

FORMULATION OF TWO-DRUG CONTROLLED RELEASE NON-BIODEGRADABLE MICROPARTICLES FOR POTENTIAL TREATMENT OF MUSCLES PAIN AND SPASM AND THEIR SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION

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Abstract: The objective of this study was to formulate stable and controlled release microparticles for simultaneous delivery and UV spectrophotometric detection in combined dosage of a non-steroidal anti-inflammatory drug (NSAID) (Nimesulide, NMS) and a spasmolytic agent (Tizanidine, TZN) to maintain plasma concentration that may increase patients compliance, improved therapeutic efficacy, The aim was also to reduce severity of upper GI side effects of NMS because of alteration in delivery pattern via slow release of drug from microparticles and to increase the benefits of spasticity and disability for spastic patients by administering TZN in a modified release formulation as these two drugs are often prescribed in combination for the management of pain associated with muscles spasm. Ethyl cellulose was used as a retardant polymer. Drug-polymer and drug-drug compatibility study were conducted by different analytical tests. Microparticles were prepared by coacervation thermal change method. The prepared microparticles were characterized for their micromeritics and drug loading. The prepared microparticles were light yellow, free flowing and spherical in shape. The drug-loaded microparticles showed 87% and 91% entrapment efficiency of NMS and TZN, respectively, and release was extended up to 10 h. The infrared spectra, differential scanning calorimetry thermograms and XRD spectra showed the stable character of both the drugs in the drug-loaded microparticles. The *in vitro* release study of microparticles was performed in phosphate buffer pH 6.8. Linearity was observed in the concentration range of 5.0–30.0 µg/mL of NMS and 0.5–3.0 µg/mL of TZN. The microparticles have a potential for the prolongation and simultaneous delivery of the NIM and TIZ. The proposed UV method for simultaneous detection can be used for routine analysis of combined dosage form.

Keywords: coacervation, ethyl cellulose, microparticles, nimesulide, tizanidine

Microencapsulation is used to modify and retard drug release. In pharmaceutical sustained release preparations, the uniqueness of microcapsules lies in the wide distribution throughout the gastrointestinal tract. This potentially improves drug adsorption and reduces side effects related to localized build-up of irritating drugs against the gastrointestinal mucosa (1).

Nimesulide (NMS) is a non-steroidal anti-inflammatory drug (NSAID). It acts as a cyclooxygenase-2 inhibitor (2). It also has other novel pharmacological features, which account for its effect in the control of pain and inflammation (3). Nimesulide attains peak serum concentrations of 1.98 to 9.85 mg/L within 1.22 to 3.17 h when given orally and extensively binds to plasma proteins (99%) having half-life 1.56 to 4.95 h, which requires

frequent higher dosing to maintain plasma concentration (4, 5).

Tizanidine (TZN) is a 2-adrenergic agonist and centrally active myotonolytic skeletal muscle relaxant with a chemical structure unrelated to other muscle relaxants. It is a new treatment for spasticity associated with multiple sclerosis, stroke and back bone injury (6). The beneficial effects of administering TZN in a controlled release formulation have been developed and presented by Smith in their clinical studies regarding spasticity and disability (7).

Ethyl cellulose (EC) is a non-biodegradable and biocompatible polymer. It is one of the extensively studied encapsulating materials for the controlled release of pharmaceuticals and was selected as the retardant material for NMS and TZN. Several researchers have investigated the utilization of ethyl

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cellulose as a polymer to microencapsulate a drug by coacervation phase separation technique (8) and emulsion solvent evaporation technique (9).

The purpose of the present work was to prepare and evaluate a pioneer combined oral controlled release microparticles of NMS and TZN using EC by coacervation thermal change method, because TZN is a useful adjunct to NSAIDs in the treatment of analgesic rebound headache and to develop a new UV analysis method for their simultaneous detection. The prepared microparticles were evaluated for drug content and *in vitro* drug release. Drug-polymer interactions in the solid state were studied by x-ray diffractometry (XRD) and Fourier transform infrared spectrophotometry (FTIR).

