Advanced PEGylation for the Development of Raloxifene Hydrochloride, BCS Class II Drug

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ABSTRACT

The aim of this study is to enhance the dissolution rate of raloxifene hydrochloride and to prepare tablets by PEGylation. The high-molecular-weight polyethylene glycols, PEG 15 000 and PEG 35 000, were used for PEGylation of raloxifene hydrochloride, a water-insoluble BCS Class II drug. The PEG conjugates were prepared with PEGs in the weight ratios of (1:1), (1:2), and (1:3.5) by using the solvent evaporation technique and the kneading technique. The conjugates were analyzed by FTIR, XRD, and DSC and subjected to dissolution studies. FTIR analysis revealed the interaction of raloxifene HCl with PEG indicating the formation of a conjugate. RLX: PEG 35 000 (1:3.5)(KM) conjugate exhibited the highest dissolution rate of 99.12% at in vitro level among all the RLX: PEG 15 000 and RLX: PEG 35 000 conjugates. The tablets of raloxifene hydrochloride were prepared by using RLX: PEG 35 000 (1:3.5) by the direct compression technique and evaluated. The prepared tablets exhibited optimum drug release characteristics of 99.12% in 60 min and the physical characteristics such as hardness (4.5 kg/cm^2), friability (less than 1%) and percent drug content (99.79 ± 0.62). The ideal drug release pattern from prepared tablets was indicated by T50 and T90 values as 29.5 and 42 min, respectively, from the dissolution data. Hence, the present studies indicated that the PEGylation of raloxifene HCl was a successful technique to enhance the dissolution rate of raloxifene HCl and to prepare its tablets.

Key words: Dissolution, FTIR, kneading technique, PEGylation, raloxifene hydrochloride, solvent evaporation, XRD

INTRODUCTION

The technology of polyethylene glycol conjugation refers to the conjugation of drug or drug moiety to polyethylene glycol (PEG) through the covalent or non-covalent interaction.[1] The technology is also called PEGylation. The word advanced PEGylation especially refers to the use of high-molecular-weight polyethylene glycols for conjugation of biologically active molecules including anticancer drugs and other small molecules.[2,3]

The PEG conjugates are of two types:

- PEG conjugates with active substances that are loose and reversible.[4]
- Drug--PEG drug conjugates formed by covalent bonding in which the drug is covalently linked to macromolecular backbone through a physiologically labile bond.[5]

PEGylation increases the hydrodynamic radius of the drugs and shields its surface toward the periphery. The enhanced hydrodynamic radius increases the solubility of conjugated molecule, protects the molecule from proteolytic degradation and phagocytosis of the drug

molecule. Conventional PEGylation that involves the use of PEGs of molecular weight below 12 000 offered limited protection against enzymatic degradation. However, it was observed that the PEGylation with PEGs having molecular weights more than 12 000 were found to be more stable in improving the pharmacokinetic and pharmacodynamics of drugs dramatically. Hence, we have selected two high-molecular-weight polyethylene glycols such as PEG 15 000 and PEG 35 000 for PEGylation. Raloxifene hydrochloride was selected as a model drug to highlight the technology of PEGylation.

Raloxifene hydrochloride (RLX) is a beta-selective beta-andregenic blocking agent, which is indicated to prevent osteoporosis in post-menopausal women. Chemically, it is [6 – hydroxy–2 (4-hydroxy phenyl)-benzothiophen-3-yl]-[4-(2-(1-piperidyl)ethoxy)phenyl]-methanone hydrochloride.[6] It is poorly soluble in water and freely soluble in methanol. It belongs to BCS class II drugs as it possesses poor aqueous solubility and good gastrointestinal permeability.[7] The molecule is not included in any official pharmacopoeia. The daily adult dose of the drug is 60 mg/day. There is a need to improve its aqueous solubility to make it as an ideal formulation possessing optimum dissolution, bioavailability, and therapeutic effect. At this juncture, PEGylation can be exploited as a choice of technology to develop the ideal formulations of raloxifene HCl. Conversion of raloxifene HCl to its PEG conjugates may produce better formulations with enhanced solubility, bioavailability, and improved pharmacokinetic profile. Hence, the objectives of the present work is to prepare conjugates of raloxifene HCl with PEG 15 000 and PEG 35 000, to select optimum RLX-PEG conjugate, to produce tablet formulations of raloxifene HCl and to evaluate the prepared tablets.

