde Type A (LTA). Scanning electron microscope and HIM were used to visualize the morphology of the LTA zeolite.

5.1 Summary

This study's objective was to use synthetic zeolite, Linde Type A (LTA) synthesized at three different temperatures 60, 80 and 105 ℃ to investigate the loading and release profiles of anticancer drug doxorubicin in-vitro in treatment of breast cancer. Characterization of control zeolite nanomaterial and drug loaded nanomaterial were determined using XRD and FTIR techniques. The XRD and FTIR techniques confirmed that the drug was loaded on all the three synthesized LTA zeolite temperatures. Different

concentrations of the drug were loaded onto equal weights of LTA zeolite until 95% loading efficiency was achieved. To further comprehend the procedure for medication release after four hours of release, the release data was also fitted to mathematical models. There were four mathematical models which were used in fitting the models, namely, zero –order, first-order, Korsmeyer-Peppas and Hixson-Crowell models. The release profile of the LTA 60 ℃ best fitted with the zero-order, first-order and Hixson –Crowell models. LTA 80 ℃ and 105 ℃ best fitted to Korsmeyer-Peppas model. A cytotoxicity study on a breast cancer cell line was conducted (MDA-MB-468), using the Alamar Blue cytotoxicity assay and observed after 48 hours of incubation.

5.2 Conclusion

The study was undertaken to explore the utility of locally sourced zeolitic material for drug delivery application. All the three temperatures of the synthetic zeolite from the different crystallization exhibited smart drug release behavior as confirmed from the mathematical fitting models. It was also observed that the doxorubicin, a cancer drug loaded on the zeolitic materials exhibited profound cytotoxic characteristics on triple negative breast cancer cell lines. This work demonstrated that Linde Type A (LTA) zeolites material can serve as drug delivery system pending further studies.

5.3 Recommendation

In-vivo studies in animal model such as rats or mice are encouraged to expand studies on the drug's release and the stability of the nanoparticle.

Further functionalization using specific cell-based receptors or antibodies would enhance the specificity of action of the nanomaterial.

Another recommendation is to explore other zeolite temperatures to see if other zeolite temperatures can maximize the drug loaded and release.

The physicochemical features of zeolites, temperature and pH of zeolites should be further explored to determine the mechanism of release.

REFERENCES

Abbasi, E., Aval, S. F., Akbarzadeh, A., Milani, M., Nasrabadi, H. T., Joo, S. W., Hanifehpour, Y., Nejaki-Koshki, K., & Pashaei-Asl, R. (2014). Dendrimers: synthesis, applications, and properties. *Nanoscale Research Letters*, *9*, 1-10.

Abankwah, G. K. J., Abdul-Hamid, K. I., Quaofio, N., & Sarbeng, K. Y. (2021). Neighbourhood satisfaction in a mining-induced displacement and resettlement (MIDR) in Ghana. *Academy of Strategic Management Journal, 20*, 2-6.

Adriana, A. C., Carmen, D., Claudiu, M., Arseniu, A. M., Rus, L. L., Butuca, A., Juncan, A. M., & Totan, M. (2020). Applications and limitations of dendrimers in biomedicine. *Journal of Molecules, 25*, 17-25.

Afadzi, M., Davies, C. D. L., Hansen, Y. H., Johansen, T. F.; Standal, Ø. K.-V.; Hansen, R., Måsøy, S.-E., Nilssen, E. A., & Angelsen, B. A. J. (2012). Effect of ultrasound parameters on the release of liposomal calcein. *Journal of Ultrasound in Medicine and Biology. 38* (3), 478-486.

Agrahari, V., & Kumar, P. (2022). Novel Approaches for Overcoming Biological Barriers. *Journal of Pharmaceutics*, *14*, 9-14.

Agyemang, A. F., Tei-Muno, N, A., Dzomeku, M, V., Nakua, K. E., Duodu, A. P., Duah, O. H., & Bentil, B. A., Agbadi, P. (2020). The prevalence and predictive factors of breast cancer screening among older Ghanaian women. *Journal of Heliyon, 6*, 3237-3741.

Ahmad, Z., Shah, A., Siddiq, M., & Kraatz, H.-B. (2014). Polymeric micelles as drug delivery vehicles. *Journal of RSC Advances, 4*, 17028-17038.

Ahmed, O. H., Sumalatha, G., & Muhamad, A. N. (2010). Use of zeolite in maize (Zea mays) cultivation on nitrogen, potassium and phosphorous uptake and use efficiency. *International Journal of Physical Sciences, 5*, 2393-2401.

Akbarzadeh, A., Rezaei-Sadabady, R., Davaran, S., Joo, S. W., Zarghami, N., Hanifehpour, Y., Samiei, M., Kouhi, M., & Nejati-Koshki, K. (2013). Liposome: classification, preparation, and applications. *Nanoscale Research letters, 8*(1),102.

Alkilani, A. Z., McCrudden, M. T. C., & Donnelly, R. F. (2015). Transdermal drug delivery: Innovative pharmaceutical developments based on disruption of the barrier properties of the stratum corneum. *Journal of Pharmaceutics,7*, 438–470.

Alqahtani, M. S., Kazi, M., Alsenaidy, M. A. & Ahmad, M. Z. (2021) Advances in Oral Drug Delivery. *Frontiers in Pharmacology 12*, 411-418.

Amandeep Kaur, N. S. & S. L. H. (2013). Design and Development of Ketoprofen Pharmacosomes for Oral Delivery, *International Research Journal of Pharmacore*, *4*(4), 111–119.

Ambade, A. V., Savariar, E. N., & Thayumanavan, S. (2005). Dendrimeric micelles for controlled drug release and target delivery. *Journal of Molecular Pharmaceutics, 2*, 264-272.

Amidon, G. L., Lennernas, H., Shah, V. P., & Crison, J. R. (1995). A theoretical basis for a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability. *Journal of Pharmaceutics Research, 12*, 413-420.

Anselmo, A. E., & Mitragotis, S. (2019).m Nanoparticles in the clinic: An update. *Journal of Bioengineering & Translational Medicine, 4*(3), 10-19.

Attia, M. F., Anton, N., Wallyn, J., Omran, Z., & Vandamme, T. F. (2019). An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. *Journal of Pharmacy and Pharmacology*, *71*, 1185–1198.

