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Research Article

Torsemide Fast Dissolving Tablets: Development, Optimization Using Box–Bhenken Design and Response Surface Methodology, *In Vitro* Characterization, and Pharmacokinetic Assessment

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Abstract. The present study planed to develop new fast dissolving tablets (FDTs) of torsemide. Solid dispersions (SDs) of torsemide and sorbitol (3:1) or polyvinylpyrrolidone (PVP) k25 were prepared. The prepared SDs were evaluated for *in-vitro* dissolution. Fourier transform infrared spectroscopy and differential scanning calorimetry for SDs revealed no drug/excipient interactions and transformation of torsemide to the amorphous form. Torsemide/sorbitol SD was selected for formulation of torsemide FDTs by direct compression method. Box–Bhenken factorial design was employed to design 15 formulations using croscarmellose sodium and crospovidone at different concentrations. The response surface methodology was used to analyze the effect of changing these concentrations (independent variables) on disintegration time (Y_1), percentage friability (Y_2), and amount torsemide released at 10 min. The physical mixtures of torsemide and the used excipients were evaluated for angle of repose, Hausner's ratio, and Carr's index. The prepared FDTs tablets were evaluated for wetting and disintegration time, weight variation, drug content, percentage friability, thickness, hardness, and *in vitro* release. Based on the *in-vitro* results and factorial design characterization, F10 and F7 were selected for bioavailability studies following administration to Albino New Zealand rabbits. They showed significantly higher C_{max} and ($AUC_{0–12}$) and shorter T_{max} than those obtained after administration of the corresponding ordinary commercial Torseretic ® tablets. Stability study was conducted for F10 that showed good stability upon storage at 30°C/75% RH and 40°C/75% RH for 3 months.

KEY WORDS: Box–Bhenken design; fast dissolving tablets; pharmacokinetic parameters; solid dispersion; sorbitol; torsemide.

INTRODUCTION

Fast dissolving tablets (FDTs) are solid dosage forms that diffuse in the oral cavity giving a fast onset of action with no need of either water or mastication (1). Recently, FDTs have drawn a great attention as a promising formulation for a major group of patients, particularly children and elderly having difficulty in swallowing of conventional tablets or capsules (2). Additionally, this dosage form is recommended in some cases such as motion sickness and sudden incidence of coughing. The growing attractiveness of FDTs is attributed to rapid disintegration, superior mouth feel, and easiness of handling (3,4).

More than 40% of the active pharmaceutical ingredients have poor water-solubility making it a challenge to develop a new formulation with acceptable drug bioavailability (5,6). Different approaches have been adopted for enhancing drug dissolution rate including formation of drug-soluble salts, diminution of particle size, formation of prodrug, conversion to amorphous forms, addition of cosolvents, complexation with cyclodextrin, and formation of solid dispersions (SDs) with hydrophilic carriers (7–11). SD is a dispersion of the active ingredient in an inert carrier in the solid form. It is prepared by different methods such as co-milling, hot melt extrusion, supercritical fluid, co-precipitation, solvent evaporation, spin coating, or solvent casting method (12). SD formation is considered as a hopeful method for enhancing *in-vitro* drug release and thus improves drug bioavailability (9). Torsemide is a weak basic loop diuretic categorized under pyridine sulfonylurea group. It is used in the treatment of

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different cases of hypertension and edema coupled with heart failure and renal and hepatic disorders. As torsemide is practically insoluble in water (13) and has not formulated before as FDTs, it was chosen as a challenge model drug in the present study. In an attempt to overcome its poor water-solubility and bitter taste, the drug was preformulated as solid dispersion with suitable water-soluble carriers and flavoring agents. Box–Bhenken factorial design is an optimization technique that is used to develop designs of acceptable formulations in a manner that save time, effort, and chemicals. Factorial design is a disciplined technique of studying the virtual significance of variables and their combined effect on different responses. Moreover, the response surface characterization is an effective method for attaining a proper model with no need for long time of trial. In the present study, Box–Bhenken factorial design was used to evaluate the effect of different concentrations of superdisintegrants as independent variables on the percentage drug released, percentage friability, and disintegration time as dependent variables (14).

MATERIALS AND METHODS

Materials

Torsemide was kindly provided by Global Napi Pharmaceuticals, Egypt. Polyvinyl pyrrolidone, K25, was supplied by FLUKA Chemika, Switzerland. Sorbitol and camphor were purchased from Sigma-Aldrich, Germany. Methyl alcohol, magnesium stearate, aspartame, and menthol were purchased from El-Nasr Pharmaceutical chemicals co., Egypt. Croscarmellose sodium and crospovidone were kindly provided by the Egyptian International Pharmaceutical Industries Co. (EPICO), Egypt. All other materials and solvents were of analytical grade.

Preparation of Solid Dispersions

SDs of torsemide with PVP K25 or sorbitol were prepared adopting co-evaporation method (15). Then, 3:1 *w/w* drug/carrier ratio was selected for the preparation of the solid dispersion based on preliminary study. The intended amounts of torsemide and the carrier were dissolved in a minimum amount of methanol. The solvent was evaporated at room temperature and the residue was kept at room temperature in a desiccator for further investigations.

Preparation of Physical Mixtures

Physical mixtures corresponding to the prepared solid dispersions were prepared using a mortar and a pestle for simple mixing for sufficient time. The resultant mixtures were retained in tightly closed containers for the following investigations.

Dissolution Study of Torsemide Solid Dispersions

Dissolution study was performed in USP type II dissolution apparatus using a dissolution medium of 900 ml phosphate buffer solution (pH 7.4) kept at 37 ± 1°C and stirred at 50 rpm. Five-milliliter samples were withdrawn at 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 min and measured spectrophotometrically at 285 nm. The amount of torsemide dissolved was then calculated. The study was carried out in triplicate.

Statistical Analysis

Statistical analysis of dissolution records was carried out using similarity factor, f_2, by comparing the test dissolution profiles with a reference (16).

Differential Scanning Calorimetry

Thermograms of the prepared torsemide solid dispersions and their corresponding physical mixtures were recorded using a differential scanning calorimetry (DSC) (DSC-60, Shimadzu, Kyoto, Japan). Then, 2–5-mg samples were placed in aluminum pans and sealed with pierced lids. The thermal behavior of the samples was investigated in temperature ranges 25–200°C by heating at 10°C/min under a purge of nitrogen.

Fourier Transform Infrared Spectroscopy

FTIR spectra of the prepared torsemide solid dispersions and their corresponding physical mixtures were investigated using FTIR spectrophotometer (Jasco, Japan). Samples were mixed with a suitable amount of potassium bromide and compressed into disks using hydraulic press and scanned from 4000 to 400 cm^{-1}.

Development of Torsemide Fast Dissolving Tablets Using Box–Bhenken Factorial Design

Based on the results of the dissolution studies, 3:1 torsemide/sorbitol SD was selected for the preparation of FDTs. Using Box–Bhenken design, 15 formulations of FDTs were designed using different concentrations of croscarmellose sodium and crospovidone as superdisintegrants. Camphor was used at different concentrations as subliming agent. According to Box-Bhenken design, three independent variables, namely croscarmellose sodium concentration (X_1), crospovidone concentration (X_2), and camphor concentration (X_3), were selected. The effect of the alteration in these independent variables on three dependent variables, namely disintegration time (Y_1), percentage friability (Y_2), and amount released at 10 min (Y_3), was studied. The design used three levels of CCs and CP: 16, 20, and 24 mg, while camphor levels were 10, 15, and 20 representing low, center, and high values (−1, 0, +1 respectively).

