PART I: FORMULATION BASED ON HYDROTROPIC AND COSOLVENT SOLUBILIZATION

The aim of the present study was to solubilize Sparfloxacina, an antibiotic drug (SFX); and Baclofen, an analgesic drug (BCF), both water insoluble drugs by the use of physiologically compatible hydrotropes and cosolvents and to attempt parenteral formulations.

The samples of SFX and BCF were used as received. Considering the sensitivity, accuracy and convenience, the UV spectrophotometric method was used for the analysis of SFX and BCF in solubilized systems and in rabbit plasma samples.

The six different hydrotropes namely ascorbic acid (AA), piperazine hexahydrate (PZH), sodium salicylate (SS), sodium benzoate (SB), p-toluene sulphonic acid (PTSA) and nicotinamide (NE) taken for present study were checked for their purity by, Calibration curves of these hydrotropes were prepared in water and plasma using UV spectrophotometric method.

The solubility determinations for SFX and BCF were carried out at 25±1°C and 37±1°C. The solubility increased upto 1798 times for SFX while only upto 78.2 times for BCF at 25±1°C. The solubility increased by increasing temperature from 25±1°C to 37±1°C. The solubilizing power of different hydrotropes could be ranked as:

For SFX: AA > PZH > SS > SB
For BCF: AA > PZH > PTSA > NE > SS > SB

From the solubility diagrams it was concluded that the increase in solubility is not the linear function of the hydrotrope concentration. The solubility of the drug was slowly increased by increasing the hydrotrope concentration, however, after a particular concentration (critical solute concentration, CSC) of the hydrotrope there was a sudden rise in the solubility.

To check the influence of pH on the solubility of SFX and BCF, phosphate buffer solutions of different pH (2.0, 4.0, 6.0, 8.0 and 10.0) were used at 25±1°C and 37±1°C. The results indicated that the enhancement in solubility of SFX or BCF was not entirely due to pH effect.

The solubility of SFX and BCF was also determined at 25±1°C and 37±1°C using various cosolvents namely polyethylene glycol 200 (PEG-200), polyethylene glycol 400 (PEG-400), polyethylene glycol 600 (PEG-600), glycerine (GL), dimethyl formamide (DMF), dimethyl acedamide (DMAE), isopropyl myristate (IPM), dioxan (D) and propylene glycol (PG). The solubility increased upto 203.3 and 24.82 times in case of
SFX and BCF, respectively. The solubilizing power of different cosolvents could be ranked as –

For SFX: Dioxan>DMF> DMAE >GL>PEG-600>IPM
For BCF: GL>PEG-600> PEG-400> PEG-200> PG

The increase in solubility for SFX and BCF was not the linear function of the cosolvent concentration.

To explain the mechanism of solubilization of SFX and BCF in presence of structurally different hydrotropes the chemical structures of drugs as well as hydrotropes were taken into consideration. The hydrogen bonding and charge transfer phenomenon were expected to play major role at lower hydrotrope concentration.

To justify the increase in solubility of SFX and BCF at lower hydrotrope concentration, UV spectra of both drugs (SFX/BCF) at different hydrotrope concentrations and phosphate buffer solutions of different pH were recorded. From the results of UV absorption spectra of SFX and BCF in different hydrotrope solutions, considering the value of shift in $\lambda_{max}$, either hypochromic shift or bathochromic shift, it can be due to minor electronic changes in the structure of drug molecules. There is no ground to assume any complex formation as the complex formation can be evidenced either by formation of new chromophores (appearance of a new peak) or merging of two peaks to generate a common peak.

In order to account for the sharp increase in drug solubility (after CSC) the solution properties of five different hydrotropes were studied over a wide concentration range i.e. from 0.3 to 1.5M. The various properties studied included specific gravity, relative viscosity, surface tension, refractive index, specific equivalent conductance, and diffusion rate.

The plots of specific gravity vs. hydrotrope concentration showed a negative deviation, in contrast the relative viscosity plots showed positive deviation. The surface tension studies showed the decrease in surface tension of resulting hydrotrope solution. The results of refractive index plots showed negative deviation. Similarly the discontinuities were observed in specific conductance plots. The equivalent conductance of all six hydrotropes showed gradual decrease over extended concentration range. Thus the study of above solution properties of the hydrotrope solutions indicated that the hydrotrope molecules probably associate into aggregates at a certain concentration.

