Abstract

This thesis entitled "**Controlled drug delivery using layered double hydroxides**" focuses on the design and development of layered double hydroxides based nanocarriers for delivery of anticancer drug and plasmid DNA vectors for cancer treatment.

Chemotherapy has become an integral part of cancer treatment in recent years. Conventional chemotherapy is based on killing of cancer cells or inhibition of their growth and reproduction. However, during the process of killing of cancer cells, chemotherapeutic agents also damage healthy tissues, causing systemic toxicity and adverse side effects such as hair loss, loss of appetite, nausea, vomiting, anemia, fatigue, loss of taste buds, destruction of the immune system etc. It is therefore desirable to develop chemotherapeutics that can either passively or actively target cancerous cells to reduce the adverse side effects while improving therapeutic efficacy of anticancer agents. Nanocarriers can passively target cancer cells through enhanced permeation and retention (EPR) effect, prolong circulation time of the loaded therapeutics, enhanced bioavailability and minimize undesired toxic effects to healthy tissues.

Layered double hydroxide (LDH) emerges recently as a promising two dimensional (2-D) nanocarrier due to its unique physical and chemical properties such as excellent anion exchange capacity, excellent biocompatibility, high drug loading efficacy, full protection of the loaded therapeutics, excellent endosome escape, stability in wider pH range, ease of preparation, low cost and biodegradability. LDHs consist of layers of a divalent metal cation such as Mg^{2+}, Zn^{2+}, Ca^{2+}, Ni^{2+}, etc., with a trivalent metal cation isomorphically substituted to give the layers a net positive charge. This extra charge is counter balanced by interlayer hydrated exchangeable gallery anions, such as Cl^{-}, NO_3^{-}, CO_3^{2-} etc. Anionic therapeutic molecules (negatively charged drugs, genetic materials, peptides, proteins etc.) can easily be intercalated into the interlayer gallery, which provides full protection against enzymatic

degradation during flowing in biological fluids and can deliver therapeutics to the targeted site. Again, theses positively charged LDH nanoparticles can easily penetrate the cellular membrane and therefore has the potential to serve as efficient intercellular delivery vehicle for drugs and nucleic acids.

In this research work, various LDH based nanocarriers have been designed and developed for controlled and safe delivery of anticancer drugs and plasmid DNA vector for cancer treatments. Detailed investigations on these developed materials have resulted in several new important findings. The important results obtained are discussed below:

A series of Mg-Al based LDHs with varying interlayer anions (NO_3^-, CO_3^{2-} and PO_4^{3-}) have been synthesized followed by successful intercalation of a model antitumor drug (raloxifene hydrochloride) into the interlayer gallery of LDHs. *In vitro* controlled drug delivery has been achieved with very fast rate in phosphate bound LDH-drug (LP-R) while sustained profile is obtained with nitrate based LDH (LN-R). *In vitro* anticancer efficacy studies indicate that drug intercalated LDHs can efficiently inhibit the growth of HeLa cells as compared to pure drug. Enhanced *in vivo* tumor suppression efficacy has been achieved in tumor bearing Balb/c mice using drug intercalated LDHs as compared to free drug. Body weight index, histopathological findings and biochemical parameter analyses strongly suggest damaged organs of mice treated with pure drug while no obvious damage has been found in mice treated with drug intercalated LDHs.

In another work, a new anticancer drug delivery vehicle has been developed after intercalating anticancer drug within the interlayer galleries of Zn-Fe based LDHs followed by embedding them in a polymer matrix to obtain polymer nanoconjugate (PN–R). *In vitro* controlled drug delivery has been achieved using drug intercalated LDHs which has been sustained further in the polymer nanoconjugate. Comparative *in vitro* cytotoxicity studies reveal that PN–R has much better *in vitro* anticancer efficacy as compared to pure drug or

drug embedded polymer conjugate (PCL-RH). Cell adhesion behavior in PN−R is found to be superior as compared to pure polymer or even from pure LDH systems. Polymer nanoconjugate also exhibits better cellular uptake than that of pure drug and pure LDH. Sustained release of drug for prolonged period has been obtained *in vivo* system using albino rats showing healthy lever and other body parts using polymer nanohybrid against damaged lever using pure drug as evident from the histological observation, lever and renal functional tests. Thus, the developed polymer LDH nanoconjugate vehicle has been found to enhance the therapeutic efficacy of anti-cancer drug while reducing its adverse side effects.

