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RESEARCH ARTICLE

Solubility enhancement of tizanidine by β-Cyclodextrin solid inclusion complexation technique

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ABSTRACT

Tizanidine is a short-acting drug for the management of spasticity. Tizanidine is an agonist at α-2-adrenergic receptor sites and presumably reduces spasticity by increasing presynaptic inhibition of motor neurons. In animal models, tizanidine has no direct effect on skeletal muscle fibres or the neuromuscular junction, and no major effect on monosynaptic spinal reflexes. The effects of tizanidine are greatest on polysynaptic pathways. The overall effect of these actions is thought to reduce facilitation of spinal motor neurons. Its poor aqueoussolubility and slow dissolution rate of the drug lead to a lack of dose proportionality and high inter and intrasubject variability. The rationale of this study was to improve the biological performance of the drug by enhancingits solubility and dissolution through complexation with β-CD. In the present study attempt has beenmade to prepare and characterize inclusion complexes of Tizanidine with β-CD and evaluation of release kinetics of the dissolution of solid inclusion complex using different models. The phase solubilityanalysis indicated the formation of 1:1 molar inclusion complex of Tizanidine with β-CD. The apparentstability constant (KC) was 37.85 M^{-1} for β-CD. The inclusioncomplexes were prepared by three different methods *viz.*, Physical, Kneading and Co-precipitation method. Theprepared complexes were characterized using FT-IR, and Differential Scanning Colorimetry (DSC). The inclusioncomplex prepared with β-CD by Kneading method exhibited significant solubility enhancementand fastest dissolution.

Key words : β-CD Tizanidine, Kneading method, Inclusion complex, Phase solubility studies

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INTRODUCTION

Cyclodextrins are cyclic oligosaccharides, containingsix, seven or eight glucopyranose units (α, β, or γ, respectively) obtained by the enzymatic degradationof starch. These are torus shaped molecules with ahydrophilic outer surface and lipophilic centralcavity, which can accommodate a variety oflipophilic drugs. Cyclodextrins are able to forminclusion complexes with poorly water-soluble drugsand have been shown to improve pharmaceuticalproperties like solubility, dissolution rate, bioavailability, stability and even palatability withoutaffecting their intrinsic lipophilicity orpharmacological properties. Out of the three parentcyclodextrins, β-cyclodextrin (β-CD) appears mostuseful as a pharmaceutical complexing agent becauseof its complexing ability, low cost and otherproperties. Natural cyclodextrins have limitedwater solubility. However, significant increase inwater solubility has been obtained by alkylation ofthe free hydroxyl groups of the cyclodextrinsresulting in hydroxyalkyl, methyl and sulfobutylderivatives. The ability of cyclodextrins to form inclusion complexes may also be enhanced bysubstitution on the hydroxyl group[1,2].

The objective of present study was to prepareinclusion complexes of Tizanidine withcyclodextrins in different molar

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ratios by differentmethods such as physical, kneading and coprecipitationmethod and increase the solubility of Tizanidine for improvement of dissolution rate andbioavailability of the drug. Also the drug release pattern was studied by applying the kinetic models to study the drug release pattern[3].

MATERIALS AND METHODS

Tizanidine was a gift from Ranbaxy Labs. India. β-cyclodextrin was gifted from Hi-Media chemicals, India. All other reagents and chemicals used were of analytical grade.

Preparation of tizanidine- -CD solid inclusion complexes[4,5] :

Solid inclusion complexes of Tizanidine with β-CD were prepared in different molar ratios 1:1, 1:2 and 1:3. (Drug: β-CD). Physical mixtures were also prepared in the same molar ratios for comparison. Before mixing both, drug and β-CD were passed through sieve # 120.

Solid inclusion complexes were prepared using methods:

- Physical mixture method
- Kneading method,
- Co-precipitation method, and
- Co-evaporation method.

The formulation chart for the solid inclusion complex is as shown in Table A :

Table A : Formulation chart of tizanidine -CD inclusion complex

Sr. No.	Formulation code	Tizanidine (%w/w)	β-Cyclodextrin(% w/w)
1.	PM_1	1	1
2.	PM_2	1	2
3.	PM_3	1	3
4.	KM1	1	1
5.	KM_2	1	2
6.	KM_3	1	3
7.	CE_1	1	1
8.	CE_2	1	2
9.	CE_3	1	3
10.	CP_1	1	1
11.	CP_2	1	2
12.	CP_3	1	3

Physical mixtures :

Physical mixtures of Tizanidine with β-CD were prepared by thoroughly mixing the two components in a mortar with spatula for 30 mins and then sieved through sieve # 100 and stored in the desiccator over fused calcium chloride to become free from moisture until further evaluation.

Kneading method :

The calculated amounts of Tizanidine and β-CD were accurately weighed, transferred to a mortar and triturated with small volume of ethanol-water (1:1, v/v) solution. The slurry obtained was kneaded for 1 hour and then dried under vacuum at room temperature in the presence of calcium chloride as a dehydrating agent. The resultant solid was pulverized and then sieved through sieve # 100.

Coprecipitation method :

The drug solution was added drop wise to aqueous solution of β-CD with constant stirring. After complete addition, the mixture was maintained at 45°C for two hour with stirring. The co-precipitated mixture was then evaporated on a water bath (Bio craft scientific systems, Agra) at 60°C for 8 hrs and further dried under vacuum at 60°C for 24 hrs.In vacuum oven (Jyoti Scientific Industry, Gwalior). The resultant solid was kept in desicator, pulverized and then sieved through sieve # 100.

Coevaporation method :

For preparation of the complex by coevaporation method, methanol and water were used as solvents. The required quantity of drug and β-CD were dissolved in methanol and water respectively. Both the solutions were mixed and solvents were evaporated by controlled heating at 45 - 50°C by buchi type vacuum rotary evaporator (Bio craft scientific systems, Agra). The resultant solid was kept in desicator, pulverized and then sieved through sieve # 100.

Evaluation of inclusion complexes [6, 7, 8]:

Drug content :

Inclusion complexes prepared by physical mixture method, kneading method, co precipitation method and co evaporation method were assayed for drug content by dissolving a specific amount of the complexes in methanol and analysed for the drug content spectrophotometrically (UV spectrophotometer, Shimadzu 1700, Japan) at 319 nm.

Saturation solubility studies :

An excess amount of solid inclusion complex was added to 5 ml of the distilled water in test tubes sealed with stoppers. The test tubes were vortex-mixed for 5 min and then centrifuged for 30 min. They were kept in a constant temperature shaking bath maintained at 37 ± 0.5°C until reaching equilibrium (48 hrs). A portion of the solution was withdrawn and then filtered with a filter paper and adequately diluted with methanol. The amount of drug solubilized was determined at 319 nm by UV-spectrophotometer (Shimadzu 1700, Japan).