MATERIALS AND METHODS

Chemicals and reagents

Nimesulide B.P. was donated by Pharvevo Pharma (Pvt.) Ltd., Karachi, Pakistan and Tizanidine was received as a gift from Raazi Therapeutics (Pvt.) Ltd., Lahore, Pakistan. Ethyl cellulose 22cp was purchased from Sigma (USA). Other chemicals such as cyclohexane and *n*-hexane were of analytical grade and supplied by Merck (Germany). UV/Vis spectrophotometer (Shimadzu, 1601, Japan) with 1 cm matched quartz cell was used for spectrophotometry.

Preparation of microparticles

A weighed amount (1 g) of ethyl cellulose was dissolved in 150 mL of heated cyclohexane at 70–80 °C with vigorous stirring (700 rpm) with the help of hot plate magnetic stirrer. Weighed amounts (1 g) of NMS and TZN were dispersed in this solution. Stirring and high temperature was maintained almost for 10 min. The temperature was then slowly reduced using cold water to induce phase separation. The product obtained by filtration was washed thrice with *n*-hexane (100 mL) at room temperature for hardness and separation of microparticles, air-dried and passed through sieve no. 40 to further separate individual microparticles, yielding a 1:1 (EC:NIM + TZN) formulation..

Multiwavelength UV spectroscopy

Using the spectral mode of spectrophotometer (Shimadzu, Japan, 1601), the spectra of NMS and TZN in methanol were measured using 376 nm (for NIM) and 230 nm (for TIZ) as two sampling wavelengths for this method. Six mixed standards of two drugs in methanol were prepared containing 0.5–3.0 µg/mL of TZN and 5–30 µg/mL of NMS. All mixed

standard solutions were scanned in multicomponent mode of spectrophotometer at 230 and 376 nm. The data from these scans were used to determine the concentration of two drugs in the sample solution (10). All mixed standards were also prepared in the phosphate buffer (pH 6.8).

Standard stock solution and calibration curve

Standard stock solutions of pure drugs were made in methanol and phosphate buffer (pH 6.8) separately, containing 100 µg/mL of TZN and 1000 µg/mL of NMS and filtered through a 0.45 µm membrane filter. Standard stock solutions of both the drugs were diluted suitably with methanol and buffer to obtain a series of known concentrations. Absorbance of series of NMS and TZN dilutions were measured at 376 and 230 nm, respectively. The absorbance values were plotted against concentrations to obtain the calibration graphs.

Validation of spectrophotometric method

The method was validated with respect to linearity, accuracy and precision. To ascertain the accuracy of the proposed method, recovery studies were carried out by addition of standard drug solution to preanalyzed sample at three different levels. The results of recovery studies were found satisfactory. Percent recovery for NMS and TZN in methanol was found in the range of 98.04% to 102.05%, respectively. The linearity of measurements was evaluated by analyzing different concentrations of the standard solutions of NMS and TZN. The Beer-Lambert's concentration range was found to be 5–30 µg/mL for NMS and 0.5–3 µg/mL for TZN. The reproducibility of the proposed method was determined by performing microparticle assay at different time intervals (morning, afternoon and evening) on the same day (intra-day assay precision) and on three different days (inter-day precision). The results of intra-day and inter-day precision are expressed in % RSD (relative standard deviation). Percent RSD for intra-day assay precision was 0.7103 (for NMS) and 0.5371 (for TZN). Inter-day assay precision was 0.7582 (for NMS) and 0.7158 (for TZN).

Dissolution studies of microparticles

In vitro dissolution testing of microparticles was carried out at 37°C in the USP XXII basket method (Pharma Test, Germany) in 900 mL of phosphate buffer (pH 6.8). Five mL of each sample was collected at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10 and 12 h with an automated sample collector after filtration through 10 µm Sinter filters. All sam-

Table 1. Goodness of fit (using R²) for mathematical models used for determination of drug release rate [Y-equation (Y = ax + b), determination coefficient (R²), and release exponent (n)].