Bae, H. Y., & Park, K. (2020). Advanced drug delivery 202 and beyond: perspectives on the future. *Journal of Advanced Drug Delivery Reviews*, *158*, 4-16.

Bae, H. Y., & Park, K. (2011). Targeted drug delivery to tumors: myths, reality and possibility. *Journal of Controlled Release*, *153*(3), 198-205.

Bae, Y. M., Park, Y. I., Nam, S. H., Kim, J. H., Lee, K., & Kim, H. M. (2012). Endocytosis, intracellular transport, and exocytosis of lanthanide-doped upconverting nanoparticles in single living cells. *Journal of Biomaterials*, *35*, 9080–9086.

Bakker-Woudenberg, I. A. (2002). Long-circulating sterically stabilized liposomes as carriers of agents for treatment of infection or for imaging infectious foci. *International Journal of Antimicrobial Agents*, *19*(4), 299-311.

Banerjee, M., Saraswatula, S., Williams, A., & Brettmann, B. (2020). Effect of purification methods on commercially available cellulose nanocrystal properties and TEMPO oxidation. *Processes*, *8*(6), 698.

Bashir, S., & Liu, L. J. (2015). *Advanced Nanomaterials and their applications in renewable energy*. Texas, USA: Elsevier Incorporated.

Bhardwaj, H., Singh, C., Pandey, M. K., & Sumana, G. (2016). Preparation and applications in food toxin detection. *Journal of Sensors and Actuators B: Chemical*, *231*, 624-633.

Britwum, R. B., Gulaid, J., & Amaning, A. O. (2000). Pattern of diseases or conditions leading to hospitalization at Korlebu Teaching Hospital, *Ghana Medical Journal*, *34*,197–205.

Bodas, D., & Khan-Malek, C. (2007). Direct patterning of quantum dots on structured PDMS surface. *Journal of Sensors and Actuators B: Chemical*, *128*, 168-172.

Boisseau, P., & Loubaton, B. (2011). Nanomedicine, nanotechnology in medicine. *Comptes Rendus Physique*,*12*, 620-636.

Bruschi, M. L. (2015). Strategies to modify the drug release from pharmaceutical systems. Texas, USA: *Elsevier Press Limited*.

Bunaciu, A. A., Udriştioiu, E. gabriela, & Aboul-Enein, H. Y. (2015). X-Ray Diffraction: Instrumentation and Applications. *Journal of Critical Reviews in Analytical Chemistry*, *45*, 289–299.

Cabral, H., Matsumoto, Y., Mizuro, K., Chen, Q., Murakami, M., Kimura, M., Terada, Y., Kano, M. R., Miyazono, K., Uesaka, M., Nishiyama, N., & Kataoka, K. (2011). Accumulation of sub-100 nm polymeric micelles in poorly permeable tumors depends on size. *Journal of Nature Nanotechnology*, *6*, 815-823.

Carey, L. A., Perou, C. M., & Livasyetal., C. A. (2006). Race,breast cancer subtypes, and survival in the Carolina breast cancer study. *The Journal of the American Medical Association*, *295* (21), 2492–2502.

Caro, J., & Noack, M. (2008). Zeolite membranes-recent developments and progress. *Journal of Microporous and Mesoporous Materials*, *15*, 185-197.

Cataldo, E., Salvi, L., & Mattii, G. B. (2021). Effects of irrigation on ecophysiology, sugar content and thiol precursors (3-S-cysteinylhexan-1-ol and 3-S-glutathionylhexan-1-ol) on Vitis vinifera cv. Sauvigon Blanc. *Journal of Plant Physiological Biochemistry, 164*, 257-259.

Cavallaro, G., Maniscalco, L., Licciardi, M., & Giammona, G. (2004). Tamoxifen-loaded polymeric micelles: preparation, physico-chemical characterization and in vitro evaluation studies. *Journal of Macromolecular Bioscience, 4*(11), 1028–1038.

Chames, P., Van Regenmortel, M., Weiss, E., & Baty, D. (2009). Therapeutic antibody: successes, limitations and hopes for the future. *British Journal of Pharmacology*, 157, 220-223.

Chandak, M. V., Lin, Y. S., Ji, W., & Higgins, R. J. (1997). Sorption and diffusion of VOCs in DAY zeolite and silicalite-filled PDMS membranes. *Journal of Membrane Science*, *133*, 231-243.

Cell Avalanche online product detail, 2014. https://cells-online .com/product/human-breast-cancer-cells-mda-mb-468/

Chauhan, A. S. (2018). Dendrimers for drug delivery. *Journal of Molecules, 23*, 938-950.

Chavez, K.J., Garimella, S.V., & Lipkowitz, S. (2010).Triple negative breast cancer cell lines: One tool in the search for better treatment of triple negative breast cancer. *Breast Disease, 32*(12):35–48.

Chenthamara, D., Subramaniam, S., Ramakrishan, S. G., Krishnaswamy, S., Essa, M. M., Lin, F. H., & Qoronfleh, M. W. (2019). Therapeutic efficacy of nanoparticles and routes of administration. *Journal of Biomaterials Research, 23*(20), 019-0166.

Chudoba, D., Jazdzewska, M., Łudzik, K., Wołoszczuk, S., Juszynska-Gał ˛Azka, E., & Koscinski, M. (2021). Description of Release Process of Doxorubicin from Modified Carbon Nanotubes. *International Journal of Molecular Science, 22*, (12), 759-766.

Clegg-Lamptey, J. N., Baako, B. N., & Badoe, E. A. (2009). *The breast, in Principles and Practice of Surgery Including Pathology in the Tropics* (4th edition). Assemblies of God Literature Centre, Accra.

Coe-Sullivan, S., Steckel, J. S., Woo, W. K., Bawendi, M. G., & Bulovic, V. (2005). Large-area ordered quantum-dot monolayer via phase separation during spin-casting. *Journal of Advanced Functional Materials*, *15*(7), 1117- 1124.

Creswell, J. W. (2003). Research design; qualitative, quantitative, and mixed methods approach (2nd Ed). London, Sage publications Inc: UK.

Dias, A. P., da Silva Santos, S., da Silva, J. V., Parise-Filho, R., Igne Ferreira, E., Seoud, O. El, & Giarolla, J. (2020). Dendrimers in the context of nanomedicine. *International Journal of Pharmaceutics, 573*,118-814.