MATERIALS AND METHODS

A pure and certified sample of raloxifene HCl was gifted by M/s. Reddys Laboratories, Hyderabad, India. PEG 15 000 and PEG 35 000 were purchased from Himedia Laboratories Pvt. Ltd, Mumbai. Dicalcium phosphate, microcrystalline cellulose, crosscarmellose, sodium starch glycolate, and aerosil were gifted by M/s. Karnataka Antibiotics and Pharmaceuticals Ltd, Bangalore, India. All other chemicals and reagents were of analytical grade.

Preparation of raloxifene-PEG (RLX-PEG) conjugates

In an attempt to prepare RLX-PEG conjugates, two methods such as the kneading method and the solvent evaporation technique were utilized. The conjugates were prepared by taking different weight ratios of RLX to PEG 15 000 as 1:1, 1:2, and 1:3.5 and RLX: PEG 35 000 as 1:1, 1:2, and 1:3.5.

Kneading method (kneading mixtures, KM)

The weighed quantities of the drug and PEG were taken in a mortar and triturated with a small volume (≈5 ml) of acetone. After proper trituration to smooth yellow moist mass, the mass was kneaded for 30 min and dried at 40 ^{0}C till the constant weight is reached. The dried mass was pulverized and sifted through 100 and the collected powder fraction was stored in 30 ml glass vials. In each case 5 g of kneading mixture was prepared.

Solvent evaporation technique (solvent evaporation mixtures, SE)

The drug-PEG conjugate was prepared by dissolving raloxifene and PEG separately in possible minimum volume of acetone (≈50 ml). The acetonic solution of a drug was poured into the acetonic solution of PEG with stirring. The solution was mixed together on a cyclone mixer for 1 h and the solvent blend was removed by evaporation in a water bath at 35 ^{0}C. Then the mass was dried at 40 ^{0}C and the solid dried mass was scrapped, pulverized, and sifted through 100.The powder fraction sieved through 100 was collected and stored in 30 ml screw capped glass vials for further use. In each case 5 g of dispersion was prepared.

