



Formulation and Evaluation of Novel FDCs of Antitubercular Drugs

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ABSTRACT

The objective of the present study was to formulate and evaluate novel FDCs of antitubercular drugs. The solid mixtures of rifampicin and sodium lauryl sulphate were prepared at molar ratio of 1:1 by co-grinding method. Characterization of Rifampicin-SLS mixture was performed using DSC, XRD and FTIR studies. In vitro dissolution studies were carried out in 0.1N HCl and phosphate buffer pH 6.8. Solid mixtures prepared by co-grinding method were found to be useful in delaying the dissolution of rifampicin in acidic medium in molar ratio 1:1. The release of rifampicin from novel fixed dose combination formulation was 0% in 0.1N HCl up to two hours and faster release within 15 minutes in alkaline pH was achieved, while Isoniazid was released completely within 20 minutes in 0.1 N HCl. A novel formulation comprising of rifampicin and sodium lauryl sulphate, prepared by co-grinding method proved effective to minimize the degradation of rifampicin in acidic environment by its retarded release pattern. Thus this approach is beneficial for the segregation of release pattern of rifampicin in alkaline environment and isoniazid in the acidic environment of the GI tract, which will lead to prevent the degradation of rifampicin alone & its interaction with isoniazid.

Key words: Rifampicin, Sodium lauryl sulphate, co-grinding, delayed release, isoniazid

INTRODUCTION

Tuberculosis (TB) has been declared a public health emergency by the World Health Organization (WHO). Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol were earlier prescribed as separate formulations in TB control. The emergence of multidrug-resistant TB has threatened the efforts of TB control. To overcome the treatment failure and increase the patient compliance, the world health organization (WHO) and International Union Against Tuberculosis and Lung Disease (IUATLD) recommended the use of four drug fixed dose combination (FDC)¹. However, concern has been expressed on the poor bioavailability of rifampicin from fixed-dose combination (FDC) products containing isoniazid and/or pyrazinamide. The World Health Organization (WHO) and International Union Against Tuberculosis and Lung Diseases (IUATLD) issued a joint statement in 1994 pointing out that anti-TB FDC products should only be used if the bioavailability of at least the rifampicin component has been demonstrated².

The probable reasons for the poor bioavailability of the rifampicin in FDCs formulation are attributed to the enhanced degradation of rifampicin in the presence of isoniazid^{3,4,5}, changes in crystalline form of rifampicin^{6,7,8}, drug adsorption by excipients^{9,10,11}. The mechanism of this degradation was proposed that the decomposing of rifampicin in acidic conditions in the absence of isoniazid stopped at the forma-

tion 3-formylrifamycin, while the reaction in the presence of isoniazid proceeded to form a hydrazone between 3-formylrifamycin and isoniazid. The previous studies indicate that rifampicin alone was degraded by 12.4% in an acidic medium in 1hr to 3-formylrifamycin and in the presence of isoniazid the degradation increased to 21.5% and also indicated that the degradation of rifampicin to 3-formylrifamycin is almost more than two times faster in the presence of isoniazid than that of rifampicin alone¹².

The reported research indicated that rifampicin bioavailability can be improved by preventing formation of 3-formylrifamycin. This can be achieved by segregating the release pattern of any one of the drugs to the GI tract at different sites¹³. If isoniazid is released to the intestine the problem persists with the release of rifampicin alone in the acidic environment leading to formation of 3-formylrifamycin. Whereas if rifampicin is released at intestine, its degradation alone as well as its interaction with isoniazid can be prevented. However, the problem associated with rifampicin (BCS Class II) less solubility in water and alkaline environment acts as a prime factor with its bioavailability. Therefore the present work was undertaken to develop a novel dosage form comprising rifampicin delayed release pattern (with sodium lauryl sulphate) and isoniazid immediate release pattern in order to prevent



interaction between two drugs as well as degradation of rifampicin alone in acidic medium. Traditionally, the stomach is by-passed by a pH-sensitive coating of the drug carrier matrix. However, an additional production step is needed for the coating, which results in a prolonged production time and further costs. A new way to overcome the passage through the stomach would be to develop an excipient, which interacts with rifampicin showing stability at the low pH of the stomach and drug release at the higher pH of the intestine. With this objective, the present study was undertaken to generate a novel rifampicin formulation that is cheap and consisting of commonly used biocompatible pharmaceutical excipient.

MATERIALS AND METHODS

Rifampicin and isoniazid were obtained as a gift sample from Lupin Ltd, Pune, sodium lauryl sulphate purchased from Loba chem, Mumbai. All other chemicals used were of analytical reagent grade.

Preparation of Rifampicin-SLS powder mixture

The rifampicin-SLS powder mixture was prepared by co-grinding method under solid-state conditions. Rifampicin and SLS (1:1 mole ratio) were ground using a mortar and pestle. Samples were ground for up to 30 minutes at ambient conditions. The prepared mixtures were stored at airtight containers till further use.

Powder X-ray diffraction studies

The x-ray diffraction study of rifampicin, sodium lauryl sulphate and ground mixture of rifampicin - sodium lauryl sulphate were measured on X ray diffractometer (Philips diffractometer, model pw 3710) using Cu K α radiation (tube operated at 40kv, 30mA). Data was collected over an angular range from 0 to 50 2 θ in continuous scan mode using a step size of 0.02 2 θ and a step time of 0.4 s.