The results of TLC studies revealed that the spots of solubilized products were separated into two spots, one of the hydrotrope and the other of drug (SFX/BCF). Both these spots were corresponding with the $R_f$ values of individual components. Hence, it can be concluded that there is no molecular interaction or complex formation between the drug (SFX/BCF) and hydrotrope molecules.
To confirm the hydrotropic behavior of selected agents as solubilizes their effect on macromolecular system was studied. All six hydrotropes (20% W/V) were found to inhibit the gel formation (gelatin solution 2%) when stored at 4°C for 12 h. Similarly all the six hydrotropes (20% W/V) were able to form starch paste (4% W/V suspension) in cold.

In order to further elucidate the solubilization mechanism of drug with hydrotropes the IR spectroscopic studies were performed for pure drug, physical mixture of drug-hydrotrope (1:1) and lyophilized solubilized products. The results of IR spectroscopic studies revealed that neither there was any change in any of the characteristic peaks of either drug or hydrotrope nor broadening or missing of any peak. This suggests that there is no indication for a fixed chemical interaction between the drug and any of the hydrotrope leading to complex formation.

Thus, the mechanism of solubility enhancement for SFX and BCF can be summarized as below:

**SFX** - The pH dependence was more for SFX which was seen by using different phosphate buffer solutions. From different studies it is evident that at lower hydrotrope concentration weak ionic interactions and at higher hydrotrope concentration the molecular aggregation seem to be the possible mechanism of hydrotropic solubilization.

**BCF** - The solubility of BCF was found to be pH dependent. From the different studies it is concluded that at lower hydrotrope concentration weak ionic interactions while at higher hydrotropes concentration the formation of molecular aggregates seems to be the possible mechanism of hydrotropic solubilization.

From the results of solubility determination studies aqueous injections of SFX and BCF using hydrotropes as well as cosolvents were prepared. Considering the pharmacokinetic data of SFX and BCF the selected doses for SFX and BCF were 3.5 mg/kg body weight and (1.5mg/kg body weight), respectively. In each formulation 0.1% w/v sodium bisulfite was added as an oxidant to preclude the chance of oxidation. These aqueous formulations were sterilized by filtration through 0.2µm single use membrane filter and filtered in clear as well as amber colored glass vials. The volume of injection in vials ranged between 1-5ml.

The lyophilization of the formulated aqueous injections of SFX/BCF (in hydrotrope solution as vehicle) was considered beneficial to increase their physical and chemical stability. Reconstitution with water for injection was although rapid (20 s), it yielded solutions with a slight opalescence particularly when small volumes were used. The opalescence could be removed by the use of lactose as the possible protective agent.

All the formulations were subjected to physical stability testing under different conditions to study the effect of light, depression in temperature and temperature
fluctuations on the stability of SFX and BCF in formulated injections. The results showed that most of the formulations were unaffected in respect of color stability at room temperature after storage for 45 days. The formulations stored in dark at room temperature were most stable against precipitation.

From the results of simultaneous physical stability programme the formulations producing promising results were selected for exhaustive chemical stability studies at 37±1°C, 45±1°C and 55±1°C for a period of 3 weeks. The results of chemical stability showed that the prepared formulations have the shelf-life of maximum of 196 days in case of SFX while 243 days for BCF.

On the basis of the results of physical and chemical stability testing the formulations SFXAA (20 mg/ml SFX in 0.3 M AA), SFXPZH (20 mg/ml SFX in 0.9 M PZH), SFXD (20 mg/ml SFX in 90:10v/v) and BCFAA (4 mg/ml BCF in 0.3 M AA), BCFPZH (4 mg/ml BCF in 0.3 M PZH), BCFS (4 mg/ml BCF in 0.3 M SS), GL (4 mg/ml BCF in 10:90 v/v) were selected for in-vitro and in-vivo evaluation.

These formulations were studied for the effect of dilution with normal saline and 5% dextrose solution in the range of 1:1 to 1:50 dilution and examined visually for the presence of any precipitate or microcrystals. In a parallel study the dilution effect was studied with the i.v. fluid flowing at the rate of 20ml/min. Dilutions of SFX/BCF formulations with normal saline or dextrose solutions did not result in immediate precipitation. All the formulations were observed to have better stability towards precipitate formation with normal saline than compared to 5% dextrose solution.

