

FORMULATION OF COATED POLYMER REINFORCED GELLAN GUM BEADS OF TIZANIDINE HCl USING FRACTIONAL FACTORIAL DESIGN

NEVINE SHAWKY ABDEL MALAK

Department of Pharmaceutics, Faculty of Pharmacy Cairo University, Kasr El Ainy street 11562, Cairo, Egypt.
Email: pharmnova@yahoo.com

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ABSTRACT

Microparticulate oral sustained release dosage form of Tizanidine HCl (TZ) a short half life water soluble drug was developed, to reduce dosing frequency, and to improve the patient compliance during the treatment of the muscle pain. A 2⁴⁻¹ fractional factorial design was employed to explore the effect of percentage of drug added in relation to polymer (X1), cross linking agent type (X2), cross linking agent concentration (X3) and curing time (X4) on the entrapment efficiency (%EE) (Y1) and the mean dissolution time (MDT) (Y2). Increasing the percentage of drug added, the concentration of cross linking agents had a negative effect on the EE and MDT while using both Zn and Ca ions significantly increased the %EE and the MDT when compared to Zn ions alone. Candidate batch with high %EE and high MDT was subjected to polymer reinforcement and coating. Beads prepared at pH 11.5 and reinforced using ethyl cellulose and coated with Eudragit® RS100 significantly increased the %EE up to 90% and extended the *in vitro* drug release for more than 8 h in a controlled manner following the Higuchi model.

Keywords: Gellan gum, Beads, Fractional factorial design, Polymer reinforced and coated, Tizanidine HCl

INTRODUCTION

Pharmaceutical invention and research are gradually more focusing on delivery systems which enhance desired therapeutic objectives while lowering side effects. Recent trends specify that multiparticulate drug delivery systems are specifically suitable for achieving controlled formulations with smallest amount risk of dose dumping¹. Multiparticulates offer greater advantages over single unit system as they disperse uniformly in GI tract, possess more predictable gastric emptying, offer flexibility and show less inter and intra individual variability in formulation process².

Over the past decades, hydrogel polymers have attracted a great deal of attention for use as potential carriers in controlled and site-specific delivery of drugs³⁻⁵.

Hydrogels are the hydrophilic, three-dimensional network structures having the natural property to absorb large quantity of water or biological fluids and they resemble those of biological tissues. The ability of hydrogels to swell in the presence of water or biological fluids regulates the release of the encapsulated drugs. By controlling the degree of swelling due to cross linking makes them potential carriers of drugs for controlled release applications⁶. Hydrogels from natural polymers, especially polysaccharides, have been widely used because of their advantageous properties over synthetic polymers such as non-toxicity, biocompatibility, biodegradability, ability to modify the properties of aqueous environments, and capacity to thicken, emulsify, stabilize, encapsulate, swell, and form gels and films⁷.

Gellan gum (GG) is an extracellular anionic heteropolysaccharide consisting of a linear structure of repeating tetrasaccharide units of glucose, glucuronic acid, and rhamnose in a molar ratio of 2:1:1. It has a characteristic temperature and ionic-dependent gelling property⁸. The mechanism of gelation involves the formation of double helical junction zones followed by aggregation of double helical segments to form a three-dimensional network by complexation with cations such as calcium and zinc and hydrogen bonding with water⁹.

Tizanidine hydrochloride (TZ) is a centrally acting α 2 -adrenergic agonist and centrally active myotonolytic skeletal muscle relaxant with short half life (2-4 hr)¹⁰. It is used for treatment of spasticity associated with multiple sclerosis, stroke, spinal cord injury, or disease. In addition it may also be a useful adjunct to NSAIDs in the treatment of analgesic rebound headache¹¹. The benefits of administering tizanidine in a modified-release formulation have been previously demonstrated, approximately 94% and 79% improvement in spasticity and disability, respectively, was observed in spastic patients¹². The main limitation for the therapeutic effectiveness of TZ is its low bioavailability 30-40%, short biological half life 2-4 hr and the fact that it undergoes first pass metabolism¹³. Thus TZ is a candidate for development of controlled release formulations.

In the earlier literature, no sustained release formulations of Gellan beads or polymer reinforced gellan gum beads containing TZ have been studied. The objective of the present research work was to develop a microparticulate oral sustained release dosage form of TZ, to reduce dosing frequency, to eliminate the dose related adverse effects and to improve the patient compliance during the treatment of the muscle pain.

Design of experiment is commonly adopted in pharmaceutical research since it provides the possibility of obtaining maximal information from a minimal number of experiments¹⁴. A fractional factorial design was adopted to study the effect of the percentage of drug added in relation to polymer weight (drug to polymer ratio), the cross linking agent concentration and type and the curing time on the entrapment efficiency and the mean dissolution time. The drug-loaded gellan beads with highest entrapment and MDT were subjected to polymer reinforced treatment using different polymers namely, ethyl cellulose, and PVA and subsequently coated with Eudragit RS100 (EU). The beads were evaluated and characterized for yield, entrapment efficiency and *in vitro* drug release. The candidate formula was further subjected to scanning electron microscopy (SEM), thermal properties, and X-ray Diffraction.

Table 1: Formulation Parameters Addressed in the Fractional Factorial Design^{2 4-1}

Parameter	Component	Units	Applied levels	
			Low level	High level
X1	percentage of drug added*	%	0.33	1
X2	Cross linking agent concentration	%	2	4
X3	Type of cross linking agent	-	ZnSO ₄	ZnSO ₄ +CaCl ₂
X4	Curing Time	Minutes (min)	15	45

- with respect to polymer weight (D:P 1:3 = 33% of polymer weight while D:P 1:1 = 100% of polymer weight)

MATERIALS & METHODS

Materials

Tizanidine hydrochloride (TZ) was kindly gifted by Amoun Company (Egypt); polyvinyl alcohol (PVA) of average molecular weight 88000 and triethyl citrate 99% MW276.29 were obtained from Acros Organics (New Jersey USA). Gellan gum (GG)P-8169, chitosan high molecular weight and ethyl cellulose N22(EC) were obtained from Sigma -Aldrich chemie GmbH (Germany). Novartis Pharma Serdalaud® tablets (2mg Novartis Pharma, Egypt), All other chemicals and reagents were of analytical grade

Design of the Experiments

A 2⁴⁺¹ fractional factorial design^{15, 16} was applied to study the effect of variables on the % entrapment efficiency (% EE) and the mean dissolution time (MDT). The percentage of drug added (in relation to polymer concentration), concentration of cross-linking agent, type of cross-linking agent and curing time were selected as four independent variables. Concentration of gellan gum was selected based on preliminary studies (data not shown). Various batches prepared by using all possible combinations of different levels of experimental variables are listed in Table1. All data were statistically analyzed using Design-Expert® software (version 7; Stat-Ease, Inc., Minneapolis, MN). Means were compared by ANOVA factorial and suitable regression models^{16, 17} were driven to enable navigation of the experimental space. Significance level was set at p< 0.05. Candidate formulation showing highest entrapment efficiency and MDT was subjected to further treatment