Preparation of Torsemide Fast Dissolving Tablets

Fast dissolving tablet were prepared by direct compression method (17). Aspartame and menthol were incorporated for enhancing taste of the tablets. The composition of the prepared tablet formulation is presented in Table I. Using the bottle method, the amount of SD equivalent to 20 mg of torsemide was mixed with the corresponding amount of the excipients for 20 min. The resultant mixture (150 mg) was

Table I. Composition of Torsemide FDTs According to Box–Bhenken Design

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
SD #20 mg TSM	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
Croscarmellose sodium	20	16	16	24	24	20	20	20	20	20	16	24	16	24	20
Crospovidone	20	16	24	16	24	16	24	20	16	24	20	20	20	20	20
Camphor	15	15	15	15	15	10	10	15	20	20	10	10	20	20	15
Aspartame	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Menthol	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mg.stearate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Lactose	61.5	69.5	61.5	61.5	53.5	70.5	62.5	61.5	60.5	52.5	70.5	62.5	60.5	52.5	61.5

All ingredients are in (mg) and total tablet weight is (150 mg)

compressed into a tablet, adopting 8 mm punch. The compression force was accustomed to produce a tablet hardness of 3–4.5 kg/cm^2. The prepared FDTs were vacuum dried at 60°C for 24 h until a constant weight was obtained to ensure complete sublimation and hence increased porosity and disintegration of the formulations.

Factorial Design Characterization

Employing Statgraphics plus software (Statpoint Tech., Inc. Warrenton, Virginia, USA), one-way ANOVA test was used to statistically analyze the response parameters at 95% level of significance ($p = 0.05$). Individual parameters were investigated using the F test and the following quadratic models:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4{X_1}^2 + B_5X_1X_2 + B_6X_1X_3 + B_7{X_2}^2 + B_8X_2X_3 + B_9X_3$$

Where Y is the measured response related to each factor-level combination; X_1, X_2, and X_3 are the studied dependent variables; B_0 is the intercept (constant); and B_1–B_9 are the regression coefficients. The equation facilitates the study of the individual effect of the dependent variables and their combined effect over the measured responses. The interaction between the main effects is symbolized as X_1X_2, X_1X_3, and X_2X_3 while ${X_1}^2$ and ${X_2}^2$ refer to the quadratic terms of the independent variables that create the curvature of the designed sample space.

Assessment of Pre-Compression Parameters of the Powder Blends

The angle of repose was measured using fixed funnel method to determine the frictional force between the drug particles. Apparent bulk and tapped density were also measured to determine the powder flowability and percent compressibility by calculating Hausner's ratio and Carr's index respectively using the e following equations:

$$\text{Hausner's ratio} = \frac{\text{tapped density}}{\text{bulk density}}$$

$$\text{Carr's index} = \frac{\text{tapped denisty-bulk density}}{\text{tapped density}} \times 100$$

Assessment of Post-Compression Parameters

Weight and Drug Content Uniformity

The weight variation and drug content uniformity tests for the prepared FDTs tablets were conducted according to USP (18) procedures.

In-Vitro Disintegration Time

The disintegration time of the prepared FDTs tablets was investigated using a modified disintegration method. Ten milliliter phosphate buffer (pH 7.4) was loaded in a Petri dish of 10 cm diameter. One tablet was placed at the center of the dish. The time taken by the tablet to disintegrate completely into fine particles was recorded (19).

Wetting Time

Ten milliliter phosphate buffer (pH 7.4) was placed in a Petri dish having an internal diameter of 10 cm. A piece of tissue paper was folded two times and moistened by putting in the dish. One milliliter of eosin dye was loaded on the upper surface of the tissue paper. One tablet was located on the center of the tissue paper and the time required for the dye color to appear on the upper surface of the tablet was determined (20).

In-Vitro Release Study

The rate of drug release from the prepared and commercial ordinary torsemide tablets Torseretic® was studied applying the same methods and conditions of the dissolution test for SDs. Five-milliliter aliquots were withdrawn at specific time intervals (2, 4, 6, 8, 10, 12, 15, 20, 30, and 45 min). The samples were measured spectrophotometrically at 285.5 nm (21).

Percentage Friability, Hardness, and Thickness

Roche Friabilator was used to measure the friability of torsemide FDTs following the IP 1996 specifications (22). The hardness of individual tablets was measured using Monsanto tablet hardness tester (23). The average hardness of ten tablets was determined. Furthermore, digimatic micrometer caliber (Mitutoyo Corporation, Japan) was used to measure the thickness of the prepared tablets.

In-Vivo Animal Study

Torsemide FDTs, F7 and F10, were selected for the animal study against Torseretic® commercial tablets based on the results of *in vitro* and factorial characterization. The study was approved by the Animal Ethics Committee of Minia University, Minia, Egypt that ensured the care and use of animals conformed to the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). Four groups each of three healthy adult Albino New Zealand rabbits with average body weight of 2.25 kg were used. Before administration of the tested tablets to the rabbits, they were kept in an animal house at room temperature with free access to water but no access to food for 24 h. The control group received no medication; another group was given the ordinary commercial torsemide tablets, Torseretic 20 mg® (Pharmed Healthcare for Utopia Pharmaceuticals, Egypt) using a stomach tube. F7 and F10 were given separately to the other two groups after being anesthetized by thiopental sodium injection to facilitate tablets disintegration and thus drug absorption in the oral cavity. After dosing, 1.5-ml blood samples were withdrawn from the marginal ear vein into pre-labeled heparin-beaded tubes at the following time points 0.166, 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 12 h. The blood samples were centrifuged at 4000 rpm for 10 min. The plasma was separated and frozen at −4°C until consequent analysis.

Chromatographic Analysis of Torsemide by HPLC

High performance liquid chromatographic system (HPLC, JASCO Corporation, Tokyo, Japan) equipped with an HPLC pump (PU-980), an automatic sampler injector, and a photodiode detector (UV/ visible) and Phenomenex-C18 reverse phase column (Germany, 250 mm × 4.6 mm, 10 μm) filled with octadecyl silane was used. Analysis was conducted at room temperature using a mobile phase of 55% *v/v* 0.01 M phosphate buffer solution (pH 3.8) and 45% *v/v* acetonitrile at a flow rate of 1 ml/min. The employed UV measuring wavelength was 288 nm. Data acquisition was achieved through computer integration software (Empower, Millennium 32V4.0, Waters Corporation). A reported simple and precise method was used for determination of torsemide in human serum (24). Calibration curve of torsemide in the rabbit plasma was constructed by preparing a stock solution of torsemide in methanol (100 μg/ml). Different concentrations of the drug, 2, 4, 6, 8, 10, 12, and 14 μg/ml, were obtained by diluting the stock solution with the blank rabbit plasma (blank plasma samples spiked with torsemide). Plasma samples (collected post administration of the tested formulations to the rabbits) were defrosted and maintained at room temperature. Then, 500 μl of either the plasma samples or the spiked rabbit plasma was transferred to a labeled tube containing 0.1 ml bumetanide (internal slandered) and 50 μl acetonitrile and shacked for 1 min. Furthermore, 2.5 ml ethyl acetate (extraction solvent) was added and shacked vigorously for 10 min. The tube was then centrifuged at 4000 rpm for 5 min. Two milliliters of the resultant supernatant were evaporated under nitrogen gas and the residue was reconstituted in 0.2 ml mobile phase. The resultant solution was shacked and filtrated through micro pore filter unit and 20 μl was injected into auto sampler vials and analyzed for the torsemide concentration by HPLC. The recovery of torsemide was established by measuring the peak heights of blank serum samples spiked with torsemide against those of their corresponding solutions in methanol. Torsemide percentage recovery, limit of detection (LOD), and limit of quantification (LOQ) were determined using the following equations.

$$\%\ \text{Recovery} = \frac{\text{Peak height of torsemide in spiked rabbit plasma}}{\text{Peak height of torsemide in methanol}} \times 100$$

$$\text{LOD} = 3.3\sigma/S \qquad \text{LOQ} = 10\sigma/S$$

Where σ is the residual standard deviation of the regression line and S is the slope of standard plot.

