A SYNOPSIS

of the Thesis

Synthesis of Nanocarriers for Stimuli responsive Drug Delivery Applications

To be Submitted
As a partial fulfilment for the award of the degree of

DOCTOR OF PHILOSOPHY

in
Chemistry

By
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December 2020

Synopsis of the Thesis

To be submitted to The Maharaja Sayajirao University of Baroda for the award of the degree of DOCTOR OF PHILOSOPHY in Chemistry.

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Title of the Thesis: "Synthesis of Nanocarriers for Stimuli Responsive Drug Delivery Applications"

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Registration No.: FOS/2041

Date of Registration: 13th February 2017

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The Thesis will be presented in form of the following chapters:

Chapter 1
Introduction

Chapter 2
β-cyclodextrin based dual-responsive multifunctional nanotheranostics for cancer cell targeting and dual drug delivery.

Chapter 3
Carbon nanotube embedded cyclodextrin polymer derived injectable nanocarrier: A multiple faceted platform for stimulation of multi-drug resistance reversal.

Chapter 4
Tumor homing dextran derived amphiphilic Self-assembling Polymer Nanoarchitectures for stimuli responsive drug delivery.

Chapter 5
Targeting the tumor microenvironment via hierarchical disassembly of curcumin vesicles: The pro-drug strategy.

Chapter: I
Introduction

Drug delivery is an interdisciplinary field of interest of pharmaceutical researchers, medical doctors and industry. A safe and targeted drug delivery will have implications for the development and success of new therapeutic strategies in cancer treatment. The researchers working on development of materials in health care sector, especially for cancer therapy are constantly facing the challenge to develop materials which can help to overcome the issue of multidrug resistance in cancer cells along with a targeted release of drug in response to stimulus. The stimuli responsive release of drug can be achieved with the assistance of stimuli responsive polymers **(figure 1)**. These polymers bear the feature of undergoing reversible changes in their microstructure in response to the changes in external environmental conditions like temperature, pH, light, ionic strength, presence of enzymes, magnetic field etc. For instance a pH responsive polymer contains pendant acidic or basic groups that either accept or donate protons in response to the environmental pH and rapid release of the drug occurs when the pH trigger point is reached, and it is ensured that the intracellular drug concentration reaches the therapeutic dose. Similarly, thermoresponsive polymers respond to the changes in the temperature.

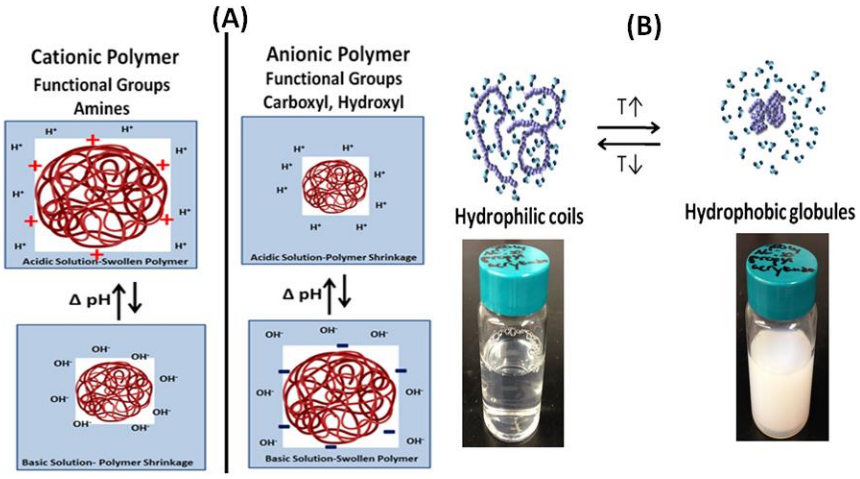


Figure 1: Illustrations of (A) pH responsive polymer undergoing swelling and shrinkage (B) thermoresponsive polymer showing coil to globule transformation in response to external environment.

Two typical phase diagrams can be used for describing the state of a polymer in solution as a function of concentration and temperature. The first type is characterized by the upper critical

solution temperature (UCST), when the transition between the single-phase and two phase regions occurs upon cooling. In the second type with the lower critical solution temperature (LCST) this transition occurs with increasing temperature. Thermally-responsive polymers undergo a coil-globule transition in aqueous solution at temperature values which is known as lower critical solution temperature (LCST). Nanoscale drug delivery devices offer an important strategy through Enhanced permeability and retention (EPR) effect. In addition, various strategies employed for active targeting of drug at the tumor sites include folate specificity, magnetic targeting, and local stimuli-triggered drug release.