Preparation of Gellan Beads

Beads were prepared by the cation-induced ionotropic gelation method⁴. A 2.5% (w/v) GG solution was prepared in deionized water with constant stirring (300 rpm) at 90 °C., the appropriate quantity of tizanidine (33.33%, or 100% of the dry mass of GG which means 1:3 and 1:1 drug to polymer ratio respectively)was then added and stirred until a homogeneous solution was formed. The homogeneous bubble-free solution was extruded drop wise into 100ml of the counter ion solution (ZnSO₄ or equal mixture of CaCl₂ and ZnSO₄) using a disposable syringe preheated in boiling water having a needle of bore size, 19G with constant stirring (200rpm). The concentration of counter ion solution was 2% or 4%. The beads were formed immediately; after the required curing time (15 or 45 min), the beads were separated by filtration, washed with double-distilled water, and air dried for 24 hours.

Preparation of Polymer Reinforced Beads

The following beads were prepared

Ethyl cellulose reinforced beads (F5EC I): the calculated amount of TZ (33.33% of dry mass of GG) was dispersed uniformly in GG (2.5% w/v) solution as previously explained in the above section and EC(2.5% w/v) was mixed and stirred for 10 min and then drop wise added to agitated counter ion solution (100 ml) containing an equal mixture of 1%CaCl₂ and 1% ZnSO₄ adjusted from pH 6 to pH 11.5(pH meter ,Schott-Gerate,GmbH, Germany) using ethylene diamine .

PVA reinforced beads (F5PVA I): the beads were prepared according to Anighotri et al¹⁷ with modification

2.5% w/v PVA was dissolved in hot deionized water at 90°C , then 2.5% GG was dispersed and stirred till obtaining a uniform dispersion , then the calculated amount of TZ(33.33% of dry mass of GG) was added stirred for 10 min and then drop wise added to agitated counter ion solution (100 ml) containing an equal mixture of 1%CaCl₂ and 1% ZnSO₄ adjusted from pH 6 to pH 11.5 (pH meter ,Schott-Gerate,GmbH, Germany) using ethylene diamine .

Coating of beads

The polymer reinforced beads prepared using ethyl cellulose and PVA namely F5EC I and F5PVA I, respectively, were further coated using Eudragit RS100 as described by Lee and Min¹⁸ with modification, and were named (F5ECII and F5PVA II) , respectively . In brief, the coating solution was prepared by adding 10% Eudragit

RS100 with 20ml acetone till complete dissolution using a stirrer at 400 rpm. Then, 5 ml triethyl citrate (TEC) as plastisizer was added to avoid aggregation and electrification of the beads and stirring was continued for 10 min. The beads were added to the above coating solution, then poured into 100 ml liquid paraffin containing 1% span 80. The solution temperature was adjusted to 40°C and stirring speed was increased to 500 rpm by 50 rpm interval every 30 min, thereafter, the stirring speed was increased to 900 rpm for reducing the aggregation of beads. The coated beads were finally formed as a result of acetone evaporation, filtered through filter paper washed three times with 50 ml n hexane to eliminate residual liquid paraffin and left to dry at room temperature

Evaluation and Characterization of Beads

Yield and Entrapment Efficiency

The prepared beads were weighed after drying, and percent yield was calculated. The entrapment efficiency within the beads was determined. Briefly, fifteen mg of the beads were crushed using a mortar and pestle and allowed to disintegrate completely in 100 ml of phosphate buffer pH6.8. The solution was filtered, diluted appropriately and drug content was determined spectrophotometrically at λ_{max}320nm¹⁹. The entrapment efficiency was calculated according to the following equation:

$$\%EE = \text{actual drug content} / \text{theoretical drug content} \times 100 \quad (1)$$

Surface Morphology

The shape and surface morphological examination of the dried beads were carried out by scanning electron microscopy (SEM-JEOL Model 8404, Japan) The beads were mounted on the standard specimen mounting stubs and were coated with a thin layer (20 nm) of gold by sputter coater unit (VG Microtech, Uckfield, East Sussex, UK).

Photographs of wet beads were taken using a digital camera Canon A70 (Japan).

Differential Scanning Calorimetric (DSC) Analysis

Thermograms of Tizanidine, plain gellan beads reinforced with ethyl cellulose, and TZ-loaded gellan beads with ethyl cellulose were obtained using Shimadzu DT -40 Thermal Analyzer, Japan. The system was purged with nitrogen gas at a flow rate of 80 ml/min, and heating was performed from 30°C to 300°C at a rate of 10°C/min.

X-Ray Diffraction (XRD) Studies

Powder X-ray diffraction patterns of Tizanidine, plain gellan beads reinforced with ethyl cellulose , and TZ-loaded gellan beads reinforced with ethyl cellulose were recorded using a Phillips X-ray diffractometer (PW1710) with a copper target at a voltage of 40 kV and a current intensity of 30 mA at a scanning speed of 1 °C per minute.

In Vitro Drug Release Studies

The release of TZ from the beads was determined using a USP 32 dissolution test apparatus I (Vankel VK 700 USA). A weighed quantity of beads equivalent to 6 mg of TZ was placed in the dissolution basket and the basket was placed in 900 mL dissolution medium, which was stirred at 100 rpm at 37±0.5°C⁹ following a pH progression method i.e pH 1.2 for first 2 h and pH 6.8 for the rest of the study.

Aliquots were withdrawn periodically, replaced with fresh dissolution medium, filtered and assayed for TZ content spectrophotometrically (UV-1601, Shimadzu, Japan) at λ_{max} 318nm for pH 1.2 and 320 nm for the rest of the samples. A mean dissolution time

(MDT)²⁰⁻²¹ was calculated to characterize the release of the drug from various formulations using the following equation:

$$MDT_{in-vitro} = \frac{\sum_{i=1}^n t_{mid} \Delta M}{\sum_{i=1}^n \Delta M} \quad (2)$$

Where, i is the sample number, n is the number of dissolution sample times, t_{mid} is the time at the midpoint between i and $i-1$ and ΔM is the additional amount of drug dissolved between i and $i-1$

Kinetics of Drug Release

In order to investigate the mechanism of drug release of the candidate formula, the data were fitted to different kinetic models: representing zero-order, first order and model proposed by

Higuchi²² The release data were also fitted to Peppas exponential model $Mt/M_{\infty} = Kt^n$, where Mt/M_{∞} is fraction of drug released after time ' t ' and

' K ' is kinetic constant and ' n ' is release exponent which characterizes the drug transport mechanism²³. The large value of the coefficient of determination (R^2) indicated a superiority of the dissolution profile fitting to mathematical equations.