Pharmacokinetic Analysis

Pharmacokinetic parameters of torsemide including maximum plasma concentration (C_{max}), time to reach the maximum plasma concentration (T_{max}), absorption rate, and elimination rate constants (K_{ab}, K_{el}) were estimated from the plasma concentration–time profile of each animal. Areas under the plasma concentration–time curves from zero to end of sampling time ($AUC_{0\text{-}12}$) were calculated using the trapezoidal method. Areas under the plasma concentration–time curve from zero to infinity ($AUC_{0\text{-}\infty}$), half-life ($T_{1/2}$), total clearance (CL_T), and apparent volume of distribution were also determined. All parameters were presented as mean values ± SE. The statistical significance of differences between the pharmacokinetic parameters of the tested torsemide FDTs and Torseretic® tablets were determined using one-way analysis of variance (ANOVA) with 95% confidence interval.

Stability Study of Torsemide FDTs

Stability study was conducted for F10. The selected formulation was stored at 30°C/75% RH and 40°C/75% RH in tightly closed bottles, wrapped within aluminum foil. After 90 days, the stored tablets were investigated for their drug content, weight variation, percentage friability, hardness, wetting time, *in-vitro* disintegration time, and amount torsemide released at 10 min. The results were compared to those obtained from the freshly prepared FDTs (23).

RESULTS

Characterization of SD

Dissolution Study

The dissolution profiles of torsemide SDs with PVP K25 or sorbitol are illustrated in Fig. 1. The results showed significant enhancement of torsemide dissolution rate from the prepared SDs compared to the untreated drug and the corresponding physical mixtures ($f_2 < 50$). Within the first 10 min, the untreated drug showed 24.8% drug dissolved while 3:1 torsemide SDs with PVP K25 or sorbitol showed 97.5 and 99.5%, respectively. On the other hand, the percentage of torsemide dissolved from the corresponding physical mixtures was 80.2 and 81.1%, respectively, within the same time period.

Differential Scanning Calorimetry

The DSC thermograms of the untreated torsemide, sorbitol, PVP K25, the prepared solid dispersions, and their corresponding physical mixtures are shown in Fig. 2a. The thermogram of the untreated torsemide exhibited an endothermic peak at 164°C corresponding to the drug melting point. PVP K25 thermogram

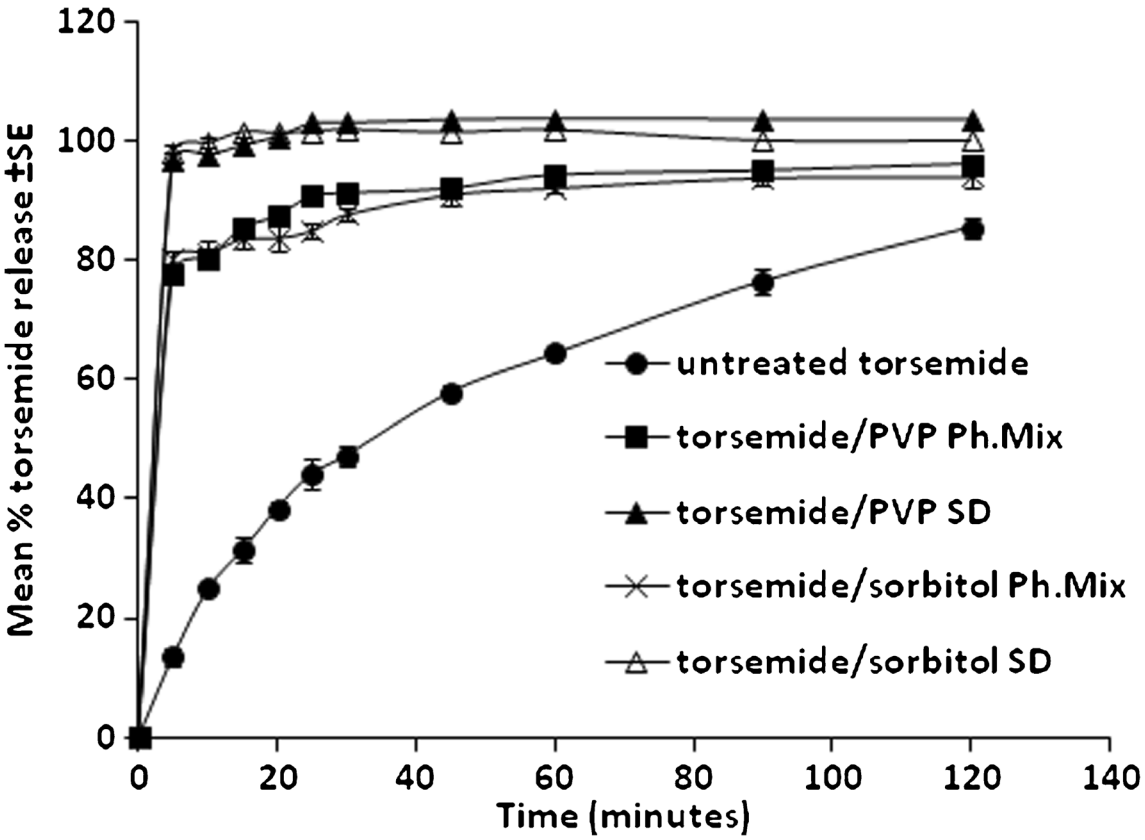

Fig. 1. Dissolution profiles of unprocessed torsemide, 3:1 torsemide/sorbitol solid dispersion, 3:1 torsemide/PVP solid dispersion, and their corresponding physical mixtures ($n = 3$)

showed a shallow, broad endotherm from 70 to 120°C while that of sorbitol showed an endotherm at 105°C corresponding to its melting point (25). DSC thermogram of torsemide/PVP K25 or torsemide/sorbitol solid dispersions exhibited disappearance of the drug melting endotherm while those of the corresponding physical mixtures showed the melting endotherm of the drug.

FTIR Spectroscopy

FTIR spectra of untreated torsemide, sorbitol or PVP K25, the prepared solid dispersions, and their corresponding physical mixtures are shown in Fig. 2b. The spectrum of untreated torsemide showed characteristic bands at 1579, 2850, 3385, and 1384 cm^{-1} corresponding to stretching of (C=O), (C=C), tertiary amine group (N-H), and sulphone group (S=O), respectively (26). The spectra of PVP K25 showed characteristic bands at 2925 cm^{-1} (C-H stretching) and 1668 cm^{-1} (amide C=O). A broad band was observed at 3410 cm^{-1} showing moisture content of the PVP K25. On the other hand, the spectrum of sorbitol showed a characteristic broad band at 3315 cm^{-1} corresponding to (-OH) stretching (27). The spectra of the torsemide/PVP K25 or torsemide/sorbitol SDs and their corresponding physical mixtures showed the characteristic bands of the drug and carriers with insignificant shift.