In the light of these facts two different strategies were employed for development of multi-stimuli responsive and multi drug carrying nanocarriers.

1. Surface functionalized multifunctional nanoconjugates

Nanoconjugates are tailored macrostructures harboring covalently-bound biologically active modules like drugs that target specific tissues and cells.

2. Amphiphilic polymeric nanovesicles.

Polymer vesicular assemblies inspired by liposomes are promising drug carriers since they resemble the structure of the cell membrane. Supramolecular interaction using dynamic covalent bonds can help to construct the vesicles. Self-assembled amphiphilic polymers are capable of loading both water insoluble and water soluble anticancer drugs simultaneously in the hydrophobic layer and hydrophilic core respectively for combinatorial therapy. Stimuli-responsive characteristic can be introduced by suitable chemical modifications.

A development of multidrug resistance and lack of targeted drug delivery are major challenges in cancer management that has withdrawn the focus of researchers. Targeted delivery of chemotherapeutic drugs specifically towards cancer cells is one of the apparent solutions to overcome the limitations of selectivity.

Objectives

- ✓ **Synthesis of stimuli responsive nanocarriers derived from biogenic polymers.**
- ✓ **Physicochemical characterization of the synthesized nanocarriers with various spectroscopic and microscopic techniques including NMR, FTIR, HRTEM, SEM, EDX etc.**

- ✓ **Investigation of the potential of these nanocarriers for encapsulating anticancer drugs (Doxorubicin Hydrochloride & Curcumin) followed by their release in response to various stimuli (pH, temperature, enzymes) in-vitro.**
- ✓ **To demonstrate the applications of these nanocarriers as targeted drug delivery systems in various cell lines followed by in-vivo studies in mice model.**

Chapter:2

Drug resistance and mutations induced by drugs can be overcome by adapting to combination chemotherapy wherein combination of two or more drugs is employed to enhance efficacy of the therapy. Thus for the researchers associated with development of drug delivery vehicles, it is imperative to design an efficient system capable of targeting multiple drugs specifically to tumor site. This approach can assist in improving the therapeutic efficacy along with a reduction in side effects. Bearing this fact in mind, multifunctional nanoconjugates possessing an assortment of various functionalities such as magnetism, florescence, cell-targeting, pH and thermo-responsive features were developed for dual drug delivery **(figure 2).**

The novelty of this work lies in a careful conjugation of each of the functionality with magnetic iron oxide nanoparticles by virtue of urethane linkages in a simple one pot synthetic approach. β-cyclodextrin (CD), an oligosaccharide having amphiphilic properties was utilized to carry hydrophobic as well as hydrophilic drug. The ultimate goal is simplified synthesis of multifunctional nanoconjugates with therapeutic and diagnostic capabilities equipped with features for targeted stimuli responsive release of multiple drugs with following features:

1. Several β-cyclodextrin (CD) units to carry high payload of both hydrophilic and hydrophobic anticancer drugs and dual delivery for use in combination chemotherapy.
2. CD modified by Maleic anhydride and Poly (N-Isopropylacrylamide) (NIPAM) for pH and thermo-responsive drug release.
3. Fluorescein for monitoring cellular uptake.
4. Folic acid for targeted drug delivery.
5. Superparamagnetism for control of intracellular movements for final clearance from the body.

