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SHORT COMMUNICATION

EUDRAGIT® FS BASED COLONIC MICROPARTICLS OF METOPROLOL TARTRATE

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Abstract: Metoprolol, a cardioselective β-blocker, is well absorbed in colon after oral administration with mean elimination half life of 3 h with bioavailability 50% due to extensive first pass effect, thus it was aimed to develop its modified release dosage form to reduce dosing frequency. Metoprolol tartrate loaded Eudragit® FS microparticles were formulated using solvent evaporation technique by varying polymer contents and then compressing into tablets. The dissolution test was performed in simulated gastrointestinal fluid. All tabletted microparticles were tested for stability after storage in accelerated conditions. As a result of various analytical tests like FTIR, XRD and DSC analyses, drug was found stable in the microparticles. Metoprolol tartrate loaded Eudragit® FS tabletted microparticles were stable in accelerated storage conditions. The release behavior of pH-dependent formulations was affected by the dissolution medium pH and the concentration of polymer used. There was a decrease in drug release rate with the increase in polymer concentration. *In vitro* drug release data (except test formulation F3) were best fitted to zero order model, which indicated the controlled release nature of formulation, while the Korsmeyer-Peppas model explored that drug release occurred according to case II relaxation transport mechanism ($n > 0.89$). Based on the results, it can be concluded that Eudragit® FS is a suitable polymer to design pH dependent microparticles using solvent evaporation technique for the release of drug in colon and T2 can be considered as an optimum formulation on the basis of model independent (f_2 test) kinetic interpretation of dissolution results ($f_2 < 50$ for T2 *versus* reference).

Keywords: metoprolol tartrate, Eudragit® FS, microencapsulation, colonic tabletted microparticles

Although the surface area of colon is small, it has prolonged retention time (1). Thus, the development of colonic drug delivery systems has recently gained considerable interest of research pharmacists. These systems are very useful for better treatment of colonic disorders such as ulcerative colitis, Crohn's disease and colonic carcinoma. Researchers are paying significant attention to colon as a portal for guiding drugs to systemic circulation (2). The colonic drug delivery approaches include the preparation of prodrugs, pH-sensitive polymer deliveries, time-dependent deliveries, bacterial degradable formulations, time pH-controlled formulations, and the use of biopolymers (3, 4).

Various drug delivery strategies have been discovered for colonic targeting on the basis of gastrointestinal features. The pH differential systems in the gastrointestinal tract (GIT) are promising because of their practicality and exploitation of the most distinctive property of the colon, pH of which provide the rationale for developing pH-dependent colonic drug delivery system (5). The pH of stomach contents is about 1.5 in the fasted state, 5.0 to 7.0 in the small intestine, and 6.0–7.2 in the colon. The pH values at the end of ileum and in the colon are significantly higher than in the upper GIT. This delivery system slowly releases drug in the upper part of the GIT, but rapidly releases drug in the colon following oral administration. Thus, delivery of lesser quantity of drug to the upper GIT but higher to the colon mitigates GI side effects and prolong the transit time in the colon (6–8).

Metoprolol tartrate is a β-blocker that is well absorbed in the colon and in the small intestine.

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Thus, this drug is considered good candidate for colonic delivery (9). It is used for the management of cardiovascular disorders such as hypertension and angina pectoris. This drug is categorized under class I of Biopharmaceutics Classification System (BCS) because it is highly soluble and highly permeable. Metoprolol tartrate is completely absorbed in intestines after oral administration and exhibits 50% bioavailability due to extensive first pass effect (10). The mean time to reach maximum plasma concentration and mean elimination half life for metoprolol tartrate after oral dosing is 2 h and 4 h, respectively. Based on these pharmacokinetic facts, metoprolol tartrate is needed to administer frequently (11, 12). These properties make metoprolol tartrate a good candidate for formulating as extended and targeted release dosage form to decrease dosing frequency up to twice a day.

Recent studies have elaborated many advantages of multi-unit dosage forms over single unit such as improved bioavailability, less local irritation, less danger of dose dumping and minimized intestinal retention of undigested polymer materials in chronic use (13). The microparticles appear to be less influenced by the physiologic factors, such as the gastric emptying, intestinal transit, as compared to tablets. Moreover, the microparticles could be widely and uniformly distributed in the GIT contents, which increase the contact between drug and GIT surface and thus improve bioavailability (13).