Characterization of Fast Dissolving Tablets

Pre-Compression Parameters of the Powder Blends

All formulations showed adequate to good flow properties as indicated by the values of angle of repose (14.84–25.96°) and Hausner's ratios (1.29 to 1.56). Carr's index showed values between 13.97 and 29.94 except F3 that exhibited a value of 36.32.

Post-Compression Characterization of Torsemide FDTs

The prepared FDTs weights were within the calculated average weight. Their drug content was in the range 89–103%. All formulations *in vitro* disintegrated in a time period between 33 to 58 s. The noticed wetting time values were between 28.83 and 52.73 s considering the shortest time for F7 and F10. The friability values were in the range of 0.435–1.101%. The tablets hardness was between 3.068 to 4.487 kg/cm^2 while the thickness was between 2.963 and 3.740 mm. The *in-vitro* release study of the prepared FDTs revealed that F7 and F10 released the highest amount of the drug (90.5 ± 2.09 and 94.1 ± 2.09%, respectively) at 10 min (data not shown). On the other hand, Torseretic® tablets showed the lowest amount of drug released at the same time period (63%).

Characterization of the Prepared Torsemide Fast Dissolving Tablets by Response Surface Methodology

Influence of Independent Variables on Disintegration Time (Y_1)

The disintegration time of torsemide FDTs (F1–F15) was recorded from 25 ± 4.582 to 34 ± 3.370 min (Table II). The following equation describes the effect of the total amounts of the superdisintegrants and subliming agent on the disintegration time:

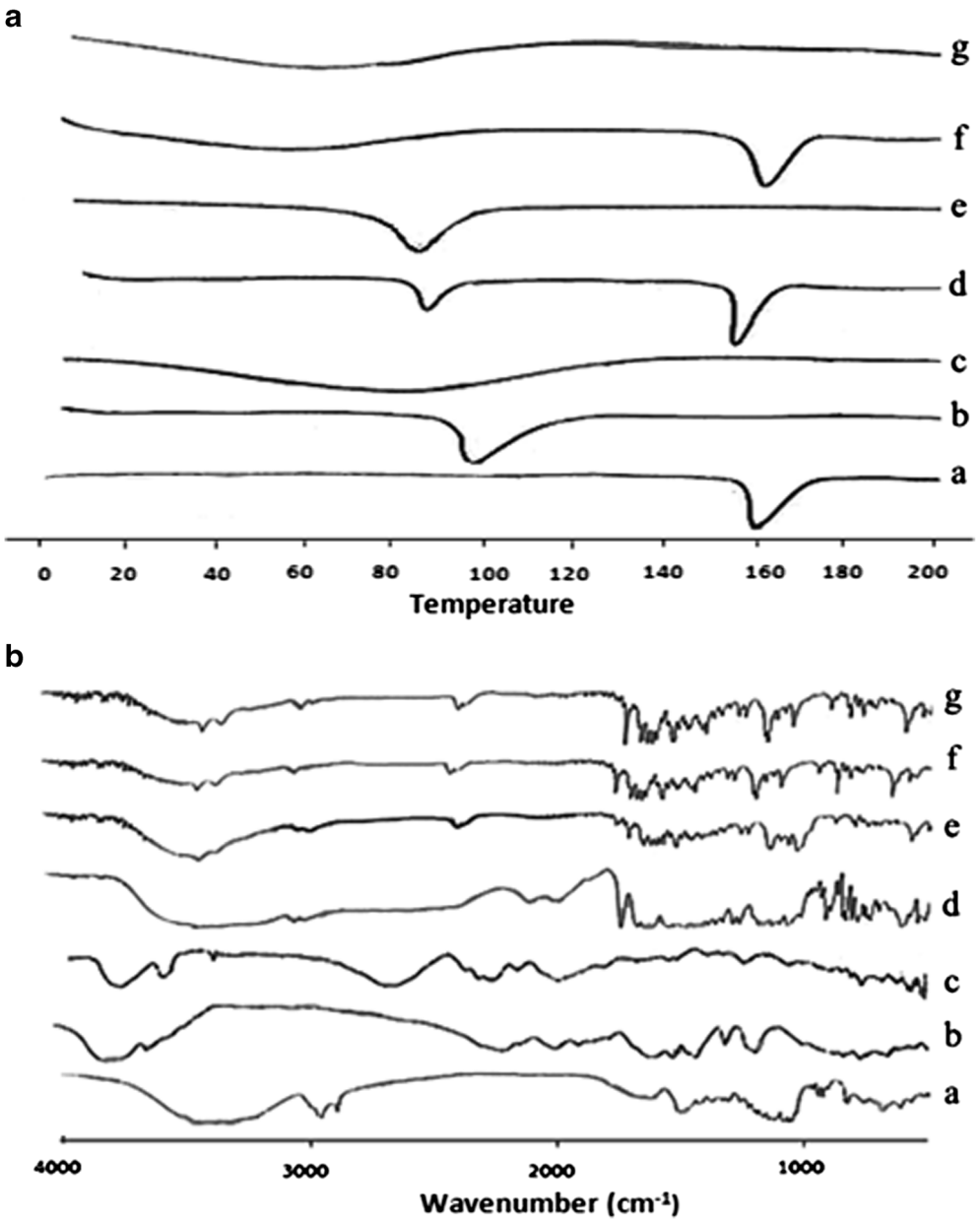

Fig. 2. **a** DSC thermograms and **b** FTIR spectra of *a* untreated torsemide, *b* sorbitol, *c* PVP K25, *d* 3:1 *w/w* torsemide/sorbitol physical mixture, *e* 3:1 *w/w* torsemide/sorbitol solid dispersion, *f* 3:1 *w/w* torsemide/PVP K25 physical mixture, and *g* 3:1 *w/w* torsemide/PVP K25 solid dispersion

$$\text{Disintegration time } (Y_1) = 50.0 + 4.05208X_1 - 7.19792X_2 + 0.55X_3 - 0.0989583{X_2}^2 - 0.03125X_1X_2 + 0.0375X_1X_3 + 0.213542{X_2}^2 - 0.0125X_2X_3 - 0.0333333X$$

The positive sign of the coefficient B_1 and B_3 refers to the increase in the disintegration time subsequent to the increase in the total amounts of CCs and camphor (the positive sign points to a synergistic effect while the negative sign indicates an antagonistic effect). On the other hand, the increase in CP concentration was followed by a decrease in the disintegration time (the coefficient B_2 has a negative sign). All tablet formulations exhibited rapid disintegration within less than 1 min which was confirmed by the *in-vitro* disintegration time test. Similar results have been attained by Shailesh *et al.* (28).

Figure 3a shows the 3D surface plot for the effect of CCs and CP concentration (X_2 and X_1 respectively) on the disintegration time (Y_1) at a constant concentration of camphor. At lower and upper levels of CP concentration, the disintegration time increased from 28 ± 0.291 to 34 ± 0.193 s. Additionally, at lower and upper levels of CCs, it had an insignificant effect on disintegration time.