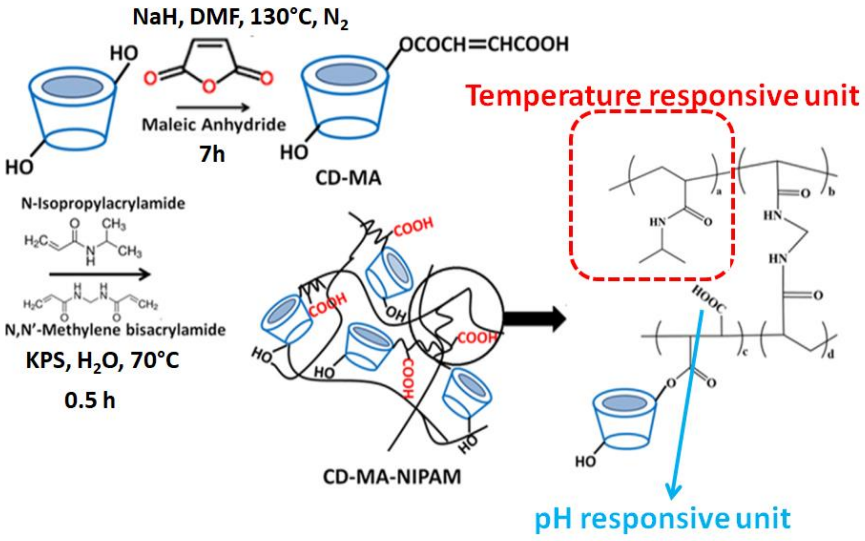


Figure 2: Schematic for synthesis of B-cyclodextrin derived pH thermal dual responsive polymer

Curcumin and DOX.HCl were used as model drugs to assess the dual drug delivery by the nanoconjugates **(figure 3).**

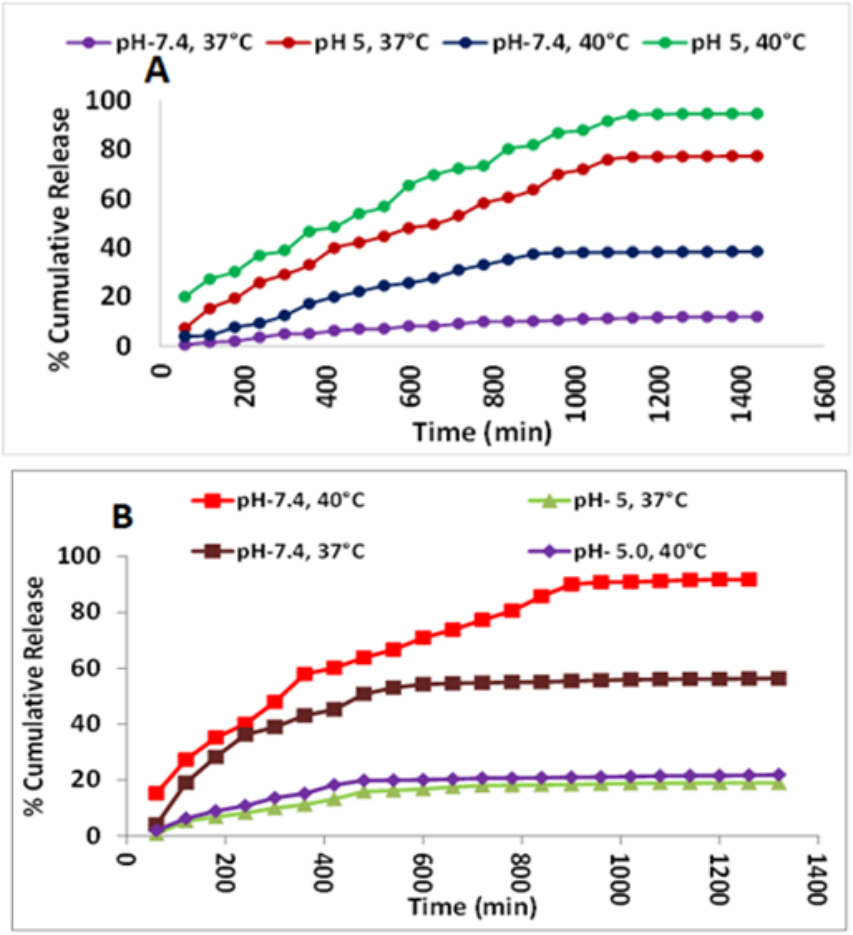


Figure 3: Cumulative drug release profile of (A) Doxorubicin hydrochloride and (B) Curcumin from the nanoconjugates

Curcumin is a potent anticancer agent devoid of side effects but is hydrophobic in nature and hence has poor bioavailability. Encapsulation in the hydrophobic cavity of cyclodextrin can enhance its bioavailability. *In-vitro* investigations carried out using cancer cell lines established the utility of the materials for targeted delivery **(figure 4)**.