Differential Scanning Colorimeter (DSC) studies

Thermal analysis of rifampicin, sodium lauryl sulphate, and ground mixture of rifampicin- sodium lauryl sulphate were recorded on a DSC (TA 60 DSC, Shimadzu Japan). The temperature axis and cell constant of DSC were previously calibrated with indium. A heating rate of 50C/min was employed over a temperature range of 25 $^{\circ}$ C-350 $^{\circ}$ C with nitrogen purging. Powder samples (2-5 mg) was weighed into an aluminum pan and analyzed as sealed with pin holes and an empty aluminum pan was used as reference.

Fourier Transform Infra-Red Spectroscopy (FTIR) Studies

Infra red (IR) spectroscopy studies of rifampicin, sodium lauryl sulphate, and ground mixture of rifampicin-sodium lauryl sulphate were recorded in a FTIR spectropho-

meter (Perkin Elmer FTIR -USA) Potassium bromide pellet method was employed and background spectrum was collected under identical conditions. Each spectrum was derived from 16 single average scans collected in the region of 400 - 4000 cm $^{-1}$ at a spectral resolution of 2 cm $^{-1}$

Preparation of Novel Rifampicin Formulation

Co grounded rifampicin-sodium lauryl sulphate mixture (equivalent to 150 mg of rifampicin) was blended with other ingredients as per the composition mentioned in Table no 1 and filled in, 0 size hard gelatin capsule.

Micrometric Properties

The flowability of rifampicin, SLS, Rifampicin-sodium lauryl sulphate and the formulation was estimated by Carr's index, Hausner ratio and the angle of repose. The loose bulk density (LBD) and tapped bulk density (TBD) of rifampicin, SLS, Rifampicin-sodium lauryl sulphate mixture and the formulation samples were determined using bulk density test apparatus. Carr's index, Hausner ratio were calculated using LBD and TBD values. The angle of repose was assessed by the fixed funnel method.

Invitro release studies

Rifampicin-sodium lauryl sulphate mixture, equivalent to 150mg of rifampicin, was taken in a basket of the USP dissolution apparatus (USP I). The stirring rate was set at 100 rpm. 0.1N HCl and pH 6.8-phosphate buffer was used as dissolution medium (900 ml) and was maintained at 37 $^{\circ}$ C. Samples of 5 ml each were withdrawn at predetermined time intervals. The collected samples were diluted suitably, and were analyzed for the rifampicin content by UV spectrophotometric at 475 nm. The volume withdrawn at each time interval was replaced with fresh quantity of the dissolution medium. In case of FDC formulation the in vitro release of rifampicin and isoniazid were determined by high performance liquid chromatography (HPLC) method.

Stability Studies of Novel Rifampicin Formulation

The stability study of formulation 1 & 2 was performed at 40 $^{\circ}$ C and 75% RH for three months. The invitro drug release was performed for 3 month.

RESULTS AND DISCUSSION

Tuberculosis is an important public health concern. Current therapeutic regimens with rifampicin, Isoniazid, Pyrazinamide, Ethambutol have proven successful in treating tuberculosis. However, therapeutic future remains problematic in some patients with relapse, multiple drug resistance and poor bioavailability of rifampicin. One of the main reasons stated for poor bioavailability of rifampicin in FDC formulation is because of degradation of rifampicin in acidic condition in the presence of isoniazid. FDC formulations that show good



dissolution show poor bioavailability or vice versa. Formulations with good dissolution ought to dissolve faster in the stomach and show significant decomposition within the period for which the drug remains in the stomach. On the other hand, formulations with poor dissolution ought to show less decomposition due to relative lower rate of dissolution, and hence a better bioavailability is seen. Minimization of contact between rifampicin and isoniazid can decrease the degradation of rifampicin. Delay of rifampicin release in the acidic medium was achieved by preparing the Rifampicin-SLS mixture in the ratio of 1:1 by co grinding method which is a relatively simple and effective method. The drug content of prepared co ground mixture was found to be in the range of 99.2 to 101.8 which indicated the satisfactory content uniformity of co ground mixture.