Table 2: Matrix of Fractional Factorial Design 2⁴⁻¹ and Results

Formula Number	Coded independent parameters				Dependent Parameters		Yield %
	X1	X2	X3	X1X2X3=X4	^a Y1 =EE%	^a Y2 =MDT*(min)	
1	-	-	-	-	14.48±0.44	38.50±0.76	70.35
2	+	-	-	+	12.32±0.74	44.00±1.97	80.95
3	-	+	-	+	9.33±0.29	20.50±1.75	67.09
4	+	+	-	-	10.67±0.75	21.00±1.12	82.22
5	-	-	+	+	18.9±1.20	55.10±0.86	71.95
6	+	-	+	-	11.25±0.68	41.60±0.88	81.52
7	-	+	+	-	14.68±0.32	36.96±0.81	65.57
8	+	+	+	+	9.6±0.80	38.55±1.77	81.45

*MDT of pure drug= 2.97±0.007 min MDT of Commercial formula=9.175 ±1.14 min + High level - Low level. a Values represent mean ± SD (n=3).

RESULTS AND DISCUSSION

In this study gellan gum beads containing TZ were prepared by a rather simple procedure using cation-induced gelation of gellan gum. It has the advantage of being carried out under very mild condition in an aqueous, organic solvent-free environment. In this work either zinc ions alone or the combination of calcium and zinc ions were used to induce cationic gelation of gellan gum. Various formulation and process variables affecting the preparation of beads and in vitro release characteristics of TZ were investigated. The characteristics of the beads are presented in Table 2 according to 2⁴⁻¹ fractional factorial design

Yield and Entrapment Efficiency

The yield ranged between 65.57% and 82.22 % (Table2). It can be observed that the beads prepared at (D:P =1:1) had higher yield than those prepared at (D:P= 1:3). Increasing the polymer ratio in the formulation lowered the product yield due to the formation of a highly viscous polymer dispersion which may be lost during the manufacturing process²⁴.

The % EE values ranged between 9.33±0.29% and 18.9±1.20% (Table2) . The entrapment efficiency values seem very low because the encapsulation process was carried out in an aqueous environment where the drug is soluble; in addition the porosity gives gellan beads a very low efficiency of incorporation^{25, 26}. (Fig.1a). The porosity and any structural defects of beads might gave low efficiency of incorporated drug²⁷.

Analysis of Factorial Design

The data of values obtained for (% EE) and mean dissolution time (MDT) were subjected to analysis of variance (ANOVA) using

statistical software Design-Expert® (version 7;Stat-Ease, Inc., Minneapolis, MN).(Table III) The equation fitted for EE and MDT was $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4$ where Y represents the measured response, X_1 - X_4 represent the independent variables β_0 is a constant, and β_1 - β_4 represent the regression coefficient computed from the responses of the formulations. $X_1 X_2$, $X_1 X_3$ and $X_1 X_4$ are the interaction terms, showing how response changes when two factors are simultaneously changed²⁸. The values of the coefficients X_1 , X_2 and X_3 are related to the effect of these variables on the response. The polynomial equations can be used to draw conclusions after considering the magnitude of the coefficient and the mathematical sign it carries (i.e., positive or negative). A positive sign of coefficient indicates that the output increases with an increase in parameter level, and negative coefficients that the output increases with a decrease in parameter level²⁹. The larger coefficient means the independent variable has more potent influence on the response. The high values of the correlation coefficients for the dependent variables indicate a good fit²⁸. Statistical models were accepted when there was no lack of fit. Table 3 shows the regression results of the measured responses when zinc and calcium ions were used as cross linking agents .

Effect of Different Variables on the %EE

Table 3 and fig 2 show that there was a negative effect of increasing the percentage of drug added and concentration of cross linking agent on the %EE and a positive effect for using a mixture of Ca and Zn ions while a non significant effect for the change in curing time . Two of the three interactions are significant which are positive interaction between percentage of drug added and crosslinking agent concentration ($X_1 X_2$) and percentage of drug added and crosslinking agent type.

Table 3: Main effects, Coefficients, Sum of Squares and p values for Full Model Terms

Model Term	Main Effect		Coefficient		Sum of Squares		p-value	
	MDT	EE	MDT	EE	MDT	EE	MDT	EE
X1- % of drug added	-2.28	-3.51	-1.14	-1.75	20.93	49.37	0.0087*	<0.0001*
X2- Cross linking agent conc.	-15.66	-3.01	-7.83	-1.50	981.54	36.16	<0.0001*	<0.0001*
X3- Type of cross linking agent	12.78	1.76	6.39	0.88	653.49	12.52	<0.0001*	0.0011*
X4- Curing Time	4.458	-0.38	2.23	-0.19	79.50	0.57	0.0001*	0.3163**
X1X2	2.16	1.31	1.08	0.65	18.62	6.85	0.0116*	0.0061
X1X3	-4.62	-2.84	-2.31	-1.42	85.084	32.18	0.0087*	<0.0001*
X1X4	5.32	0.342	2.66	0.17	113.72	0.46	<0.0001*	0.3636**