De Lucena, M. P. S., Oliveira, A. C. J., Goncalves, D. V., Lucas, M. O. L, Moura, A. S. P., Santiago, G. R., Azevedo, D. C. S., & Bastos- Neto, M. (2022). LTA zeolite characterization based on pore type distribution. *Journal of Industrial & Engineering Chemistry Research*, *61*(5), 2268-2271.

Dreaden, E. C., Alkilany, A. M., Huang, X., Murphy, C. J., & El-Sayed, M. A. (2012). The golden age: gold nanoparticles for biomedicine, *Journal of Chemical Society Review, 41*, 2740-2779.

Drummond, D. C, Meyer, O., Hong, K., Kirpotin, D. B., & Papahadjopoulos, D. (1999). Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. *Journal of Pharmacology Review, 51*, 691-743.

Duan, X., & Li, Y. (2013). Physicochemical characteristics of nanoparticles affect circulation, biodistribution, cellular internalization, and trafficking. *Journal of Nano.Micro Small*, *9*, 1521-1532.

Edmund, D. M., Naaeder, S. B., Tettey, Y., & Gyasi, R. K. (2013). Breast cancer in Ghanaian women: what has changed? *American Journal of Clinical Pathology*, *140*, 97–102.

Feng, S., & Chien, S. (2003). Chemotherapeutic engineering: application and further development of chemical engineering principles for chemotherapy of cancer and other diseases, *Chemical Engineering Science*, *58*, 4087–4114.

Ferrari, R., Sponchioni, M., Morbidelli, M., & Moscatelli, D. (2018). Polymer nanoparticles for the intravenous delivery of anticancer drugs: The checkpoints on the road from the synthesis to clinical translation. *Journal of Nanoscale*, *10*, 22701–22719.

Gao, X. H., & Dave, S. R. (2007). Quantum dots for cancer molecular imaging. Bio-Applications of Nanoparticles, Germany*: Springer Nature Link, 620*, 57-73.

Gao, X., Yang, L., Petros, J. A., Marshall, F. F., Simons, J. W., & Nie, S. (2005). In vivo molecular and cellular imaging with quantum dots. *Journal of Current Opinion in Biotechnology*, *16*(1), 63-72.

Gaucher, G., Dufrense, M.-H., Sant, V. P., Kang, N., Maysinger, D., & Leroux, J.-C. (2005). Block copolymer micelles: preparation, characterization and application in drug delivery. *Journal of Controlled Release*, *109*, 169-188.

Gexin, C., Derek, E.B., Rajwant S. B., Yushan S. Y., & Sharon L. W. (2009). Initial bacterial deposition on bare and zeolite-coated aluminum alloy and stainless steel. *Journal of Langmuir, ACS Publications*, *25*, (3), 1620–1626.

Gerion, D. (2006). Fluorescence imaging in biology using nanoprobes. *Nanosystem Characterization Tools in the Life Sciences*, 1st Edition, Kumar, C.S.S.R., Edition, Wiley-VCH, Weinheim, Germany, 1-37.

Ghartey, N. F., Anyaful, A., Eliason, S., Adamu, M. S., & Debrah, S. (2016). Pattern of breast cancer distribution in Ghana: A survey to enhance early detection, diagnosis, and treatment. *International Journal of Breast Cancer*, *9*, 308 - 364.

Ghezzi, M., Pescina, S., Padula, C., Santi, P., Del Favero, E., Cantu, L., & Nicoli, S. (2021). Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. *Journal of Controlled Release*, *332*, 312-336.

Gopinath, A., & Wilson, M. (2020). Factors affecting drug absorption and distribution. *Journal of Anaesthesia and Intensive Care Medicine, 21*, 223–228.

Grimuado, M. A., Pescina, S., Padula, C., Santi, P., Concheiro, A., Alvarez-Lorenzo, C., & Nicoli, S. (2019). Topical application of polymeric nanomicelles in ophthalmology: a review on research efforts for the noninvasive delivery of ocular therapeutics. *Journal of Expert Opinion on Drug Delivery*, *16*(4), 397-413.

Gupta, U., & Perumal, O. (2014). Chapter 15- Dendrimers and its biomedical applications. *Journal of Natural and Synthetic Biomedical Polymers*, *10*, 243-257.

Heath, J. R. (2015). Nanotechnologies for biomedical science and translational medicine. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(47), 14436–14443.

He, H., Liang, Q., Shin, M. C., Lee, K., Gong, J., Ye, J., & Yang, V. (2013). Significance and strategies in developing delivery systems for bio-macromolecular drugs. *Frontiers of Chemical Science and Engineering*, *7*,496–507.

Hedaya, M. A. (2012). Extravascular routes of drug administration. *Journal of In Basic Pharmacokinetics, 595*. 570 -580.

Hobday, C. L., Rogge, S. M. J., Evans, J. D., & Bunzen, H. (2021). Perspective on the influence of crystal size and morphology on the properties of porous framework materials. *Frontiers in Chemistry, 9*, 772-859.

Hofheinz, R. D., Gnad-Vogt, S. U., Beyer, U., & Hochhaus, A. (2005). Liposomal encapsulated anti-cancer drugs. *Journal of Anticancer Drugs*, *16*, 691–707.

Hoshyar, N., Gray, S., Han, H., & Bao, G. (2016). The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction. *Journal of Nanomedicine (London, England), 11*(6), 673-692.

Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., & Snyder, N. (2014). Drug resistance in cancer: an overview. *National Library of Medicine, 6,* 1769-1792.

Hosokawa, H., & Oki, K. (2003). Synthesis of nanosized a-type zeolites from sodium silicates and sodium aluminates in the presence of a crystallization inhibitor. *Journal of Chemical Letters, 32*, 586-606.

Iftitahiyah, V. N., Prasetyoko, D., Nur, H., Bahruji, H., & Hartati, H. (2018). Synthesis and characterization of zeolite NaX from Bangka Belitung kaolin as an alternative precursor. *Malaysian Journal of Fundamental and Applied Sciences*, *14*(4), 414-418.

IIT JEE Study Material for Physics, Chemistry and Maths, Zeolites (2003).

Imran, M., Shah, R. M., & Shafiullah. (2018). Chapter-10 Amphiphilic copolymers-based micelles for drug delivery. *Journal of Design and Development of New Nanocarriers*, *12*, 365-400.