In-vitro drug release :

USP type II rotating paddle method was used to study the drug release from the oral tablet at 50 rpm. A weighted amount of inclusion complexes equivalent to 20 mg drug was placed in a non-reacting muslin cloth that had smaller mesh

size than that of inclusion complexes. The muslin cloth was tied with a nylon thread to avoid the escape of any inclusion complexes. In order to produce digestive physiological phase, 900 ml of dissolution medium with different pH environments at 37± 0.5 ^{0}C was performed. The dissolution medium with the pH of 1.2 was changed to 7.4 after 2 hours and continued for up to 24 hours. At suitable intervals, samples were withdrawn, and filtered through what man filter paper no. 42 and analysed after appropriate dilution by UV double beam spectrophotometer at 319.0 nm. Studies were performed and the mean cumulative percentage of drug was calculated and plotted against time. During the drug release studies, all the formulations were observed for physical integrity at different time.

***In vitro* drug release kinetics studies[9, 10] :**

The results of *in-vitro* release profile obtained for all the formulations were plotted in models of data treatments as follows :

- Cumulative per cent drug released versus time (zero-order kinetic model).
- Log cumulative per cent drug remaining versus time (first-order kinetic model).
- Cumulative per cent drug released versus square root of time (Higuchi's model).

When the data was plotted, it yields straight line indicating that the drug was released by diffusion mechanism the slope is equal to 'K' (Higuchi, 1963). So, the drug release pattern shows Higuchi model.

Formulation and evaluation of the tablets[11, 12] :

The solid inclusion complex batch with the best solubility and dissolution properties was formulated into tablet dosage form. The blend was evaluated for different flow properties like angle of repose, bulk density, tapped density and Carr's index. Then the blend was compressed into tablets using multistation tablet compression machine and tablets were evaluated as follows :

General appearance :

It includes evaluation of size, shape, colour, odour, taste, surface texture, physical flow, consistency and legibility of any identifying markin. Tablets' visual identity and over all 'elegance' are essential for customer acceptance.

Uniformity of weight :

To study weight variation test according to USP the test was run by weighing 20 tablets individually, calculating the average weight, and comparing the individual tablet weight to the average. The tablets meets the USP weight variation tests if not more than two tablets are outside the percentage limit shown in the Table B and if no tablet differs by more than two times the percentage limit.

Table B : Weight variation tolerances of tablets

Average weight of tablets (mg)	Maximum % difference allowed
80 mg or less.	10
80 mg - 324 mg.	7.5
More than 324 mg	5

Thickness of tablets :

The crown thickness of individual was measured using Vernier Callipers. Ten individual tablets from each batch were used for the test and the average thickness was calculated.

Hardness :

Hardness of tablet was determined by using Monsanto hardness tester. The test was conducted on three tablets from each and average values were calculated.

Friability :

Friability was determined using Roche's friabilator. Apre-weighed sample of 10 tabletswas placed in the friabilatorand operated at 25 rpm for 4 mins. Then tabletswere de-dusted and reweighed to calculate friability.

Drug content uniformity :

Crushed 10 tablets and powder equivalent to 20 mg of Tizanidine was dissolved in phosphate buffer 7.4.pH. Drug content was calculated by measuring absorbance of above test sample at wavelength 319 nm in UV spectrophotometer (Shimadzu 1700, Japan).

Disintegration time :

Disintegration time of the prepared tablets was determined by using disintegration test apparatus with six tablets and distilled water kept at 37 ± 0.5^{0}C as a dissolution medium. A digital stopwatch was used to measure the disintegration time to the nearest second.

***In-vitro* dissolution studies :**

In-vitro dissolution study of formulated tablet containing solid inclusion complex was performed using USP dissolution test apparatus II (paddle type) in SGF (pH 1.2) and PBS (pH 7.4). Also drug release of formulated tablets was compared with drug release pattern of marketed tablets.

***In-vivo* studies in rats[13, 14] :**

Nine albino rat (100mg) obtained from the animal house, Institute of pharmacy, Bundelkhand University, Jhansi were used in this study. Animal were not studies until after two week of environmental adjustment period.

Dose calculation for rat :

Drug dose for the rat was calculated on the basis of body surface area (Conversion of Animal Doses to Human Equivalent

Doses Based on Body Surface Area).

$$\text{HED (mg/kg)} = \text{Animal dose (mg/kg)} \times \frac{\text{km for rat body}}{\text{km for human body}}$$

Human equivalent dose (HED) :

A dose in humans anticipated to provide the same degree of effect as that observed in animals at a given dose.

Km :

Correctionfactor for converting mg/kg dose to mg/m^2 dose (Where Km for human body is 37 and for rabbit is 6).

The orally human adult (Average body Wt. 70 kg) single dose of Tizanidine conventional dosage form as 24 mg per day .So single dose of Tizanidine-β-CD inclusion complexes for oral route was also 24 mg as selected.

So HED =24mg/70kg =0.34mg/kg,

$$\frac{\text{Km for rat body}}{\text{Km for human body}} = \frac{6}{37} = 0.16$$

So from above HED and correction factor (ratio) value, I determined single oral dose of Tizanidine for rat (body Wt. 100 mg) was 0.21mg.

Plasma drug concentration study :

The crossover study required three albino rat were used in group for three groups, namely: Group I received Tizanidine-β-CD inclusion complexes (DSK_1), Group II received Marketed drug, and Group III received plain drug tablets.

All tablet formulations, an equivalent amount of 0.21 mg Tizanidine were given to the rat and the blood sample were taken at 15, 30, 45, 60, 90, and 120 min. after dose administration. The experiment was carried out on the same rat, in which at least one week passed between each application in order to obtained complete washout of the drug.

For the collection of blood sample the rat tail artery was dilated by topical application of an alcohol swab. Blood sample were collected by mean of a 1 ml syringe fitted with a gauge needle. The needle with the level in the upright position was inserted at a 25 ^{o} to 30^{o} angle into the tail beside the artery. The needle was lowered until it was almost flush with the skin and aimed directly into the artery. Blood sample of 0.5 ml were collected in the specific time intervals. The blood samples were collected in clean 2 ml centrifuge tubes without anticoagulants. The blood was allowed to clot and the serum was separated by placing the tube in a centrifuge 15 minutes at 2000 rpm. 100µl serum samples were taken and mixed with 1ml of acetonitrile, the serum containing acetonitrile were vertexes and filtered, and then 100 µl of deprotenized serum sample were taken by micro pipette and diluted up to 3000 µl with phosphate buffer saline pH 7.4. The mixture was the firstly vertexes the centrifuged at 2000 rpm for 5 min. and supernatant was filtered through what man filter paper no.1. The plasma drug concentration of Tizanidine-β-CD inclusion complexes was analysed by UV spectrophotometer at 319.0 nm.

Pharmacokinetic parameters were calculated by non-compartmental analysis also called as Model independent analysis using Graph pad prism 5.02, software Inc., and Graph pad in stat. Peak plasma concentration (C_{max}) and time of its occurrence (t_{max}) were read directly from the plasma concentration time profile. Area under concentration time curve (AUC_{0-t}) was calculated according to trapezoidal rule (the method involves dividing the curve by a series of vertical lines into a number of trapezoids, calculating separately the area of each trapezoid and adding them together).