*significant ** non significant

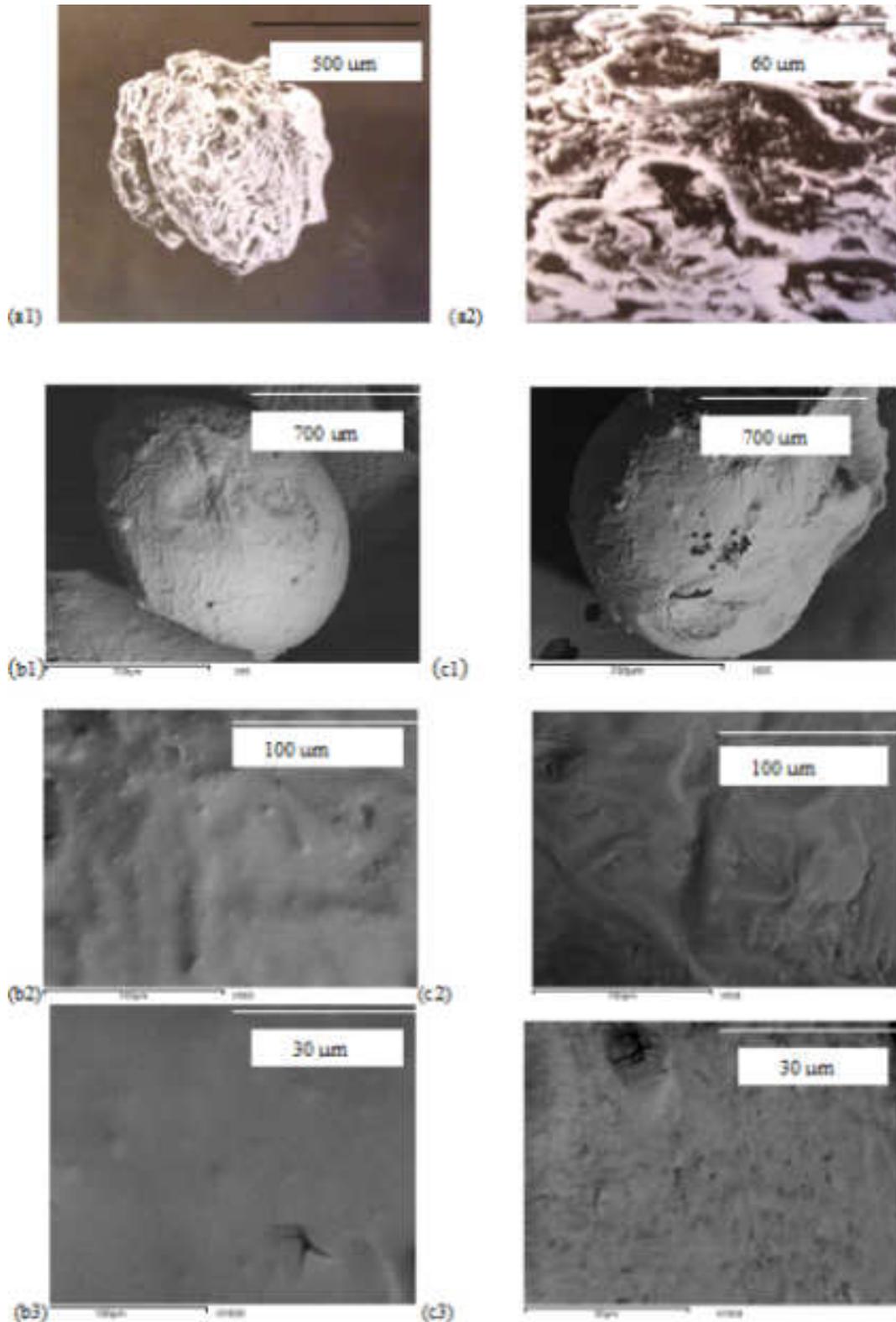


Fig. 1: SEM of gellan gum beads at magnification 50x (a1) , 300x (a2) , gellan gum beads reinforced with PVA and coated with Eudragit RS100 at magnification 65x (b1) , 500x (b2), 1500x(b3), gellan gum beads reinforced with EC and coated with Eudragit RS100 at magnification 65x(c1) , 500x (c2), 1500x(c3)

There are two non significant terms (factor X4 and X1X4 $p > 0.1$) in the full model which are not needed to support hierarchy, so model reduction may improve results³⁰. Removing the non significant terms led to a model with $R^2 = 0.90873$ higher than that explained by the full model $R^2 = 0.8867$. The appropriateness of the selected mathematical model was ensured by a high correlation coefficient,

the absence of a lack of fit of the model equation to the data³¹. Hence this model can be used to navigate the design space.

The equation in terms of actual factors for the reduced model for EE when using Zn and Ca was

$$EE = + 27.788 - 15.339X1 - 2.80X2 + 1.95X1X2 \quad (3)$$

Effect of the percentage of drug added on the %EE

The beads prepared at higher drug quantity had lower entrapment efficiency than those prepared at low drug quantity. At higher drug levels, the amount of gum may be insufficient to hold the increased drug amount suggesting that the quantity of gellan becomes insufficient to entrap the drug. Similar results were obtained by Patil et al³² when studying gellan beads of diclofenac sodium. Similarly, Manjanna et al²⁴ and Narkar et al³³ found a decrease in % EE with an increase in drug amount added.

Increasing the polymer concentration consequently makes the actual drug loading higher due to the increase in hydrophobicity, leading to better precipitation of polymer at the boundary phase of the droplets. The increase in the viscosity as a result of the increase in polymer concentration can impede drug mobility which was observed as an increase in the % EE³⁴. Another reason may be that in case of D:P of 1:1 there were equal quantities of gellan gum and tizanidine in the dispersion and it is possible that the cationic drug could partly mask the negatively charged groups of the gellan gum, which were no longer available to the Ca and zinc counter-ions.

Effect of the cross linking agents concentration on the %EE

An increase in the concentration of cross linking agent from 2 to 4% led to a significant decrease in drug %EE. It was previously found that higher cross linking agent concentration led to less drug encapsulated. This may be attributed to an increase in the porosity of the beads²⁶ and more extended gel bead shrinkage during gelation³⁵. Gelation usually occurs radially from the surface to the center of the beads as gelation proceeds water is expelled due to cross links produced by the cations, therefore, a more cross linked structure may show a larger water loss. Dissolved portion of the drug in the beads may be lost from the gellan beads during curing by simple diffusion and the expulsion of water will cause connective loss of drug molecules³⁵.

Effect of the cross linking agents type on the %EE

Concerning the type of cross linking agent, calcium and zinc ions produced higher entrapment efficiency compared to zinc ions alone. This may be due to greater extent of interaction when both calcium and zinc salts were used rather than zinc sulfate alone.

Effect of the curing time on the %EE

Regarding the curing time both 15 min (low) and 45min (high) value of curing time didn't differ significantly. This may be due that after 15 minutes formation of tight junction between calcium and zinc

ions and the active sites on the glucuronic acid chain took place consequently the drug was entrapped in highly bound calcium and zinc gellan gum resulting in no further diffusion of drug in the curing medium. Rastorgi et al³⁶ found that cross linking time of 10 minutes was found to be sufficient for good entrapment efficiency. Increasing the cross linking greater than 10 minutes, caused no change in entrapment efficiency.

In Vitro Drug Release Studies

Fig. 3 shows the release profile of TZ from the prepared beads. An equivalent amount of the pure drug and the commercial formula were illustrated in all the release figures for comparison.

The powder drug and the commercial formula showed very rapid release when compared to the prepared gellan gum beads.