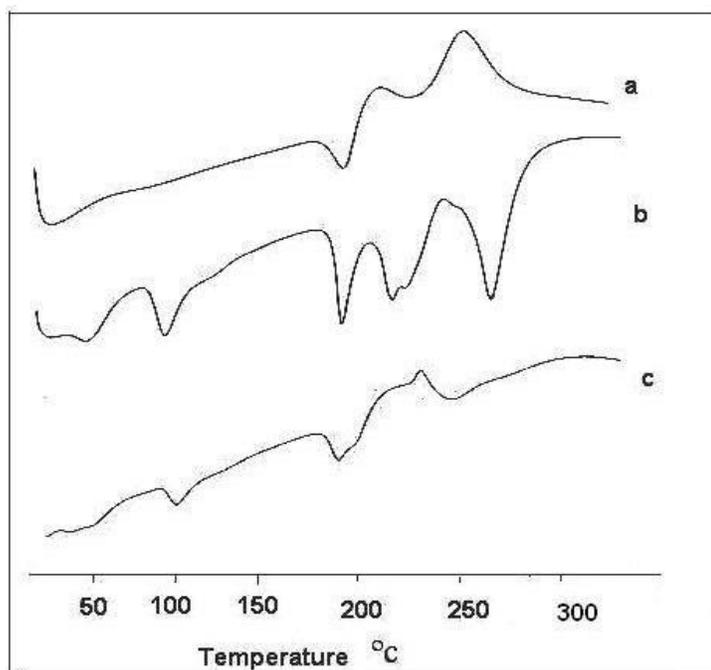


Figure.1. DSC thermo grams of (A) rifampicin, (B) sodium lauryl sulphate (C) Rifampicin- sodium lauryl sulphate mixture.

The X-ray powder diffraction pattern of pure rifampicin

cin exhibited its characteristic diffraction peaks (2 theta) at 9.33, 11.1 which indicated the presence of crystalline polymorph II of rifampicin. The X-ray powder diffraction pattern of sodium lauryl sulphate exhibited characteristic peaks at 20.59, 20.83, which revealed indicated the presence of some crystallinity. The X-ray powder diffraction pattern of ground mixture of rifampicin –sodium lauryl sulphate indicated the characteristic peaks of sodium lauryl sulphate at 20.5 while the characteristic peaks of rifampicin were of significantly lower intensity. Rifampicin therefore existed in a less crystalline state in the ground mixture as compared to rifampicin alone. (Figure 2).

The presence or absence of characteristic peak asso-

DSC studies of rifampicin showed melting endothermic peak at 196.68°C, immediately followed by recrystallization to form I exothermic peaks at 218.06°C and finally decomposed at 262.32°C. Similarly SLS showed five endothermic peaks at 100.09°C (loss of water), 191.88°C, 218.02°C, 224.96°C and 270.85°C. DSC studies of co-ground mixture rifampicin –SLS mixture showed endothermic peak of rifampicin at 101.69°C, 187.07°C, 223.89 °C and 247.37°C. Since the DSC peak value of co-ground mixture was broader and away from the melting point of rifampicin, it may be concluded that there is a formation of complex between rifampicin and sodium lauryl sulphate. (Figure 1).

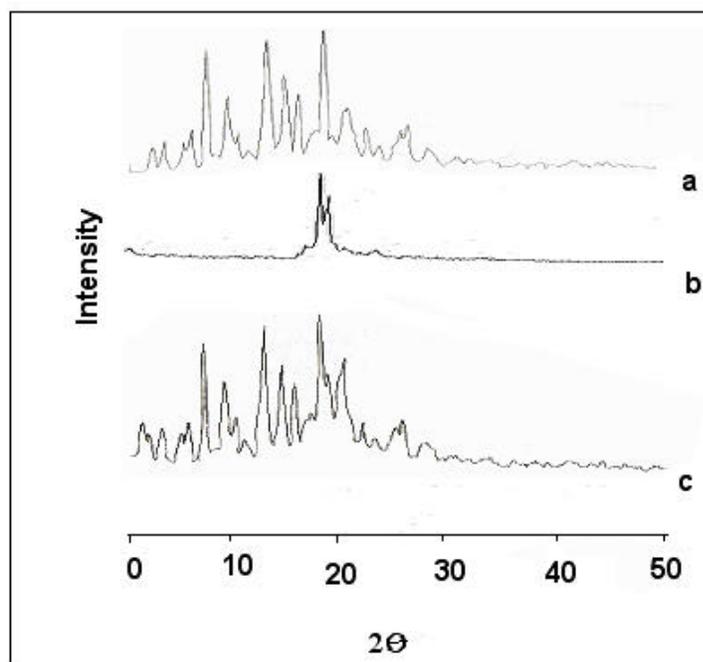


Figure.2. pXRD patterns of (A) rifampicin, (B) sodium lauryl sulphate (C) Rifampicin- sodium lauryl sulphate mixture.

ciated with specific structural group of drug molecule was noted. The FTIR studies showed that the significant peaks of rifampicin were acetoxy C=O vibration at 1711.91 cm⁻¹, furanone C=O absorption at 1734.04 cm⁻¹, amide NH-C=O at 1655.24 cm⁻¹ and C=C vibration at 1566.47 cm⁻¹ and N-rifampicH₃ at 2884.42 cm⁻¹. Sodium lauryl sulphate showed two significant peaks i, e SO₂ stretch at 1220 cm⁻¹ (asymmetric) and 1084 cm⁻¹ (symmetric). The freshly prepared co-ground rifampicin –sodium lauryl sulphate mixture showed characteristic peaks of two carbonyl peaks acetoxy C=O at 1711.91 cm⁻¹ and furanone C=O absorption at 1734.4 cm⁻¹ of rifampicin shifted to 1726.99 cm⁻¹.