Table II. Box–Bhenken Design Layout Showing Factor Combinations and Response Parameters of Torsemide FDTs

	Croscarmellose sodium		Crospovidone		Camphor		Disintegration time (s) (Y_1)	% Friability (Y_2)	% Amount torsemide released after 10 min (Y_3)
	Level	Amount (mg)	Level	Amount (mg)	Level	Amount (mg)			
F1	0	20	0	20	0	15	29.0	0.850	75.1
F2	−1	16	−1	16	0	15	28.0	0.934	59.13
F3	−1	16	+1	24	0	15	29.0	0.501	82.8
F4	+1	24	−1	16	0	15	33.0	0.570	80.92
F5	+1	24	+1	24	0	15	32.0	0.953	89.1
F6	−1	16	0	20	−1	10	27.0	0.473	74.2
F7	+1	24	0	20	−1	10	26.0	0.770	90.5
F8	0	20	0	20	0	15	28.0	0.668	73.1
F9	−1	16	0	20	+1	20	25.0	0.773	78.6
F10	+1	24	0	20	+1	20	27.0	0.955	94.1
F11	0	20	−1	16	−1	10	28.0	0.580	72.8
F12	0	20	+1	24	−1	10	33.0	0.840	85.3
F13	0	20	−1	16	+1	20	30.0	0.874	84.0
F14	0	20	+1	24	+1	20	34.0	0.339	89.4
F15	0	20	0	20	0	15	29.0	0.740	74.5

At fixed CP concentration, CCs and camphor had a significant effect on disintegration (Fig. 3b). Figure 3c revealed that at fixed CCs concentration, CP had a positive effect on the disintegration time, whereas camphor had an insignificant effect. ANOVA test revealed that there was a statistically significant correlation between disintegration time and X_2 at the 95% confidence level ($p < 0.05$). The *R*-squared statistic pointed to that the fitted model elucidated 92.0247% variability in the disintegration time. Furthermore, the standard deviation of the residuals was 0.763763 which can be employed to put up prediction limits for new

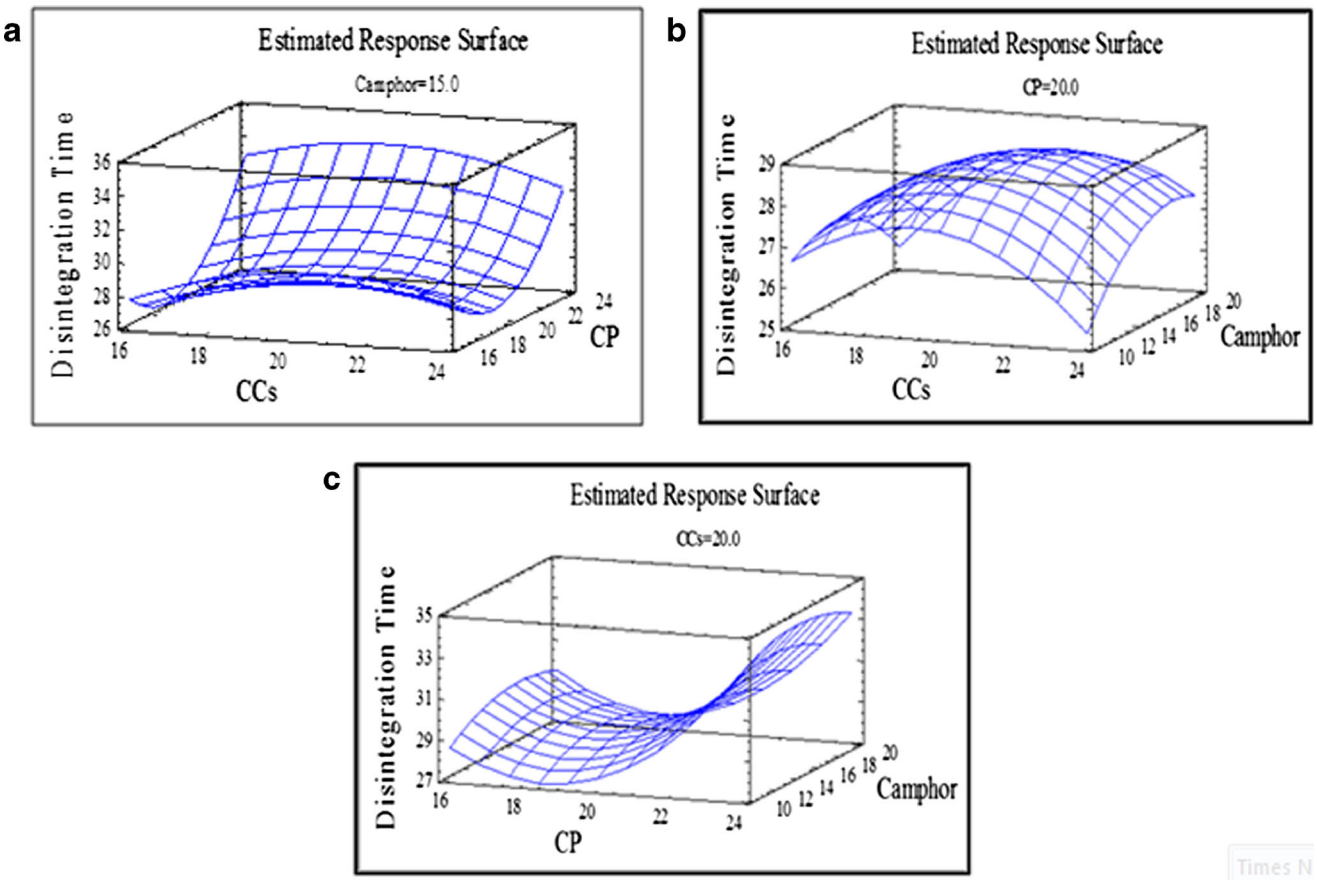

Fig. 3. Three dimensional response surface plot for the effect of **a** amount of CCs (X_1) and CP (X_2), **b** amount of CCs (X_1) and camphor (X_3), and **c** amount of CP (X_2) and camphor (X_3) on disintegration time (Y_1) of torsemide FDTs

observations. The mean absolute error (MAE) of 0.388889 was the middling value of the residuals. There might be some suggestion for serial correlation as indicated by the Durbin-Watson (DW) value (<1.4). Additionally, CP had a significant effect on disintegration time with an F value of 61.93 ($p < 0.001$). In deciding whether the model can be simplified, the highest p values of CCs and camphor concentrations were 0.6629 and 0.3970, respectively. It was concluded that the percentage of CCs and camphor had statistically insignificant effect on disintegration at 90% or higher confidence level, since the p value was ≥0.10 while B_4 and B_6 coefficients were significant with p value ≤0.05.

Influence of Independent Variables on the Percentage Friability (Y_2)

The percentage friability of torsemide FDTs (F1–F15) was recorded from 0.339 ± 0.729 to 0.995 ± 0.991% (Table II). The following equation describes the effect of the total amounts of the superdisintegrants and subliming agent on the percentage friability:

$$\%\ \text{Friability}\ (Y_2) = 0.90525 + 0.315135X_1 - 0.0481146X_2 - 0.31965X_3 + 0.00301042 X_2{}^2 + 0.01275X_1X_2 - 0.0030625\ \ X_1X_3 - 0.00383333X_2{}^2 - 0.0103125\ \ X_2X_3 - 0.00162333\ X_3{}^2$$

The positive sign of the coefficient B_1, B_4, and B_5 points to the decrease in the percentage of friability following the increase in the total amounts of CP and camphor. On the other hand, percentage friability increased as CCs concentration increased. Friability test revealed that all the prepared formulations had good mechanical resistance as indicated by the friability values (<1%). Similar results were obtained for sumatriptan succinate and rizatriptan fast dissolving tablets (29). The p value estimated by ANOVA analysis ($p \geq 0.10$) referred to the presence of a statistically insignificant correlation between the percentage friability and the chosen independent variables at confidence level of 90% or higher. Moreover, the R-squared statistic pointed to that the fitted model elucidated 80.5312% variability in the percentage friability. According to the standard error of the estimation, the standard deviation of the residuals was 0.141409. The mean absolute error (MAE) was 0.0666444 which is the middling value of the residuals. Given that the DW value is larger than 1.4 (1.82189), most likely, there was no serious autocorrelation in the residuals. In deciding whether the model can be simplified, the highest p values of the independent variables were 0.6051 and 0.7441 for camphor concentration and $X_3{}^2$, respectively. It was concluded that CP, CCs, and camphor concentration had statistically insignificant effect on friability at confidence level of 90% or higher ($p > 0.10$).