The plasma levels of SFX formulations (3.5 mg/kg body weight) and BCF (1.5 mg/kg body weight) were studied in rabbits after i.v. administration. In a parallel study SFX tablet (3.5mg/kg body weight, equivalent of SFX) and BCF tablet (1.5mg/kg body weight, equivalent of BCF) were administered orally. The C_{max} value for SFX ranged from 27.29 to 22.67 µg/ml as against 4.45 µg/ml on oral administration. For BCF formulation the C_{max} 7.05 to 6.10 against 1.78 µg/ml on oral administration.

Similarly the analgesic activity of different selected formulations of BCF was determined by tail flick method using analgesiometer. Formulations BCFAA, BCFPZH, BCFS and BCFGL showed higher analgesic activity than orally administered marketed suspension of BCF.

Based on the values of AUC_{0-10} it may be concluded that the SFX and BCF injection formulations have higher bioavailability as compared with the oral formulations at the same dose level. The significantly higher C_{max} values from attempted formulations appear to be therapeutically promising. It is implied that the injection
formulation of SFX and BCF should result in dose reduction and attainment of higher $C_{\text{max}}$ values as compared to conventional mode of drug administration i.e. oral route.

PART II: FORMULATION BASED ON METAL-LIGAND COMPLEXATION

The objective of the present work is two fold. Firstly, it seeks to investigate the structure and behaviour of complexes of an important broad spectrum antibiotic drug, sparflxacin (SFX) and an analgesic drug, baclofen (BCF) with some life essential metal ions viz. Fe(II), Co(II) and Zn(II), and to know the change in antibiotic and analgesic activity of drugs due to complexation with the above said metals and quantitative and qualitative analysis of these drugs using polarography and amperometry.

The polarographic (Direct current polarography, DCP and Differential pulse polarography, DPP) and amperometry methods were used for the qualitative and quantitative estimation of SFX and BCF in pharmaceutical formulations i.e. (Sparx-100mg, SFX 100mg) and (Lioresal -20mg, BCF 20mg) tablet.

In 0.1M ammonium tartrate (pH 6.0 ±0.1) SFX produces well defined polarographic wave/peak with $E_{1/2}$ /$E_p$ = -1.45/1.49 V vs. SCE. Its wave height was found to be proportional to the concentration of SFX.

The plot of E vs log i/i_d-i was a straight line showing the reduction to be reversible. The determination of SFX content of the tablet was carried on by calibration method and standard addition method.

The results of the polarographic analysis on the tablet (Sparx-100mg, SFX100mg) showed the presence of 99.95 mg of SFX per tablet, which is in good agreement as claimed by the manufacturer (100 mg/tablet).

In 0.1 M lithium chloride (pH 2.9±0.1) BCF produces well defined polarographic wave/peak with $E_{1/2}$ = -1.49 V vs. SCE. Its wave height was found to be proportional to the concentration of BCF. The plot of E vs log i/i_d-i was a straight line showing the reduction be reversible. The determination of BCF content of the tablet was carried out by calibration method and standard addition method.

The results of the polarographic analysis of the tablet (Lioresal-20mg, BCF 20mg) showed the presence of 19.92 mg of BCF per tablet, which is in good agreement as claimed by the manufacturer (20 mg/tablet).

From the data on polarographic, amperometric studies, elemental analysis and infra red measurements mentioned, it is quite clear that metal complexes of SFX and BCF show stoichiometric ratio of 1:1.
In 0.1 M KCl at pH 5.0 the metal and their complex with SFX/BCF ligand were found to be reversibly reduced involving two electrons, which was evidenced by the plot of $\log i/(i_s-i)$ vs E. The reduction was found to be diffusion controlled which was evidenced by plot $i_s$ vs $\sqrt{h} \text{corr.}(h=\text{height of mercury column})$.

On gradual increase of SFX/BCF concentration the half wave potential of metal ion shifted to more electronegative value and the diffusion current also decreased there by showing complex formation between metal (II) with SFX/BCF.