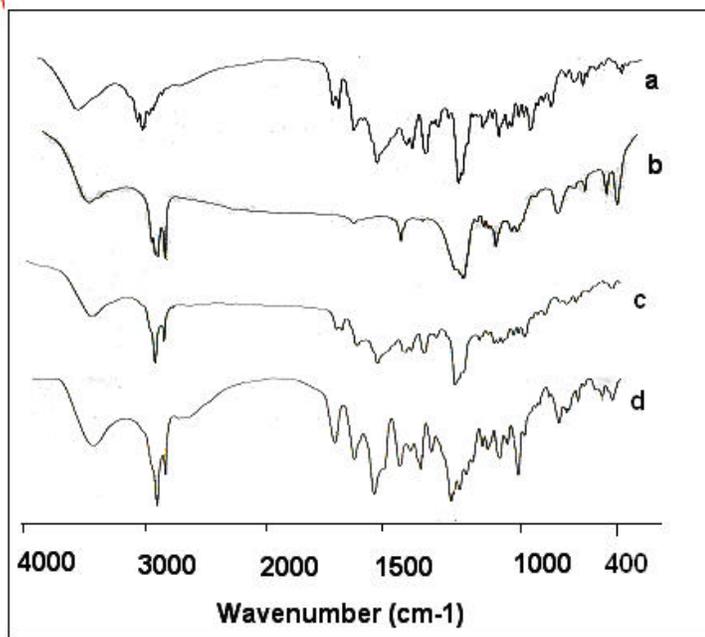


Figure. 3. FTIR patterns of (A) rifampicin, (B) sodium lauryl sulphate (C) Rifampicin- sodium lauryl sulphate mixture (before dissolution studies) (d) Rifampicin- sodium lauryl sulphate mixture (after dissolution studies) in 0.1N HCl.

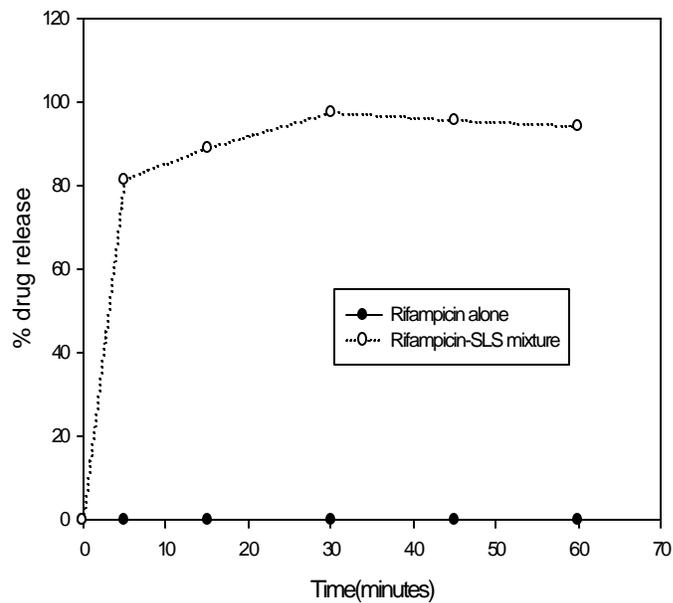


Figure.4. In-vitro release of rifampicin from (●) surfactant complex and (○) rifampicin (alone)

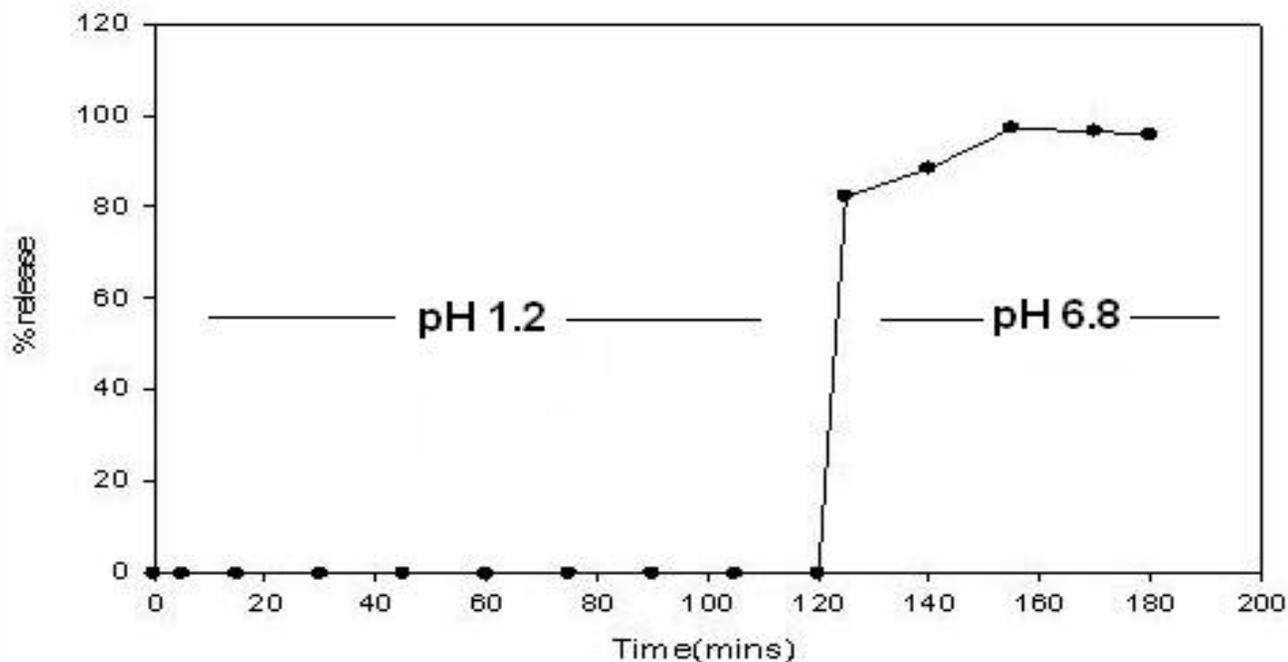


Figure.5. In-vitro release of rifampicin from its surfactant complex in 0.1N HCl followed by pH 6.8 phosphate buffer.

