Chapter 9

SUMMARY AND CONCLUSIONS

I am turned into a sort of machine for observing facts and grinding out conclusions.

Charles Darwin
9.1. Introduction

As stated earlier, cancer is one the most dangerous diseases that cause a high mortality rate in the world. Some conventional therapies are in practices that treat cancer when diagnosed earlier. These therapies include chemotherapy, radiotherapy, biopsies etc. However, these treatments have numerous side effects as they are not locally operated. They cause a serious harm to the normal cells along with destruction of cancerous cells.

Magnetic fluid hyperthermia (MFH) is a modern concept of cancer hyperthermia therapy utilizing MNPs for to treat a tumor. MNPs are locally injected to a tumor and given external AC magnetic field so that the MNPs start to vibrate and generate heat. Rise in temperature in the range of 42-44°C kills tumor cells and is harmless for the normal cells.

9.2. Competent Components of Thesis

Fe$_3$O$_4$ MNPs are of great interest for biomedical applications owing to their small size, superparamagnetic behavior, biocompatibility and low cytotoxicity. Hence superparamagnetic Fe$_3$O$_4$ MNPs were chosen for the proposed study. They were synthesized using alkaline precipitation method, which is a slight modification of conventional co-precipitation method. The synthesized MNPs were with desirable properties to be used for hyperthermia therapy, viz. size below 20 nm, high magnetization value, superparamagnetic behavior, high SAR value and low cytotoxicity.
As synthesized iron oxide MNPs were coated with a biocompatible biopolymer, chitosan. The effect of coating of chitosan on MNPs was thoroughly studied. It shows increases colloidal stability than that of bare MNPs. Also, increase in SAR value (118.8 Wg\(^{-1}\)) and lowered cytotoxicity made the CS coated MNPs more suitable for hyperthermia therapy application than the bare ones.

Again, bare iron oxide MNPs were coated with acrypol, which is nothing but polyacrylic acid. Capping of MNPs with AP lead to a dramatically increase in colloidal stability of MNPs. Here also increase in SAR values (95.8 Wg\(^{-1}\)) and lowering of cytotoxicity were obtained after AP coating on MNPs.

Oleic acid coated MNPs are most extensively studied for biomedical applications. However, a disadvantage related with OA is its hydrophobicity, due to which OA coated MNPs show no dispersion stability in water and other physiological media. Hence, in order to render them hydrophilicity, OA coated MNPs are further capped with CS. Resulting MNPs showed highly water dispersibility confirming hydrophilic nature. These particles also showed increased SAR value (113.4 Wg\(^{-1}\)) and low cytotoxicity than that of bare MNPs.

Hence, the study proved that the bare as well as CS, AP and OA-CS coated Fe\(_3\)O\(_4\) MNPs have a great potential to be used for hyperthermia therapy application, owing to their nano size, high magnetization and SAR values, superparamagnetic behavior, high colloidal stabilities and negligible cytotoxicity.
The flow chart for the synthesis of Fe$_3$O$_4$ MNPs via alkaline precipitation and their surface functionalization for hyperthermia therapy application are shown below:

- **Synthesis of Fe$_3$O$_4$ NPs by alkaline precipitation method**
- **Surface functionalization of Fe$_3$O$_4$ NPs with Chitosan, Acrypol and Oleic acid-Chitosan**
- **Study of effects of coating on structural, magnetic and colloidal properties of Fe$_3$O$_4$ NPs**
- **Study of effects of coating on induction heating ability of Fe$_3$O$_4$ NPs**

**Achievements**

- Superparamagnetic Fe$_3$O$_4$ NPs were prepared using a simple method of alkaline precipitation.
- Surface functionalization of Fe$_3$O$_4$ NPs was done with Chitosan, Acrypol and Oleic acid-chitosan.
- Hydrophobic oleic acid coated Fe$_3$O$_4$ NPs were further capped with chitosan to impart hydrophobicity to them.
- Surface modified MNPs showed improved colloidal stability and SAR value and lowered cytotoxicity.

**Surface functionalized Fe$_3$O$_4$ NPs showed higher potential to be used for hyperthermia therapy application owing to their superparamagnetic behavior, high magnetization, improved colloidal stability, increased SAR values and lower cytotoxic effects.**
9.3. Major Conclusions

- Fe$_3$O$_4$ NPs were successfully synthesized by alkaline precipitation method.
- Co-precipitation method was more simplified and made cost effective for synthesis of MNPs.
- Synthesized Fe$_3$O$_4$ NPs fulfilled all the requisites to be used for cancer hyperthermia therapy application which include particle size less than 20 nm, high magnetization, superparamagnetism, colloidal stability, high SAR value and non-cytotoxicity.
- The surface of Fe$_3$O$_4$ NPs was modified successfully with the CS. Nanofluids of CS coated Fe$_3$O$_4$ NPs were successfully prepared in water for biomedical applications showed improved colloidal stability and lower cytotoxicity.
- The surface of Fe$_3$O$_4$ NPs was modified successfully with the AP. Nanofluids of AP coated Fe$_3$O$_4$ NPs were successfully prepared in water for biomedical applications showed improved colloidal stability and lower cytotoxicity.
- Hydrophobic OA- Fe$_3$O$_4$ NPs were rendered hydrophilicity by further attachment of CS forming core-shell structure.
- The aim of attaining the high SAR and maximum temperature rise at relatively low concentration of NPs was successfully attempted with the Fe$_3$O$_4$ NPs within the biological safety and physiologically tolerable range.
- Under induction heating experiments, it is found that the hyperthermia temperature (42-44°C) cannot be achieved at concentration 2 mg mL$^{-1}$ of bare
MNPs under applied field of 167.6 Oe while it is achieved at the applied field of 251.4 Oe.

- The SAR value can be increased by surface functionalization of Fe₃O₄ NPs with CS, AP and OA-CS. The maximum SAR is obtained at 335.2 Oe for CS coated MNPs was 118.85 Wg⁻¹, for AP coated MNPs it was 95.8 Wg⁻¹ and for OA-CS coated MNPs it was 113.4 Wg⁻¹.

- *In vitro* cytotoxicity study of Fe₃O₄ NPs on L929 cell line using MTT assay for 48 h of incubation showed the non-cytotoxicity with the concentration up to 10 mg mL⁻¹.

- Surface functionalization of Fe₃O₄ NPs with biocompatible polymers lowered their cytotoxicity making them more biocompatible.

### 9.4. Future Scope of thesis

From this work it is revealed that the surface modified Fe₃O₄ NPs show suitable hyperthermia temperature rise (42-44°C) and very non-toxicity with the L929 cell lines which opens the door for its *in vivo* application. *In vivo* application of Fe₃O₄ NPs will be next step to assess the biological performance of the NPs. Also, acrypol coated MNPs show very promising results and hence need to study further for their different biomedical applications.