Models	Parameters	Microparticles	
		TZN	NMS
Zero order	Y-equation	9.2724x + 10.798	8.2888x + 10.105
	R ²	0.9597	0.9712
First order	Y-equation	0.2978x + 2.2862	0.2833x + 2.2724
	R ²	0.5604	0.5474
Higuchi	Y-equation	31.86x – 8.9599	28.164x – 7.0176
	R ²	0.9768	0.9726
Hixon-Crowell	Y-equation	-0.2683x + 4.6241	-0.22x + 4.5995
	R ²	0.9971	0.9852
Korsmeyer-Peppas	Y-equation	0.7989x + 2.8721	0.5905x + 3.002
	R ²	0.9855	0.9849
	n (release exponent)	0.8	0.6

Table 2. Physical characteristics of microparticles.

Characteristics		
Particle size ^a (mean diameter in μm)		174.36 \pm 8.72
Entrapment efficiency ^b (%)		94.56 \pm 34
Solvation (%)		158.34
$t_{60\%}$ (M \pm SD) (h)	TZN	5
	NMS	6
% yield	practical	96.45 \pm 0.68
	theoretical	100.00

^a Average of hundred microcapsules \pm SD. ^b Average of three batches \pm SD

ples were analyzed by UV-spectrometry and the percentage of drug release at different sampling intervals was calculated (11).

Particle size analysis

Microparticles prepared were sieved through combined sieve system (British Standard) to determine their size.

Percentage yield

The yield was calculated according to the expression (12):

$$\text{Yield \%} = (\text{weight of microparticles} / \text{total weight of drug and polymer}) \times 100$$

Drug content estimation

For NMS and TZN, drug loaded microparticles (100 mg) were powdered and suspended in 100 mL of methanol. Drugs were extracted in 20 mL of phosphate buffer pH 6.8 and coating polymeric material was separated out through the 0.45 μm Whatman filter paper. The polymer was washed three times (7 mL each time) with phosphate buffer pH 6.8 and all filtrates were mixed and assayed spectrophotometrically after suitable dilution at 230 and 376 nm for TZN and NMS, respectively. The concentration of the drugs in the filtrate was determined from the calibration curves and encapsulation efficiency for microcapsules was calculated. Each determination was made in triplicate.

Encapsulation efficiency (%) = $(Da/Dt) \times 100$
 where Da = actual drug loading and Dt = theoretical drug loading. Physical properties of microparticles are shown in Table 2.

Micromeritics Studies

Tap density

Tap density was measured by employing the conventional tapping method (13) using 10 mL measuring cylinder and the number of tappings was reduced to 100 as it was sufficient to reach a plateau condition. Tap density was calculated by the following formula:

$$\text{Tap density} = \frac{\text{weight of microcapsules/volume of microcapsules after 100 tappings}}{\text{volume of microcapsules after 100 tappings}}$$

Compressibility index

Compressibility index was calculated by the following formula:

$$Ci = \left\{ \frac{\text{initial volume} - \text{final volume}}{\text{initial volume}} \right\} \times 100$$

Hausner ratio

The Hausner ratio, another index of flowability of microcapsules, was calculated according to the expression:

$$\text{Hausner ratio} = \frac{\text{volume before tapping (V1)}}{\text{volume after tapping (V2)}}$$

Angle of repose

Angle of repose was measured by passing microcapsules through a funnel on the horizontal surface. The height (h) of heap formed and radius (r) of cone base (heap) were measured. The angle of repose (θ) was calculated by the following formula (13):

$$\theta = \tan^{-1} h / r$$

where 'r' is the radius and 'h' is the height. Rheological properties are shown in Table 3.

Fourier transform infrared spectrophotometry (FTIR)

In order to study the interaction between NMS, TZN and EC, FTIR (Shimadzu, FTIR-8400) analy-

sis was conducted to verify the occurrence of chemical bonds between the drug and the polymer. The samples were scanned in the IR range from 500 to 4000 cm^{-1} and carbon black was used as a reference. The detector was purged carefully with clean dry helium gas to increase the signal level and reduce moisture.