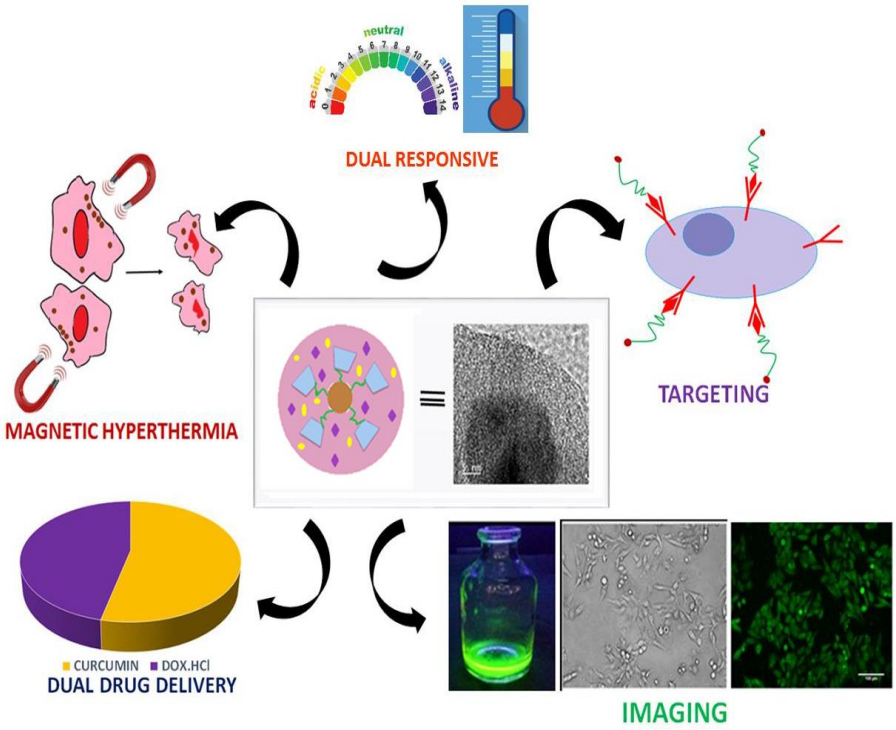


Figure 4: Multiple facets of therapy and diagnosis that can be achieved by employing the synthesized carriers for cancer management

Chapter 3

Carbon nanotube (CNT) embedded cyclodextrin polymer derived injectable nanocarrier: A multiple faceted platform for stimulation of multi-drug resistance reversal.

A combination of cocktail chemotherapy (CCT), photothermal therapy (PTT) and inhibition of angiogenesis was investigated as an effective approach to combat major challenges of multidrug resistance and non-targeted drug delivery encountered in conventional cancer therapy **(figure 5&6)**. For this the dual stimuli responsive polymer was conjugated to CNTs. The resulting nanocarriers were characterized by various spectroscopic and microscopic techniques.