lauryl sulphate peaks at 1220 cm^{-1} (asymmetric), 1084 cm^{-1} (symmetric) does not exhibited significant change. However after dissolution study in acidic medium, N-C-H peak of rifampicin at 2884.42 cm^{-1} and SO_2 stretch at 1084 cm^{-1} (symmetric) of sodium lauryl sulphate disappeared from the IR spectra of Rifampicin- SLS mixture. This may be due to the

formation of ion-pair complex between rifampicin and sodium lauryl sulphate. (Figure3).

The results of dissolution studies showed that (figure 4), there was no release of rifampicin from its sodium lauryl sulphate mixture as compared to rifampicin alone, whereas it was completely released in pH 6.8 phosphate buffer. (Figure5). It may be due to formation of ion-pair interaction be-



tween rifampicin and sodium lauryl sulphate. Rifampicin, a zwitterionic compound¹⁴ at low pH the basic piperazine nitrogen group of rifampicin becomes protonated with positive charge leading to cationic nitrogen having strong tendency to form ion-pairs with lauryl sulfate anion of the surfactant. (Figure 6) The drug - SLS ion-pairs formed are hydrophobic in nature and tends to precipitate in acidic media. While in alkaline pH, rifampicin started to release from the SLS mixture

of rifampicin in alkaline media is attributed to wetting and solubilization properties of sodium lauryl sulphate as compared to rifampicin alone.

The results of loose bulk density (LBD) and tapped bulk density (TBD) are presented in Table no 1. These parameters were used to assess the packability of crystals. The pure drug powder was more bulky, which was indicated by the lowest LBD value of 0.474gmL⁻¹ for rifampicin and of 0.495 gmL⁻¹ for Isoniazid. The TBD value of 0.770 gmL⁻¹