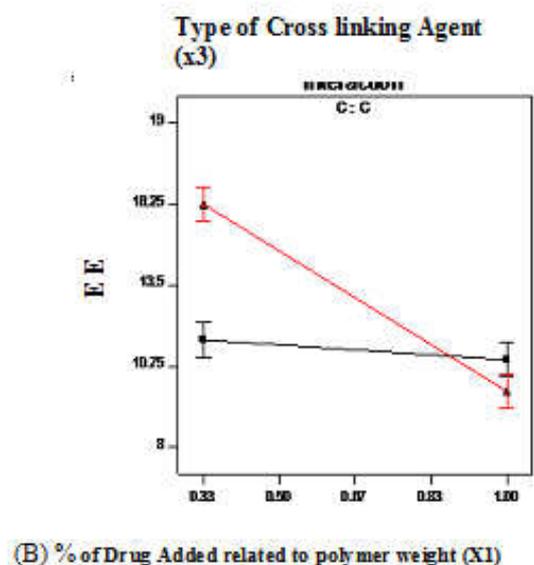
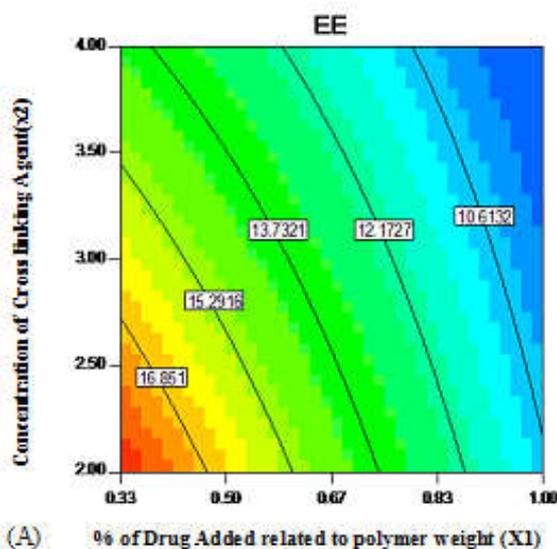
It was previously published that the disintegration of the calcium gellan beads are dependent on the pH of the dissolution medium and on the solubility of the drug entrapped in the beads²⁶. During the first two hours, a high percentage released was observed which may be due to high solubility of TZ in 0.1 N HCl when compared to pH 6.8 dissolution medium. The rapid drug release from gellan matrices in acid medium could be explained change in matrix properties in contact with the acid the calcium and zinc ions in gellan beads were totally discharged in acid environment and the carboxylic groups were shifted to unionized forms. This might alter the matrix properties including the release characteristics of the incorporated drug²⁶. The swelling of gellan matrices in phosphate buffer resulted from exchange of hydrogen ion with sodium ion of the dissolution medium²⁶. An initial burst release was observed which may be due to unequal distribution of the drug within the gellan beads resulting in slightly higher concentration of the drug at the surface²⁶. The values of MDT varied between 20.50±1.75min to 55.1± 0.86 min while MDT of the powder drug and commercial formula were only 2.97±0.007 min and 9.175 ±114 min, respectively. (Table 2)

Effect of Different Variables on the MDT

The equation in terms of actual factors for MDT when using Zn and Ca was

$$+85.45-35.8X1 -9.97X2-0.2X4+3.22X1X2+0.53 X1X4 \quad (4) \quad R^2= 0.992859$$

The ANOVA analysis for MDT (Table 3 and Fig.2) show that the MDT of the encapsulated drug was governed by all the four variables. A negative effect of increasing drug concentration and cross linking agent concentration and a positive effect for using a mixture of Ca and Zn ions and the change in the curing time were obtained.



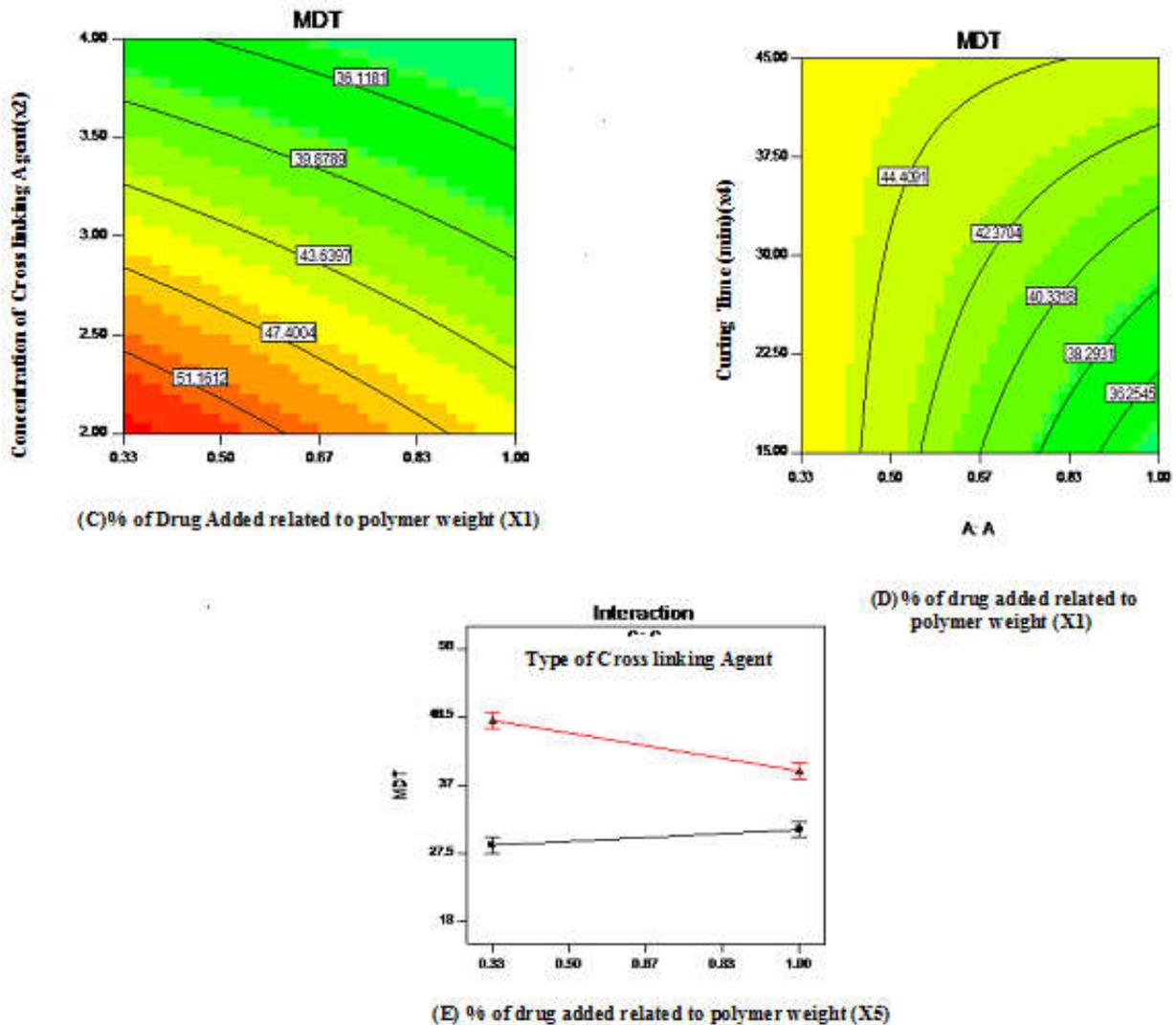


Fig. 2: Interaction plot representing the effect of different factors on the % EE and MDT (A) percentage of drug added related to total weight of polymer (x1) and concentration of cross linking agent (x2) on % EE (B) percentage of drug added related to total weight of polymer (x1) and type of cross linking agent (x3) on % EE ■ using ZnSO₄ ▲ using equal mixture of CaCl₂ and ZnSO₄. (C) Percentage of drug added to total weight of polymer (x1) and concentration of cross linking agent (x2) on MDT (D) percentage of drug added to total weight of polymer (x1) and curing time (x4) (E) percentage of drug added to total weight of polymer (x1) and type of crosslinking agent (x3) ■ using ZnSO₄ ▲ using equal mixture of CaCl₂ and ZnSO₄

Effect of the percentage of drug added on the MDT

The beads prepared at higher drug quantity (D:P =1:1) had lower MDT when compared to those prepared at low drug quantity (D:P=1:3). This may be due at higher polymer concentration compared to the drug, so there was an increase in gel layer thickness that acted as a barrier for the penetration medium thus retarding the diffusion of the drug from the beads. Similar results were obtained by Rajinkanth and Mishra³⁷ who found that as the gellan concentration of the prepared beads increased, the rate and extent of drug release was decreased significantly ($p < 0.01$). This could be attributed to increase of gellan matrix density and increase in diffusion path length which the drug molecules have to traverse. Similarly it was previously published that the release rates were slower for formulations containing lower amount of drug in the beads⁶.