Microencapsulation, in this context, is widely used for the development of multi-unit formulations such as microparticles. It is a technique that involves the application of a thin coating to individual core materials (solid, liquid or gas) that have an arbitrary particle size range between 5 and 5000 μm (14). The application of this rapidly expanding coating technique is not only limited to the pharmaceutical field but also other sciences such as: to modify release, impart stability to drug molecules, mask unacceptable taste and odors, improve bioavailability and to develop targeted drug delivery (14). Solvent-evaporation technique, a simple method of microencapsulation, involves the emulsification of a drug-polymer solution with another medium in which the drug and polymer are insoluble. The technique has been widely used to develop microparticles of a variety of compounds using several different polymers (15–23).

Eudragit polymers are pH-dependent co-polymers that are available in various ionic grades. These have been approved by the Food and Drug Administration for drug delivery systems, therefore, Eudragit® FS was employed to encapsulate metoprolol tartrate. In addition, the eudragits also behave as both binders and as coating materials (7). Eudragit® FS is a potential pH-dependent carrier for colonic drug delivery, which prevents drug release in stomach and small intestine. It is a copolymer of methacrylic acid, methyl methacrylate and methyl acrylate (8). The literature survey showed no drug delivery system involving Eudragit® FS, therefore, present study may stimulate new research in this field employing this new grade of Eudragit.

Based on these considerations, this study was designed to develop metoprolol tartrate-Eudragit® FS extended and targeted release pH-dependent formulations i.e., tabletted microparticles followed by the *in vitro* evaluation of the prepared formulations.

EXPERIMENTAL

Materials

Metoprolol tartrate was procured from Novartis Pharma-Pakistan. Eudragit® FS was obtained from Rohm Pharma, Germany. Metoprolol tartrate tablets (Mepressor® 200 mg, Novartis Pharma-Pakistan) was purchased from the market. Light liquid paraffin, acetone, span 80, *n*-hexane, and other chemicals of analytical grade were purchased from Merck, Germany and were used without any further purification.

Preparation of metoprolol-loaded microparticles

Metoprolol tartrate loaded Eudragit® FS microparticles were developed by solvent evaporation technique. Metoprolol tartrate (1 g) and Eudragit® FS (1, 1.5 or 2 g) were dissolved in acetone (20 mL) using magnetic stirrer (stirring speed 450 rpm) for the preparation of 1:1, 1:2 and 1:3 drug-polymer ratio solutions. Light liquid paraffin (40 mL) solution containing span 80 (0.2 g) was added to this drug-polymer solution with continuous stirring for 4 h at room temperature. The resultant microparticles were obtained by vacuum filtration after complete removal of acetone by stirring. The microparticles were washed three times with *n*-hexane (100 mL) and dried in an oven at 30°C.

Compatibility analysis

To analyze any possible chemical interaction between drug and polymer during the process of microencapsulation, Fourier transform infra-red (FTIR) spectroscopy, x-ray diffractometry (X-RD) and differential scanning calorimetry (DSC) analytical tests were performed for pure metoprolol, Eudragit® FS and microparticles (T2 microparticles before compression into tablets).

X-ray diffractometer (Bruker D8 Discover, Germany) was used to elaborate crystalline behavior of metoprolol, before and after encapsulation with Eudragit® FS. The α-radiation source was CuK with Ni-filter. The x-ray diffractometer was run at tube voltage of 35 KV, current of 35 mA and scanning rate of 5°/min, over a range of 8–60° diffraction angle (2Θ) (23).

The SDT Q600, USA was employed for DSC analysis of metoprolol and metoprolol-loaded microparticles. A small amount (approximately 5 g) of the samples was directly placed in aluminum pan and the lids were crimped. The temperature range for the analysis was 25–200°C at a rate of 20°C/min, under the flow of nitrogen at a rate of 25 mL/min. Th calibration of this appratus was carried out with indium before performing analysis (23).

The compatibility of metoprolol in microcapsules was analyzed by FTIR spectroscopy. The spectra were obtained using FTIR Midac 2000, USA. The samples for metoprolol and metoprolol-loaded microparticles were prepared by KBr disk method. The scanning range was 500–4500 cm^{-1} and the resolution was set at 2 cm^{-1} (23).

Morphology study and particle size determination

Scanning electron microscopy (Philips-XL-20, Netherlands) was used for the morphological study of microparticles. After mounting the microparticles directly onto the sample stub, a thin (200 nm) coating of gold was applied to assess their morphology under reduced pressure (0.133 Pa).