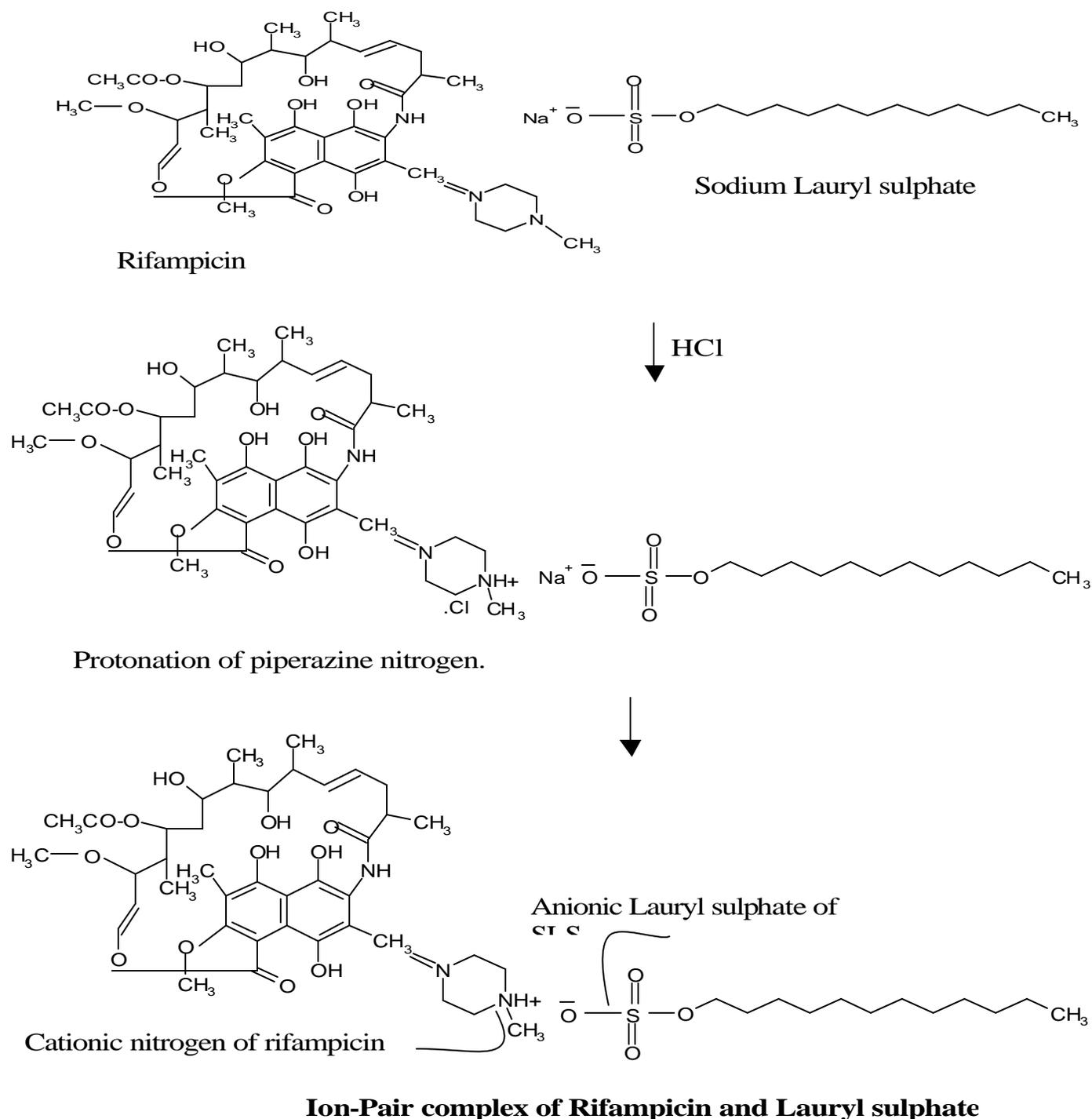


Figure .6. Proposed reaction mechanism of rifampicin and sodium lauryl sulphate interaction

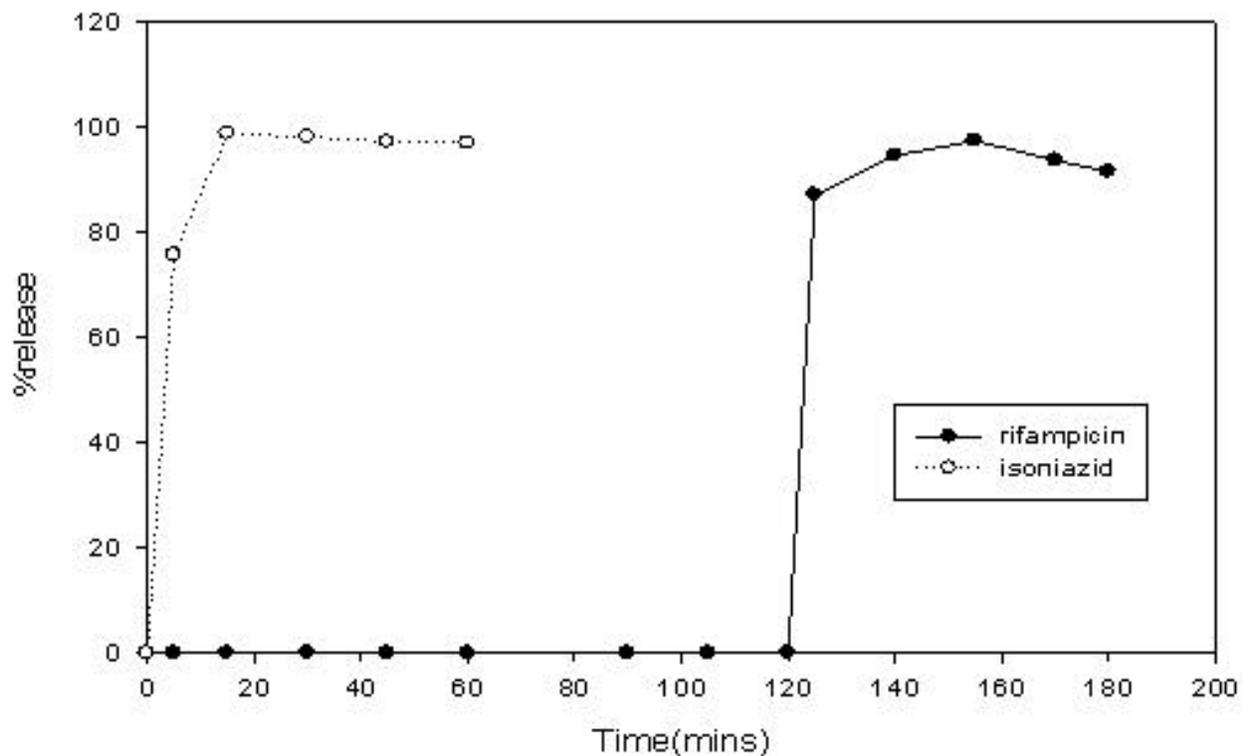


Figure.7. In-vitro release of rifampicin and isoniazid from novel FDC formulations in 0.1N HCl followed by pH 6.8 phosphate buffer