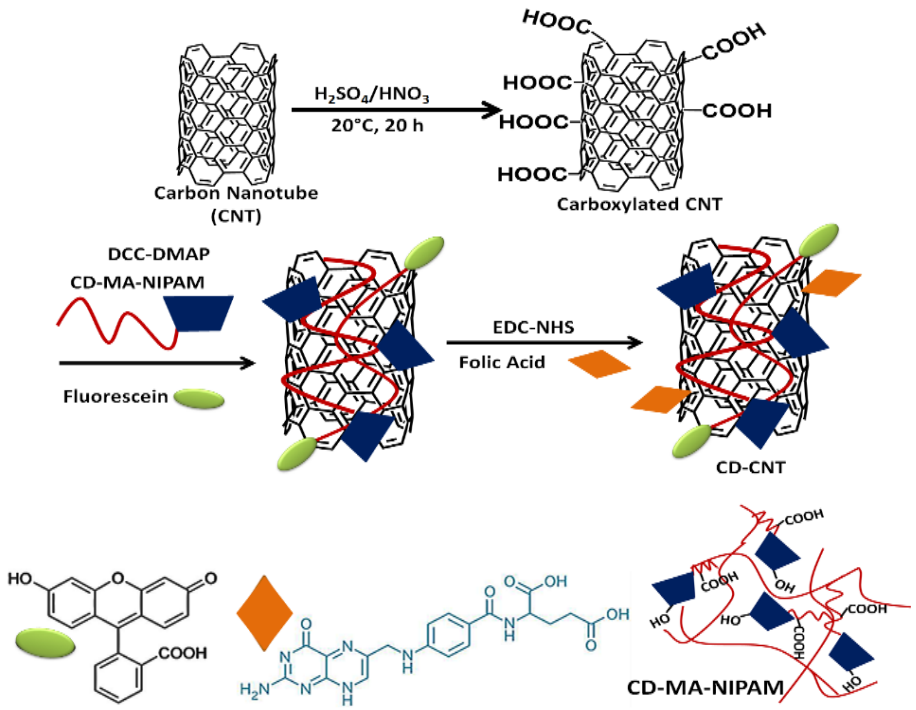


Figure 5: Schematic for the synthesis of CNT derived multifunctional nanoconjugates.

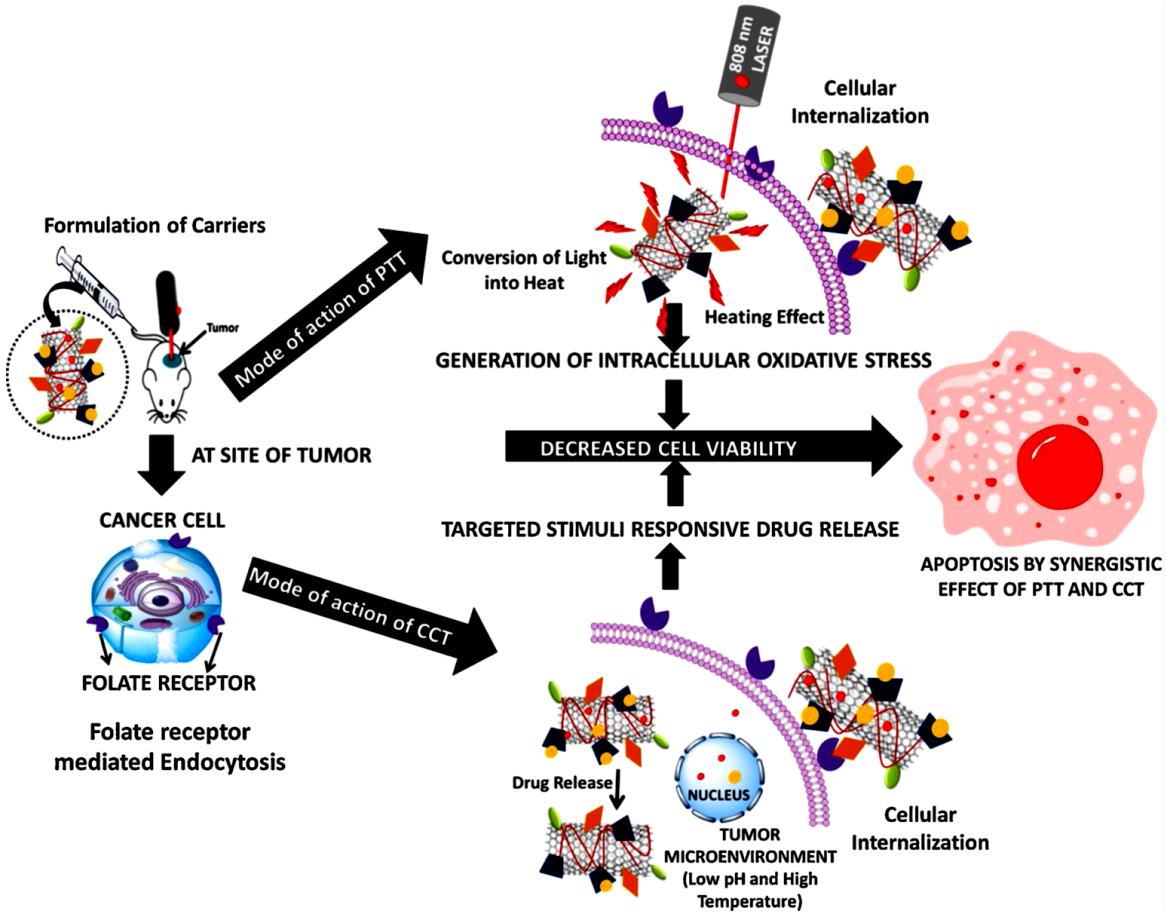


Figure 6: Probable mechanism of action of the nanoconjugates in inducing cell death at the cellular level.