X-ray diffractometry studies (XRD)

Samples of NMS, TZN, EC and drug loaded microparticles were prepared by pulverizing in a mortar. The XRD patterns of samples were recorded using a Philips PW 1830 X-ray diffractometer to find out any change in the crystallinity of drug during microencapsulation.

Kinetic models

Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism of drug release from the ethyl cellulose microsphere (Table 1). The kinetic models used were:

$$Q_t = k_0 \times t \quad (\text{zero-order equation})$$

$$\ln Q_t = \ln Q_0 - k_1 \times t \quad (\text{first-order equation})$$

$$Q_t = K \times S \times \sqrt{t} = k_h \times \sqrt{t} \quad (\text{Higuchi equation based on Fickian diffusion})$$

Hixson-Crowell cube root equation describes drug release from systems showing dissolution-rate limitation and does not dramatically change in shape as release proceeds.

$$M_0^{1/3} - M_t^{1/3} = 1 - k_{HC} t$$

where, M is the amount of drug release in time t, M_0 is the initial amount of drug in the microsphere and k_0 , k_1 , and k_h are rate constant of zero order, first order and Higuchi rate equations, respectively. In addition to these basic release models, there are several other models as well. One of them is Korsmeyer-Peppas equation.

$$M_t / M_\infty = k \times t^n$$

where M_t is the amount of drug release at time t and M_∞ is the amount released at time $t = \infty$, thus M_t / M_∞ is the fraction of drug released at time t, k is the

Table 3. Rheological properties of microparticles

Rheological property	Value
Bulk density (g/mL)	0.214
Tapped density (g/mL)	0.250
Compressibility index (%)	16
Angle of repose ($^\circ$)	23.57
Hausner's ratio	1.190

Table 4. Position of XRD peaks in drug/ polymer/ microparticles.

Name	2 θ value of peaks
EC	10.828
NIM	10.996, 11.923, 19.554, 21.655, 22.961
TIZ	11.921, 13.931, 17.353, 19.635, 20.846
Microparticles	10.828, 11.923, 17.353, 19.554, 19.635, 20.846, 22.961

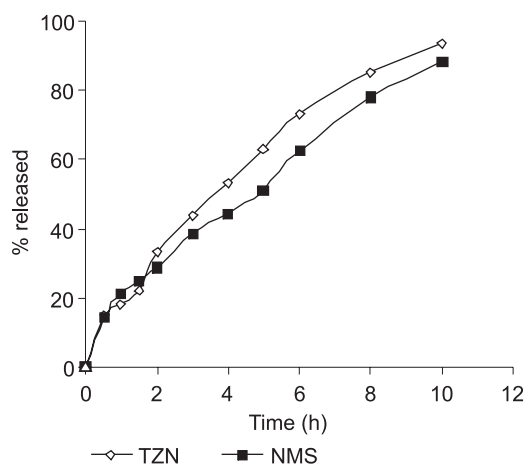


Figure 1. Dissolution profile of NMS and TZN in microparticles

kinetic constant, and n is the diffusion exponent which can be used to characterize both mechanism for both solvent penetration and drug release. Determining the coefficient of determination assessed fitness of the data into various kinetic models (14).

RESULTS AND DISCUSSION

A matrix microparticles system for sustained release of two drugs has been formulated. For this type of system, it is important that the presence of one drug species may not alter the physicochemical properties of the other (15).

The mean particle size was $174.36 \pm 8.72 \mu\text{m}$ (average of hundred microparticles). The entrapment efficiency of NMS and TZN in the microparticles was 87% and 91%, respectively. This difference in entrapment efficiency could be attributed to their slightly different solubilities in phosphate buffer pH 6.8. The percentage yield of microparticles was about 96.45%.

The microparticles showed an angle of repose 23.57 that is less than 30 ($n = 3$), which showed free flowing nature of microparticles. The bulk and tapped density (0.214 g/mL and 0.250 g/mL, respectively) showed good packability of microparticles. Compressibility index was 16% showing excellent compressibility. The Hausner ratio was 1.19.