Li-Al based LDH has been synthesized for the delivery of plasmid gene into mammalian cancer cell. Li-Al based LDH demonstrates high loading capacity of DNA and can release the loaded DNA in a controlled manner. The developed gene delivery carrier has been found to provide remarkable protection against DNase I and also can protect the vector from thermal damage. This vehicle also demonstrated excellent cellular uptake performances. Successful gene transfection has been achieved by using the developed LDH based nanocarrier. Further, it is also found that the developed Li-Al LDH has efficiently induced GFP-p53 mediated apoptosis in HeLa cells to prove its efficacy

The thesis is divided into six chapters.

Chapter I includes literature review on the significance of controlled drug delivery systems and their structural characteristics mentioning the improvement of therapeutic efficacy.

Chapter II describes the experimental section, methodology used to prepare various LDHs and characterization details.

Chapter III deals with the synthesis and characterization of Mg-Al based LDHs and tailoring of drug release rate for effective cancer treatment.

Chapter IV describes the development of layered double hydroxides-polymer nanoconjugate for the enhancement of cellular uptake and controlled delivery of hydrophobic anticancer drug.

Chapter V deals with the development of Li-Al based LDHs as gene delivery vehicle for cancer treatment.

Chapter VI includes the conclusion and future scope of the present work.

List of Publications:

1. Layered double hydroxides as effective carrier for anticancer drugs and tailoring of release rate through interlayer anions; **Sudipta Senapati**, Ravi Thakur, Shiv Prakash Verma, Shivali Duggal, Durga Prasad Mishra, Parimal Das, T. Shripathi, Mohan Kumar, Dipak Rana, Pralay Maiti; **J. Control. Release 2016,224, 186-198.**
2. Engineered Cellular Uptake and Controlled Drug Delivery Using Two Dimensional Nanoparticle and Polymer for Cancer Treatment
   Sudipta Senapati, Arun Kumar Mahanta, Dipak Rana and Pralay Maiti
   (**Communicated**)
3. Layered Double Hydroxides as an effective Gene delivery carrier; **Sudipta Senapati** and Pralay Maiti **(Manuscript under preparation)**.
4. Biodegradable poly (ε-caprolactone) as controlled drug delivery vehicle of vancomycin for the treatment of MRSA infection; Alok Rai, **Sudipta Senapati,** S K Saraf and Pralay Maiti; **J. Mater. Chem. B 2016, 4, 5151-5160**.
5. Controlled drug release through regulated biodegradation of poly (lactic acid) using inorganic salts; Sunil Kumar, Shikha Singh, **Sudipta Senapati**, Akhand Pratap Singh, Biswajit Ray and Pralay Maiti; **Int. J. Biol. Macomol.** 2017,104, 487-497.
6. Novel shape memory behaviour in IPDI based polyurethanes: Influence of nanoparticle
   Satyam Srivastava, Arpan Biswas, **Sudipta Senapati**, Biswajit Ray, Dipak Rana, VK Aswal and Pralay Maiti; **Polymer 2017, 110, 95-104.**

7. Optical Studies of Poly (9, 9-di-(2-ethylhexyl)-9H-fluorene-2, 7-vinylene) and its Nanocomposites; S Layek, Mihir Ghosh, KS Reddy, **Sudipta Senapati**, P Maiti and Subrata Sinha; **J. Appl. Spectrosc. 2015, 82, 868-874**.

Book Chapters:

1. Crystal-Melt Phase Change of Food and Biopolymers, Glass Transition and Phase Transitions in Food and Biological Materials; **Sudipta Senapati**, Dipak Rana and Pralay Maiti; **John Wiley & Sons, March 2017.**