They exhibited multidrug delivery with high magnitude of drug encapsulation efficiency (>90%) and a sustained release over 30 h under tumor microenvironment stimulations and having potential of mild photothermal therapy.

To the best of our knowledge, the strategy of chemo-photothermal combination therapy in amalgamation with angiogenesis inhibition has not been reported. The excellent antiangiogenic potential of the carriers loaded with both DOX and Curcumin has been demonstrated ex-ovo by CAM (Chorioallantoic membrane Assay) for the first time. The in-vivo experiments demonstrate that the injectable formulation of these carriers have a profound influence on the decrement of MMP-9 which is associated with tumor progression and metastasis. This shows the potential of the carriers to combat cancer by the synergistic effect of combinatorial CCT-PT and anti-angiogenesis.

Chapter 4

Tumor homing dextran derived amphiphilic Self-assembling Polymer Nanoarchitectures for stimuli responsive drug delivery.

The pharmaceutical industries associated with designing new drug molecules for cancer treatment are constantly facing the issue of poor therapeutic performance. Even after investing huge amount of time (nearly 20 years) and capital (approximated cost of 500 million USD) for development of new drug molecules the issues of poor drug solubility, bioavailability and adverse side effects still remain a matter of concern to researchers. Nanotechnology derived formulations for delivering existing drugs thus emerged as a promising strategy to improvise their efficacy. Despite of various advancements in the design of synthesis, such materials bear certain disadvantages like residual toxicity. These challenges led the researchers towards the pursuit of developing biomaterial derived “smart polymeric nanocarriers”. Owing to their wholesome non-toxicity, these bio-macromolecular carriers have emerged as much efficient drug carriers. This argument is supported by the fact that out of various nanomedicines approved by regulatory bodies for cancer management, approximately 40% are either protein polymer conjugates or liposomes (Eg. Doxil, which is a liposomal formulation of Doxorubicin). Thus the development of such bio-macromolecular smart polymers with improvised efficacy and their effective clinical translation are the key steps in repositioning various anticancer drugs for more patient compliant treatment.

Towards this goal, we have strategized the synthesis of dextran derived random amphiphilic co-polymers **(figure 7)**. Amphiphilic polymers possess stimuli responsiveness due to the

presence of both hydrophilic and hydrophobic monomers in their skeleton. Additionally, they can self-assemble in into various nanosized morphologies by the virtue of various functionalities associated with the respective monomers. Interestingly such architectures are promising candidates as drug carriers owing to their long term stability, enhanced drug loading and excellent permeability.