Evaluation of RLX-PEG conjugates

In vitro drug release studies
Dissolution studies of pure drugs and all the RLX-PEG conjugates were carried out in triplicate with the USP XXI dissolution testing apparatus (Paddle type) (Electrolab, India) at stirring speed of 50 rpm.[8] Water containing 0.1% Tween-80 was used as a dissolution medium and was maintained at 37±1 ºC. Samples of each preparation equivalent to 60 mg of drug were added to the dissolution medium. The sample aliquots each of 5 ml were withdrawn at appropriate time intervals, filtered through a 0.45 μ membrane filter (Millipore, nylon discs). The initial volume of the dissolution medium was maintained by replacing with 5 ml of the medium to maintain sink conditions. The filtered aliquots were suitably diluted and assayed for its drug content at λ_{max} of 285 nm by measuring the absorbencies against solvent blank using the UV-visible spectrophotometer (systronics, India). Each test was performed three times. The mean percent of drug dissolved and the SD were calculated and represented in Figures 1 and 2.
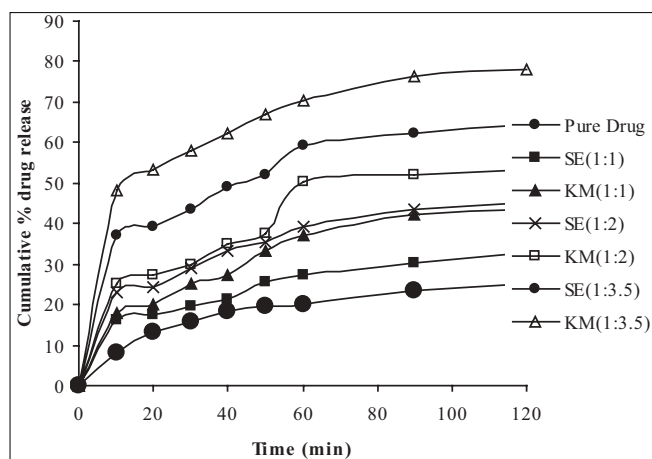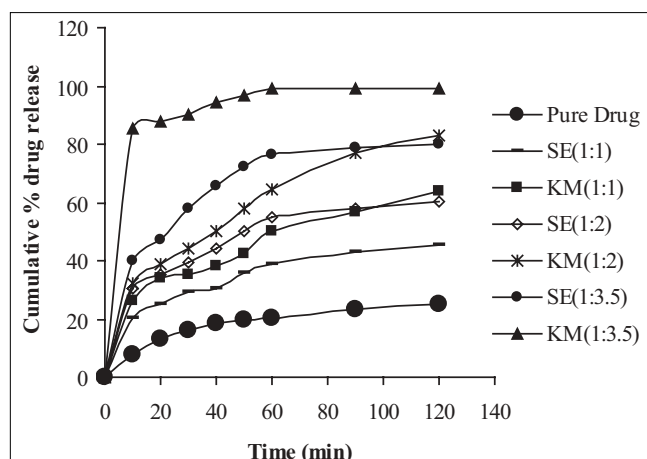
Figure 1: Dissolution profiles of pure raloxifene HCl and RLX: PEG 15 000 conjugates

Drug-PEG conjugate confirmation studies by FTIR

FTIR spectra of pure drug, PEG 35 000, and the promising RLX-PEG conjugate that exhibited highest dissolution rate were obtained on a Perkin-Elmer 841 model FTIR Spectrometer equipped with a DTSG detector. Samples were prepared by the KBr pressed pellet technique. The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

Powder X-ray diffraction analysis

Powder X-ray diffraction patterns of pure drug, PEG and the promising RLX-PEG conjugate were recorded using X-ray diffract meter(Scintag XGEN). The cross sections of the samples were held in place on quartz plate and subjected to CuKα radiations. The samples were analyzed at room temperature over a range of 0-5^0 at an angle of 2θ with a sampling interval of 0.02^0. The scanning rate was 2 ^{0}C/min.

Preparation of raloxifene HCl tablets by direct compression

Raloxifene HCl 60 mg tablets were prepared by using the selected formulation i.e. RLX: PEG 35 000 (1:3.5) (KM) which exhibited a significant improvement in the dissolution at *in vitro* level among all the RLX–PEG conjugates. The various formulations were tried to select the best tablet with good dissolution profile and mechanical properties. The selected tablet formulation that satisfied all the official parameters is given in Table 1. All the ingredients were sifted through 20. The sifted material was loaded into a polyethylene bag and mixed for 10 min. The micromeritic properties of the prepared blend are found satisfactory. Then the blend was compressed with 12 mm concave round punches using 16-station rotary punch machine (Clit Jemkay, CTD 3-16) until desired hardness was obtained. One station only was used and other stations were made dummy.

Figure 2: Dissolution profiles of pure raloxifene HCl and RLX: PEG 35 000 conjugates

Table 1: Formulation of PEGylated raloxifene tablets

Ingredients	% of the ingredient	Quantity per tablet (mg)
Drug: PEG 35 000 (1:3.5)	67.5	270
Micro crystalline cellulose	21	84
Dicalcium phosphate	2.5	10
Sodium starch glycolate	3.5	14
Talc	0.75	3
Croscarmellose	3.5	14
Magnesium stearate	0.75	3
Aerosil	0.5	2

Total weight = 400 mg

Table 2: Physical Parameters of PEGylated raloxifene HCl tablets

Parameter	Values (mean ± S.D)
Average weight (mg)	399.2±0.2
Drug content (%)	99.79±0.62
Hardness (kg/cm^2)	4.5±0.07
Friability (%)	0.49±0.61
Disintegration time (min)	7.5±0.25
Thickness (mm)	4.6±0.15