To study the composition and formation constant of the complex plots of $\Delta E_{1/2}$ (shift in the E$_{1/2}$) = $(E_{1/2})_c - (E_{1/2})_s$ against log C$_x$ (logarithm of the concentration of the ligand) was drawn. The plot was linear showing the formation of single complex species in solution. Ligand treatment of the observed polarographic data revealed 1:1 metal-SFX and 1:1 metal-BCF, complex formation with formation constants $\log \beta_1 = 3.92$ (Fe(II)-SFX), $\beta_1 = 4.0$ (Co(II)-SFX) and $\beta_1 = 4.13$ (Zn(II)-SFX) and $\log \beta_1 = 3.2$ (Fe(II)-BCF), 3.29 (Co(II)-BCF) and 3.33 (Zn(II)-BCF), respectively.

Each metal ion gives a well defined polarographic wave in 0.1 M potassium chloride (KCl) at pH 5.0. The diffusion current was found to be proportional to be its concentration. SFX/BCF does not produce any wave under said experimental conditions. The plateau potential for the polarographic wave of metal (Fe(II)) i.e. -1.60 Co(II) i.e. -1.40 and Zn(II) i.e. -1.2 V vs SCE) was applied for carrying out amperometric titration. Metal was taken as the titrate and the drug solution was taken as titrant. The current volume plots resulted in L shaped curve. The end point as located by graphical method revealed metal to drug ratio of 1:1 which is in agreement with the author’s observations on the metal:ligand complexation equilibrium using polarographic method.

This procedure has been used for the estimation of the SFX and BCF in a pharmaceutical formulation i.e. tablet Sparx-100 and Lioresal 20 mg tablet. The results of amperometric titrations were in close agreement with that claimed by the manufacture.

The ir spectra of the titled drugs and their complexes with some biometal viz Fe, Co and Zn were recorded in 4000-400 cm$^{-1}$ region. The observations of IR peaks clearly indicate the involvement of oxygen of carboxylic group and nitrogen of N-H group in complex formation.

The solubility determinations for SFX and BCF were carried out at 25±1°C in metal-ligand complex. The solubilizing power of different metal complex could be ranked as:

For SFX: Zn(II)> Co(II)>Fe(II)

For BCF: Zn(II)> Co(II)>Fe(II)
The complexes used in the present study were found effective with promising results against a number of bacteria. A good number of reproducible results have been observed. Some of the important features of the inhibition due to complexes under study have been discussed here.

The antibacterial studies on the metal-drug complexes, showed that complex of Fe(II), Co(II) and Zn(II) with SFX drug under study were found to be active against bacteria.

The reported LD$_{50}$ for SFX is 20.0 ± 12 mg/kg in mice. The LD$_{50}$ of the Fe(II), Co(II), and Zn(II) complex against Balb-c mice was found to be 21.7 ±0.24, 22.0 ±0.15, 22.5 ±13 mg/kg body weight.

The statistical treatment of the observed data i.e. standard deviation and coefficient of variation for different mice groups never exceeded 1.0 % and 2.5%, respectively. It clearly reveals the reliability of the observed data.

Analgesic activity was measured by 'tail flick' method using analgesiometer. Complexes were administered orally as suspension in distilled water. The activity was found to be in order: Zn(II)-BCF < Co(II)-BCF < Fe(II)-BCF.

The analgesic studies on the metal-drug complexes showed that complex of Fe(II), Co(II) and Zn(II) with SFX and BCF drug understudy were found to be more effective comparison to BCF.

From the results of pharmaceutical study of metal (Fe(II), Co(II) and Zn(II) with BCF it could be concluded that all the drug complexes with some life essential metal i.e. Fe(II), Co(II) and Zn(II) more effective and non-toxic in nature as compared to the respective drugs. Thus Fe(II)-SFX, Co(II)-SFX, Zn(II)-SFX, Fe(II)-BCF, Co(II)-BCF and Zn(II)-BCF may be recommended as more potent drugs in lieu of the drug taken for present study.

**CONCLUSION**

The present work was undertaken with a two fold objectives:

(i) To solubilize sparflaxacin (an antibiotic drug) and baclofen (an analgesic drug) by use of physiologically compatible hydrotropes and cosolvents and to attempt parenteral formulations; and

(ii) To investigate the structure and behaviour (in-vitro & in-vivo) of complexes of sparflaxacin and baclofen with life essential metal ions and their analysis by polarography and amperometry.
Sparfloxacin and baclofen were successfully solublized using various hydrotropes and cosolvents and their parenteral formulations were developed.

The metal complexes of sparfloxacin and baclofen having better biological activity were synthesized and successfully estimated by polarography and amperometry.

Both the approaches have excellent potential for clinical application and further extensive studies.