Statistical analysis :

Data are expressed as the means ± standard deviation (SD) of the mean (Calculated by Graph Pad Instant 3.0) and statistical analysiswas carried out employing the one-way analysis of variance(ANOVA) by using the software PRISM (Graph Pad). A value of $P < 0.05$ was considered statistically significant.

Stability studies[7, 9] :

Stability of a pharmaceutical product may be defined as a capability of a particular formulation, in a specific container, to remain within its physical, chemical, microbiological, therapeutic and toxicological applications. Stability studies were carried out according to ICH and WHO guidelines to assess the drug and formulation stability. The prepared tablets containing solid inclusion complexes (DSK1) were selected for stability studies on the basis of *in-vitro* drug release and their physical properties.

The selected tablets containing solid inclusion complexes (DSK_1) were sealed in aluminium foil packaging coated inside with polyethylene and were stored in humidity chamber at accelerated (50 ± 2°C/75 ± 5% RH) and ambient (25 ± 2°C/60% RH) conditions for a period of 60 days. Samples were withdrawn at 0, 15, 30 and 60 days periods. These samples were analysed for percentage drug content, hardness, friability, weight gain/ loss and *in-vitro* dissolution.

Accelerated stability testing :

The deterioration of active ingredients in pharmaceutical dosage forms may takes place by hydrolysis, ring cleavage, decarboxylation, oxidation, reduction, recemerization and photolysis. Predictions were based on Arrhenius explanation, which could be applied to enumerate the effect of temperature on degradation rate. The degradation rate constant (K) at various elevated temperatures are obtained by plotting some function for residual drug concentration against time. From the slope of the plot, the degradation rate at that particular temperature is obtained.

RESULTS AND DISCUSSION

Following Table 1 shows the solubilities of drug Tizanidine in different solvents.

Table 1 : Solubility studies of Tizanidine in different solvents at 25^{0}C

Sr. No.	Solvent	Solubility
1.	Water	Insoluble
2.	Methanol	Soluble
2.	Ethanol	Soluble
3.	Hydrogen chloride	Soluble
4.	Dichloromethane	Very soluble

Partition co-efficient (log P)

- n-octanol/water : 0.7
- n-octanol/SGF : 1.12

Table 2 : Phase solubility studies of inclusion complex

Sr. No.	Conc. of b-CD (mol/lit × 10^{-3})	Amount of drug (µg)	Conc. of drug (mol/lit × 10^{-5})	Enhancement ratio
1.	2	69.01	3.6	1.00
2.	4	100.38	3.8	1.81
3.	6	128.05	5.8	2.76
4.	8	148.05	7.3	3.47
5.	10	188.05	9.0	4.28
6.	12	225.86	10.8	5.14
7.	14	253.31	12.1	5.76

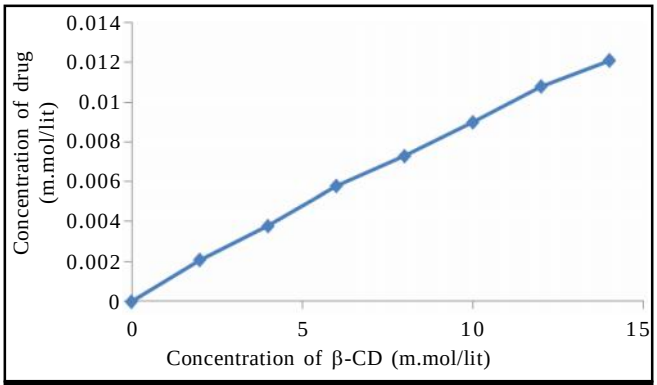

Fig. 1 : Phase solubility diagram for Tizanidine and -CD

Phase solubility studies :

Evaluation of Tizanidine- -CD solid inclusion complexes [11-15] :

Tizanidine-β-CD solid inclusion complexes were prepared by kneading method, co-precipitation method and co-evaporation method in different molar ratios (drug to β-CD). Physical mixtures were also prepared in the same molar ratios for comparison and packed for further study.

Drug content :

The percentage of drug content for all the formulations was found to be between the range of 96.5 ± 1.42% and 98.3 ± 0.76% (n=3).

Aqueous solubility :

At the end of 48 hours aqueous solubility of Tizanidine was calculated and reported as shown in Table 3.

Table 3: Aqueous solubility of pure drug and formulations

Sr. No.	Formulations	Aqueous solubility (µg/ml)
1.	Pure drug	145 ± 1.45
2.	DSP_1	266 ± 1.56
3.	DSP_2	291 ± 2.70
4.	DSP_3	328 ± 3.19
5.	DSK_1	508 ± 4.32
6.	DSK_2	526 ± 5.89
7.	DSK_3	530 ± 6.12
8.	DSE_1	436 ± 2.45
9.	DSE_2	458 ± 3.92
10.	DSE_3	476 ± 4.12
11.	DSC_1	453 ± 1.83
12.	DSC_2	468 ± 2.21
13.	DSC_3	498 ± 2.68

Results have been expressed as mean ± S.D. (n=3)

In-vitro release studies :

Table 4 : *In-vitro* drug release study (in S.G.F.) of pure drug & formulations DSP_1, DSP_2, DSP_3

Sr. No.	Time (min)	Cumulative percentage drug release			
		Pure drug	DSP_1	DSP_2	DSP_3
1.	0.0	0.00	0.00	0.00	0.00
2.	15	5.21±0.45	14.22±0.05	12.52±0.63	9.08±0.46
3.	30	11.14±0.14	31.21±0.73	26.20±0.56	20.68±0.61
4.	45	16.52±0.37	38.01±1.24	31.90±0.41	29.86±0.30
5.	60	22.39±0.65	44.20±0.12	38.48±1.08	36.69±0.92
6.	90	29.10±0.07	56.08±0.17	49.61±0.28	44.86±1.23
7.	120	33.85±0.19	64.41±0.43	53.99±0.02	50.48±0.98

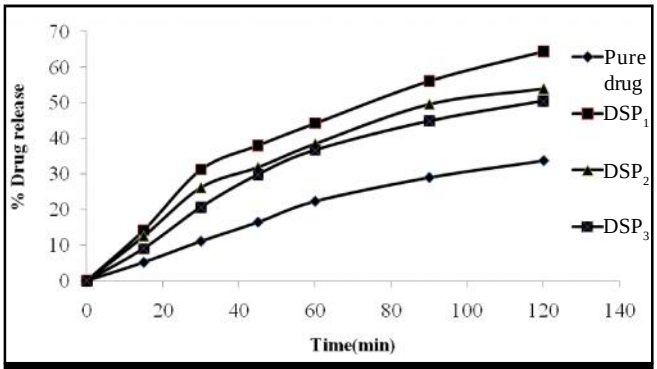

Fig. 2 : *In-vitro* release profile of pure drug & formulations (DSP_1, DSP_2, DSP_3)