The $t_{60\%}$ (time in hours in which 60% of the drug is released) of microparticles was 6 and 5 h for

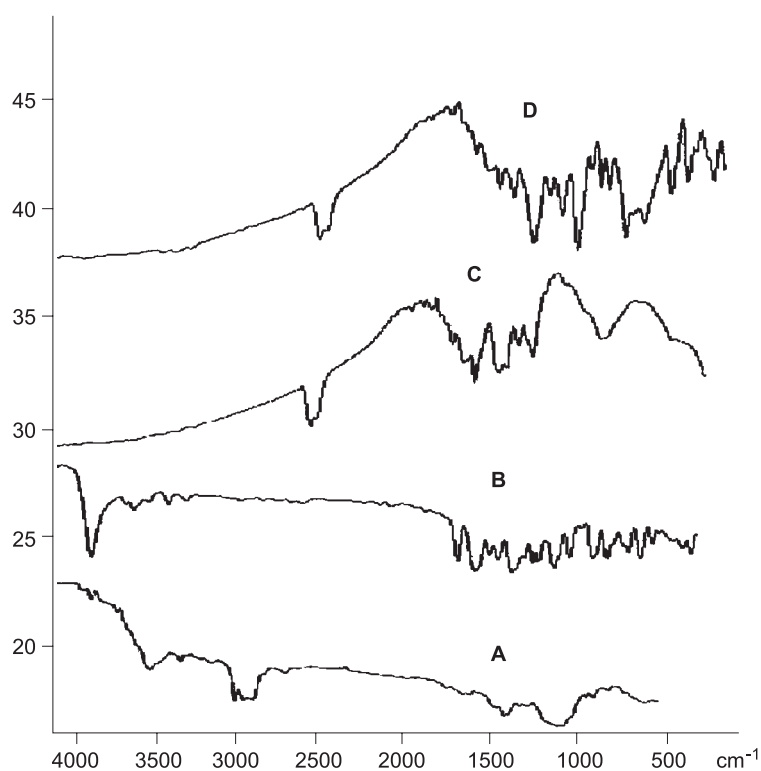


Figure 2. FTIR spectra of (A) EC, (B) NMS active, (C) TZN active, (D) NMS + TZN microparticles

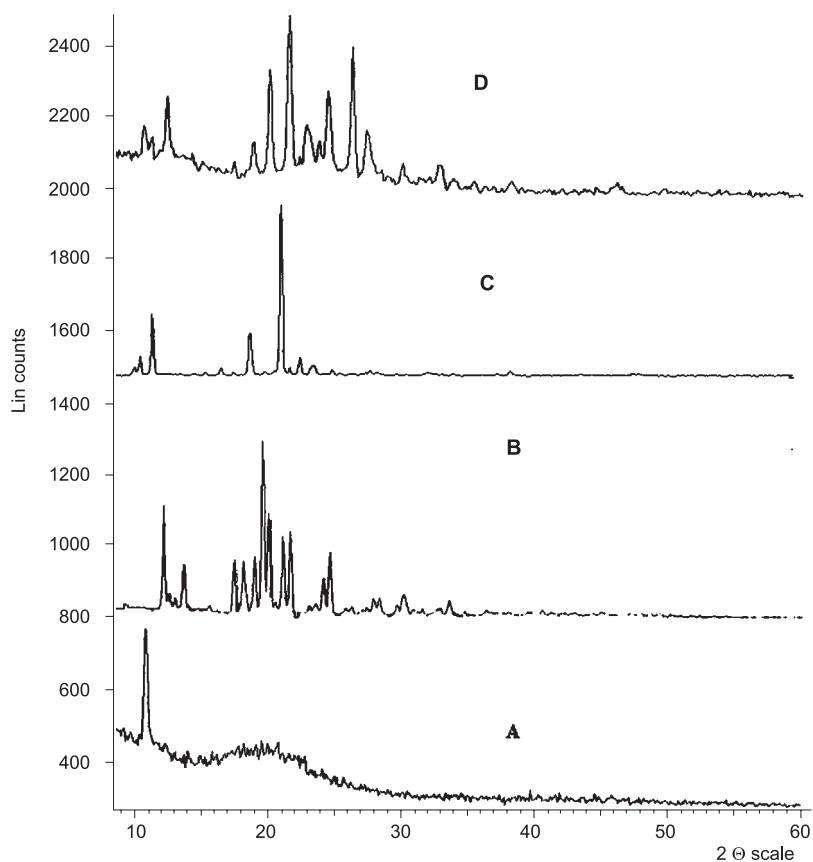


Figure 3. XRD spectra of (A) EC, (B) TZN, (C) NMS active, (D) NMS + TZN microparticles (on x-axis 2θ scale, y-axis Lin counts)

