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Research Article

Production and Stability Evaluation of Modified-Release Microparticles for the Delivery of Drug Combinations

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Abstract. Production and evaluation of novel formulations of tizanidine and tramadol microparticles was the chief purpose of this project. Microparticles of both drugs were prepared separately via temperature change method. To extend the release of formulations, ethyl cellulose was employed. Higuchi, zero-order, first-order, and Korsmeyer–Peppas kinetic models were applied to appraise the mechanism and mode of drugs release. Higuichi model was found to be best for all release profiles. Stability of microparticles at 40°C/75% RH over a 3-month duration was determined by Fourier transform infrared (FTIR), X-ray diffractometry (XRD), and drugs assay. Microparticles were compatible and stable as no significant differences were observed when subjected to drug assay, FTIR, and XRD during accelerated stability studies.

KEY WORDS: microparticles; stability evaluation; sustained-release (SR) combination; tizanidine; tramadol.

INTRODUCTION

Tizanidine (TZD; Fig. 1) is α2-adrenergic agonist and centrally active myotonolytic skeletal muscle relaxant with a chemical structure unrelated to other muscle relaxants. Several published uncontrolled clinical studies, as well as ongoing clinical trials, suggest that tizanidine is effective in relieving pain associated with a range of disorders such as myofascial pain (1), refractory pain, and neuropathic pain chronic tension-type headache and chronic daily headache (2).

Tramadol (TmH; Fig. 1) is a centrally acting analgesic having both opioid and non-opioid effects. TmH acts as an opiate agonist through selective binding to the μ-opioid receptor and weak inhibition of norepinephrine and serotonin uptake (3). It is administered when non-steroidal anti-inflammatory drugs fail to mitigate pain. It is readily absorbed after oral administration, and its half-life is 6.3 h (4).

The benefits of administering tizanidine in a modified-release formulation have been established and demonstrated by researchers in their clinical studies. Approximately 94% and 79% improvement in spasticity and disability, respectively, was observed in spastic patients. TZD may also be a useful adjunct to NSAIDs in the treatment of analgesic rebound headache.

Sustained-release combination of TmH and TZD has not been developed so far. Patients have to take conventional tablets three to four times a day. To improve patient's compliance, SR combination was developed and characterized for its stability. Phase separation by temperature change was adopted for the development of microparticles, and Fourier transform infrared (FTIR) and X-ray diffractometry (XRD) were used to asses the compatibility and stability. This new SR combination will be useful to improve compliance in patients requiring adjuvant therapy of analgesic and muscle relaxant.

MATERIALS AND METHOD

Tizanidine HCl (Raazee Therapeutics, Pakistan), Tramadol HCl (AGP, Pakistan), ethyl cellulose 22 cP (BDH Chemicals Ltd., Poole, UK), cyclohexane (Merck, Germany), *n*-hexane (Merck, Germany), and methanol (Merck, Germany) were used in this study.

Preparation of Drug Microparticles

Microencapsulation based on phase separation by temperature change was employed to formulate the microparticles of TmH and TZD separately. Firstly, ethyl cellulose (EC) was dissolved in boiling cyclohexane by heating it at 80°C, and then TZD was added in polymer solution with continuous stirring at 1,000 rpm on a magnetic stirrer (Velp Scienifca, Germany), and stirring was continued for 30 s to disperse drug evenly. Afterwards, the temperature was lowered rapidly by placing the flask in ice bath with continuous stirring to induce phase separation. The formed microparticles were washed with distilled water to remove any unencapsulated TZD. Finally, the microparticles were rinsed with *n*-hexane to prevent aggregation by making their surface harder. In the end, the microparticles were dried for 10 min at 40°C in an oven (Mammert, Germany), and dried microparticles were stored in an amber-colored, air-tight glass bottle. Similarly, microparticles of TmH were also produced and preserved until characterization.