Jahromi, P. L., Ghazali, M., Ashrafi, H., & Azadi, A. (2020). A comparison of models for the analysis of the kinetics of drug release from PLGA-based nanoparticles. *Journal of Heliyon*. *6*, 345-351 .

Jana, D., Adam, V., Kizek, R., & Hubalek, J. (2009). Quantum dots-characterization, preparation and usage in biological systems. *International Journal of Molecular Sciences*, *10*(2), 656-673.

Jain, K. K. (2020). An overview of drug delivery systems. *In Methods in Molecular Biology*,*437*, 1–54.

Janaszewska, A., Lazniewska, J., Trzepinski, P., Marcinkowska, M., & Klajnert-Maculewicz, B. (2019). Cytotoxicity of dendrimers. *Journal of Biomolecules*, *9*, 330-430.

Jeevanandam, J., Barhoum, Ahmed., Chan, Y. S., Dufrense, A., & Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilsten Journal of Nanotechnology*, *9*, 1050-1074.

Jiang, G. B., Quan, D., Liao, K., & Wang, H. (2006). Preparation of polymeric micelles based on chitosan bearing a small amount of highly hydrophobic groups. *Journal of Carbohydrate Polymers*, *66*, 514-520.

Ji, W., Zhang, S., Zhao, P., Feng, N., Lan, L., Zhang, X., Sun, Y., Li, Y., & Ma, Y. (2020). Green synthesis method and application of NaP zeolite prepared by coal gasification coarse slag from Ningdong, China. *Journal of Applied Sciences*, *10*(8), 2694-3000.

Joens, M. S., Huynh, C., Kasuboski, J. M., Ferranti, D., Sigal, Y. J., Zeitvogel, F., Obst, M., Burkhard, C. J., Curran, K. P., Chalasani, S. H., Stern, L. A., Goetze, B., & Fitzpatrick, J. A. J. (2013). Helium Ion Microscopy (HIM) for the imaging of biological samples at sub-nanometer resolution. *Journal of Scientific Reports*, *3*, 3514-3854.

Johnston, M. J., Semple, S. C., Klimuk, S. K., Ansell, S., Maurer, N., & Cullis, P. R. (2007). Characterization of the drug retention and pharmacokinetic properties of liposomal nanoparticles containing dihydrosphingomyelin. *Biochimica et Biophysica Acta*, *5*, 1121–1127.

Joly, F., Ray-Coquard, I., Fabbro, M., Donoghoe, M., Boman, K., & Sugimoto, A. (2011). Decreased hypersensitivity reactions with carboplatin-pegylated liposomal doxorubicin compared to carboplatin-paclitaxel combination: Analysis from the GCIG CALYPSO relapsing ovarian cancer trial. *Journal of Gynecologic Oncology*, *122*(2), 226–232.

Julbe, A., & Drobek, M. (2016). Zeolite A type. In: *Drioli, E., Giorno, L. Encyclopedia of Membranes. Germany:* Springer Nature Link.

Jurásek, B., Bartůněk, V., Huber, Š., & Kuchař, M. (2019). X-Ray powder diffraction – A non- destructive and versatile approach for the identification of new psychoactive substances. *Talanta*, *195*, 414–418.

Kaur, A., Sharma, N., & Harikumar, S. L. (2013). Design and development of ketoprofen pharmacosomes for oral delivery. *Journal of Pharmacophore*, *4*, 229-540.

Khan, A. R., Liu, M. W., & Zhai, G. (2017). Progress in brain targeting drug delivery system by nasal route. *Journal of Controlled Release*, *268*, 364-389.

Khaleque, A., Alam, M. M. D., Hoque, M., Mondal, S., Haider,B. J., Xu, B., Johir, M. A. H., Karmakar, K. A., Zhou, J.L., Ahmed, B. M., & Moni, A. M. (2020). Zeolite synthesis from low-cost materials and environmental applications: A review. *Journal of Environmental Advances*, *2*, 100-119.

Khan, I., Saeed, K., & Khan, I. (2017). Nanoparticles: properties, applications and toxicities. *Arabian Journal of Chemistry*,*12*,1878-5352.

Kolhatkar, R., Lote, A., & Hiren, K. (2008). Active tumor targeting of nanomaterials using folic acid, transferrin and integrin receptors. *Journal of Current Drug Discovery Technologies*, *3*,197-206.

Kok-Yong, S., & Lawrence, L. (2015). Drug Distribution and Drug Elimination. In Basic Pharmacokinetic Concepts and Some Clinical Applications. *Intech Open Access, 10*, 5772-5992.

Kshirsagar, N. A., Pandya, S. K., Kirodian, G. B., & Sanath, S. (2005). Liposomal Drug Delivery System from Laboratory to Clinic. *Journal of Postgraduate Medicine*, *51*(5), 5–15.

Kulthe, S.S., Choudari, Y. M., Inamdar, N. N., & Mourya, V. (2012). Polymeric micelles: authoritative aspects for drug delivery. *Journal of Designed Monomers and Polymers*, *15*(5), 465-521.

Kumar, S., & Gupta, S. K. (2012). Natural polymers, gums and mucilages as excipients in drug delivery. *Polimery W Medycynie*, *42*, 191–197.

Kyotani, T., Ma, Z., & Tomita, A. (2006). Template synthesis of novel porous carbons using various types of zeolites. *Journal of Carbon, 7*, 1451-1459.

Larysa, R., Frédéric, K., & Kévyn, J. (2022). Open Sorption Systems. *Encyclopedia of Energy storage System, Elsiever Limited*, *1*, 526-541.

Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Elst, V. L., & Muller, R. N. (2010). Magnetic iron oxide nanoparticle: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Journal of Chemical Reviews*, *110*(4), 2574-2574.

Lee, P. I., & Li, J. X. (2010). Evolution of oral controlled release dosage forms. *Journal of Oral Controlled Release Formulation Design and Drug Delivery: Theory to Practice*, *2*, 21–31.

Lehman, S. E., & Larsen, S. C. (2014). Zeolite and mesoporous silica nanomaterials: greener syntheses, environmental applications and biological toxicity. *Journal of Environmental Science: Nano*, *1*, 200-213.