Table 5. Calibration data for NIM and TZN

Parameters	For NIM	For TZN
Recovery (%)	98.04–102.05	98.04–102.05
Beer's law limit ($\mu\text{g/mL}$)	5–30	0.5–3
Intraday precision (%RSD)	0.7103	0.5371
Interday precision (%RSD)	0.7582	0.7158
Correlation coefficient (r^2)	0.9986	0.9992

NIM and TZN, respectively. The release of TZN was found to be higher as compared to NMS release. This may be due to the higher solubility of TZN than NMS in the dissolution medium. Dissolution studies show a sustained release up to 11 h from microparticles. From the release kinetics (Table 1, Figure 1)

it can be observed that in the first three models the best-fit release kinetics model was Higuchi plot followed by zero order and first order as shown by R^2 values of these equations. The Higuchi square root equation describes the release from systems where the solid drug is dispersed in an insoluble matrix,

and the rate of drug release is related to the rate of drug diffusion (16, 17). This confirms that EC microparticles were released in a Higuchian diffusion fashion.

The dissolution data were also plotted in accordance with the Hixson-Crowell cube root law. The applicability of the formulation to the equation indicated a change in surface area and diameter of the particles with the progressive dissolution of the matrix as a function of time.

The corresponding plot (log cumulative percent drug release vs. time) according to Korsmeyer-Peppas equation indicated a good linearity. The release exponent n is $0.45 < n < 0.89$, which appears to indicate a coupling of the diffusion and erosion mechanism so-called anomalous diffusion and may indicate that the drug release is controlled by more than one process i.e., erosion and diffusion. Reddy et al. observed similar results with a matrix tablet of nicorandil with an n value of 0.71 and they also considered the corresponding n values to indicate an anomalous release mechanism (18). The physico-chemical compatibility of the drugs and the polymer was established through the following tests (Figs. 2-4). Results from FTIR, and XRD studies do not show any interaction or degradation.

FTIR studies

The FTIR spectra (Fig. 2) of the NMS indicated the presence of bands at about $906\text{-}640\text{ cm}^{-1}$ (C-H aromatic), 1078 cm^{-1} (S=O), 1159 cm^{-1} (C-O-C ether linkage), 1077 cm^{-1} (CH_3 C-H bending), 1516 and 1340 cm^{-1} (NO_2), 2929 cm^{-1} (CH_3 C-H stretching) and 3286 cm^{-1} (N-H). The FTIR spectrum of EC indicates characteristic absorption bands for -C-O-C- stretching vibration at 1052 cm^{-1} and at this at 1384 cm^{-1} corresponds to C-H bending. FTIR spectra of NMS and TZN loaded microparticles of EC also showed the same peaks with small changes in position and height. This evidenced the conversion of crystalline to amorphous form of drug and could be attributed to the in-situ complex formation between polymer and drugs.

XRD studies

X-ray diffraction is done to determine the crystalline nature of a compound and the XRD patterns of NMS, TZN, EC and drugs loaded microparticles in Figure 3 confirm the presence of crystalline structure of NMS and TZN in the microparticles. Positions of peaks in drug/polymer/ microparticles are shown in Table 4.

Disappearance of sharp peaks of drugs in diffractogram of microparticles indicates polymorphic

modifications i.e., habit modification from crystalline to amorphous form. On the other hand, the intensity of peaks is changed. This is probably due to the poor crystal perfection or different preferred orientations of the crystals in the sample holder because of their different crystal habits. Therefore, the abundance of the planes exposed to the X-ray source would have been altered, producing the radiation in the relative intensities of the peaks. NMS and TZN being entrapped into the EC offer different preferred orientations resulting in a different pattern but confirming its presence as a crystalline substance.