Evaluation of tablets

The prepared tablets were evaluated for disintegration,[9] hardness,[10] friability,[11] and drug content. Five tablets collected at random were determined for hardness by using Monsanto hardness tester. The mean values are presented in Table 2. The disintegration time of tablets was determined by using the thermionic tablet disintegration test apparatus of USP standard. The results are given in Table 2. The friability test was carried out by using Roche Friabilator (Veego Instruments Ltd, India). Ten tablets were collected at random from a batch and their initial average weight (W_1) was denoted. Then the tablets were placed in the rotating chamber and subjected to combined effects of abrasion and shock with revolving plastic chamber at
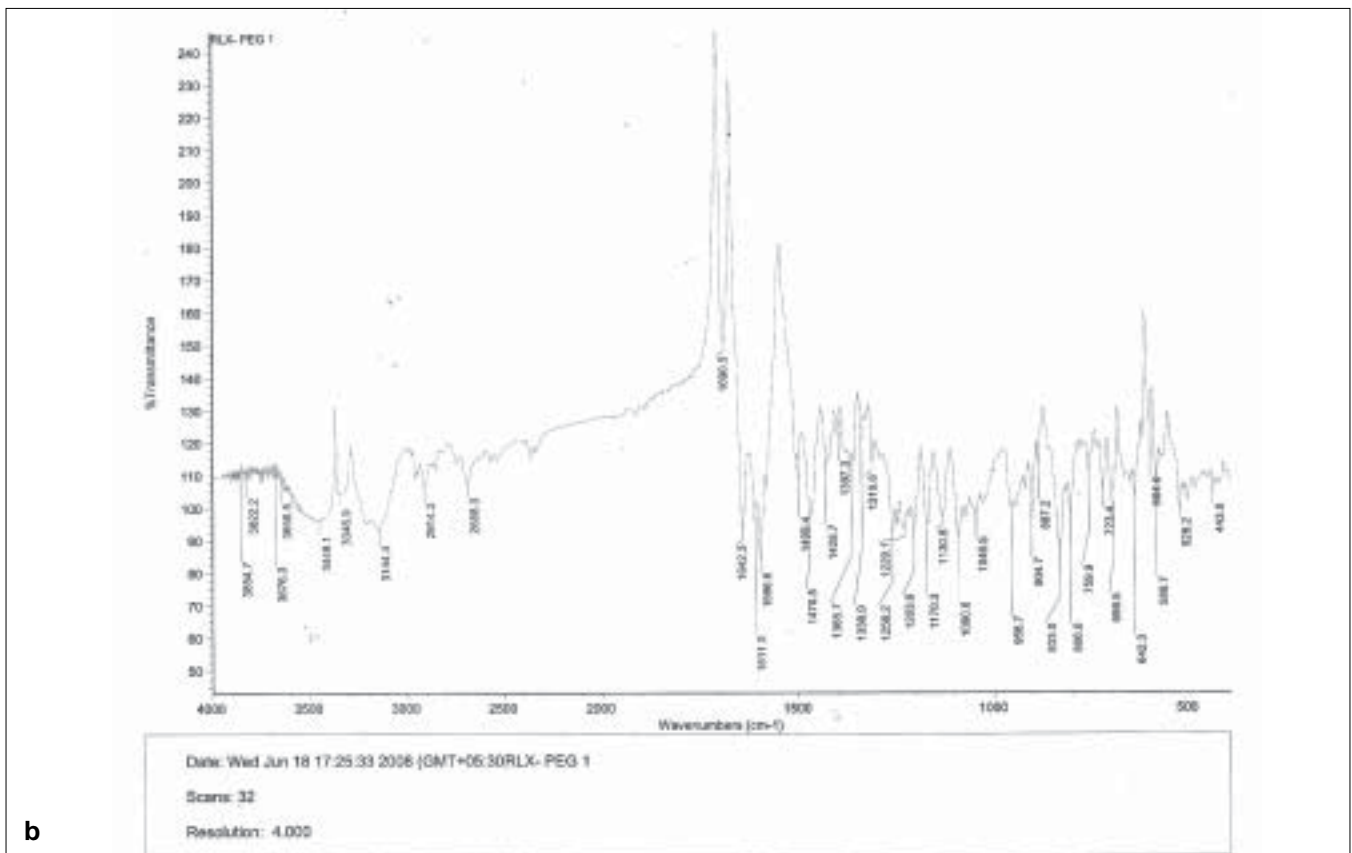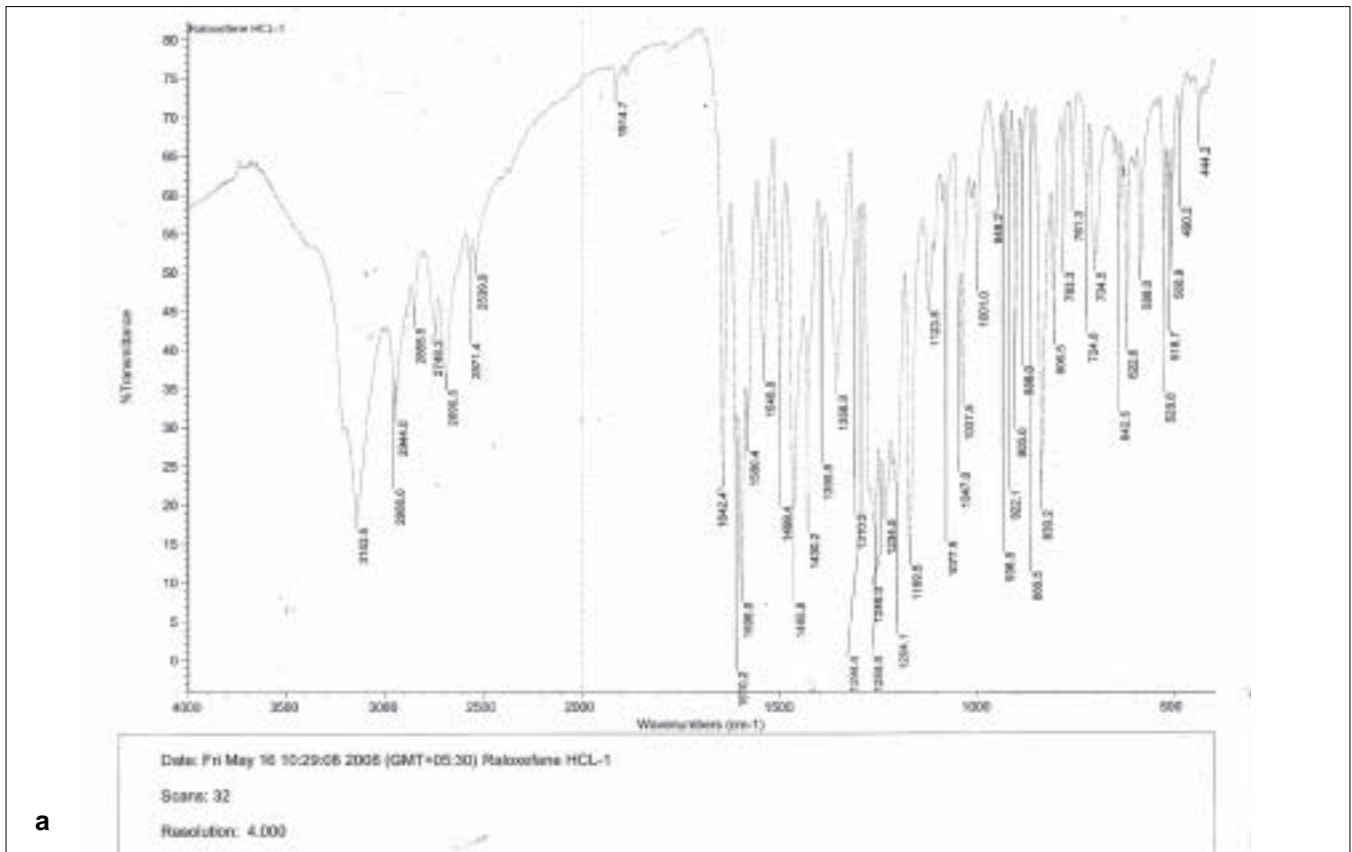
Figure 3: FTIR spectrum of (a) pure RLX HCl; (b) RLX: PEG 35 000 (1:3.5) (KM)
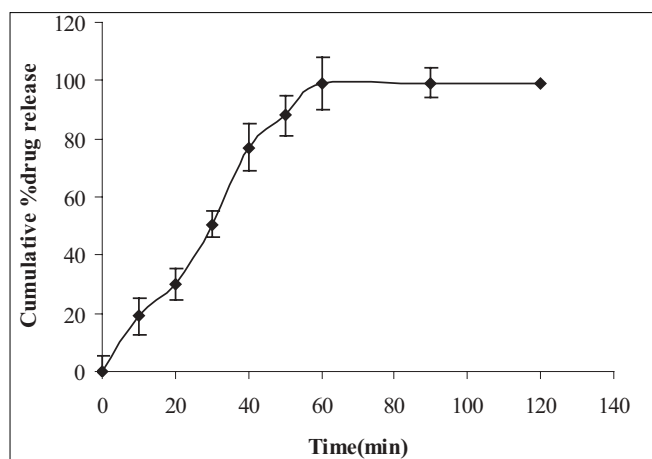
Figure 4: Dissolution profile of PEGylated raloxifene HCl tablets