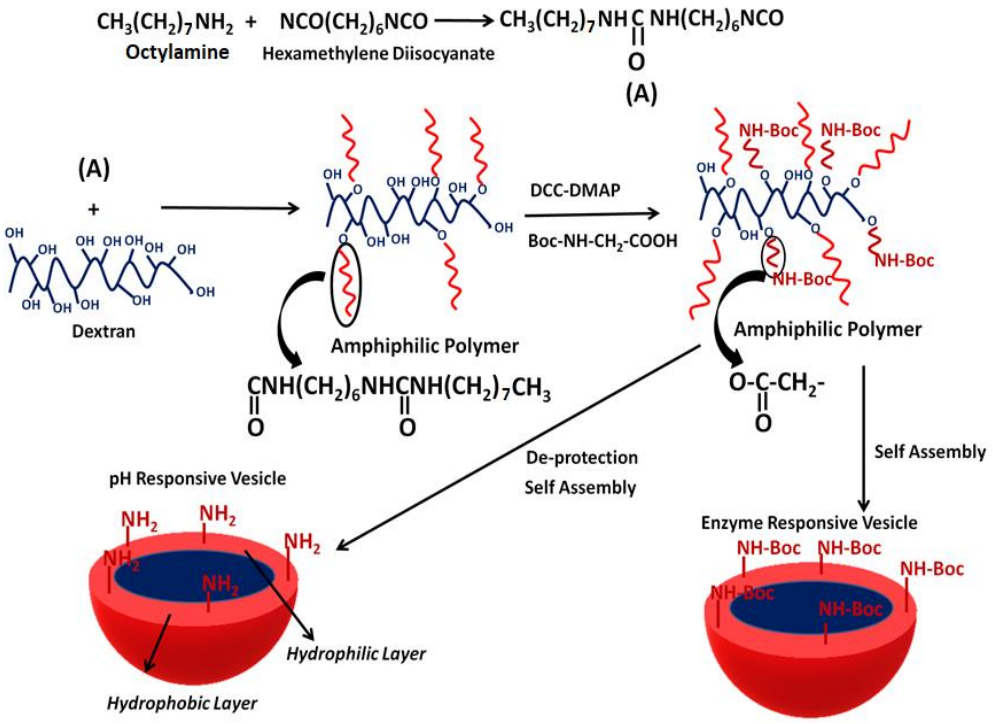
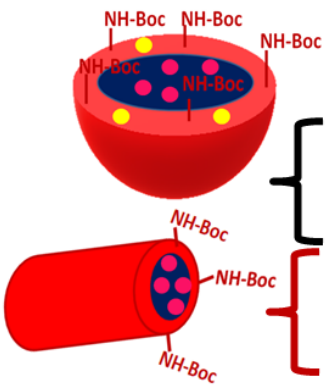
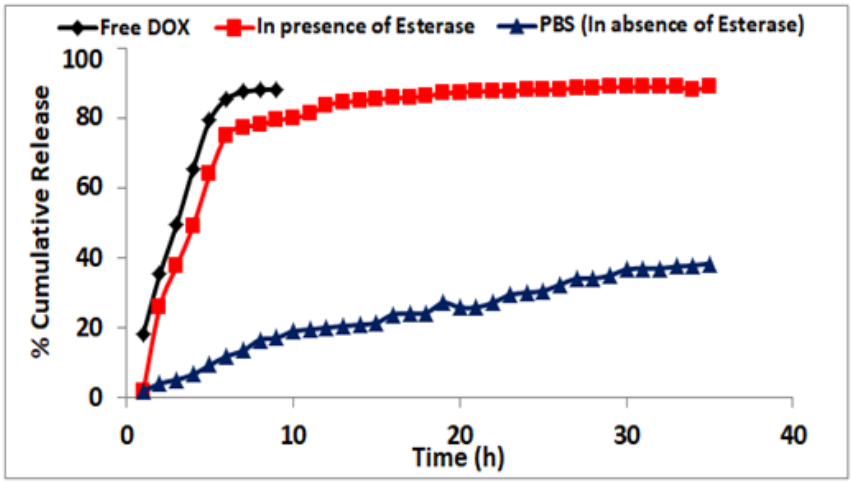


Figure 7: Schematic for the synthesis of amphiphilic polymer and their subsequent self assembly into spherical vesicles.

Two different polymers were synthesized having dextran as the hydrophilic component, one of the polymer had octylamine as the hydrophobic unit whereas in the other curcumin was selected as the hydrophobic unit. Moreover both these polymer variants were conjugated with amino group protected glycine that rendered dual (pH and enzyme) responsivity to the carriers. The variation of the hydrophobic component demonstrated interesting diversity in size which in turn affected both the drug loading and release behaviours. The octylamine system exhibited spherical vesicles and curcumin system produced tubustecan vesicles. The polymers were characterized by various spectroscopic and microscopic techniques whereas the vesicular morphology was established with FTIR, fluorescence, dynamic light scattering, and electron microscopic studies.



Carrier	% EE	%LC	% EE	%LC
	DOX.HCL		Curcumin	
$DEX\text{-}OA_{PR}$	40	3.9	36	3.6
$DEX\text{-}OA_{ER}$	42	4.2	38	3.9
$DEX\text{-}CUR_{PR}$	53	5.2	-----	-----
$DEX\text{-}CUR_{ER}$	55	5.0	-----	-----