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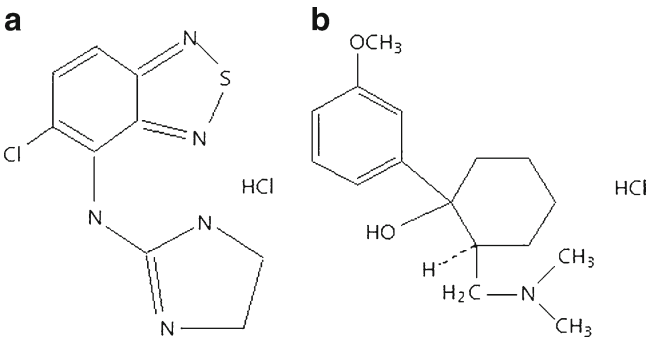

Fig. 1. **a** Structural formula of TmH. **b** Structural formula of TZD

Drug Loading

The TZD content of microparticles was determined by dissolving microparticles (theoretically equivalent to 4 mg of TZD) in 5 ml of methanol to dissolve polymer and drug. Then, addition of 20 ml of distilled water was done to precipitate hydrophobic EC. After the filtration of insoluble EC, final volume was made to 100 ml with distilled water. The final solution was diluted 100 times and analyzed by double beam UV-spectrophotometer (Shimadzu 1601, Japan) at 227 nm. The absorbance of reference drug was also determined. The percentage of drug loading in microparticles was calculated by dividing absorbance values from microparticles to the absorbance value of 4 mg reference TZD subjected with the same dilution factor. The same procedure was adopted for TmH with the exception of wavelength (271 nm). In the end, it was observed that the average drug loading was 73% and 81% for TZD and TmH, respectively.

Size and Morphology of Microparticles

Morphology of microparticles was studied using scanning electron microscope (Hitachi, S 3000H, Japan). For scanning purposes, microparticles were dappled on a double-sided adhesive tape attached to an aluminum stub. Excess of microparticles was removed and the stub sputter was coated with gold using a vacuum evaporator to turn them into electrically conductive microparticles. The coated microparticles were inspected under SEM at 25 kV to disclose the surface quality. For size determination of microparticles, an optical microscope coupled with monitor display (Nikon eclipse E200, Japan) was selected. Size was directly measured from the monitor display. An average of 100 microparticles was considered as mean value.

Dosage Form Development

TZD microparticles (equivalent to 4 mg active drug) and TmH microparticles (equivalent to 100 mg active drug) were mixed and filled in the same gelatin capsules and named as CMs (capsule-contained microparticles).

In Vitro Drug Release from CMs

In Vitro Drug Release of Microparticles and CMs

Dissolution of microparticles of TmH and TZD and CMs was carried out separately. *In vitro* drug release was determined using USP apparatus-II, paddle method, (Pharma Test, Germany) at 50 rpm. To simulate gastrointestinal conditions, the dissolution medium (900 mL) consisted of 0.1 N hydrochloric acid for initial 2 h and then replaced to phosphate buffer, pH 6.8, from 3 to 10 h. Temperature of dissolution medium was maintained at 37±0.5°C. Sink conditions were attained by enclosing the formulation in stainless steel sieves placed at the bottom of dissolution vessel. Samples (5 mL) were collected at regular intervals of 0, 0.5, 1.0, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, and 10.0 h with an automated collector, and recovered volume was replaced with fresh volume stored at 37±0.5°C. All the samples were diluted to 100 mL with distilled water and analyzed at 227 nm for TZD and 271 nm for TmH using a double-beam UV/visible spectrophotometer. The drug release evaluation from microparticles and CMs was conducted in triplicate.

Stability Evaluation

According to the ICH guidelines for stability studies, CMs were kept in an incubator (Sanyo MIR-162, Japan) maintained at controlled conditions, i.e., 40±2°C plus 75±5% relative humidity. The samples of CMs were withdrawn and evaluated for stability by conducting drug assay, FTIR, and XRD after 1, 2, and 3 months. For the drug assay, a CM was opened and microparticles were dissolved in methanol (10 mL). Then, 20 mL of distilled water was added to precipitate EC. It was filtered via a 0.45-μm cellulose acetate membrane to remove insoluble polymer and filtrate volume was made 100 mL with distilled water. After 100 times of dilution again, the final solution was analyzed by a double-beam UV spectrophotometer at 227 nm for TZD and 271 nm for TmH. The absorbance of working standard was also determined under the same conditions and percent drug assay was calculated by dividing the absorbance of CM to the absorbance of working standard. Drug assay was conducted in triplicate and the average of three CMs assay was considered as mean value.

X-Ray Diffraction

Characteristic crystalline nature of pure drugs and CMs was evaluated by X-ray powder diffractometer (Bruker D8 Discover, Germany) using CuKα radiation source with nickel filter. The scanning rate was 5° per minute over a range of 8–60°, and the tube voltage was 35 kV with 35-mA current.

Fourier Transform Infrared Spectroscopy

Chemical interactions between drugs and polymer were studied by FTIR spectroscopy. The spectra were recorded for TZD, TmH, EC, and drug-loaded CMs using FTIR (Midac 2000, USA). All the samples were prepared in KBr disks. The scanning range was 500–4,500 cm^{-1} with a resolution of 2 cm^{-1}.