Light microscope (XSZ-150A, Ningbo, China) was used for the determination of microparticle size. The light microscope consisted of a microscope stage, a digital camera and a computer. The suspension of microparticles was placed on a glass slide and its photomicrographs were taken with digital camera. The microparticulate diameter was determined from image analysis.

Tabletting of microparticles

The tabletted microparticles [drug/polymer ratios of 1:1, 1:1.5 and 1:2 (w/w), T1, T2 and T3 named as tabletted microparticles or test formulations containing 1, 1.5 and 2 g polymer, respectively] were prepared by direct compression of microparticles single punch tablet machine (Emmay, Pakistan) so that each tablet (test) formulation contained metoprolol tartrate equivalent to 200 mg of metoprolol. The standard hardness of test tablets was achieved by varying compression force. Based on *in vitro* studies, these test tablets were compared with reference sustained release (SR) tablet, Mepressor 200 mg SR (Batch No. 457X, Expiry date: 30.06.2011, Novartis-Pharma, Pakistan).

Tablet evaluation

Weight variation, tablet hardness, friability, disintegration and dissolution tests for reference and test tablets were performed according to USP (21). Six test tablets were tested for disintegration in 0.1 M HCl solution for 2 h by using USP basket rack assembly and then in phosphate buffer pH 6.8. The disintegration tests for reference tablets were also performed.

Encapsulation efficiency and yield

An accurately weighed quantity of drug loaded microparticles containing drug equivalent to 100 mg of metoprolol tartrate was dissolved in 10 mL of methanol by vortexing for about 24 h for complete extraction of metoprolol tartrate. After the filtration of solution, the filtrate was analyzed by HPLC as described previously. Encapsulation efficiency (%) were determined by dividing actual drug loading with theoretical drug loading and multiplied by 100, while drug loading (%) was calculated by dividing amount of drug with the amount of microparticles and multiplied by 100. The product yield (%) was determined by dividing amount of microparticles with the total initial amount of drug plus polymer. Each determination was made in triplicate.

Dissolution test by sequential pH change method

The reference and test formulations were passed through the dissolution test by sequential pH change method (21). To simulate gastrointestinal transit conditions, dissolution conditions were: USP dissolution apparatus II at temperature 37 ± 5°C and 50 rpm in three dissolution media [pH 1.2 (0.1 M HCl) for 2 h, pH 4.5 (phosphate buffer) for 2 h and pH 7.0 (phosphate buffer)] for 8 h with a final volume up to 900 mL. Dissolution samples (5 mL) were withdrawn at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h, filtered through a 0.45 μm filter and analyzed at 273 nm using UV/Vis spectrophotometer (1601, Shimadzu, Japan). The formulations were observed visually to assess any physical changes to the particles occurred during the dissolution testing process. All dissolution studies were performed in triplicate.

Drug release kinetics

To assess the mode of *in vitro* drug release from the formulations, the release profiles were analyzed using zero-order, first-order, Higuchi and

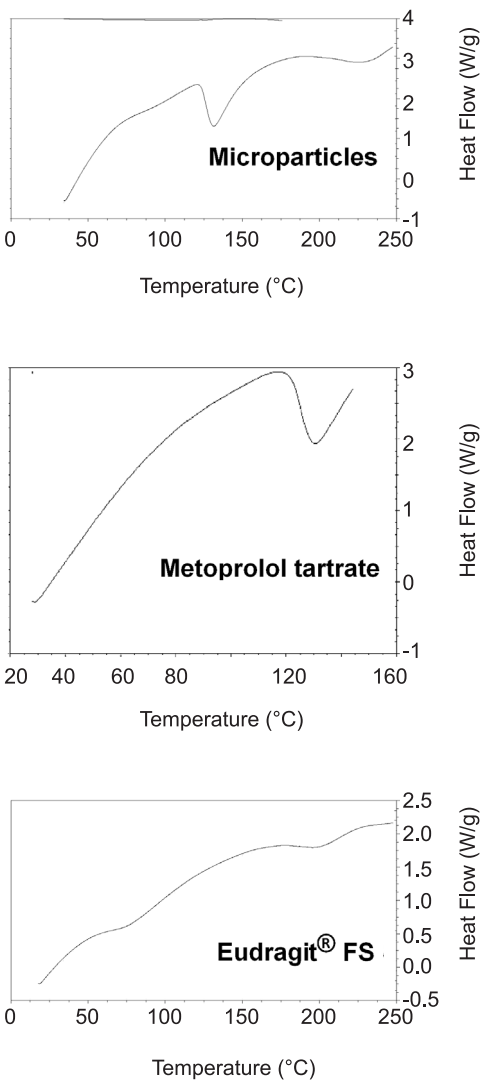

Figure 1. DSC thermograms of metoprolol tartrate, Eudragit® FS and microparticles (T2)

