4.0 SUMMARY & CONCLUSION

Fulvic and humic acids act as complexing agents similar to hydroxy-propyl-β-cyclodextrin having an exterior hydrophilic and an interior which is hydrophobic. It entraps the bioactive constituents of shilajit and is responsible for their stability in natural habitat. Humic and fulvic acid have polymeric structure having large number of voids and pores with a number of functional groups which are capable of forming inclusion complexes. The interior of these complexing agents are thus capable of forming inclusion complexes with non-polar solutes and drug molecules with low bioavailability. These drug molecules can be entrapped in the hydrophobic interior so as to increase their solubility, dissolution and stability, thereby enhancing their bioavailability.

Aspirin is a very old drug but it still has a very high market value. It possesses antipyretic, anti-inflammatory, analgesic and anti-aggregatory activity. The acetylsalicylic acid molecule has a carboxyl group and an ester group. The ester group can be easily hydrolyzed, which reduces its medical value and causes side effects in humans. A strategy was designed to inhibit the hydrolytic decomposition and at the same time enhance the dissolution of aspirin inside the void of humic acid and fulvic acid isolated from shilajit shilajit.

The method reported by Ghosal et. al., 1989 for the extraction of humic and fulvic acid was standardized. However, the method has drawback as the yield of fulvic acid obtained was very low. Also the quality was very poor and it also was not completely soluble in water. Hence an improved method for the extraction of fulvic acid using ion-exchange resins was developed and standardized. The developed method gave higher yield of pure amorphous fulvic acid. Fulvic acids are characterized by FT-IR, HNMR, and fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) in electrospray negative ion mode.

The present study was an attempt to extract fulvic and humic acids from shilajit and develop an oral dosage form of aspirin through complexation with fulvic and humic acids and compare it with HP-β-CD-aspirin complex. The aim of the complexation was to increase the solubility and dissolution rate as well as permeability of aspirin and to improve the stability of aspirin.
Following is the summary of the results obtained:

1. In solid state the complexes of aspirin with fulvic acid were prepared by three methods viz; solvent evaporation, freeze drying and spray drying whereas complexes of aspirin-humic acid were prepared by solvent evaporation and freeze drying technique. However, aspirin-HP-β-CD complex was prepared only by spray drying technique.

2. The complexes were characterized by using differential scanning calorimetry (DSC), X-Ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and proton nuclear magnetic resonance (1H NMR) methods.

3. Aspirin-fulvic acid system 1:1 spray dried complex and as aspirin-humic acid system 1:2 freeze dried inclusion complex were optimized.

4. Aspirin-HP-β-CD system 1:1 spray dried complex was optimized according to phase solubility study.

5. Inclusion complex formation resulted in the production of an amorphous powder with improved solubility, dissolution, permeability and stability of aspirin.

6. Spray dried complexes (aspirin-FA) in molar ratio 1:1 and freeze dried complex of aspirin-humic acid in molar ratio 1:2 have greater solubility, dissolution, permeability and stability of aspirin than solvent evaporated complexes.

7. Spray dried complex (1:1, aspirin-HP-β-CD) showed maximum improvement in solubility, dissolution, permeability and stability of aspirin as compared to complexes prepared with humic and fulvic acid.

8. It is evident from the results that the complexation showed a significant increase in the solubility of the drug, with the maximum increase in solubilization is observed in the case of spray dried (1:1) ASA- HP-β-CD complex (57 times), 1:1 ASA-FA spray dried complex (43 times) and 1:2...
ASA-HA freeze dried complex (12 times) as compared to aspirin alone in 0.1 N HCl.

The dissolution data indicated only 31.32% release was obtained with aspirin alone at 30 minutes and a maximum of 99.7% release was obtained from 1:1 spray dried fulvic acid complex in 25 minutes. The study clearly demonstrates that when aspirin is complexed with fulvic acid there is a significant increase in the dissolution rate of the drug.

The dissolution of the aspirin-humic acid complexes prepared by using different techniques in molar ratio 1:1 and 1:2 were shown to cause a significant increase in the release of drug. A significant release was observed by 1:2 freeze dried complex of humic acid complex as compared to aspirin alone.

Maximum release was shown by 1:1 spray dried complex of HP-β-CD among all the complexes of fulvic and humic acid.

The overall profile of ASA degradation in the complexes like fulvic acid, humic acid and HP-β-CD were studied at 40 ± 2 °C and 75 ± 5% RH for 120 days as indicated by the rate of appearance of salicylic acid. However, content of salicylic acid, 2.35%, 4.31% and 6.2% were determined after 120 days in 1:1 spray dried of ASA-HP-β-CD complex, 1:1 spray dried complex of ASA-FA and 1:2 ASA-HA freeze dried complex respectively. It was concluded that a significant improvement in aspirin stability were observed with fulvic acid, humic acid and HP-β-CD when compared to aspirin alone.