Table 5 : *In-vitro* drug release study (in S.G.F.) of pure drug & formulations DSK_1, DSK_2, DSK_3

Sr. No.	Time (min)	Cumulative percentage drug release			
		Pure drug	DSK_1	DSK_2	DSK_3
1.	0.0	0.00	0.00	0.00	0.00
2.	15	5.21±0.45	38.25±0.49	36.14±0.26	33.52±0.22
3.	30	11.14±0.14	58.62±0.52	52.34±0.14	54.05±0.79
4.	45	16.52±0.37	71.00±0.21	68.75±0.57	61.52±1.21
5.	60	22.39±0.65	80.06±0.83	74.52±0.77	69.18±0.82
6.	90	29.10±0.07	88.18±1.11	81.46±0.72	78.38±0.41
7.	120	33.85±0.19	93.64±0.34	86.38±0.29	81.40±0.87

Results have been expressed as mean ± S.D. (n=3)

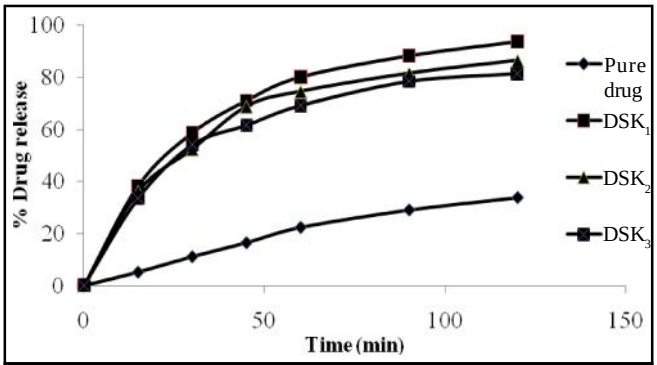

Fig. 3 : *In-vitro* release profile of pure drug & formulations (DSK_1, DSK_2, DSK_3)

Table 6 : *In-vitro* drug release study (in S.G.F.) of pure drug & formulations DSE_1, DSE_2, DSE_3

Sr. No.	Time (min)	Cumulative percentage drug release			
		Pure drug	DSE_1	DSE_2	DSE_3
1.	0.0	0.00	0.00	0.00	0.00
2.	15	5.21±0.45	35.64±1.25	38.92±0.02	27.24±0.40
3.	30	11.14±0.14	57.48±0.06	56.63±0.37	43.86±0.67
4.	45	16.52±0.37	69.16±0.67	68.19±0.19	58.06±0.29
5.	60	22.39±0.65	74.65±0.16	77.96±0.01	67.53±0.84
6.	90	29.10±0.07	85.48±1.27	85.28±0.85	72.38±0.20
7.	120	33.85±0.19	89.53±0.78	92.76±0.64	81.24±0.35

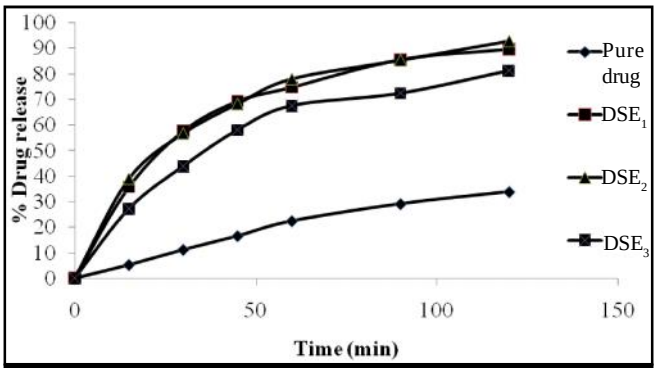

Fig. 4 : *In-vitro* release profile of pure drug & formulations (DSE_1, DSE_2, DSE_3)

Table 7 : *In-vitro* drug release study (in S.G.F.) of pure drug & formulations DSC_1, DSC_2, DSC_3

Sr. No.	Time (min)	Cumulative percentage drug release			
		Pure drug	DSC_1	DSC_2	DSC_3
1.	0.0	0.00	0.00	0.00	0.00
2.	15	5.21±0.45	32.68±1.31	30.41±0.31	33.55±0.16
3.	30	11.14±0.14	55.61±0.83	51.11±0.95	49.15±0.72
4.	45	16.52±0.37	63.25±0.85	61.29±0.70	58.77±0.60
5.	60	22.39±0.65	71.77±0.27	69.36±0.49	66.32±0.60
6.	90	29.10±0.07	79.75±0.53	76.62±0.42	73.59±1.30
7.	120	33.85±0.19	85.88±0.72	81.31±0.45	77.25±0.96

Results have been expressed as mean ± S.D. (n=3)

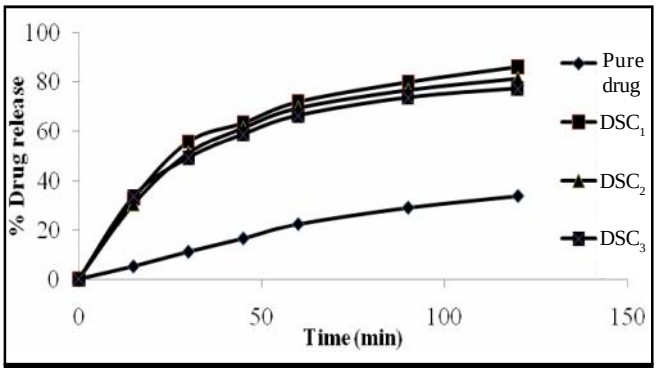

Fig. 5 : *In-vitro* release profile of pure drug & formulations (DSC_1, DSC_2, DSC_3)

Table 8 : *In-vitro* drug release study (in PBS pH 7.4) of pure drug & formulations DSP_1, DSP_2, DSP_3

Sr. No.	Time (min)	Cumulative percentage drug release			
		Pure drug	DSP_1	DSP_2	DSP_3
1.	0.0	0.00	0.00	0.00	0.00
2.	15	8.32±0.51	16.27±0.15	14.64±1.19	10.71±0.95
3.	30	14.51±0.14	31.70±0.59	28.32±0.87	24.24±0.17
4.	45	18.32±0.91	42.03±0.90	33.98±0.41	33.38±0.35
5.	60	21.62±0.48	46.22±0.43	42.59±0.55	38.62±0.51
6.	90	29.43±0.79	59.14±0.51	54.73±0.76	49.19±0.77
7.	120	35.42±0.72	67.41±0.29	60.04±0.71	53.47±0.91

Results have been expressed as mean ± S.D. (n=3)