RESULTS AND DISCUSSION

Size and Morphology of Microparticles

Microparticles of TZD and TmH were sphere-shaped with even surfaces (Fig. 2). Rough and porous surface is

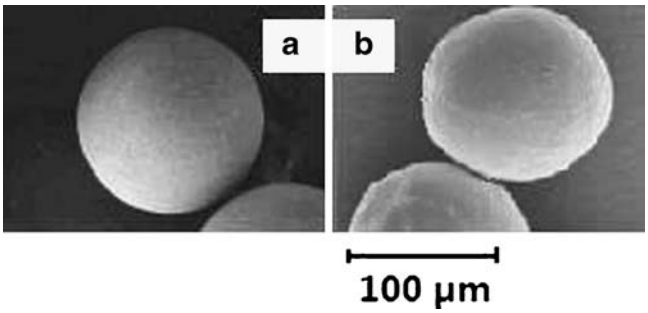

Fig. 2. Scanning electron microscopy of TZD micropaticles (**a**) and TmH microparticles (**b**)

generally caused by evaporation of solvent. As the microparticles were prepared by temperature change employing rapid cooling, their surface remained smooth. The mean particle sizes were 124±8.43 and 115±11.65 µm for TmH and TZD, respectively. Perhaps, the greater diameter of TmH microparticles may be due to the higher molecular weight of TmH.

In Vitro Drug Release Profile of Microparticles and CMs

The release profile of the microparticles could not be studied because it was difficult to make them sink in the dissolution medium. Because of the low density of a large fraction of microparticles, they remained on the surface of the medium, which could affect the true assessment of release profile. In order to attain sink condition for microparticles, stainless steel sieves were employed. The effect of dissolution media on the release behavior was statistically insignificant because solubility of EC, TmH, and TZD is independent of pH. After 10 h, 88.39% and 92.87% of TZD and TmH were released from their microparticles, respectively (Fig. 3).

In the case of CMs, the effect of the gelatin shell on the release profile was considered to be insignificant because it readily dissolved in the release medium. As microparticles were fine particles, drugs could easily be diffused out of them. Moreover, both drugs were soluble in dissolution medium (0.1 N HCl and 6.8 phosphate buffer) and were looking for a passage to come out, but EC formed an impermeable hydrophobic layer around the drugs, thus drug release was retarded. Drug release was facilitated when EC swelled up and channels were created through which drugs were leached out in dissolution medium. After 10 h, 87.59% and 95.19% of TZD and TmH were released from CMs, respectively (Fig. 3).