The Arrhenius plots of the stability data at the three storage temperatures for the optimized complexes of aspirin with fulvic acid, humic acid and HP-β-CD were obtained by extrapolation, the $K$ value at 25°C for the optimized complex of aspirin with fulvic acid, humic acid and HP-β-CD were found to be $7.27 \times 10^{-5}$, $9.83 \times 10^{-5}$ and $6.78 \times 10^{-5}$ which corresponds to shelf-lives of 3.98, 2.94 and 4.27 years, respectively. The results revealed that the presence of the fulvic acid, humic acid and HP-β-CD moieties as complexing agent has decreased the rate of aspirin decomposition and improved its shelf-life.
14 It was found that aspirin stability increases exponentially with decreasing pH. Additionally, the graph of log \( k_{\text{app}} \) versus pH were linear, with solutions of higher pH having higher \( k_{\text{app}} \) values, therefore lower stability. Maximum log \( k_{\text{app}} \) was observed with aspirin alone, however it were decreased with complexation with fulvic acid, humic acid and HP-\( \beta \)-CD. So, stability were improved after complexation.

15 Complete degradation (100 %) was observed in case of hydrogen peroxide hydrolysis. Spray dried (1:1) fulvic acid complex was found to be stable as compared to aspirin alone. However, spray dried (1:1) HP-\( \beta \)-CD complex provide maximum protection against forced degradation.

16 The permeation of aspirin from aspirin-fulvic acid complex (1:1) prepared by spray drying was found to be significantly higher (about 8 times) as compared to aspirin alone. However, maximum permeability was observed with aspirin - HP-\( \beta \)-CD (1:1) spray dried complex, about 9 times more as compared to aspirin alone.

17 The anti-inflammatory effect of aspirin caused an inhibition of 22.92 % after four hours while their optimized complexes of fulvic acid, humic acid and HP-\( \beta \)-CD inhibited edema 35.4%, 60.4% and 70.8% respectively after four hours of treatment. However, maximum of 93.3% of inhibition was observed by spray dried aspirin-FA (1:1) at 3 hours. It was concluded that humic and fulvic acid reduces inflammation and can be used as complexing agent comparable to cyclodextrin.

18 The spray dried complex prepared with fulvic acid (1:1) gave the lowest score of ulcer index; 0.48 ± 0.08 as compared to aspirin alone 1.12 ± 0.08. Spray dried complex of aspirin with HP-\( \beta \)-CD (1:1) showed an intermediate effect (score: 0.53 ± 0.16). Freeze dried complex of aspirin with humic acid in the molar ratio 1:2 also showed significant reduction (0.63 ± 0.10) in ulceration as compared to aspirin alone.

19 Histopathological sections of stomach mucosa: Group I- Control (1% CMC)-- shows normal mucosa with no ulcer in the epithelium lining Group II- Aspirin – A number of hemorrhagic lesions could be clearly seen Group III- Spray
SUMMARY & CONCLUSION

Dried aspirin-fulvic acid (1:1) complex does not show any lesion on stomach of rats as compared to aspirin alone. Group IV & V. No lesions could also be observed when aspirin complexed with humic acid and HP-β-CD.

20 Tablets prepared with aspirin-fulvic acid (1:1 spray dried complex) and aspirin-HP-β-CD have greater dissolution as compared to marketed formulation containing aspirin in an uncomplexed form.

21 The optimized tablets of aspirin-fulvic acid complex and aspirin-HP-β-CD were subjected to accelerated stability studies to ascertain the chemical and physical stability of formulations. The optimized tablets were kept at 40°C ± 0.5°C and 75% ± 5% RH. No significant changes in properties like hardness and disintegration time of formulation was observed.

22 Molecular modeling has shown to aspirin stable in fulvic acid, humic acid and β-cyclodextrin. It revealed that humic/fulvic acids have ability for inclusion complexation as cyclodextrin.

4.1 Achievements

- A novel complexing agent / bioavailability enhancer in the form of humic and fulvic acid was extracted from shilajit.

- Investigation with the complexation of aspirin with humic acid and fulvic acid and also HP-β-CD has been carried out successfully.

- Among the various complexes prepared, spray dried aspirin-fulvic acid (1:1), freeze dried aspirin-humic acid (1:2) and spray dried aspirin-HP-β-CD (1:1) gave the best results in terms of solubility, dissolution, permeability and stability of drug.

- The above complexation enhanced the anti-inflammatory and ulcer protection effect of aspirin as compared to aspirin in uncomplexed form.

- The optimized formulation of aspirin-fulvic acid is stable and is capable of being marketed.
Technology has been developed which can be commercialized for improved formulation of aspirin.

Fulvic and humic acids appear to have complexing properties similar to β-cyclodextrins and hence capable of being used as such.