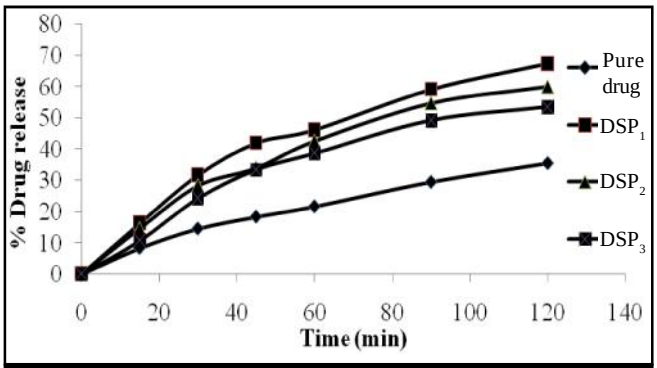

Fig. 6 : *In-vitro* release profile of pure drug & formulations (DSP_1, DSP_2, DSP_3)

Table 9 : *In-vitro* drug release study (in PBS pH 7.4) of pure drug & formulations DSK_1, DSK_2, DSK_3

Sr. No.	Time (min)	Cumulative percentage drug release			
		Pure drug	DSK_1	DSK_2	DSK_3
1.	0.0	0.00	0.00	0.00	0.00
2.	15	8.32±0.51	40.70±0.50	36.93±0.82	34.49±0.93
3.	30	14.51±0.14	57.39±0.22	51.40±0.90	52.96±0.87
4.	45	18.32±0.91	71.94±0.56	67.72±0.22	61.76±0.73
5.	60	21.62±0.48	81.43±0.70	75.17±0.40	72.01±0.61
6.	90	29.43±0.79	91.23±0.40	85.20±1.38	82.20±0.84
7.	120	35.42±0.72	94.47±0.46	89.30±0.58	85.99±0.78

Results have been expressed as mean ± S.D. (n=3)

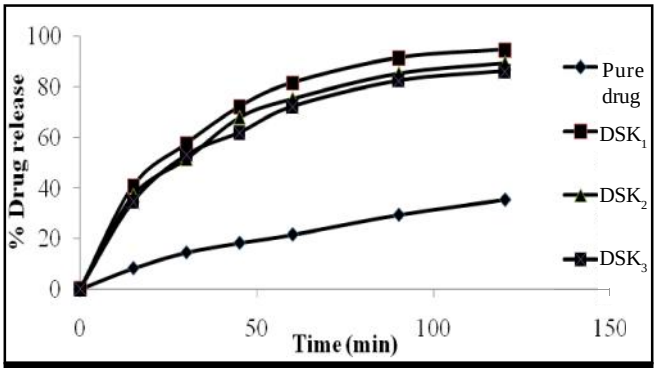

Fig. 7 : *In-vitro* release profile of pure drug & formulations (DSK_1, DSK_2, DSK_3)

Table 10 : *In-vitro* drug release study (in PBS pH 7.4) of pure drug & formulations DSE_1, DSE_2, DSE_3

Sr. No.	Time (min)	Cumulative percentage drug release			
		Pure drug	DSE_1	DSE_2	DSE_3
1.	0.0	0.00	0.00	0.00	0.00
2.	15	8.32±0.51	37.27±0.27	34.68±0.64	32.72±0.56
3.	30	14.51±0.14	47.02±0.72	54.480.44	49.85±0.28
4.	45	18.32±0.91	67.95±0.83	60.92±0.46	59.51±1.38
5.	60	21.62±0.48	74.37±0.75	67.66±0.93	65.01±0.62
6.	90	29.43±0.79	84.05±0.53	76.24±0.16	72.46±0.86
7.	120	35.42±0.72	87.85±0.66	82.62±0.50	78.74±0.80

Results have been expressed as mean ± S.D. (n=3)

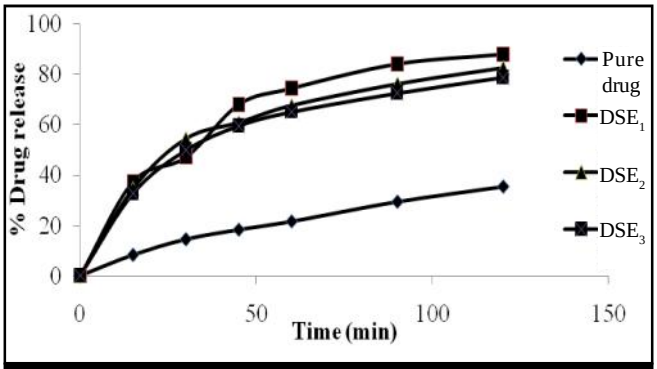

Fig. 8 : *In-vitro* release profile of pure drug & formulations (DSE_1, DSE_2, DSE_3)

Table 11: *In-vitro* drug release study (in PBS pH 7.4) of pure drug & formulations DSC_1, DSC_2, DSC_3

Sr. No.	Time (min)	Cumulative percentage drug release			
		Pure drug	DSC_1	DSC_2	DSC_3
1.	0.0	0.00	0.00	0.00	0.00
2.	15	8.32±0.51	30.50±0.15	27.30±0.75	32.70±0.63
3.	30	14.51±0.14	48.40±0.17	44.60±0.94	53.50±0.84
4.	45	18.32±0.91	59.60±0.88	54.00±0.97	63.30±0.62
5.	60	21.62±0.48	67.30±0.41	61.70±0.46	70.80±0.77
6.	90	29.43±0.79	77.60±0.48	71.10±0.41	76.40±0.80
7.	120	35.42±0.72	82.80±0.65	76.90±0.79	80.60±0.29

Results have been expressed as mean ± S.D. (n=3)

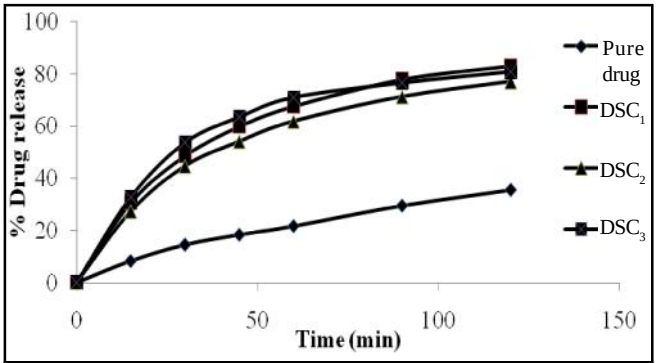

Fig. 9 : *In-vitro* release profile of pure drug & formulations (DSC_1, DSC_2, DSC_3)

Characterization of inclusion complexes:

Differential scanning colorimetry study (DSC):