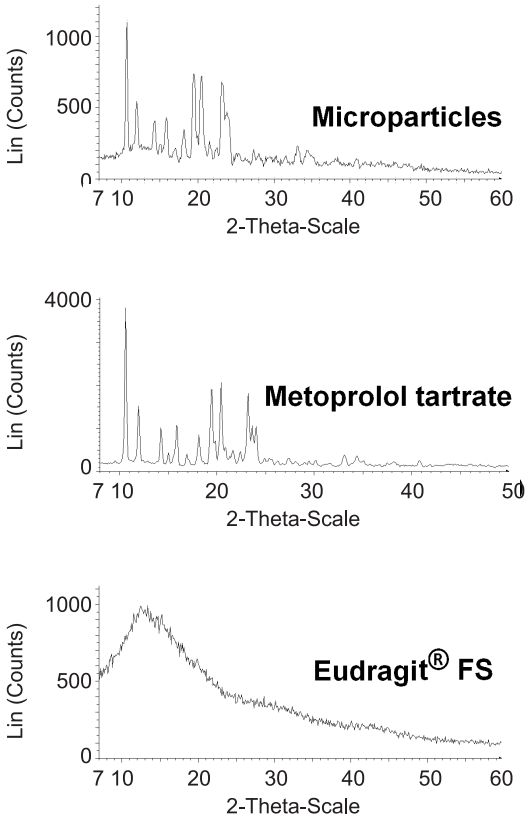

Figure 2. XRD profiles of metoprolol tartrate, Eudragit® FS and microparticles (T2)

Korsmeyer–Peppas models (22–24). The best-fit model was identified by calculating the regression coefficients (R^2), where the highest R^2 value elaborates the best fit. Model independent analysis of dissolution data was also conducted by calculating similarity factor, f_2. The value of f_2 from 50 to 100 indicates sameness between the two compared dissolution profiles.

Stability studies

The optimum formulation was filled in 10 amber colored glass bottles and stored at accelerated stability conditions (40 ± 2°C/75% ± 5% RH) for 6 months. One bottle was used each month for the investigation of dissolution behavior and drug contents of stored formulation.

Mathematical and statistical analysis

The experimental results were narrated as the mean ± SD. The significance of variation between different parameters was evaluated by one way analysis of variance (ANOVA) using software, SPSS version 13.0. The level of significance was set at 0.05.

RESULTS AND DISCUSSION

Eudragit® FS microparticles loaded with metoprolol tartrate were prepared by solvent evaporation (15). Since Eudragit® FS and metoprolol tartrate were both soluble in acetone, an emulsion of acetone in light liquid paraffin was used. Microparticles were formulated with Span 80 as surfactant, which is extensively used in the formulation of microspheres by solvent evaporation (15). Additionally the influence of the concentration of Eudragit® FS on

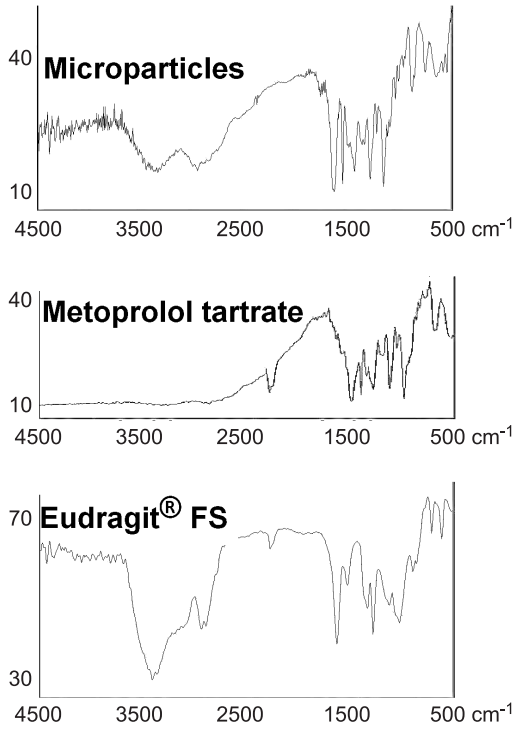

Figure 3. FTIR spectra of metoprolol tartrate, Eudragit® FS and microparticles (T2)