Lei, C., Cao, Y., Hosseinpour, S., Gao, F., Liu, J., Fu, J., Staples, R., Ivanovski, S., & Xu, C. (2021). Hierarchial dual-porous hydroxyapatite doped dendritic mesoporous silica nanoparticles based scaffolds to promote osteogenesis in vitro and in vivo. *Journal of Nano Research*, *14*(3), 770-777.

Li, H. C., Zhou, Q. F., Liu, W., Yan, B., Zhao, Y., & Jiang, G. B. (2008). Progress in the toxicological researches for quantum dots. *Journal of Science in China Series B*, *51*, 393-400.

Li, M., Du, C., Guo, N., Teng, Y., Meng, X., & Sun, H. (2019). Composition Design and Medical Application of Liposomes. *European Journal of Medicinal Chemistry*, *164*, 640–653.

Li, J., Li, Z., Zhou, T., Zhang, J., Xia, H., Li, J., He, J., He, S., & Wang, L. (2015). Positively charged micelles based on a triblock copolymer demonstrate enhanced corneal penetration. *International Journal of Nanomedicine*, *10*, 6027-6037.

Liu, T. C., Zhang, H. L., Wang, J. H, Wang, H. Q., Zhang, Z. H., Hua, X. F., Cao, Y. C., Luo, Q. M., & Zhao, Y. D. (2008). Study on molecular interactions between proteins on live cell membranes using quantum

dot-based fluorescence resonance energy transfer. *Journal of Analytical and Bioanalytical Chemistry, 391*, 2819-2824.

Liu, H., Zang, C., Fenner, M. H., Possinger, K., & Elstner, E. (2003). PPARgamma ligands and ATRA inhibit the invasion of human breast cancer cells in vitro. *Journal of Breast Cancer Research and Treatment, 79*(1), 63-74.

Li, Z. (2003). Use of surfactant-modified zeolite as fertilizer carriers to control nitrate release. *Journal of Microporous and Mesoporous Materials, 61*, 181-188.

Lizik, A., & Musial, W. (2019). Conductomeric evaluation of the release kinetics of active substances from pharmaceutical preparations containing iron ions. *Journal of Materials, 12*, 730-832.

Lu, R.M., Hwang, Y.C., Liu, I. J., Lee, C.C., Tsai, H.Z., Li, H.J., & Wu, H.C. (2020). Development of therapeutic antibodies for the treatment of diseases. *Journal of Biomedical Science, 27*(1), 129-329.

Lu, Y., & Park, K. (2013). Polymeric micelles and alternative nanosized delivery vehicles for poorly soluble drug. *International Journal of Pharmaceutics, 453*(1), 198-214.

Ma, Q., Song, T. Y., Yuan, P., Wang, C., & Su, X. G. (2008). QDs-labeled microspheres for the adsorption of rabbit immunoglobulin G and fluoroimmunoassay. *Colloid Surf. B-Biointerfaces, 64*, 248-254.

Malik, S., Muhammad, K., & Waheed, Y. (2023). Emerging Applications of Nanotechnology in Healthcare and Medicine. *Journal of Molecules, 28*, 6624-8182.

Matea, C. T., Mocan, T., Tabaran, F., Pop, T., Mosteanu, O., Puia, C., Iancu, C., & Mocan, L. (2017). Quantum dots in imaging, drug delivery and sensor applications. *International Journal of Nanomedicine, 12*, 5421-5431.

Makhmalzade, B. S., & Chavoshy, F. (2018). Polymeric micelles as cutaneous drug delivery system in normal skin and dermatological disorders. *Journal of Advanced Pharmaceutical Technology & Research*, *9*, 2-8.

Manocha, B., & Margaritis, A. (2010). Controlled release of doxorubicin from doxorubicin/γ-polyglutamic acid ionic complex. *Journal of Nanomaterials*, *12*, 1-8.

Mansoori, M., Agrawal, S., Jawade, S., & Khan, M. (2012). A review on liposome. International *Journal of Advanced Research in Pharmaceutical & Bio-sciences*, *2*(4), 453–464.

McCusker, L. B., & Baerlocher, C. (2001). Zeolite structures. Introduction to Zeolite Science and Practice, Studies in Surface Science and Catalyst, London: *Elsevier Press Limited*, 37-67.

Mikhail, A. S., & Allen, C. (2009). Block copolymer micelles for delivery of cancer therapy: transport out the whole body, tissue and cellular levels. *Journal of Controlled Release*, *138*(3), 214-223.

Mintova, S., Mo, S., & Bein, T. (1998). Nanosized alpo4-5 molecular sieves and ultrathin films prepared by microwave synthesis. *Journal of Chemical Mater*, *10*, 403-506.

Mishra, N., Rana, K., Seelam, S. D., Kumar, R., Pandey, V., Salimath, B. P., & Agsar, D. (2021). Characterization and cytotoxicity of pseudomonas mediated rhamnolipids against breast Cancer MDA-MB-231 cell line. *Journal of Front Bioengineering Biotechnology*, *9*,869-1732.

Moghimi, S. M., Hunter, A. C., & Murray, J. C. (2001). Long circulating and target-specific nanoparticles: theory to practice, *Journal of Pharmacological Reviews*, *53*, 283–318.

Mohamed, W. A. A., El-Gawad, H. A., Mekkey, S., Galal, H., Handal, H., Mousa, H., & Labib, A. (2021). Quantum dots synthetization and future prospect applications. *Journal of Nanotechnology Reviews, 10*, 1926-1940.

Molavi, O., Xiong, X.-B., Douglas, D., Kneteman, N., Nagaka, S., Pastan, I., Chu, Q., Lavasanifar, A., & Lai, R. (2013). Anti- CD30 antibody conjugated liposomal doxorubicin with significantly improved therapeutic efficacy against anaplastic large cell lymphoma. *Journal of Biomaterials, 34*(34), 8718-8725.

Mollazadeh, S., Mackiewicz, M., & Yazdimamaghani, M. (2021). Recent advances in the redoxresponsive drug delivery nanoplatforms: A chemical structure and physical property perspective. *Journal of Materials Science and Engineering: C, 118*, 111-536.

Müller, R. H., Mäder, K., & Gohla, S. (2000). Solid Lipid Nanoparticles (SLN) for Controlled Drug Delivery - a Review of the State of the Art. *European Journal of Pharmaceutics and Biopharmaceutics, 50* (1), 161–177.