100 rpm. After completion of rotations, the tablets were reweighed (W_2). The percent loss in weight or friability (f) was determined.

Estimation of drug content

Drug content estimation was carried out by collecting 10 tablets from each batch at random. The tablets were powdered and the fine powder equivalent to 60 mg of the drug was transferred into a 10 ml volumetric flasks. The volume was made with water containing 0.1% Tween-80 and sonicated for 10 min. The sample was filtered through 0.45 μm millipore filter paper and estimated for the drug content at λ_{max} of 285 nm by measuring the absorbencies against blank using the UV-visible spectrophotometer (systronics, India).

In vitro drug release studies

In-vitro drug release studies of prepared raloxifene tablets were carried out using the USP XXI rotating basket apparatus (Electrolab, India). Water containing 0.1% Tween-80 was used as a dissolution medium and was maintained at 37±1°C. The stirring speed was maintained at 50 rpm. Aliquot samples were withdrawn at various time intervals, filtered, suitably diluted, and assayed at 285 nm by measuring the absorbencies against blank using the UV-visible spectrophotometer (Systronics, India). The initial volume of the dissolution medium was maintained by replacing with 5 ml of the medium to maintain sink conditions. Each test was performed in triplicate. The mean percent of drug dissolved and the SD were calculated.

Release kinetics raloxifene HCl tablets

The analysis of the drug release mechanism from the prepared Raloxifene HCl tablets was evaluated by

using a model-dependent approach. The dissolution data were fitted to popular release models such as zero order ($Q = Q_0 - k_0 t$) and first order (Log C $= \text{Log } C_0 - Kt/2.303$). To assess the extent of drug release mathematically the official parameters such as T_{50} and T_{90} were calculated by taking the time points of 50% and 90% of the drug dissolved.