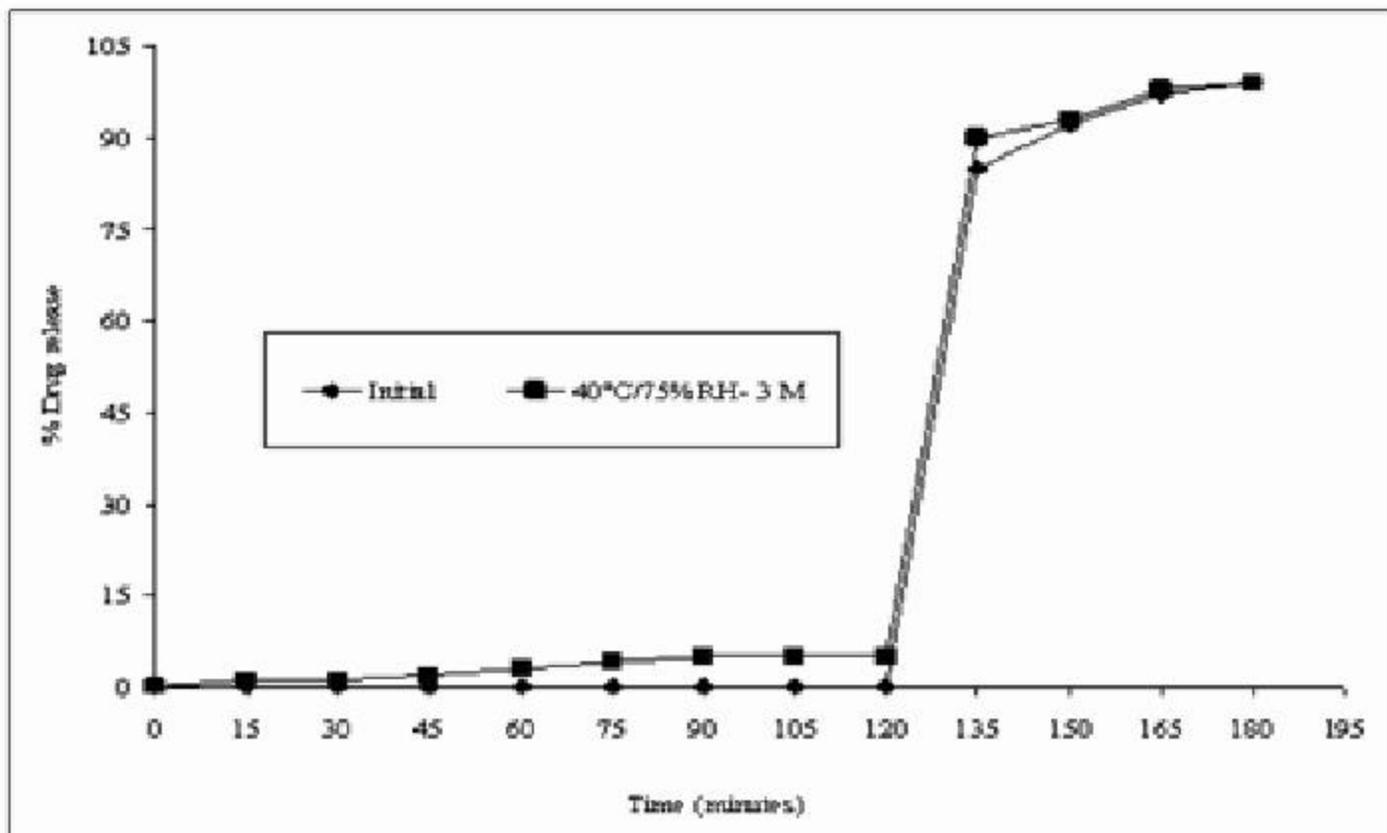


Figure.8. Comparative in vitro release of rifampicin (Formulation 1)



for rifampicin and 0.762 gmL⁻¹ for Isoniazid indicates the high intergranular space between the particles. Powder from the formulation 1 and 2 exhibited higher LBD of 0.512 gmL⁻¹ and 0.530 gmL⁻¹ respectively. These results revealed good packability of prepared formulation as compared to pure drug alone. The results of Carr's index and the Hausner ratio of formulation 1 and 2 in comparison to pure drug are presented in table no1. These parameters were used to assess the flow and compressibility properties of the prepared formulation. Carr's index of pure drug was 38.2 and 27.11 for rifampicin

and isoniazid respectively which indicated the poor compressibility. Hausner ratio of pure drug was 1.62 and 1.37 for rifampicin and isoniazid respectively which expressed indicates poor flow properties. Similarly, the angle of repose observed for rifampicin and isoniazid i.e. 33.3° and 30.9° respectively indicates poor flow property. On the other hand, prepared formulations exhibited low Carr's index and Hausner ratio indicates good flow and compressibility properties. The results of dissolution studies of the prepared novel FDCs for-