Nadeem, J., & Dirk, L. (2022). Nanoparticle classification, physicochemical properties, characterization and applications: a comprehensive review for biologists. *Journal of Nanobiotechnology, 20*, 917-1489.

Nakhaei, P., Margiana, R., Bokov, O. D, Abdelbasset, W. K., Kouhbanani, M. A. J., Varma, R. S., Faroogh, Jarahian, M., & Beheshtkhoo, N. (2021). Liposomes: structure, biomedical applications and stability parameters with emphasis on cholesterol. *Frontiers in Bioengineering and Biotechnology*, *9*, 705-886.

National Institutes of Health (2022). Drug delivery systems. *U.S. Department of Health & Human Services,* U.S.A.

Navya, P. N., Kaphle, A., Srinivas, S. P., Bhargava, S. K., Rotello, V. M., & Daima, H. K. (2019). Current trends and challenges in cancer management and therapy using designer nanomaterials, *National Library of Medicine*, *6*(1), 626-766.

Nayak, A. K., Ahmad, S. A., Beg, S., Ara, J. T., & Hasnain, M. S. (2018). Chapter-12 drug delivery: present, past, and future of medicine. Applications of Nanocomposite Materials in Drug Delivery, USA: *Woodhead Publishing Series in Biomaterials*, 255-282.

Noble, G. T., Stefanick, J. F., Ashley, J. D., Kiziltepe, T., & Bilgicer, B. (2014). Ligand-targeted liposome design: Challenges and fundamental considerations. *Trends Biotechnology*, *32* (1), 32–45.

Owen, S. C., Chan, D. P. Y., & Shoichet, M. S. (2012). Polymeric micelle stability. *Journal of Nano Today*, *7*, 53-65.

Pandita, D., Poonia, N., Kumar, S., Lather, V., & Madaan, K. (2014). Dendrimers in drug delivery and targeting: Drug-dendrimer interactions and toxicity issues. *Journal of Pharmacy and Bioallied Sciences, 6*, 139–150.

Parhiz, H., Khoshnejad, M., Myerson, J. W., Hood, E., Patel, P. N., Brenner, J. S., & Muzykantov, V. R. (2018). Unintended effects of drug carriers: Big issues of small particles. *Advanced Drug Delivery Reviews*, *130*, 90–112.

Park, K. (2017). The drug delivery field needs a well- diversified technology portfolio. *Journal of Controlled Release*, *245*,177-180.

Park, K. (2014). Controlled drug delivery systems: Past forward and future back. *Journal of Controlled Release*, *190*, 3–8.

Park, H., Otte, A., & Park, K. (2022). Evolution of drug delivery systems: From 1950 to 2020 and beyond. *Journal of Controlled Release*, 53-65.

Patel, J., Basu, B., Dharamsi, A., Garala, K., & Raval, M. (2011). Solubility of aceclofenac in polyamidoamine dendrimer solutions. *International Journal of Pharmaceutical Investigation*, *1*(3), 135-145.

Perez-Botella, E., Valencia, S., & Rey, F. (2022). Zeolites in adsorption processes: State of the art and future prospects. *Journal of Chemical Review*, *122*(24), 17647-17695.

Petersen, G. H., Alzaghari, S. K., Chee, W., Sankari, S. S., & La-Beck, N. M. (2016). Meta-analysis of clinical and preclinical studies comparing the anticancer efficacy of liposomal versus conventional non-loposomal doxorubicin. *Journal of Controlled Release*, *28*,255-264.

Portioli, C., Bovi, M., Benati, D., Donini, M., Perduca, M., Romeo, A., & Bentivoglio, M. (2017). 68, Novel functionalization strategies of polymeric nanoparticles as carriers for brain medications. *Journal of Biomedical Materials Research - Part A, 105*(3), 847–858.

Prabahar, K., Alanazi, Z., & Qushawy, M. (2021). Targeted drug delivery system: advantages, carriers and strategies. *Indian Journal of Pharmaceutical Education and Research, 55*(2), 346-353.

Punia, H., Bhadu, S., & Tokas, J. (2020). Nanotechnology and nanomedicine: going small means aiming big. *Advances in Biochemistry & Application in Medicine, 2*, 1882-1892.

Ramirez, H. Y., Florez, J., & Camacho, A. S. (2015). Efficient control of coulomb enhanced second harmonic generation from excitonic transitions in quantum dot ensembles. *Journal of Physical Chemistry Chemical Physics, 17*(37), 23938-23946.

Rimmington, F. (2020). Pharmacokinetics and pharmacodynamics. *Southern African Journal of Anaesthesia and Analgesia, 26*(6), 153–156.

Rimoli, M. G., Rabaioli, M. R., Melisi, D., Curcio, A., Mondello, S., Mirabelli, R & Abignente, E. (2007). Synthetic Zeolites as a new tool for drug delivery. *Journal of Biomedical Materials Research Part A, 87*,156-164.

Ríos-Reyes, C.A., Reyes- Mendoza, G.A., Henao-Martínez, J.A., Williams, C.D.; Dyer, A. (2021). First Report on the Geologic Occurrence of Natural Na–A Zeolite and Associated Minerals in Cretaceous Mudstones of the Paja Formation of Vélez (Santander), Colombia. *Journal of Crystals, 11*, 218-300.

Rocha, M., Chaves, N., & Bao, S. (2017). Nanobiotechnology for breast cancer treatment. *Intech Open Access, 508,* 577-629.

Safari, F., Houshmand, B., & Nejad, A. E. (2019). Application of zeolite, a biomaterial agent, in dental science*: Journal of Regeneration, Reconstruction & Restoration (Triple R),30,* 3-4.

Saltzman, W. M. (2001). *Drug delivery: Engineering principles for drug therapy*. USA: Oxford University press.

Sapsford, K. E., Pons, T., Medintz, I. L., & Mattoussi, H. (2006). Biosensing with luminescent semiconductor quantum dots. *Journal of Sensors*, *6*, 925-953.

Saravanakumar, K., Swapna, P., Nagaveni, P., Vani, P., & Pujitha, K. (2015). Transdermal drug delivery system: A review. *Journal of Global Trends in Pharmaceutical Sciences*, *6*, 2485–2490.

Servatan, M., Zarrintaj, P., Mahmodi, G., Kim, S. K., Ganjali, R. M., Saeb, R. M., & Mozafari, M. (2020). Zeolites in drug delivery: Progress, challenges and opportunities, USA: Elsiever Limited

Senior, J., & Radomsky, M., (2000). *Sustained-Release Injectable Products* (1st edition).USA: CRC press.