RESULTS AND DISCUSSION

RLX-PEG conjugates

RLX-PEG conjugates with PEG 15 000 and PEG 35 000 were prepared in the different weight ratios of (1:1), (1:2), and (1:3.5) by using the solvent evaporation technique and the kneading technique. All the conjugates were subjected to *in vitro* dissolution testing to select the optimized RLX: PEG conjugate possessing enhanced *in vitro* dissolution rate. The dissolution profiles of RLX: PEG 15 000 and RLX: PEG 35 000 conjugates are shown in Figures 1 and 2. Dissolution of raloxifene HCl was increased in all the PEG conjugates prepared by both the solvent evaporation technique and by the kneading technique when compared to the dissolution of a pure drug. PEG conjugates prepared by the kneading method exhibited a better dissolution profile than conjugates prepared by the solvent evaporation technique. This may be due to the uniform distribution of drug in the polymer crust at a molecular level in a highly dispersed state upon grinding. Dissolution of raloxifene HCl was increased upon increasing the concentration of PEG as well as molecular weight of PEG. According to the results evident from dissolution profiles, RLX: PEG 35 000 (1:3.5) (KM) was proved to possess the highest dissolution rate of 99.18% in 60 min. Hence, this conjugate was further studied by FTIR and XRD and utilized in the preparation of tablets.

FTIR spectra obtained for pure raloxifene HCl, PEG35000, and the selected PEGylated raloxifene HCl conjugate, i.e. RLX: PEG 35 000 (1:3.5) (KM) are shown in Figure 3a-b. The FTIR spectrum of pure raloxifene HCl in Figure 3a shows its characteristic peaks at 3142.5 due to phenolic –OH group, 1642 due to C=O stretching, 1596 due to C=C stretching, 2914 due to aromatic C-H, 806 due to thiophene C-H, and 1258 due to C-O stretching. The FTIR spectrum of PEG 35 000 revealed its characteristic absorption peaks at 3418 due to aliphatic –OH group and at 1099 due to primary alcohol. The FTIR spectrum of RLX: PEG 35 000 (1:3.5) (KM) conjugate shown in Figure 3b showed new absorption peak at 3448 which may be due to the attachment of hydroxyl group of PEG to amine group of piperidine. This indicated the formation of conjugate

between raloxifene HCl and polyethylene glycol.

X-ray diffractogram of pure raloxifene HCl was complicated with number of peaks showing the crystalline state of the drug. The XRD of PEG 35 000, showed only two sharp peaks indicating the amorphous nature of the polymer. The powder X-ray diffractogram of PEGylated raloxifene HCl was less intensified with considerable reduction in the peaks. This indicated the complete conversion of crystalline nature of raloxifene HCl into amorphous nature upon conjugation with PEG.

To assess the effective application of PEGylation in the formulation development, the tablets of raloxifene HCl were formulated using the RLX: PEG 35 000 (1:3.5) (KM) conjugate which exhibited the highest dissolution rate at *in vitro* level. Various formulations were tried and the selected formulation is shown in Table 1. The prepared tablets possessed good physical characteristics as shown in Table 2. The disintegration time (7.5 ± 0.25), hardness (4.5 ± 0.07 kg/cm^2), friability (0.49 ± 0.61), and the percent drug content (99.79 ± 0.62) were all found satisfactory.

In vitro dissolution profile of raloxifene HCl tablets

In vitro dissolution profiles of raloxifene HCl tablets are presented in Figure 4. The drug release characteristics were found to be satisfactory in such a way that there was 99.12% of drug release in 60 min.

Release kinetics

The dissolution profiles were mathematically tested to assess the release kinetics by fitting the data into zero-order, first-order and Hixon-Crowell cube root models. The correlation coefficient value ($r = 0.9765$) in case of first-order plot proved that the release kinetics followed the first-order mechanism. The official dissolution parameters such as T_{50} and T_{90} were 29.5 and 42 min, respectively, indicating the ideal drug release pattern of the prepared tablets.

CONCLUSION

The PEG conjugates of raloxifene HCl are useful to enhance the dissolution rate of raloxifene HCl, a poorly water-soluble drug. The RLX: PEG 35 000 conjugates prepared by the kneading technique in the weight ratio of 1:3.5 exhibit highest dissolution characteristics. The tablets of raloxifene HCl prepared by using RLX: PEG 35 000 (1:3.5) (KM) conjugate successfully produces the tablets possessing optimum physical and drug release characteristics that satisfy the official criteria required for optimum therapeutic effect.

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