UV studies

The proposed method for simultaneous estimation of NMS and TZN in combined dosage form was found to be simple, accurate, rapid and economical rather than complex and time consuming HPLC methods. The wavelengths selected for quantitation were 230 nm for TZN and 376 nm for NMS. The linearity for detector response was observed in the concentration range of $5.0\text{-}30.0\text{ }\mu\text{g/mL}$ for NMS and $0.5\text{-}3.0\text{ }\mu\text{g/mL}$ for TZN. Thus, lowest limit of quantitation of NMS and TZN was $5\text{ }\mu\text{g/mL}$ and $0.5\text{ }\mu\text{g/mL}$, respectively. The concentration of the individual drug present in the mixture was determined against the calibration curve in quantitative mode. The values of coefficient of variance were satisfactorily low and recovery was close to 100% indicating reproducibility of the method. Based on the results obtained, it was found that the proposed method is accurate, precise, reproducible and economical and can be employed for routine quality control of NMS and TZN in combined formulation.

CONCLUSION

This study showed that the thermal change coacervation technique is an appropriate method to microencapsulate Nimesulide and Tizanidine into ethyl cellulose. No strong chemical interaction between drugs and polymer was found, except the conversion from crystalline to amorphous form and polymorphism. These results may suggest the potential application of ethyl cellulose microparticles as a suitable sustained release combined drug delivery system for Nimesulide and Tizanidine. Since no UV spectrophotometric method is reported for simultaneous estimation of NIM and TIZ from combined dosage form, this developed method can be used for routine analysis of two drugs without prior separation.

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REFERENCES

1. Baldha R.G., Patel Vandana B., Mayank B.: *Int. J. ChemTech. Res.* 1, 233 (2009).
2. Kulkarni S.K.: *Curr. Sci.* 83, 1442 (2002).
3. Li S.P., Kowalski C.R., Feld K.M., Grim W.M.: *Drug Dev. Ind. Pharm.* 14, 353 (1988).
4. Philip A.K., Pathak K.: *AAPS PharmSciTech.* 7 (3), E1 (2006).
5. Sajeev C., Vinay G., Archana R., Saha R.N.: *J. Microencapsul.* 19, 753 (2002).
6. Saravanam G., Bhasker K., Srinivasa R.G., Dhanaraju M.D.: *J. Microencapsul.* 20, 289 (2003).
7. Shariff A., Manna P.K., Paranjothy K.L.K., Manjula M.: *Pak. J. Pharm. Sci.* 20, 1 (2007).
8. Shovarani K.N., Goundalkar A.G.: *Indian J. Pharm. Sci.* 56: 45 (1994).
9. Siepmann J., Peppas N.A.: *Adv. Drug Deliv. Rev.* 48, 139 (2001).
10. Schwartz J.B., Simonelli A.P., Higuchi W.I.: *J. Pharm. Sci.* 57, 274 (1984).
11. Lee H.J., Lee M.H., Shim C.K.: *Arch. Pharm. Res.* 7, 33 (1984).
12. Reddy KR, Mutalik S, Reddy S.: *AAPS PharmSciTech.* 4 (4), E61 (2003).
13. Singla AK, Chawla M, Singh A.: *J. Pharm. Pharmacol.* 52: 467 (2000).
14. Olive G.; Rey E.: *Drugs* 46, 73 (1993).
15. Perucca, E.: *Drugs*, 46, 79 (1993).
16. Smith TR.: Low-dose tizanidine combined with long acting NSAIDs for analgesic rebound headache: a retrospective review. *Worldwide Pain Conference, San Francisco* (2000).
17. USP30-NF25. The official compendium of standards. 1 May 2007, the United States Pharmacopoeial Convention, Rockville 2007.
18. Wagstaff A.J., Bryson H.M.: *Drugs* 53, 435 (1997).

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