Sephra, N. M. (2012). Multiple applications of Alamar blue as an indicator of metabolic function and cellular health in cell viability bioassays. Journal of Sensors, *12*(9), 12347-12360.

Sharma, A., & Kakkar, A. (2015). Designing dendrimer and miktoarm polymer based multi- tasking nanocarriers for efficient medical therapy. *Journal of Molecules, 20,* 16987–17015.

Sharma, A. K., Bhandari, R., Sharma, C., Dhakah, S. K., & Pinca-Bretotean, C. (2022). Polymer matrix composites: A state of the art review. *Journal of Materials Today: Proceedings*, *5*, 2330-2333.

Sherje, A. P., Jadhav, M., Dravyakar, B. R., & Kadam, D. (2018). Dendrimers: A versatile nanocarrier for drug delivery and targeting. *International Journal of Pharmaceutics, 548,* 707–720.

Shih, T., & Lindley, C. (2006). Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies. *Journal of Clinical Therapeutics*, *28*(11), 779-802.

Shin, W. K., Cho, J., Kannan, A. G., Lee, Y. S., & Kim, D. W. (2016). Cross-linked composite gel polymer electrolyte using mesoporous methacrylate-functionalized SiO_2 nanoparticles for lithium-ion polymer batteries. *Journal of Scientific Reports*, *6*, 263-332.

Szostak, R. (1998). Molecular sieves, principles of synthesis and identification. Van Nostrand Reinhold Electrical / Computer Science and Engineering Series. 1st Edition. Netherlands: Springer Publishing.

Tardi, P. G., Boman, N. L., & Cullis, P. R. (1996). Liposomal doxorubicin. *Journal of Drug Targeting, 4*(3), 129-140.

Thomas, S. A., Kidwell, M. K., Oppong, K. J., Adjei, K. E., Osei-Bonsu, E., Boahene, A., Jiagge, E., Gyan, K., & Merajver, D. S. (2017). Breast cancer in Ghana: Demonstrating the need for population-based cancer registries in low- and middle-income countries. *Journal of Global Oncology, 3*, 700-765.

Thürmann, P. A. (2020). Pharmacodynamics and pharmacokinetics in older adults. *Current Opinion in Anaesthesiology, 33*, 109–113.

Tiwari, G., Tiwari, R., Bannerjee, S., Bhati, L., Pandey, S., Pandey, P., & Sriwastawa, B. (2012). Drug delivery systems: An updated review. *International Journal of Pharmaceutical Investigation, 2*(1), 2-11.

Tomalia, D. A., & Frechet, J. M. J. (2002). Discovery of dendrimers and dendritic polymers: a brief historical perspective. *Journal of Polymer Science, Part A Polymer Chemistry*, *40*, 2719–2728.

Torchilin, V. P. (2005). Targeted pharmaceutical nanocarriers for cancer therapy and imaging. *Journal of AAPS PharmaSci*., *9*(2), 128-147.

Trivedi, R., & Kompella, U. B. (2010). Nanomicellar formulations for sustained drug delivery: strategies and underlying principles. *Journal of Nanomedicine), 5*(3), 485-505.

Troung, N. P., Whittaker, M. R., Mak, C. W., & Davis, T. P. (2015). The importance of nanoparticle shape in cancer drug delivery. *Expert Opinion on Drug Delivery*, *12*, 129-142.

Tyagi, S. (2016). Nanoparticles-an overview of classification and applications. *Journal of Pharmaceutics and Nanotechnology*,*5*,2347-7857.

Vargason, A. M., Anselmo, A. C., & Mitragotri, S. (2021). The evolution of commercial drug delivery technologies. *Journal of Nature Biomedical Engineering*, *5*, 951-967.

Versypt, A. F. (2015). Modeling of controlled-release drug delivery from autocatalytically degrading polymer microspheres. USA: University of Illinois Library.

Vilasceca, M., Mintova, S., Karaghiosoff, K., Metzger, T. H., & Bein, T. (2004). *Journal of Applied Surface Science*, *226,* 1-5.

Vyas, M., Simbo, D. A., Mursalin, M., Mishra, V., Bashray, R., & Khatik, G. L. (2020). Drug delivery approaches for doxorubicin in the management of cancers. *Current Cancer Therapy Reviews, 16*, 320-331.

Wagner, K.-U. (2022). Know thy cells: commonly used triple-negative human breast cancer cell lines carry mutations in RAS and effectors. *Journal of Breast Cancer Research*, *24*, 44-50.

Wambang, N., Schifano-Faux, N., Aillerie, A., Baldeyrou, B., Jacquet, C., & Bal-Mahieu, C. (2016). Synthesis and biological activity of ferrocenyl indeno [1, 2-C]isoquinolines as topoisomerase II inhibitors. *Journal of Bioorganic and Medicinal Chemistry*, *4*, 651-660.

Wang, B., Chen, L., Sun, Y., Zhu, Y., Sun, Z., An, T., Li, Y., Lin, Y., Fan, D., & Wang, Q. (2015). Development of phenylboronic acid-functionalized nanoparticle for emodin delivery. *Journal of Materials Chemistry. B*, *3*(18), 3840-3847.

Wang, J., Li, B., Qui, L., Qiao, X., & Yang, H. (2022). Dendrimer-based drug delivery systems: history, challenges, and latest developments. *Journal of Biological Engineering*, *16*, 16-18.

Wang, Y., Li, H., Wang, L., Han, J., Yang, Y., Fu, T., & Li, J. (2021). Mucoadhesive nanocrystal- in-microspheres with high drug loading capacity for bioavailability enhancement of silybin. *Colloids and Surfaces B: Biointerfaces*, *198*. 111-461.

Wang, X., Zhang, H., & Chen, X. (2019). Drug resistance and combating drug resistance in cancer. *Journal of Cancer Drug. Resistance, National Library of Medicine*. *2*, 141–160.

Wenten, I. G., Dharmawijaya, P. T., Aryanti, P. T. P., Mukti, R. R., & Khoiruddin. (2017). LTA zeolite membranes: current progress and challenges in pervaporation. *Journal of RSC Advances*, *7*, 29520-29539.