Effect of the cross linking agents concentration on the MDT

Regarding the decrease in MDT at high cross linking concentration. Increased porosity of the beads when compared to lower

concentration might be the reason. Rastogi et al³⁶ found that an increase in CaCl₂ concentration from 2.5 to 5% led to a decrease in release rate of rifampicin from gellan microgel, similarly Basu et al³⁸ found that beads prepared with 5% (w/v) CaCl₂ showed increase in the release rate than the beads prepared with 2% (w/v) CaCl₂; this was attributed to increase in the porosity and swelling ratio of the beads with increase in the concentration of the counter ion³⁷.

Effect of the cross linking agents type on the MDT

Concerning the cross linking agent type, both calcium and zinc ions gave higher MDT when compared to zinc ion alone this may be due to greater extent of interaction when both calcium and zinc ions were used rather than zinc ion alone. Chan et al³⁹ reported that a combination of calcium and zinc ions could produce more sustained release from alginate microspheres compared to calcium ions alone.

Effect of the curing time on the MDT

Regarding the curing time, 45 min gave higher MDT when compared to 15 min. Similar results were obtained by Babu et al²⁶.

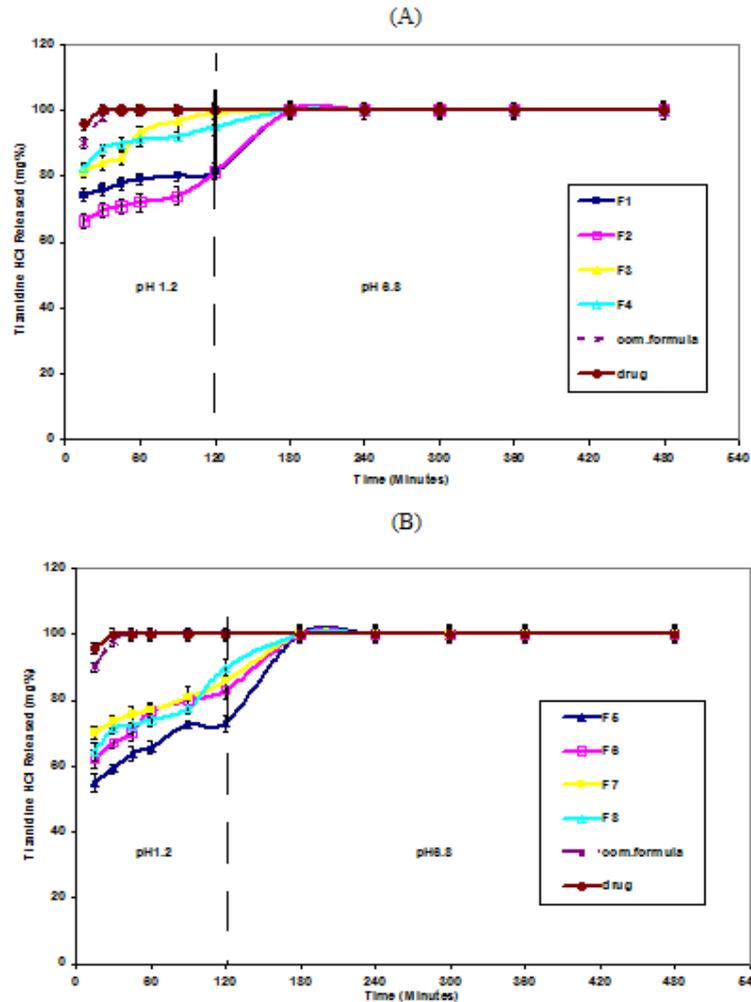


Fig. 3: Release profile of TZ from different gellan gum beads formulations (A) F1-F4 (B)F5-F8

Based on the above characterization, the entrapment efficiency and the release of TZ from the prepared beads didn't offer satisfactory results. Therefore, further modifications were performed to beads belonging to F5 (showing highest EE and MDT) with the aim of increasing EE and extending TZ release. For improving the EE the modifications included change in pH of counter-ion solution from pH6 to 11.5 and polymer reinforcement using ethyl cellulose and PVA. For extending TZ release, the polymer reinforced beads were further coated with Eudragit RS100.

Ethyl cellulose is a hydrophobic polymer previously used to reinforce theophyllin microbeads⁴⁰. PVA is a widely used hydrophilic polymer because of its processability, strength and pH as well as its temperature stability. It was previously used with gellan gum in order to control the release of Carvedilol¹⁷. The evaluation of the beads could be shown under the following headings

Surface Morphology

Photographs of wet gellan gum beads and ethyl cellulose reinforced gellan gum beads are shown in Fig.4. The wet gellan gum beads

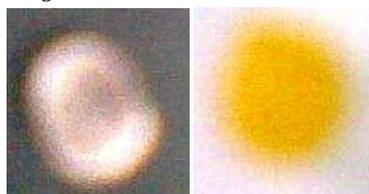


Fig. 4: Photographs of wet gellan gum beads prepared at pH6 (A) and wet ethyl cellulose reinforced gellan gum beads prepared at pH 11.5(B).

produced at pH 6 showed transparent appearance when compared to those reinforced with ethyl cellulose and prepared at pH 11.5. They showed a yellowish globular body as indication of good entrapment. It was previously found that the pH of the counter ion media appeared to have a significant effect on the morphology of the beads⁶. Cephalixin beads prepared at high pH 9 had smooth surfaces, while those prepared in pH 5 had a porous structure. This was attributed to a more rapid approach of calcium and zinc ions to the surface of the GG droplet which possessed higher anionic character at higher pH and thus a dense matrix is formed⁶.

SEM of Gellan beads prepared at pH6 and polymer reinforced and coated gellan beads prepared at pH11.5 are shown in Fig.1.

Gellan beads had predominately spherical shape with porous structure while polymer reinforced beads appeared lustrous with dense uniform coat matrix free from pores.

The increased uniformity of the gel coat at higher pH may be attributed to viscosity and cohesivity of gel⁴¹.