Figure 4 shows the 3-D surface plots for the effect of CCs (X_1), CP (X_2), and camphor (X_3) concentration on percentage friability (Y_2). At fixed concentration of CP and at lower levels of CCs, the percentage of friability increased from 0.770 ± 0.034 to 0.840 ± 0.423 while at the upper level of camphor, the percentage of friability increased from 0.740 ± 0.672 to 0.773 ± 0.0672 (Fig. 4a). Therefore, CCs and camphor concentrations had insignificant effect on the percentage friability. At fixed concentration of camphor and at lower and upper levels of CP concentration, the percentage friability increased from 0.580 ± 0.129 to 0.953 ± 0.009. On the other hand, at lower and upper levels of CCs concentration, percentage friability significantly decreased from 0.934 ± 0.342 to 0.668 ± 0.452. Thus, CCs and CP concentrations had a significant effect on the percentage of friability (Fig. 4b). At fixed concentration of CCs, Fig. 4c shows that the percentage friability increased from 0.473 ± 0.007 to 0.740 ± 0.672 at lower and higher levels of CP concentration. On the other hand, it decreased from 0.770 ± 0.034 to 0.473 ± 0.007 at low and higher levels of camphor concentration. Therefore, CP and camphor concentrations did not exhibit any significant effect on percentage friability.

Influence of Independent Variables on the In-Vitro Amount Torsemide Released at Ten Minutes (Y_3)

Table II shows that the uppermost and lowest percentage torsemide released at 10 min (Y_3) was 94.10 ± 1.25 and 59.13 ± 3.54%, respectively.

The following equation describes the effect of the total amounts of the superdisintegrants and subliming agent on the percentage torsemide released at 10 min (Y_3):

$$\text{Amount released at 10 min}\ (Y_3) = 31.9062 + 0.443229X_1 + 4.7601X_2 - 6.445X_3 + 0.163411X_2{}^2 - 0.242031X_1X_2 - 0.01X_1X_3 + 0.071224X_2{}^2 - 0.08875X_2X_3 + 0.300083X_3{}^2$$

The positive sign of the coefficient B_1, B_2, B_4, B_7, and B_9 points to the increase in percentage torsemide released at 10 min following the increase in the total amounts of CCs and CP. On the other hand, the initial increase in camphor concentration (from −1 to 0) resulted in a decrease in the percentage torsemide released. However, further increase (from 0 to +1) resulted in increase in the amount drug released which was represented by the negative sign of the coefficient B_3. All FDTs exhibited fast release that was supported by the results of the *in-vitro* release study.

Figure 5a shows the 3-D surface plots for the effect of CCs (X_1) and CP (X_2) concentration on the amount torsemide released (Y_3) at 10 min. At lower and upper levels of CCs or CP concentration, the amount torsemide released increased from 59.13 ± 0.231 to 90.5 ± 0.672 and from 59.13 ± 0.231 to 80.92 ± 0.331, respectively. Therefore, at fixed concentration of camphor,

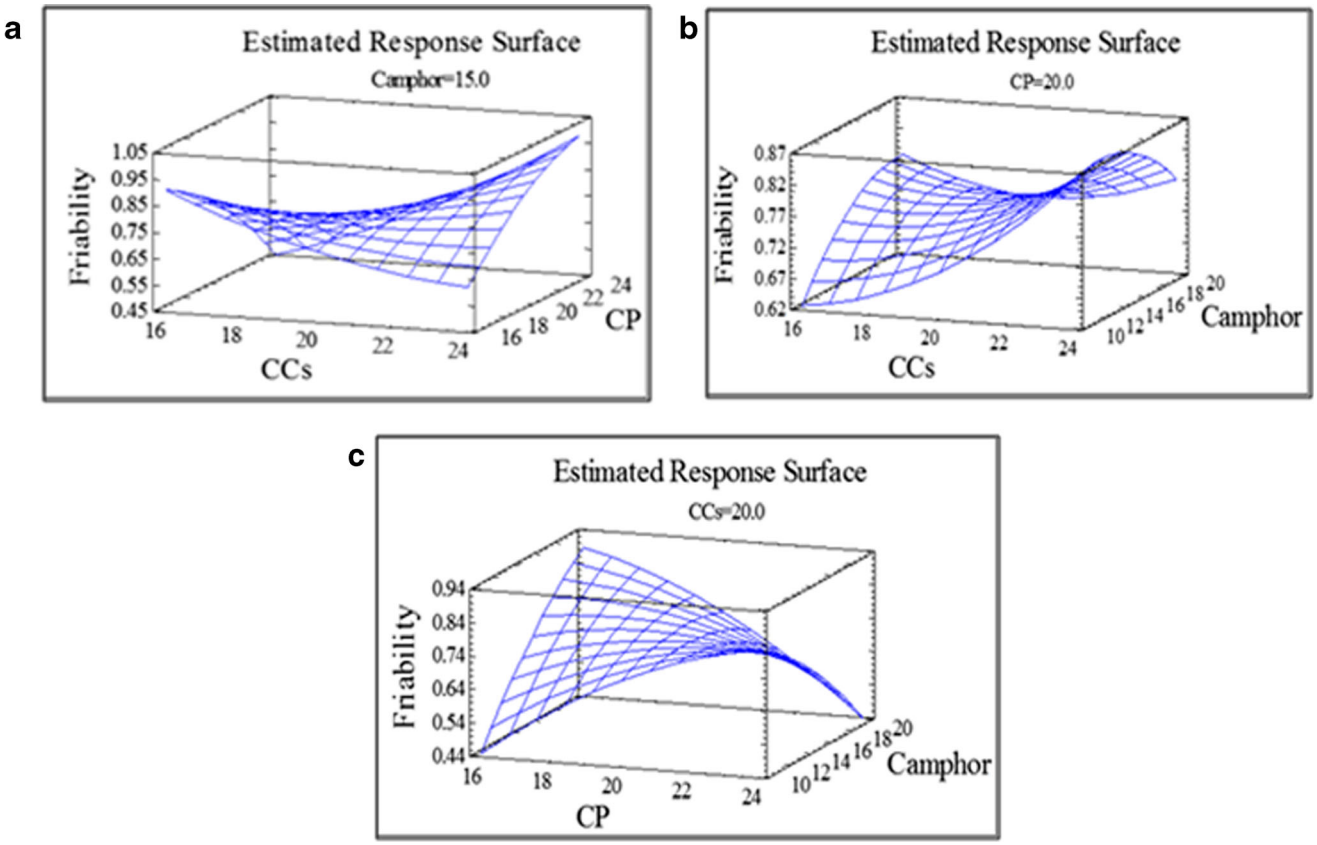

Fig. 4. Three dimensional response surface plot for the effect of **a** amount of CCs (X_1) and CP (X_2), **b** amount of CCs (X_1) and camphor (X_3), and **c** amount of CP (X_2) and camphor (X_3) on the percentage friability (Y_2) of torsemide FDTs