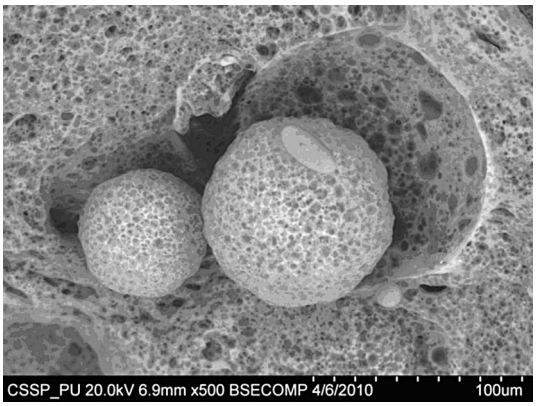

Figure 4: Scanning electron microscopic photo of microparticles (T2 before compression into tablets)

the release profile was probed (18). The drug release data and the encapsulation efficiency (%) for metoprolol tartrate of the resulting microparticles were compared.

Compatibility analysis

The DSC thermograms, x-ray diffraction patterns and FTIR spectra of the microparticles, metoprolol tartrate and Eudragit® FS are shown in Figures 1–3. Differential scanning calorimetry was used to characterize the physical state of metoprolol tartrate within the microparticles (Fig. 1). A sharp endotherm was observed for metoprolol tartrate at approximately 135°C, indicating its melting transition point. This peak was observed also in the thermogram of the microparticles, indicating that the nature of metoprolol tartrate remained intact in the form of microparticles. The DSC results were further verified by X-RD tests. XRD patterns indicate that the crystalline nature of metoprolol tartrate in the microparticles was not different from its original crystal, however, there was significant reduction in peak intensities, which suggest that the extent of metoprolol tartrate crystallinity was reduced by Eudragit® FS (Fig. 2). In addition, FTIR spectra elaborated that the principal FTIR peaks observed in the spectra of metoprolol tartrate were in close resemblance to those in the spectra of the microparticles (Fig. 3).

Morphology study and particle size determination

Surface morphology of scanning electron microscopic photo of the T2 microparticles (before compression into tablets) is shown in Figure 4. The microparticles prepared were spherical and discrete (except few agglomerated microparticles) with plugged porous surface. Average size (n = 3) was 56 ± 4.5 µm, 59 ± 6.3 µm and 102 ± 7.9 µm for T1, T2 and T3 microparticulate formulations, respectively.

Encapsulation efficiency and yield

The average encapsulation efficiency (n = 3) of Eudragit® FS microparticles loaded with metoprolol tartrate was, determined as described above, 78.6 ± 6.7%, 78.1 ± 9.5% and 69.7 ± 7.3% for T1, T2 and T3 microparticulate formulations, respectively. The product yield was approximately 87% for all microparticulate formulations.

Tablet evaluation

The weight variation, hardness, friability and disintegration (Table 1) for reference and test formulations were within allowed limits of USP (21). Average disintegration time for T1, T2, T3 and reference tablets was found to be 8 min, 8.3 min, 8.9 min and 10 min, respectively. The difference in the mean disintegration time of test formulations may be due to the difference in the concentration of polymer. Different disintegration time for reference and test tablets may be due to the difference in excipi-

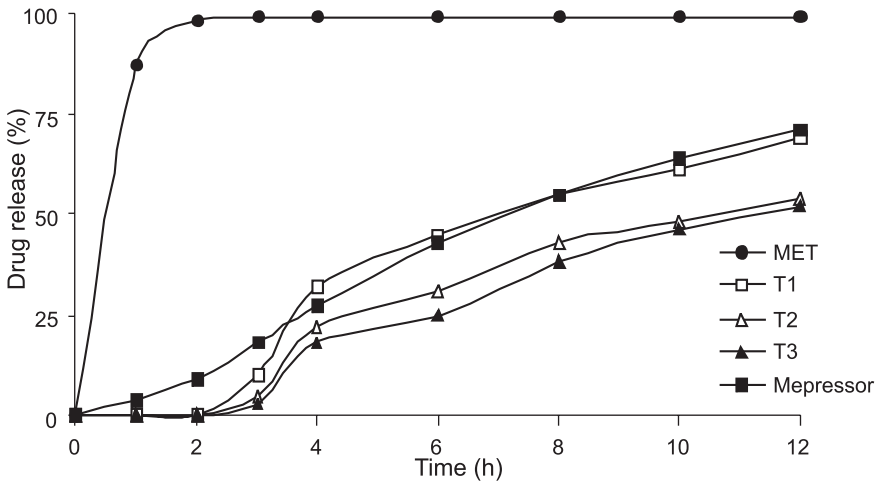

Figure 5: Effect of the Eudragit® FS concentration on the release profiles of metoprolol tartrate from test tablets in comparison to reference tablets and pure metoprolol tartrate (MET)

