SUMMARY AND CONCLUSION

Combined drug loaded chitosan and alginate-chitosan nanoparticles were prepared by ionotropic gelation method. Quantitative analysis of INH and PYZ was performed by preparing the calibration curve in phosphate buffer (pH 7.4) in concentration range of 2-10 µg/ml at 262 and 268.5 nm respectively. Isobestic point (252 nm) was determined for simultaneous estimation of drugs (INH and PYZ) from combine drug dosage form. The isobestic point was estimated from overlay spectrum of both the drugs in concentration range 2-10 µg/ml. In study 3^2 factorial design was used as an optimization tool through which effect of polymers on various characterstics of nanoparticles were studied. In formulation the effect of concentration of polymers on particle size, zeta potential, entrapment efficiency, loading capacity and *in vitro drug* release were studied.

Among the batches of chitosan nanoparticles the optimized batch (8N) showed average particle size: 414.3 ± 27.13nm, zeta potential: +26.52 ± 0.67mV, PDI: 0.296, entrapment efficiency: 55.29 ± 0.06% and 63.14 ±0.29%, loading capacity 11.99 ±0.78% and 14.6 ± 0.24% for INH and PYZ respectively. The *in-vitro* drug release was performed in 0.1 N HCl (1.2 pH) for 2h and in phosphate buffer (7.4pH) for 14h. The cumulative release of INH and PYZ from optimized batch (8N) was 33.55 ± 1.09 and 55.21 ± 0.87% respectively at 1.2 pH for 2 h while the cumulative % release was found to be 84.82± 2.54 and 61.48 ± 1.52 % of INH and PYZ at pH 7.4. The *in-vitro* drug release was best explained by zero order with fickian release at pH 7.4. The study revealed that on increasing chitosan and sodium tripolyphosphate concentration the particle size of nanoparticles increased. Increase in chitosan concentration lead to increased positivity i.e. zeta potential of nanoparticles, while increase in sodium tripolyphosphate reduced positivity i.e. zeta-potential. The increase in polymer concentrations also lead to increase in entrapment and loading efficiency to certain concentration and beyond that entrapment and loading efficiency decreases.

Among the batches of alginate-chitosan nanoparticles the optimized batch (3S) showed average particle size: 539 ± 23.3nm, zeta potential: -26.4 ± 0.55mV, entrapment efficiency

70.2 ± 0.24% and 73.4 ± 0.21%, loading capacity 23.14 ±1.23% and 19.54 ± 0.67% for INH and PYZ. The *in-vitro* drug release in 0.1 N HCl was 9.38 ± 0.92% (INH) and 8.48 ± 0.34% (PYZ) at 1.2 pH for 2h, while cumulative % release was found to be 74.53 ± 2.53% (INH) and 57.87 ± 2.04% (PYZ) in phosphate buffer (7.4pH) for 10 h. The *in vitro* drug release was best explained by first order with Fickian diffusion. The study revealed that increase in chitosan and sodium alginate concentration lead to increase in particle size of nanoparticles. On increasing the chitosan concentration, the negativity i.e. zeta potential of nanoparticles decreased, while negativity of particles increased with increase in alginate concentration. The entrapment and loading efficiency both increased on increasing polymer concentration to certain concentration and beyond that decreased.

Both optimized batches 8N and 3S were analysed for antimycobacterial screening through MABA (Microplate alamar blue assay) at CDRI (Central drug Research Institute), Lucknow. The study concluded that sodium alginate nanoparticles had MIC_{90} at 25µg/ml.

On the basis of MABA study and ability to maintain its integrity along with entrapped drug capacity in 0.1N HCl, the 3S was considered as best batch among polymeric nanoparticles for ligand attachment i.e. to achieve specific targeting towards macrophages. The folic acid /D-mannose ligands were incorporated in sodium alginate nanoparticle and further evaluated for macrophage phagocytosis and cytotoxicity test at Aakaar biotech Private limited Laboratory, Lucknow and IITR (Indian Institute of Toxicological Research), Lucknow respectively. The results of uptake study and toxicity study concluded that folic acid decorated INH and PYZ loaded sodium alginate nanoparticles at concentration of 100µg/ml, incubation time 96 h showed significant macrophage phagocytosis and cell viability. The stability study of INH and PYZ loaded and folic acid ligand decorated (3S2) alginate chitosan nanoparticles showed more stability at 5 ± 3°C compared to storage at room temperature for 6 month.

PUBLICATIONS

- Kalpana, Dwivedi Harinath. 2018. Fabrication and characterization of chitosan nanoparticles: A controlled release approach towards tuberculosis chemotherapy. *Drug delivery letters.*Vol.8, 209-216.

- Kalpana Kushwaha and Harinath Dwivedi. 2018. Interfacial Phenomenon based biocompatible alginate-chitosan nanoparticles containing Isoniazid and Pyrazinamide. Pharmaceutical Technology. Vol 6, 209-217.

POSTER PRESENTATION

- Research paper presented on topic "*Macrophage targeting: A Novel approach for tuberculosis*" in **International Pharmaceutical Conference**, 2^{nd}-3^{rd} Feb, **2015** on **Nanoformulations and Translational Research: Small Getting Bigger** organized at **BBAU**, Lucknow.
- Presented a poster on topic "*Ligand based macrophage targeting: A novel approach*" in DBT sponsored **National seminar**, 20^{th}-21^{st} September **2016** in **Recent advances and emerging challenges in biological techniques** organized at **DGPG College**, Kanpur.
- Presented a poster on topic "*Tuberculosis: A challenges for society and healthcare system*" in **National conference** 3^{rd}-4^{th} feb **2017** in **Frontier Research in Natural Products Oppurtunities and Challenges** organized by Department of Pharmaceutical science and technologyat AKS university, Satna.
- Oral presentation on topic "Nanoparticles: New potential carrier for drug delivery system" in **Symposium on** 19^{th} August **2017** in **Trends in biosciences :Recent developments and future prospects (TRENDBIO-2017)** organized at IBSBT, CSJMU, Kanpur.

- Presented a poster on topic '*The role of nanotechnology in cancer prevention*" in **International conference**, 5^{th}-7^{th} Oct **2017** in **FAC Con -2017 International conference on fight against cancer** organized at UIHS, CSJMU, Kanpur.
- Presented a poster on topic '*Importance of Pharmacovigilance for Antitubercular Drug Safety'*, in AKTU, U.P sponsored **International seminar** on **Herbal Pharmacovigilance: Current Scenario and Future Strategies**. 31^{st} August **2018** organized by I.T.S College of Pharmacy, Delhi NCR.
- Attended AKTU sponsored **National seminar**, on 6^{th}-7^{th} October **2018** on **Empirical research on alternative medicine: Impingement on health care system**, organized by United Institute of Pharmacy, Allahabad.