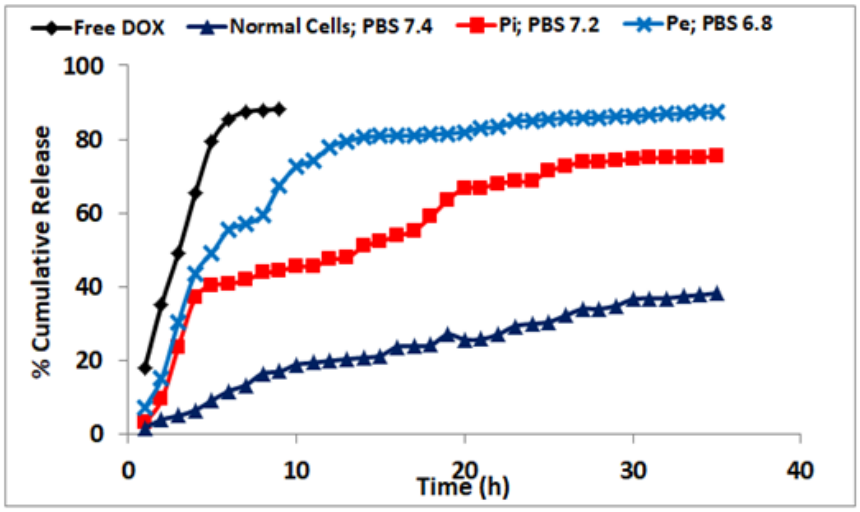


Figure 8: Drug loading and release behaviour from the nanoarchitectures.

Due to tubular morphology the curcumin derived vesicle was capable of encapsulating more cargo of drug as compared to spherical ones **(figure 8)**. Further they also demonstrated a "needle like cellular internalization" thus accounting for a more pronounced cell death in vitro and enhanced tumor regression in vivo.

Chapter 5

Targeting the tumor microenvironment via hierarchical disassembly of curcumin vesicles: The pro-drug strategy.

The efficacy of drug delivery gets drastically affected in the body as the carrier has a tendency to face several barriers. Certain barriers include rapid drug leakage in-vivo, insufficient circulation of blood, limitations encountered by the carrier in tumor penetration etc. Thus it becomes imperative to rationally develop sophisticated delivery systems that can transform via adaptation towards physiological conditions and in response to tumor related stimuli (pH, temperature, enzymes etc.). These types of delivery agents ensure a targeted drug release and with appropriate designing strategy they can also be made to carry multiple drugs thus eliminating the issue of multidrug resistance.

Hence a rational pH sensitive and hierarchically degrading vesicle containing labile groups was synthesized. The presence of pH gradient inside various cell organelles of the cancer cells was employed to attain a desirable degradation and subsequent drug release. A pro-drug strategy was employed for further enhancing the pharmacological response of curcumin by incorporating it in the polymeric backbone via appropriate chemical modifications.

The hydrophilic end of the copolymer was functionalised with active cancer targeting ligand Biotin which binds strongly to the biotin receptors widely over-expressed on cancer cell surfaces. The resulting amphiphilic copolymer can self-assemble into a stable vesicle in an aqueous environment, acting as an efficient nanocarrier for other cancer chemo-therapeutic drugs (e.g. Doxorubicin Hydrochloride). As a result, both the chemotherapeutic agent (DOX.HCl) and the chemo sensitizer (Curcumin) can be efficiently delivered into the target cancer cell at the same time.

Conclusion

The objective of the work was to develop some nanocarriers which can carry high payload of cancer drug and trigger an on demand delivery. Several stimuli responses such as pH, temperature, light and enzyme alone and in combination were taken into account for combinatorial therapy. Targeting ligands and pro-drug strategy were employed for delivery of solitary/multiple drugs to address MDR issue. Efforts were dedicated to enhance the bioavailability of the natural anticancer agent curcumin. All results were evaluated in vitro on various anticancer cell lines (MCF-7 breast cancer & Hela cervical cancer cell lines) as well as in vivo rat model induced with liver cancer. Thus the anticancer potential of the nanocarriers was demonstrated and validated as desired.

References

1. Jayakannan, M. et al. *Biomacromolecules*, 2017, *18*, 113
2. Zhang et al. ACS Applied Materials & Interfaces, 2017, 9, 9986
3. Chattopadhyay et al. *ACS Appl. Bio Mater.*2019, 21, 533
4. C. Du, Y. Ding, J. Qian, R. Zhang and C. Dong, *J. Mater. Chem. B*, 2019, 3451
5. Thakore, S. et al.*Carbohydrate Polymers*, 2019, 206, 694
6. Shunmugam, R. et. Al.*ACS Appl. Nano Mater.*, 2020, 3, 2104

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