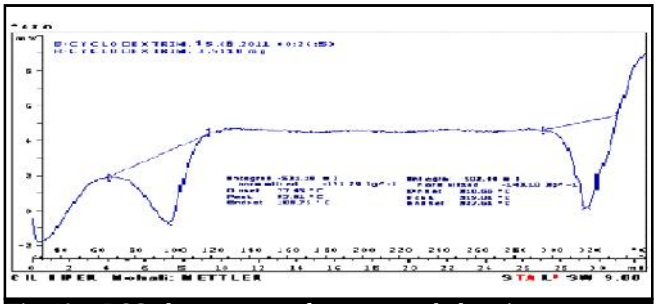

Fig. 10 : DSC thermogram of pure -cyclodextrin

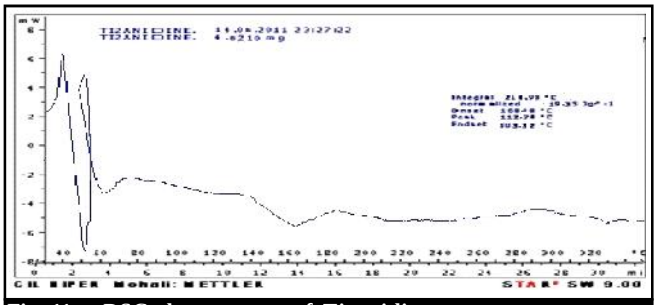

Fig. 11 : DSC thermogram of Tizanidine

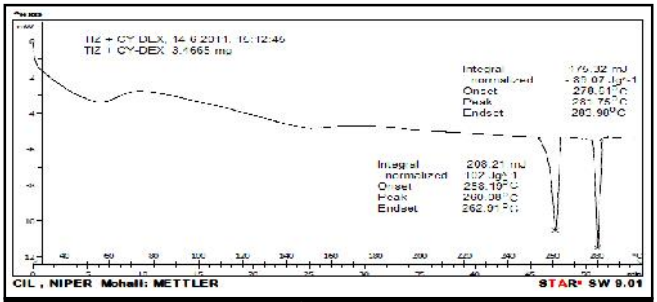

Fig. 12 : DSC thermogram of pure Tizanidine & -cyclodextrin (DSK_1)

Powder X-ray diffraction study (PXRD):

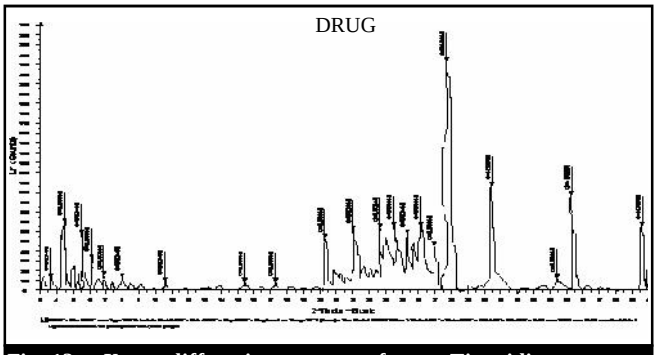

Fig. 13 : X-ray diffraction pattern of pure Tizanidine

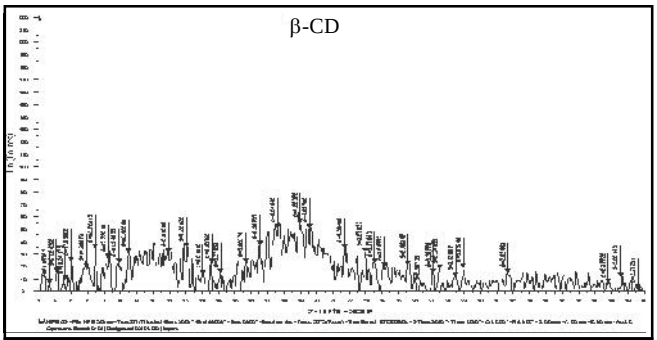

Fig. 14 : X-ray diffraction pattern of pure -cyclodextrin

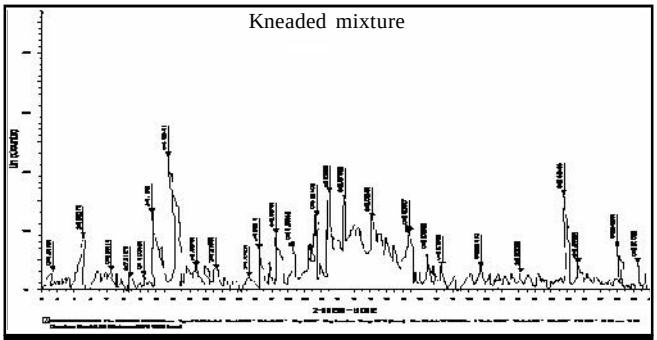

Fig. 15 : X-ray diffraction pattern of formulation DSK_1

Kinetics of drug release:

Kinetics of drug release in SGF (pH 1.2):

Zero order kinetic treatment of release data of solid inclusion complexes:

Table 12 : Zero order kinetic treatment of release data

Formulation code	Equation of the line	Correlation co-efficient (r^2)
DSP_1	y=0.5149x+8.9682	0.926
DSK_1	y=0.6895x+25.932	0.784
DSE_1	y=0.6615x+24.829	0.781
DSC_1	y=0.6326x+23.029	0.790

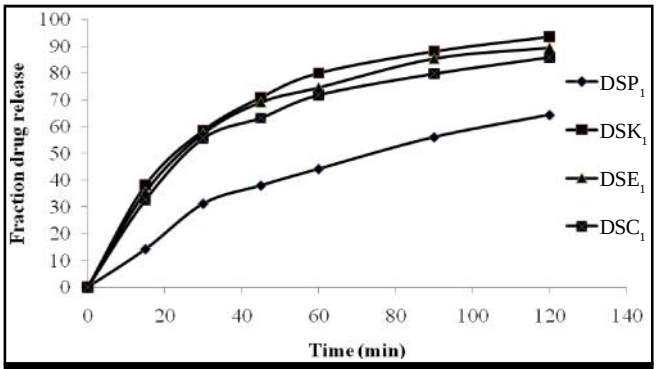

Fig. 16 : Zero order kinetic treatment of formulations DSP_1, DSK_1, DSE_1 and DSC_1

First order kinetic treatment of release data of solid inclusion complexes :

Table 13: First order kinetic treatment of release data

Formulation code	Equation of the line	Correlation co-efficient (r^2)
DSP_1	y=-0.0037x+1.9754	0.985
DSK_1	y=-0.0097x+1.9358	0.990
DSE_1	y=-0.008x+1.9157	0.971
DSC_1	y=-0.0068x+1.9143	0.962

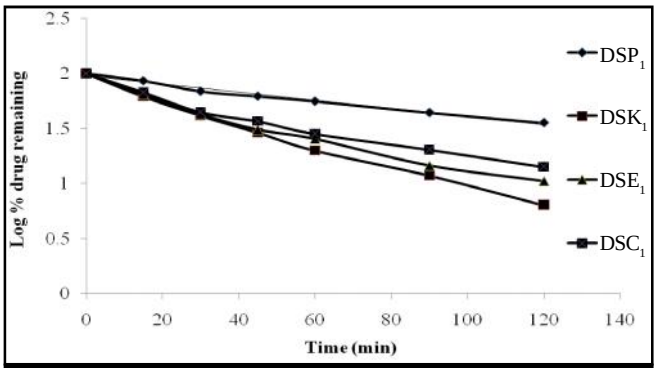

Fig. 17 : First order kinetic treatment of formulations DSP_1, DSK_1, DSE_1 and DSC_1

Higuchi's square root kinetic treatment of release data of solid inclusion complexes :