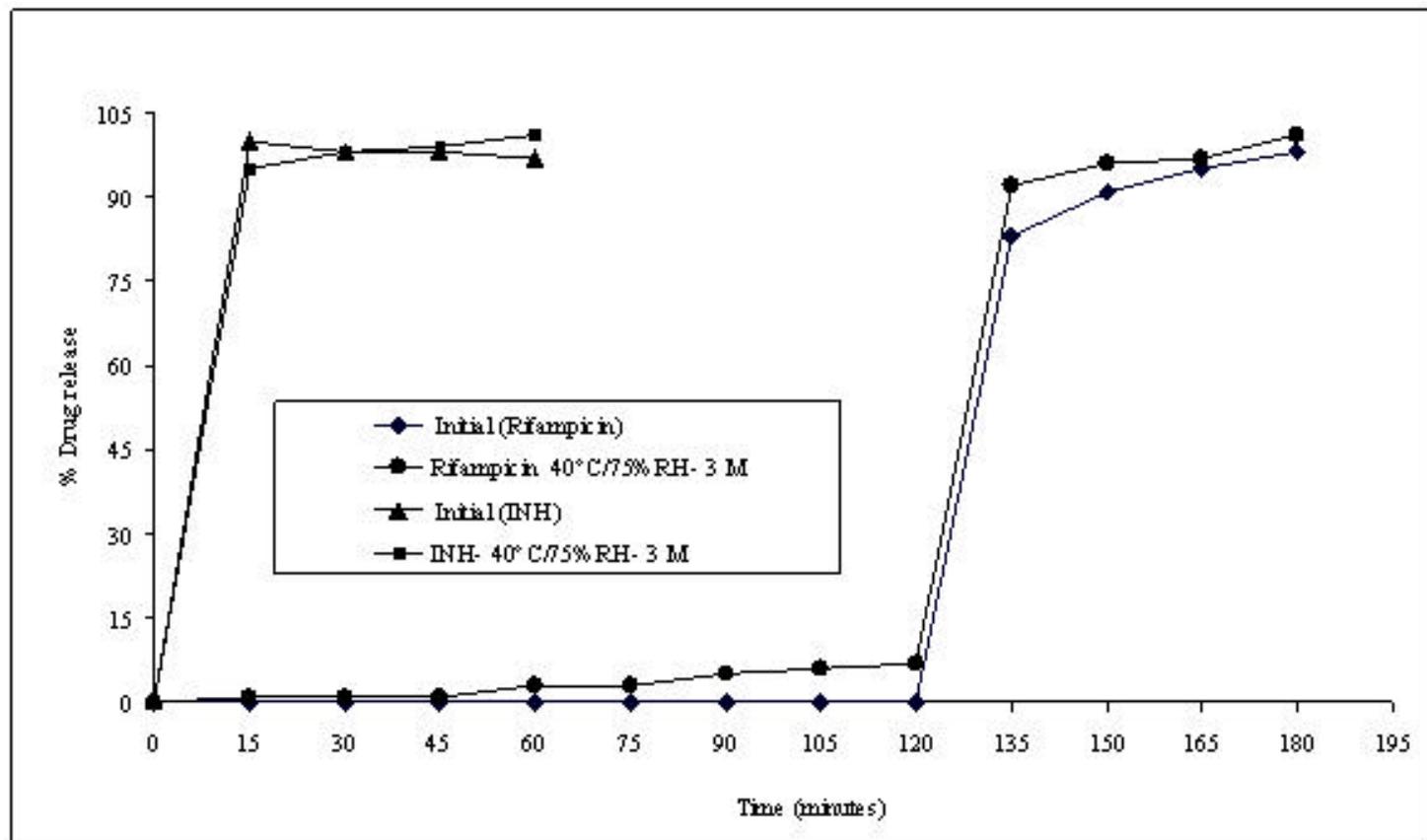


Figure.9. Comparative in vitro release of rifampicin and isoniazid. (Formulation 2)

Table.1. Micrometric Properties of drugs and formulations(n=3)

| Formulations | LBD (g mL ⁻¹) | TBD (g mL ⁻¹) | Carr's index (%) | Hausner's ratio | Angle of repose (°) |
|--------------|---------------------------|---------------------------|------------------|-----------------|---------------------|
| Rifampicin | 0.474±0.009 | 0.770±0.008 | 38.2±0.461 | 1.62±0.020 | 33.3±0.21 |
| Isoniazid | 0.495±0.012 | 0.762±0.013 | 35.01±0.05 | 1.53±0.014 | 36.9±0.548 |
| F1 | 0.512±0.003 | 0.652±0.0136 | 21.54±0.21 | 1.27±0.06 | 24.7±0.017 |
| F2 | 0.530±0.007 | 0.679±0.009 | 21.88±0.04 | 1.28±0.013 | 26.9±0.43 |

Table.2. Composition of formulations.

| Ingredients | Formulation 1 mg/capsule | Formulation 2 mg/capsule |
|------------------------|-----------------------------|-----------------------------|
| Rifampicin-SLS complex | 203 | 203 |
| Isoniazid | - | 75 |
| Starch | 63.75 | 55 |
| Talc | 5.5 | 10.5 |
| Magnesium Stearate | 2.75 | 1.7 |

mulation exhibited 0% release of rifampicin in 0.1N HCl up to two hours and significant release within 15 minutes in alkaline pH, whereas isoniazid was released completely within 20 minutes in acidic medium (figure 7). The results of the short-term stability study showed in Figure 8, 9. There was no appreciable difference in drug dissolution behavior. Thus this approach proves to be beneficial for the segregation of re-



release pattern of rifampicin in alkaline environment and isoniazid in the acidic environment of the GI tract.

The prevention of degradation of rifampicin alone and in the presence of isoniazid was achieved by segregating the release pattern of rifampicin and isoniazid by using a cheap, commonly used biocompatible pharmaceutical excipient sodium lauryl sulphate. Thus, this approach can be successfully employed for formulation of novel FDCs and for improvement of bioavailability of rifampicin.

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