Entrapment Efficiency

In all the two polymers used, there was a significant increase in %EE when compared to gellan gum beads alone (Table4).

For ethyl cellulose and PVA reinforced beads, the % EE increased from 18.9 ± 1.2 to 90.5 ± 3.39 and 63.1 ± 2.90 , respectively. Increasing the polymer concentration and changing pH of the media from 6 to 11.5 led to a significant increase in % EE ($p < 0.001$). Since the pKa of tizanidine hydrochloride is 9.42⁴², its solubility rapidly decreased above this value. The difference in EE is due to the properties of the drug soluble or insoluble at the two pH values⁴³. At pH 6 the drug is soluble, when the beads were prepared with counter ion solution adjusted at pH 11.5, a yellow halo was observed inside the transparent beads, which was a precipitate of the drug (Fig.4). It was previously found that for amoxicillin, higher drug entrapment for beads was obtained at alkaline pH due to decreased solubility³³. Similarly, Agnihotri et al⁶ found an increase in EE of cephalexin by increasing pH from 5 to 9; this was attributed to the degree of ionization of carboxyl groups of gellan gum in different pH media. So another reason for increasing in % EE of TZ may be that at pH 6 there is less number of carboxylate groups on gellan gum, then zinc and calcium ions are less likely to crosslink and form a dense matrix that can entrap TZ successfully⁶.

Beads reinforced with ethyl cellulose showed higher entrapment than those reinforced with PVA, this may be due to hydrophobic nature of ethyl cellulose compared to PVA

In Vitro Drug Release Studies

Release profile of TZ from polymer reinforced beads is shown in figure (5).

Although, there was a significant increase in MDT, $p < 0.05$ (Table4) and a reduction in TZ release from all the prepared polymer

reinforced gellan gum beads compared to gellan gum beads alone due to increasing the polymer concentration that acted as a barrier to the penetration of the medium, therefore suppressed the diffusion of TZ from the beads, they still didn't offer the desired results so the beads were further coated not only to retard drug release and erosion but also to overcome the possible defects of outer surface with defects and pores.

Therefore, it was necessary to further retard the release rate by coating the PVA and ethyl cellulose gellan gum beads with Eudragit RS100. Eudragit RS 100 is referred to as ammoniomethacrylate copolymer of acrylic and methacrylic acid esters with a low content of quaternary ammonium groups. Since Eudragit RS 100 films are only slightly permeable, protection against rapid beads disintegration and drug release retardation through the film could be achieved⁴⁴.

It is evident that, coating the beads with Eudragit RS 100 caused a decrease in the extent of drug release (Table 4 and figure 5), the coated ethyl cellulose beads F5 ECII gave highest MDT, remained intact and were able to retard the release for more than 8 hours probably due to the presence of an extra diffusion barrier on the beads in addition to the hydrophobic nature of ethyl cellulose. Eudragit RS decreased the beads permeability and increased the MDT. In F5 ECII the release was lower compared to reinforced beads F5ECI. It was previously reported that although the release rate of drug can be changed by polymeric reinforcement of beads, coated beads may be more useful not only to retard drug release and erosion but also to overcome the possible defects of outer surface of beads with cracks and pores. The porosity and any structural defects give a fast release and low efficiency incorporating drug⁴⁵.

The release data of F5ECII was studied and was found to follow Higuchi model $R^2 = 0.984$ (Table5), value of n was equal to 0.47 which indicated Fickian or diffusion controlled release²⁴.

Table 4: Composition of Polymer Reinforced and Coated Polymer Reinforced GG Beads & Results

Formulation	Composition	Counter ion	Coating solution	%EE	MDT (min)
F5ECI	0.25gGG, 0.25 EC, 0.0833 g TZ in 10 ml deionized water	100 ml of (1% CaCl ₂ and 1% ZnSO ₄) adjusted to pH11.5	-	90.5±3.394	70.57±1.46
F5PVAI	0.25gGG, 0.25PVA, 0.0833 g TZ in 10 ml deionized water	100 ml of (1% CaCl ₂ and 1% ZnSO ₄ adjusted to pH11.5	-	63.1±2.900	64.81±1.41
F5ECII	0.25gGG, 0.25 EC, 0.0833 g TZ in 10 ml deionized water	100 ml of (1% CaCl ₂ and 1% ZnSO ₄ adjusted to pH11.5	2gm Eudragit RS100 in 20 ml acetone and 5ml triethyl citrate	88.5±2.446	158.83±0.97
F5PVAII	0.25gGG, 0.25PVA, 0.0833 g TZ in 10 ml deionized water	100 ml of (1% CaCl ₂ and 1% ZnSO ₄ adjusted to pH11.5	2gm Eudragit RS100 and 5ml triethyl citrate	62.0±2.99	114.14±2.65

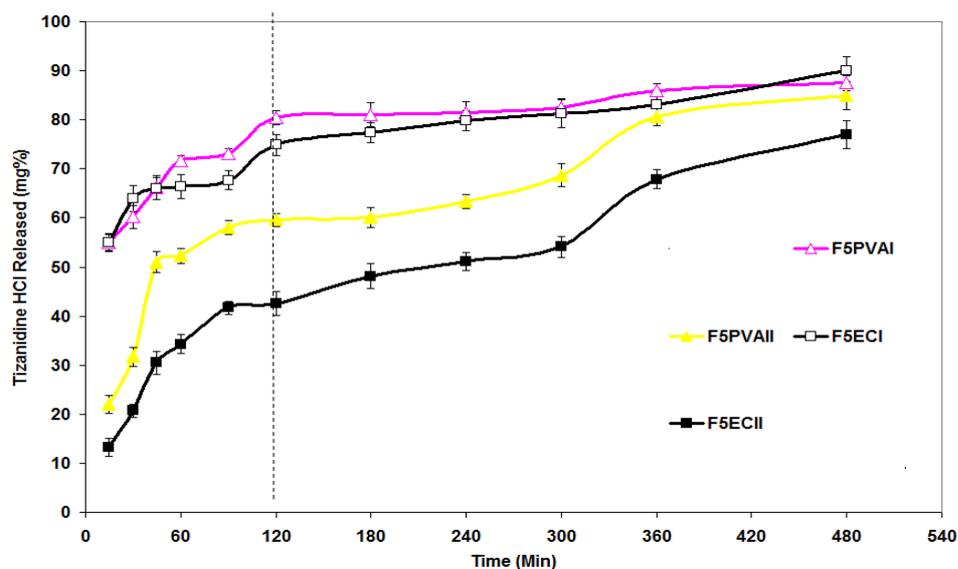


Fig. 5: Release profile of TZ from polymer reinforced gellan gum beads (F5ECI, F5PVAI) and polymer reinforced gellan gum beads and coated with Eudragit RS100 (F5ECII, F5PVAII)

Differential Scanning Calorimetric (DSC) Analysis

Fig (6) shows the DSC thermograms of TZ (a), placebo ethyl cellulose reinforced gellan gum beads (b) ethyl cellulose reinforced gellan gum beads loaded with TZ (F5 ECI). (c) In the thermogram of TZ, a sharp endothermic peak was observed at 287.63° C indicating the melting point of TZ. Placebo beads have shown broad endothermic peak between 50° C and 100° C due to a partial loss of residual humidity, and at 150° C indicating the melting temperature of ethyl cellulose and a peak between 250° C and 290° C, at about 284° C indicating the melting temperature gellan gum , beads loaded with TZ showed a peak at 286° C corresponding to a shift in the peak corresponding to gellan gum and disappearance of the peak of TZ indicating significant decrease in crystallinity of drug in formulation which could favor the increase in entrapment efficiency.