Table 14 : Higuchi's square root kinetic treatment of release data

Formulation code	Equation of the line	Correlation co-efficient (r^2)
DSP_1	y=6.1319x-3.3113	0.983
DSK_1	y=8.8582x+5.4016	0.968
DSE_1	=y8.5014x+5.1128	0.966
DSC_1	y=8.0953x+4.3941	0.968

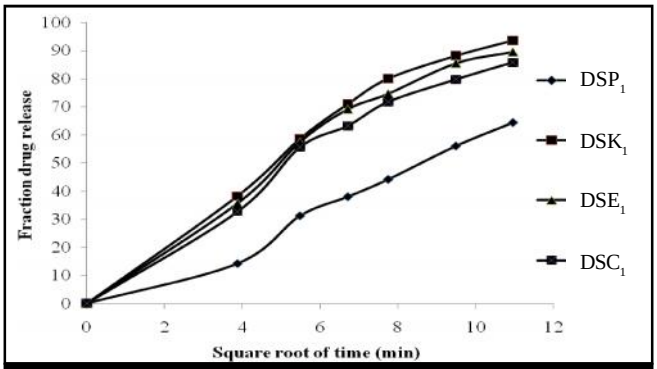

Fig. 18 : Higuchi's square root kinetic treatment of formulations DSP_1, DSK_1, DSE_1 and DSC_1

First order kinetic treatment of release data of solid inclusion complexes :

Table 16: First order kinetic treatment of release data

Formulation code	Equation of the line	Correlation co-efficient (r^2)
DSP_1	y=-0.004x+1.973	0.985
DSK_1	y=-0.0106x+1.9444	0.989
DSE_1	y=-0.0076x+1.9253	0.965
DSC_1	y=-0.0063x+1.9307	0.970

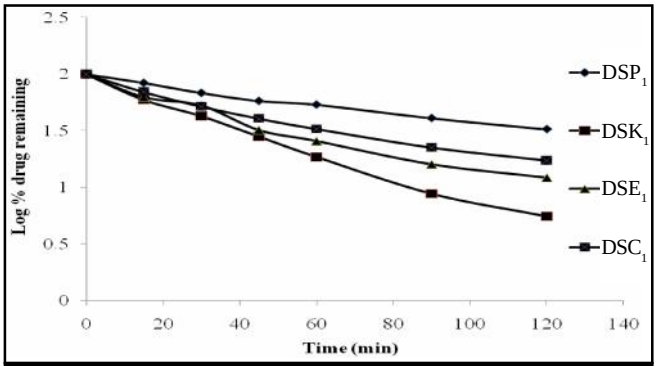

Fig. 20 : First order kinetic treatment of formulations DSP_1, DSK_1, DSE_1 and DSC_1

Kinetics of drug release in PBS (pH 7.4):

Zero order kinetic treatment of release data of solid inclusion complexes :

Table 15 : Zero order kinetic treatment of release data

Formulation code	Equation of the line	Correlation co-efficient (r^2)
DSP_1	y=0.5363x+9.9577	0.921
DSK_1	y=0.7005x+26.428	0.785
DSE_1	y=0.6615x+22.912	0.803
DSC_1	y=0.5902x+23.548	0.755

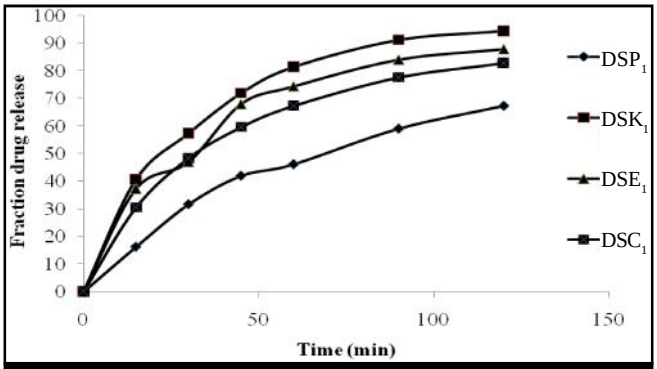

Fig. 19 : Zero order kinetic treatment of formulations DSP_1, DSK_1, DSE_1 and DSC_1

Higuchi's square root kinetic treatment of release data of solid inclusion complexes :

Table 17: Higuchi's square root kinetic treatment of release data

Formulation code	Equation of the line	Correlation co-efficient (r^2)
DSP_1	y=6.4131x-2.9977	0.986
DSK_1	y=8.9934x+5.6057	0.969
DSE_1	y=8.3975x+3.8509	0.969
DSC_1	y=7.8781x+2.5181	0.982

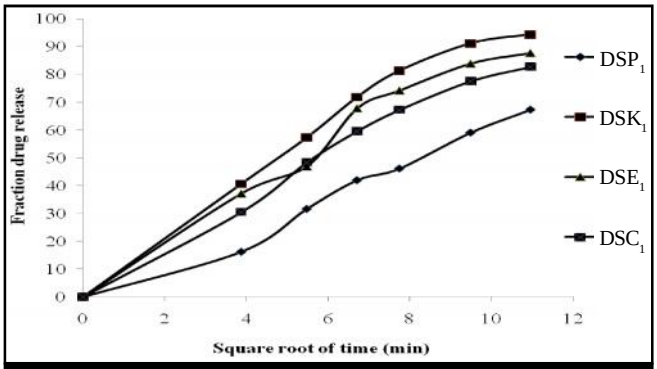

Fig. 21 : Higuchi's square root kinetic treatment of formulations DSP_1, DSK_1, DSE_1 and DSC_1

Preparation of the tablets containing inclusion complexes:

Micromeritic properties of solid inclusion complexes :

Solid inclusion complexes prepared by kneading method (DSK_1) were studied for physical properties to judge its tableting ability. Various parameters used for evaluation.

Table 18 : Micromeritic properties of solid inclusion complexes (DSK_1)

Sr. No.	Micromeritic property	Determined value	Flow property
1.	Angle of repose (º)	29±1	Good
2.	Bulk density	1.13±0.08	Fair
3.	Tapped density	1.32±0.10	Fair
4.	Compressibility index	15±1	Good
5.	Hausner's ratio	1.1±0.43	Passable

Results have been expressed as mean ± S.D. (n=3)

Micromeritic properties of blend powder:

Solid inclusion complexes prepared by kneading method (DSK_1) and other ingredientswere mixed properly for 15 min in a glass mortar and then various physical parameters were determined.