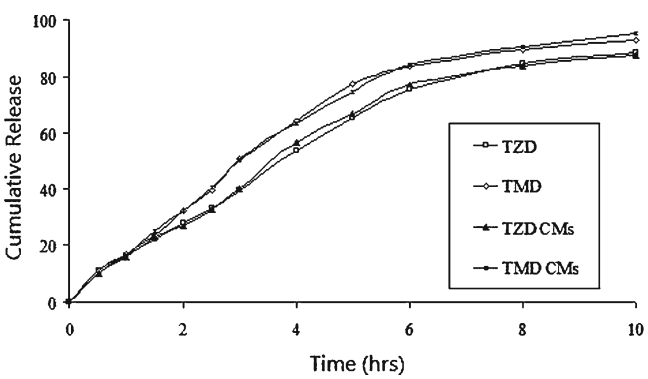

Fig. 3. Percentage cumulative *in vitro* drugs release profile from CMs

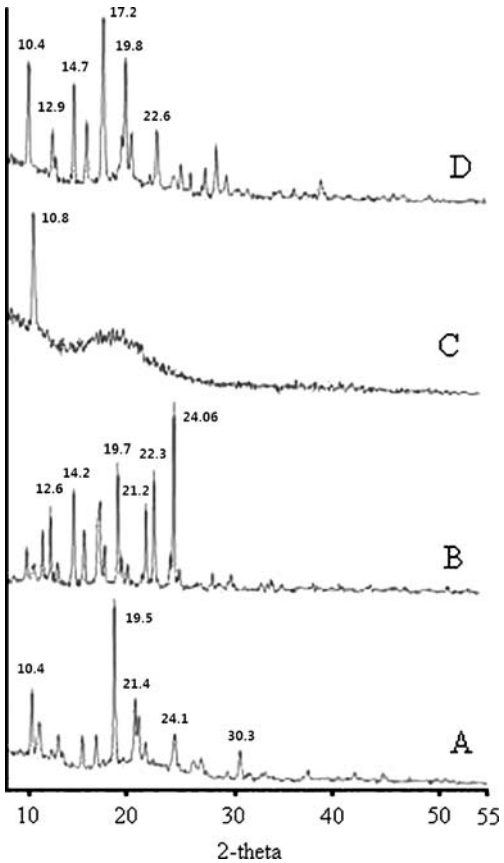

Fig. 4. X-ray powder diffraction patterns of TZD (*A*), TmH (*B*), EC (*C*), and CMs (*D*)

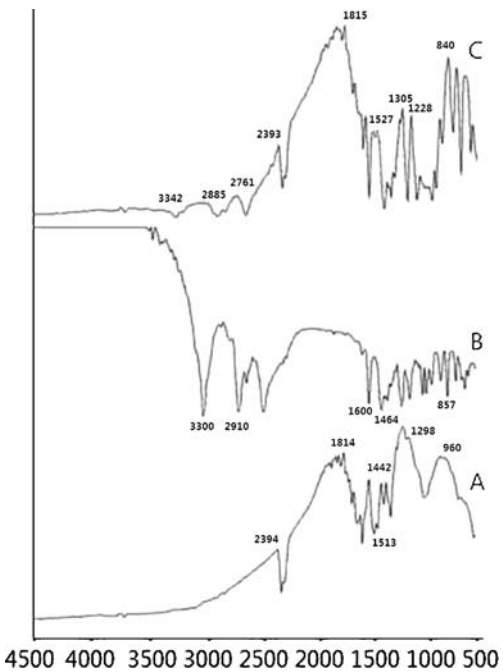

Fig. 5. Fourier transform infrared spectra; *A* TZD, *B* TmH, *C* CMs

Table I. Values of Release Rate Constant and Correlation Coefficient

	Zero-order		First-order		Higuchi equation		Korsmeyer–Pappas	
Drugs	R^2	K_o (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	n
TmH	0.898	9.949	0.553	0.309	0.951	35.069	0.608	1.09
TZD	0.930	9.366	0.582	0.310	0.954	32.489	0.626	1.083

X-Ray Diffractometry

Every crystalline substance has a specific crystallographic pattern which can be used for its recognition similar to fingerprint. The model drugs, polymer, and CMs were subjected to XRD. TmH and TZD showed characteristic concentrated peaks depending upon their crystalline nature. Intense peaks were observed for active drugs, but the intensity of peaks was reduced when they were encapsulated in polymer, which indicated reduced crystallinity (Fig. 4). The decrease in crystallinity confirmed the physical stability of TmH–TZD–EC combination (5–7).

Fourier Transform Infrared Spectroscopy

In the FTIR spectrum of TmH, the characteristic peaks of aromatic ring stretching at 1,600 cm^{-1}, aliphatic CH stretching at 2,900 cm^{-1}, aromatic CH stretching at 3,050 cm^{-1}, and OH shoulders at 3,300 cm^{-1} were seen.

The FTIR spectrum of TZD showed the C–N amide stretching peaks (1566, 1507, 1560 and 1513 cm^{-1}) and the C–C aromatic stretching peaks (1614, 1507, 1442, 1626, 1513 and 1453 cm^{-1}).

In the case of CMs, similar peaks with slight shift were observed. The slight shift was because of the overlapping and broadening of more than one peak (Fig. 5). There was no new band detected in the FTIR spectrum of CMs, indicating that there was no interaction between TmH–TZD–EC combinations. Therefore, both drugs were chemically stable in the new formulation (8–10). Prolonged release of drugs was the result of the physical barrier of polymer network, not a chemical interaction.

Kinetic Behavior Assessment of CMs

The dissolution data of CMs was fitted to commonly used models, i.e., zero-order, first-order (11), Higuchi (12), and Korsmeyer–Peppas (13,14), to determine the pattern and mechanism of drug release. The drug release constant (k) and regression coefficient (R^2) obtained from zero-order, first-order, Korsmeyer–Pappas, and Higuchi models are shown in Table I. Drug release kinetics indicated that release of TZD and TmH was best supported by Higuchi's model, i.e., based on Fickian diffusion, as it presented the highest values of linearity (TZD=0.954 and TmH=0.951),