the concentrations of CCs and CP had a significant effect on the amount drug released at 10 min. Figure 5b reveals that the amount drug released at ten minutes increased from 73.10 ± 0.009 to 89.10 ± 0.177 at lower and upper levels of CCs concentration. On the other hand, the amount drug released showed initial decrease followed by increase upon changing from the lower to the upper levels of camphor concentration. So, at fixed concentration of CP, the concentrations of CCs rather than camphor concentration significantly influenced the amount torsemide released at 10 min. Figure 5c reveals that the amount torsemide released increased from 72.8 ± 0.354 to 84 ± 0.050 at lower and upper levels of CP concentration. Conversely, the amount drug released exhibited initial decrease followed by increase upon moving from the lower to the higher levels of camphor concentration. Consequently, at fixed concentration of CCs, CP concentrations rather than camphor concentrations significantly affect the amount torsemide released.

According to the estimates of ANOVA test regarding the p values for CCs and CP (<0.05), a statistically significant correlation was found between the amount torsemide released at 10 min and the independent variables X_1 (CCs) and X_2 (CP) at the 95% confidence level. Moreover, the R-squared statistic pointed to that the fitted model elucidated 98.1204% variability in the released amount of torsemide at 10 min. According to the standard error of the estimation, the standard deviation of the residuals was 2.08556 which could be employed to assemble prediction limits for new observations. The mean absolute error (MAE) presenting the average value of the residuals was 1.07378. The DW value of 2.27646 revealed that CCs and CP concentration significantly influence the amount torsemide released at 10 min with F values of 116.43 and 60.78, respectively ($p < 0.001$).

Pharmacokinetic Analysis

Chromatographic analysis of torsemide was accomplished with proper peak shape, resolution, and retention time (4.5 min). The results of the validation of the employed chromatographic method showed percent recovery of 92.51367 ± 1.7, LOD and LOQ of 0.059 and 0.199 μg/ml, respectively. Figure 6 shows the torsemide plasma concentrations profiles *versus* time that obtained after administration of F7, F10, or Torseretic ® tablets to Albino New Zealand rabbits. Table III shows the pharmacokinetic parameters of F7 and F10 in comparison with those of Torseretic ® tablets. The peak plasma concentrations (C_{max}) of torsemide after administration of F7, F10 were significantly higher than that attained after administration of Torseretic ® tablets ($p < 0.05$). Regarding the $AUC_{0\text{-}12}$, F7 and F10 achieved higher values than did Torseretic ® tablets ($p < 0.05$). Furthermore, the investigated FDTs showed shorter T_{max} than did the commercial tablets Torseretic ®.

Stability Study

Table IV shows the results of the stability study for F10. No considerable changes were observed in the drug content, weight variation, percentage friability, hardness, wetting time, *in-vitro* disintegration time, and amount torsemide released at 10 min after storage for 90 days at 30°C/75% RH and 40°C/75% RH.

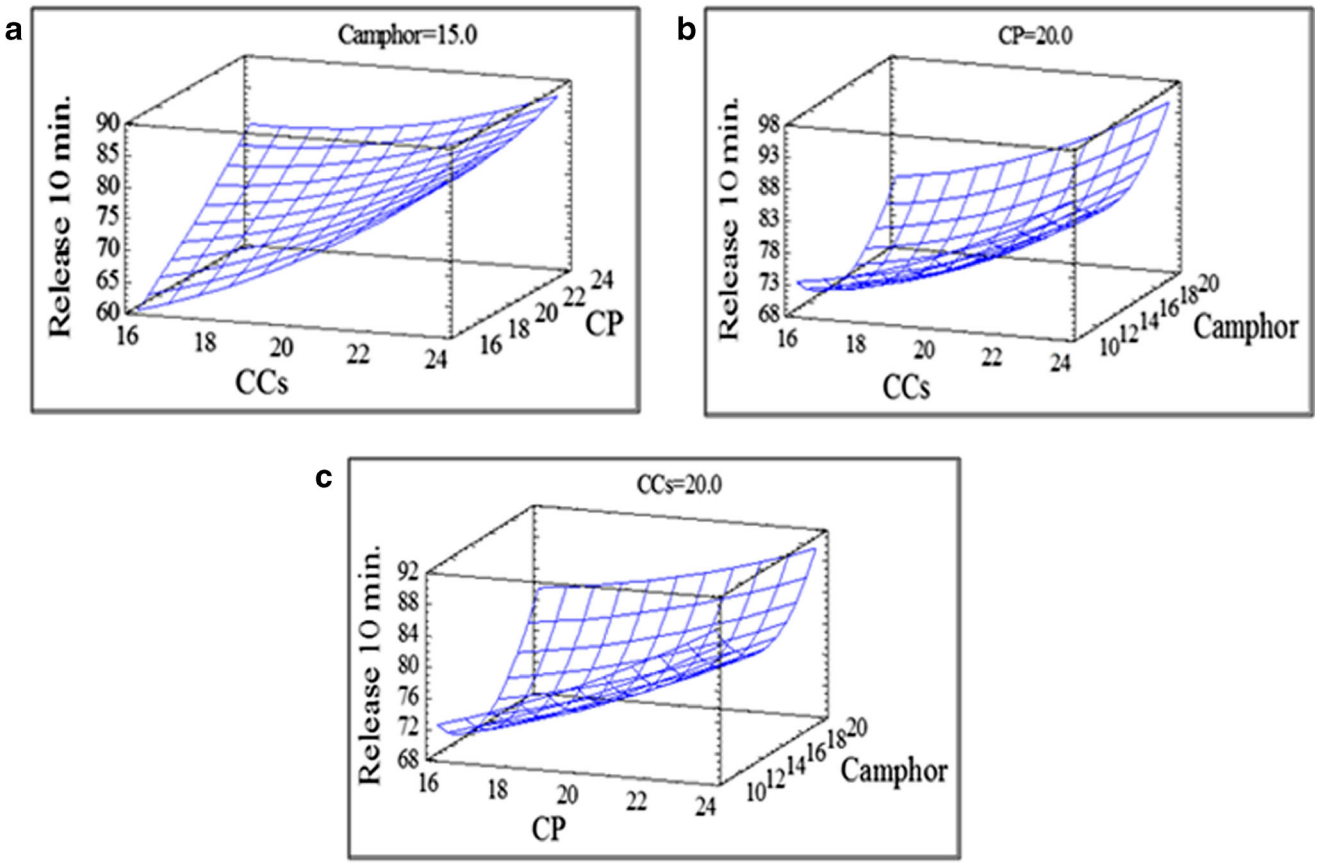

Fig. 5. Three-dimensional response surface plot for the effect of **a** amount of CCs (X_1) and CP (X_2), **b** amount of CCs (X_1) and camphor (X_3), and **c** amount of CP (X_2) and camphor (X_3) on amount drug released at 10 min (Y_3) from torsemide FDTs