Table 19 : Micromeritic properties of blend powder

Sr. No.	Micromeritic property	Determined value	Flow property
1.	Angle of repose (º)	28±2	Good
2.	Bulk density	0.88±0.12	Fair
3.	Tapped density	1.01±0.74	Fair
4.	Compressibility index	13±0.87	Good
5.	Hausner's ratio	1.4±0.20	Passable

Results have been expressed as mean ± S.D. (Passable n=3)

Evaluation of the prepared tablets :

Table 20 : Evaluation of tablets containing inclusion complexes (DSK_1)

Sr. No.	Evaluation parameters	Calculated value
1.	Appearance	smooth, convex surface
2.	Average weight (mg)[a]	248.93
3.	Thickness (mm)[b]	5.7±0.03
4.	Hardness (kg/cm^2)[b]	6.5±0.4
5.	Friability (%)[c]	0.85±0.8
6.	Drug content (%)[b]	96.35±2.78
7.	Disintegration time (min)[d]	49.14±0.76

Results have been expressed as mean ± S.D. (a-n=20; b-n=5; c- n=10; d-n= 6)

***In-vitro* dissolution comparison of formulated and marketed tablets of tizanidine :**

Table 21 : Comparative *in-vitro* drug release profiles of conventional tablets containing Tizanidine hydrochloride and tablets containing DSK_1 in S.G.F. and phosphate buffer pH 7.4

Sr. No.	Time (min)	Cumulative percentage drug release			
		Conventional tab. containing Tiz.HCL		Tab. containing DSK_1	
		S.G.F. (pH 1.2)	phosphate buffer pH 7.4	S.G.F. (pH 1.2)	Phosphate buffer pH 7.4
1.	0.0	0.00	0.00	0.00	0.00
2.	15	13.02±3.71	19.33±5.34	38.25±0.49	40.70±0.50
3.	30	42.85±1.83	53.30±4.44	57.62±0.52	58.39±0.22
4.	45	62.06±2.02	61.54±1.67	71.00±0.21	71.91±0.56
5.	60	72.61±2.66	73.22±5.74	80.06±0.83	81.41±0.70
6.	90	75.78±3.33	76.15±4.62	88.18±1.11	91.23±0.40
7.	120	78.97±1.96	77.72±3.57	93.64±0.34	94.47±0.46

Results have been expressed as mean ± S.D. (n=3)

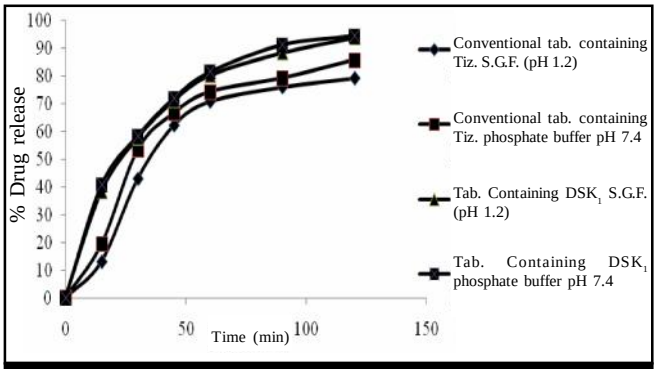

Fig. 22 : Comparative *in-vitro* drug release profiles of conventional tablets containing Tizanidine hydrochloride and tablets containing DSK_1 in S .G.F. and phosphate buffer pH 7.4

Table 22 : Plasma drug concentration studies of plain drug (control), Marketed drug (Tizanidine hydrochloride) and DSK_1 formulation in albino rat

Sr. No.	Time (min)	Plasma drug concentration of Tizanidine tablet formulation (µg/ml)		
		Plain drug	Marketed drug (Tiz. hydrochloride)	DSK_1
1.	0.0	0±0.00	0±0.00	0±0.00
2.	15	1.1±0.10	0.8±0.12	0.8±0.21
3.	30	2.3±0.30	1.7±0.11	2.0±0.32
4.	45	1.5±0.30	2.6±0.31	3.5±0.21
5.	60	0±0.00	1.3±0.02	2.6±0.19
6.	90	-	0±0.00	1.8±.02
7.	120	-	-	0±0.00

All value represent as mean ± SD (n = 3) and values are overall significant ($p<0.01$)

Table 23 : Pharmacokinetic parameter of Tizanidine tablet formulation

Sr. No.	Formulation	Cmax (µg/ml)	AUC (ng.min/ml)
1.	Plain drug	2.3±0.30	73.20±5.46
2.	Marketed drug (Tizanidine hydrochloride)	2.6±0.31	105.75±12.87
3.	DSK_1	3.5±0.21	201±21.45

All value represent as mean ± SD (n=3)

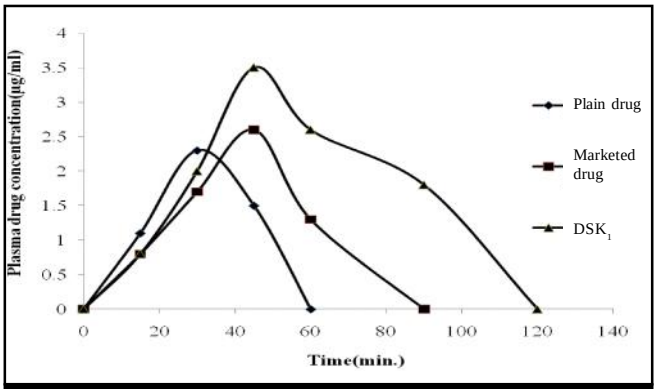

Fig. 23 : Comparison of plasma concentration of Tizanidine tablets after oral administration in optimized DSK_1 formulation, marketed drug and plain drug

Accelerated stability studies:

Table 24 : Effect of storage at room temperature (25 ± 2ºC) on the properties of tablets at the end of different time intervals

Parameters	Time (in days)			
	0	15	30	60
Hardness (kg/cm^2)	6.5±0.4	6.5±0.4	6.5±0.4	6.5±0.4
Friability (%)	0.85±0.8	0.85± 0.8	0.85±0.8	0.85±0.8
Drug content (%)	96.35±2.78	96.35± 2.78	96.35±2.78	96.35±2.78
% *in-vitro* drug release (after 120 min)	93.64±0.34 (SGF) 94.47±0.46 (7.4pH)	93.64±0.34 94.47±0.46	93.64±0.34 94.47±0.46	93.64±0.34 94.47±0.46
Weight gain/loss (w/w)	0.00	0.00	0.00	0.00

Results have been expressed as mean ± S.D. (n=3)

Conclusion:

So we can conclude that the Tizanidine β-CD complex can be formulated and evaluated in order to enhance the solubility and bioavailability of the drug Tizanidine.

Table 25 : Effect of storage at elevated temperature (50 ± 2ºC) on the properties of tablets at the end of different time intervals

Parameters	Time (in days)			
	0	15	30	60
Hardness (kg/cm^2)	6.5±0.4	6.5±0.4	6.5±0.4	6.5±0.4
Friability (%)	0.85±0.8	0.85±0.8	0.85±0.8	0.85±0.8
Drug content (%)	96.35±2.78	96.35±2.78	96.35±2.78	96.35±2.78
% *in-vitro* drug release (after 120 min)	93.64±0.34(SGF) 94.47±0.46 (7.4pH)	93.64±0.34 94.47±0.46	93.64±0.34 94.47±0.46	93.64±0.34 94.47±0.46
Weight gain/ loss(w/w)	0.00	0.00	0.00	0.00

Results have been expressed as mean ± S.D. (n=3)

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