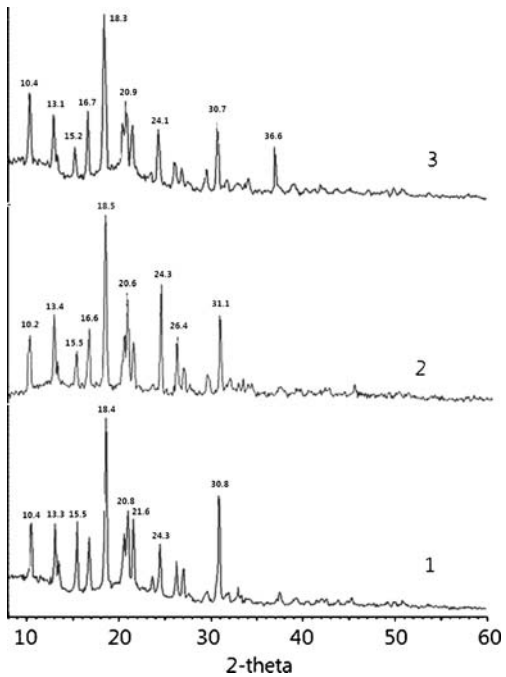

Fig. 6. X-ray powder diffraction patterns after first (*1*), second (*2*), and third (*3*) months of accelerated stability studies

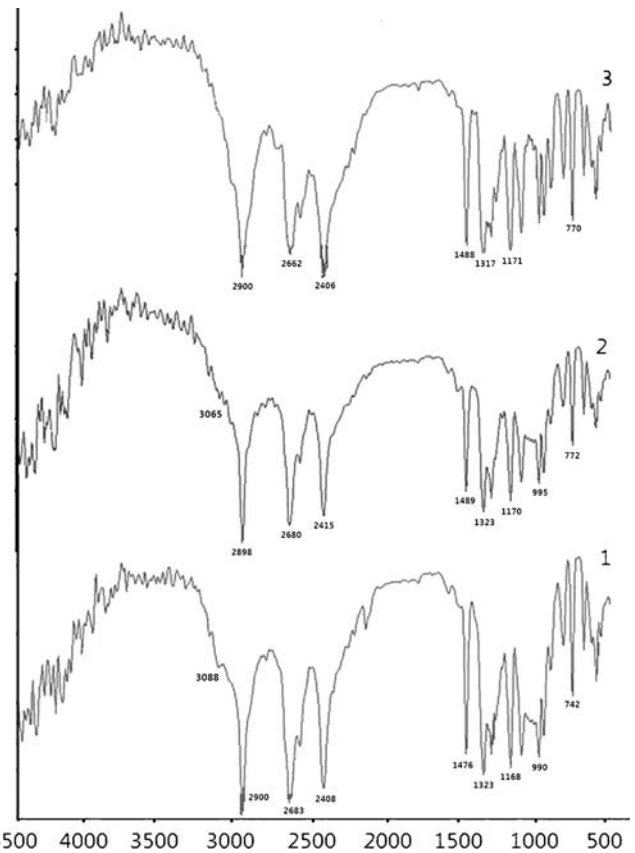

Fig. 7. Fourier transforms infrared spectra after first (*1*), second (*2*), and third (*3*) months of accelerated stability studies

but a close relationship was also noted with zero-order kinetics (TZD=0.930 and TmH=0.898). Korsmeyer's plots indicated an *n* value of 1.08 and 1.09 for TZD and TmH, respectively, which was indicative of case II transport.

Stability Evaluation

ICH guidelines for stability studies of new drug products were followed. Pakistan lies in zone 4; therefore, CMs were evaluated by keeping them in extreme controlled environment, i.e., 40±2°C and 75±5% RH for 3 months. Drug assay, FTIR, and XRD were employed as test parameters for the assessment of stability.

No significant change in XRD and FTIR spectra were observed (Figs. 6 and 7). Active drugs were also subjected to the same conditions as CMs, and drug assay of CMs was determined by its comparison with the reference one. The drug assay was also within the product specification range. The drug assay was 101.20%, 98.99%, 98.21%, and 96.57% for TZD and 99.90%, 97.99%, 97.43%, and 94.77% for TmH, after zero, first, second, and third months of stability studies, respectively.

CONCLUSION

Coacervation via temperature change method can be employed for the development of new formulations of TZD and TmH. EC retarded the release from CMs for about 12 h. The stability and compatibility of combination of TmH with TZD was demonstrated by FTIR, XRD, and content assay for both drugs. This SR combination may be given to improve a patient's compliance by reducing dosing frequencies.

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