ents. Similarly, hardness of all tablets was in a range of 6.3 ± 1.2 – 7.1 ± 1.1 kg/cm^2 which is according to the compendial requirements. The friability for all tablets was less than 0.5% (w/w). All the tablets showed low weight variation (less than ± 3.0%). The average thickness for test tablets ranged from 3.87 to 3.89 mm. The results fulfilled the requirements of USP (9).

Drug release kinetics

The influence of Eudragit® FS ratio in the encapsulated formulation on the release profiles of metoprolol tartrate microparticles is shown in Figure 5. Then *in vitro* release results were also evaluated by various model dependent and independent approaches. The Eudragit® FS concentration played an important role in regulating the release behavior of metoprolol tartrate microparticles. Metoprolol tartrate was released approximately 1.0% in pH 1.2 dissolution medium until 2 h for T1, T2 and T3. After changing the medium to pH 4.5, drug release was about 32%, 22% and 18% for T1, T2 and T3, respectively, after dissolution of 4 h. Then, the dissolution medium pH was switched to pH 7.0. More than 71%, 54% and 52% drug release from T1, T2 and T3 formulation, respectively, was achieved in pH 7.0 medium after dissolution of 12 h. The *in vitro* release data for T1, T2 and T3 were compared by f_2 test to investigate the influence of polymer concentration. According to results, T1 *versus* T2 and T2 *versus* T3 are similar to each other while T1 *versus* T3 are different from each other. As the polymer ratio was increased from T1 to T3, the acid-resistant property of Eudragit® FS matrix increased and eventually the release of metoprolol tartrate from formulations decreased. This can be attributed to the fact that the higher polymer concentration produced larger particles with proportionately less drug, so that the polymer quantity was changed and thus release was reduced. Almost none of the drug released from formulations in pH 1.2 and pH 4.5 dissolution media. In contrast, the cumulative drug release was about 56–71% in pH 7.0, regardless of the polymer concentration. This could be due to the fact that Eudragit® FS was pH sensitive copolymers that started to be dissolved from pH 6.0 (13). In pH 7.0 medium, Eudragit® FS could be dissolved and the channels were then quickly created in the coating membrane and thus allowing for the higher drug release (13, 22).

Based on similarity factor f_2 (a model independent approach), release profile of T1 was similar (f_2 = 55) to that of reference formulation. By contrast, dissolution data of T2 and T3 were dissimilar (f_2 < 50) from that of reference formulation. On the other hand, best fit kinetic model to the dissolution data of test formulations was zero order followed by Higuchi's and then first order model. Zero order model describes the concentration independent release of drug. The release of drug from reference tablets occurred by Higuchi's model followed by zero and first order models. The mode of drug release from reference, T2 and T3 was case II relaxation transport while from T1 was anomalous type.

Stability studies

The percentage residual drug content of tabletted microparticles (T2) stored at accelerated stability condition was 99.1, 98.9, 99.0, 98.2, 98.3 and 97.7 % at 1st to 6th month, respectively, indicating non-significant ($p > 0.05$) decrease in drug contents. No significant ($p > 0.05$, $f_1 < 0.5$, $f_2 > 99.0$ for all comparisons) difference was found in the release behavior of tabletted microparticles, which indicates the reliability and reproducibility of the manufacturing process employed. Also, the release kinetics remained unaltered for up to three months of storage, and there were no changes in the tablet characteristics regarding hardness, friability, weight variation and disintegration suggesting that metoprolol tartrate is stable in the tabletted microparticles for the above mentioned period.

CONCLUSION

Eudragit® FS is a suitable polymer to design pH dependent microparticles using solvent evaporation technique for the release of drug in colon. The results showed that the polymer concentration influenced the encapsulation efficiency and particle size of the microparticles and the polymer concentration has the greatest influence on the encapsulation efficiency. The tabletted microparticles showed good stability in accelerated storage conditions. Based on model independent (f_2 test) kinetic interpretation of dissolution results ($f_2 < 50$ *versus* reference), T2 can be considered as an optimum formulation.

Acknowledgment and conflict of interest

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