World Health Organization (2008), Breast cancer in Ghana, Retrieved from Google, September, 20, 2020. https://www.scirp.org.

Xu, P., Zuo, H., Chen, B., Wang, R., Ahmed, A., Hu, Y., & O, J. (2017). Doxorubicin-loaded platelets as a smart drug delivery system: An improved therapy for lymphoma. *Scientific Reports, 7*, 426-632.

Xue, T., & Yang, L. (2021). Zeolite-based materials for the catalytic oxidation of VOCs: A mini review. *Journal of Frontier in Chemistry*, *9*. 581-751.

Yadav, H. S. K., Almokdad, A. A., Shaluf, S. I. M., & Debe, M. S. (2019). Polymer-based nanomaterials for drug delivery carriers. *Nanocarriers for Drug Delivery*, *19*, 531-536.

Yan, W., Richard, I., & Kurtuldu, G. (2020). Structured nanoscale metallic glass fibres with extreme aspect ratios. *Journal of Nature Nanotechnology*. *15*, 875–882.

Yang, R., Wei, T., Goldberg, H., Wang, W., Cullion, K., & Kohane, D. S. (2017). Getting Drugs Across Biological Barriers. *Journal of Advanced Materials*, *29*, 65-96.

Yanru, X., Mingming, Y., Liyuan, Z., Fanling, M., & Liang, L. (2017). Recent progress on nanoparticle-based drug delivery systems for cancer therapy. *Journal of Cancer biology & Medicine*, *14*(3), 228-241.

Youcef, L. D., Lopez-Galindo, A., Verdugo-Escamilla, L., & Belaroui, L. S. (2020). Synthesis and characterization of zeolite LTA by hydrothermal transformation of a natural Algerian palygorskite. *Journal of Applied Clay Science, 193*, 105-290.

Yue, B., Liu, S., Chai, Y., Wu, G., Guan, N., & Li, L. (2022). Zeolites for separation: Fundamental and applications. *Journal of Energy Chemistry*, *71*, 288-303.

Yoo, J., Park, C., Yi, G., Lee, D., & Koo, H. (2019). Active targeting strategies using biological ligands for nanoparticle drug delivery systems. *Cancers, 11*(5), 506-640.

Yun, Z., Haiping, H., Qianming, G., & Lijun, J. (2013). 1D nanomaterials: synthesis, properties, and applications. *Journal of Nanomaterials*, *2013* 1687-4110.

Zahra, R. S., Seyed, M. Z. D., & Aniseh, K. K. (2019). Two-dimensional nanomaterials, *Intech Open Access*, *85*, 923-993.

Zelle, S. G. Nyarko, K. M., & Bosu, W. K. (2012). Costs, effects and cost-effectiveness of breast cancer control in Ghana, Journal of Tropical Medicine and International Health, *17*(8), 1031-1043.

Zhang, Y., Huang, Y., & Li, S. (2014). Polymeric micelles: nanocarriers for cancer-targeted drug delivery. *Journal of AAPS PharmaSciTech*, *15*(4), 862-871.

Zhao, N., Woodle, M. C., & Mixson, J. A. (2018). Advances in delivery systems for doxorubicin, *Journal of Nanomedicine Nanotechnology*, *9*(5), 519-600.

Zhaoqing, L., Jizhen, H., Songfeng, E., Jiaoyang, L., Lianmeng, S., Cheng, Y., Fengfeng, J., & Meiyung, Z. (2020). All cellulose composites prepared by hydroxyethyl cellulose and cellulose nanocrystals through the crosslink of polyisocyanate. Journal of Carbohydrate Polymers, *15*(250), 116-919.

Zhong, S., & Pochan, D. J. (2010). Cryogenic transmission electron microscopy for direct observation of polymer and small- molecule materials and structures in solution. *Journal of Polymer Reviews, 50,* 287- 320.

Zhou, C., Alshameri, A., Yan, C., Qui, X., Wang, H., & Ma, Y. (2013). Characteristics and evaluation of synthetic 13X zeolite from Yunnan's natural halloysite. *Journal of Porous Materials*, *20*, 587-594.

Zhu, G., Qui, S., Gao, F., Wu, G., Wang, R., Li, B., Fang, Q., Li, Y., Gao, B., Xu, X., & Terasaki, O. (2001). Synthesis of aluminophosphate molecular sieve AIPO4-11 nanocrystals. *Journal of Microporous and Mesoporous Materials*, *50,* 129-40.

APPENDICES

APPENDIX A

DOXORUBICIN DRUG STOCK CONCENTRATION CALCULATION

Doxorubin Stock Concentration in vial is **50 mg/ 25 ml w/v**

- To calculate for **2 mg/ml** using $C\ 1\ V\ 1 = C\ 2\ V\ 2$

  (2 mg/50 mg) x 25 ml = **1 mg/ml**

- To calculate for **4 mg/ml** using $C\ 1\ V\ 1 = C\ 2\ V\ 2$

  (4 mg/50 mg) x 25 ml = **2 mg/ml**

APPENDIX B

DRUG LOADING DATA COLLECTION FOR LTA 60 ℃, 80 ℃ AND 105 ℃

Sample	Before Loading UV [ABS]	After Loading UV [ABS]	Differences UV [ABS]
LTA 60 ℃	2.632	0.020	2.612
LTA 80 ℃	2.632	0.024	2.608
LTA 105 ℃	2.632	0.018	2.614

APPENDIX C

DRUG RELEASE DATA COLLECTION FOR LTA 60 ℃

Time [HRS]	Absorbance [ABS]
0	0.022
0.5	0.028
1	0.030
2	0.034
3	0.080
4	0.177
6	0.047
8	0.047
18	0.006
20	0.006
22	0.005
24	0.018

APPENDIX D

DRUG RELEASE DATA COLLECTION FOR LTA 80 ℃

Time [HRS]	Absorbance [ABS]
0	0.071
0.5	0.134
1	0.153
2	0.158
3	0.113
4	0.200
6	0.037
8	0.099
18	0.019
20	0.014
22	0.008
24	0.012

APPENDIX E

DRUG RELEASE DATA COLLECTION FOR LTA 105 ℃

Time [HRS]	Absorbance [ABS]
0	0.043
0.5	0.059
1	0.092
2	0.094
3	0.060
4	0.066
6	0.050
8	0.055
18	0.012
20	0.015
22	0.006
24	0.027