DISCUSSION

The enhancement of the dissolution rate of torsemide from its solid dispersions with PVP K25 or sorbitol over the corresponding physical mixtures and the untreated torsemide might be attributed to changes in the physical form of the drug, diminution of the drug particle size, improving drug wettability, avoiding drug particles coalescence, or due to all the mechanism together (30). Disappearance of the drug melting endotherm in the DSC thermograms of its solid dispersions with PVP K25 and sorbitol indicates the conversion of torsemide from the crystalline to the amorphous form in these solid dispersions. Many studies have reported the inhibition effect of PVP for the crystallization of the drugs in their SDs (31). This result explains the enhancement of the dissolution rate of torsemide from these solid dispersions. The similarity between the FTIR spectra of torsemide/PVP K25 or torsemide/sorbitol SDs and their corresponding physical mixtures points to the absence of interaction between torsemide and sorbitol or PVP K25 in the prepared solid dispersions. The values of angle of repose, Hausner's ratio, and Carr's index support good flowability and compressibility of all the powder blends of FDTs formulations. Disintegration times of the prepared formulations were acceptable according to the USP criteria (32). The long wetting or disintegration time of some formulations (around 50 s) might be attributed to the lower concentration of the superdisintegrants in some formulations. Moreover, higher concentrations of superdisintegrants might lead to formation of a highly viscous layer surrounding the tablets and hindering the penetration of the disintegration medium to the tablet bed (33). F10 showed the highest amount of torsemide released at 10 min due to high concentration of CP which has low tendency for gel formation while having the ability for rapid absorption of water by capillary and swelling

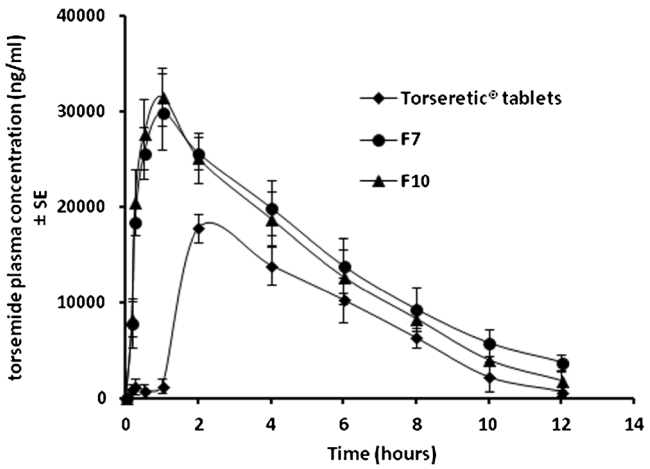

Fig. 6. Mean plasma concentration–time curves of torsemide after oral administration of F7, F10, or Torseretic ® tablets to Albino New Zealand rabbits

Table III. Pharmacokinetic Parameters of Torsemide FDTs (F7 and F10) Compared with Ordinary Commercial Torseretic ® Tablets After Oral Administration of F7, F10, or Torseretic ® Tablets to Albino New Zealand Rabbits

Pharmacokinetic parameters	F7	F10	Torseretic ® tablets
C_{max} (μg/ml)	29.95 ± 0.32	31.55 ± 0.02	17.811 ± 0.12
T_{max} (h)	1 ± 0.09	1 ± 0.03	2 ± 0.046
K_{abs} (h^{-1})	1 ± 0.56	1 ± 0.11	0.41 ± 0.56
$T_{1/2\ (abs)}$ (h)	0.690 ± 0.32	0.692 ± 0.87	1.65 ± 0.03
$AUC_{0\text{-}12h}$ (μg h/ml)	16,0732.30 ± 0.034	16,3491.9 ± 0.45	93,510.61 ± 0.47
$AUC_{0\text{-}\infty}$ (μg h/ml)	16,0732.3 ± 0.034	16,3491.9 ± 0.45	93,510.61 ± 0.47
Cl_T (ml/min)	0.0018 ± 0.56	0.0020 ± 0.08	5.4 ± 0.48

($n = 3$, mean ± SD)

mechanism and thus increase the internal pressure resulting in fast disintegration (34). The higher amount torsemide released from F7 and F10 was attributed to the rapid wetting and disintegration of these formulations. The response surface methodology characterization of the prepared torsemide FDTs revealed that there was a positive correlation between the disintegration time (Y_1) and croscarmellose sodium (X_1) or camphor (X_3) concentration, a negative correlation between the disintegration time (Y_1) and crospovidone (X_2) concentration, a positive correlation between the percentage of friability and crospovidone (X_2) or camphor (X_3) concentration, a negative correlation between the percentage of friability (Y_2) and croscarmellose sodium (X_1) concentration, a positive correlation between the amounts of torsemide released at 10 min (Y_3) and croscarmellose sodium (X_1) or crospovidone (X_2) concentration, and a negative correlation between the amounts of torsemide released at 10 min (Y_3) and camphor (X_3) concentration. The higher values of C_{max} and $AUC_{0\text{-}12}$ and the shorter T_{max} of F7 and F10 compared to Torseretic ® tablets indicate superior bioavailability of torsemide from the investigated FDTs over the ordinary commercial tablets. The absence of changes in the *in-vitro* characterization of F10 after storage at different stress conditions supports good stability of F10 at the end of the 90th days.

CONCLUSION

SD formation of torsemide with PVP K25 or sorbitol enhanced the dissolution rate of the drug with a superior enhancing effect for sorbitol. The enhanced dissolution was primarily due to change in the drug physical form rather than solubilizing effect of the used carriers. The FDTs prepared from 3:1 *w/w* torsemide/sorbitol SD exhibited satisfactory physicochemical properties. The results of Box–Bhenken factorial design and response surface methodology revealed that the amounts of superdisintegrant and camphor significantly influence the disintegration time, percentage friability, and *in-vitro* release of torsemide from fast disintegrating tablets. Box–Bhenken factorial design was used successfully to statistically optimize the formulation parameters of torsemide FDTs. The use of two superdisintegrants in addition to a subliming agent in the formulation resulted in tablets with established properties. F7 and F10 were chosen as the optimal formulations as they best fulfilled the specified requirement for fast dissolving tablets in BP. Moreover, *in-vivo* animal study revealed that F7 and F10 had an enhanced bioavailability of torsemide compared to the ordinary commercial tablets. Storage of F10 under different stress conditions for 90 days showed non-considerable changes in the *in-vitro* characterization of the tablets supporting a good stability of these formulations.

Table IV. Characterization Properties of F10 After Storage Under Different Conditions for 3 Months Compared with the Corresponding Freshly Prepared Tablets

Parameters	Time of sampling			
	Week 0	Week 12		
		25°C	30°C +RH 75%	40°C +RH 75%
Weight (mg ± SE)	149.23 (±2.405)	148.90 (±1.562)	149.00 (±1.233)	148.11 (±1.410)
Drug content (% ± SE)	95.28 (±0.010)	94.87 (±0.010)	94.76 (±0.045)	93.18 (±0.013)
Disinteg. time (s ± SE)	27 (±1.386)	26.5 (±4.214)	26.12 (±2.301)	25.87 (±2.042)
Wetting time (s ± SE)	29 (±1.069)	28.3 (±1.587)	28.45 (±0.443)	28.07 (±1.907)
Amount TSM released at 10 min (% + SE)	94.1 (±0.006)	93.80 (±0.009)	93.76 (±0.846)	92.26 (±0.013)
Friability (% ± SE)	0.955 (±0.991)	0.964 (±1.973)	0.980 (±0.783)	1.002 (±1.757)
Hardness (kg/cm^2 ± SE)	4.487 (±0.138)	4.463 (±0.057)	4.362 (±0.365)	4.152 (±0.487)

($n = 3$, mean ± SD)

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