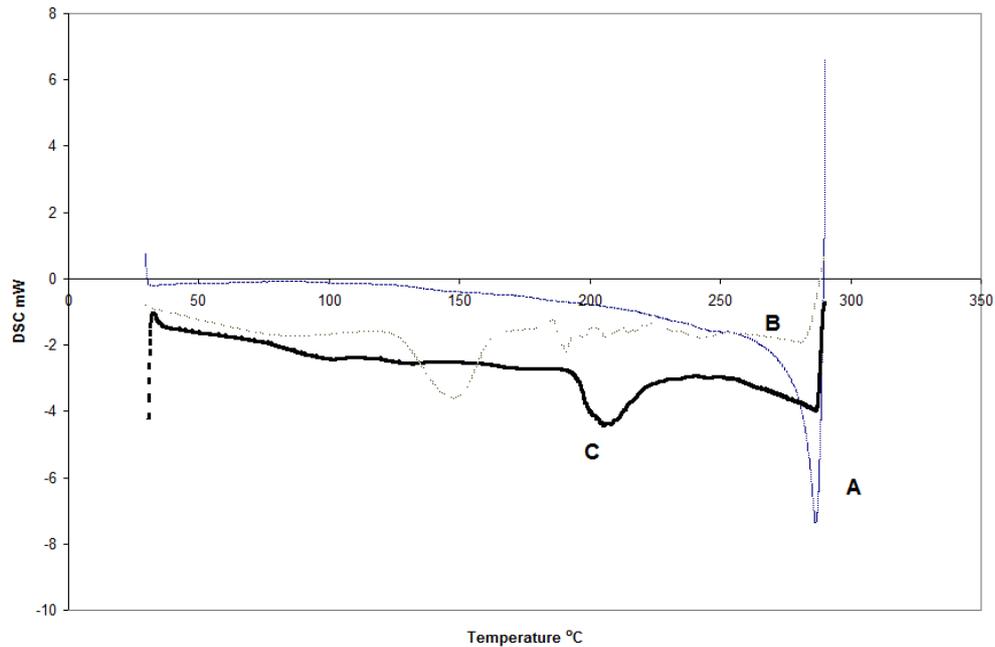


Fig. 6: DSC thermograms of (a) pure TZ ; (b) placebo ethyl cellulose reinforced gellan gum beads, (c) ethyl cellulose reinforced gellan gum beads loaded with TZ

X-Ray Diffraction (XRD) Studies

Fig. 7. shows the XRD diffractograms of TZ, placebo ethyl cellulose reinforced gellan gum beads and ethyl cellulose reinforced gellan gum beads loaded with TZ (F5 ECI). The diffractogram of TZ exhibited a series of intense peaks at 2θ 0-55° the most indicative are 10.57°, 12.311°, 21.67°, 23.95°, 24.79°, 27.18°, 32.017° and 45.46°. XRD of placebo beads exhibited amorphous character. The XRD of TZ loaded gellan beads reflects the character of the polymer and shows the absence of characteristic peaks of TZ, indicating significant reduction in crystallinity of drug in the prepared beads. It could be suggested that the drug is embedded in the polymers matrices or homogeneously distributed through it. The results are in good agreement with those obtained from DSC studies.

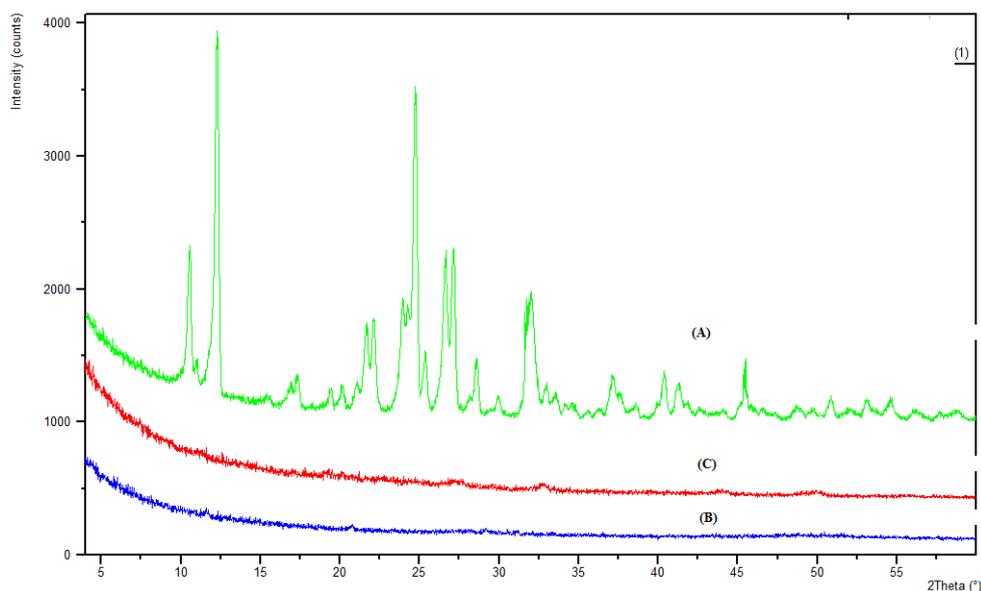


Fig.7:X-ray diffraction pattern of a) pure TZ ; (b) placebo ethyl cellulose reinforced gellan gum beads, (c) ethyl cellulose reinforced gellan gum beads loaded with TZ

Table 5: Kinetic Treatment of the Release Data of the Drug from F5ECII (Ethyl Cellulose Reinforced Gellan Gum Beads loaded with TZ and Coated with 10%Eudragit RS100)

Release Order	Slope*	Intercept**	R ²
Zero Order	0.124	23.206	0.957
First Order	-0.001	1.914	0.979
Diffusion	3.301	5.464	0.984
Peppas	0.475*	0.636**	0.971

*n value ** k

CONCLUSION

The present study gave an overview of formulating TZ in a microparticulate oral sustained release dosage form according to 2⁴-1 fractional factorial design using gellan gum by the inotropic gelation technique. This study shows that increasing the concentration of drug added, the concentration of cross linking agents had a negative effect on the EE and MDT while using both Zn and Ca ions had a positive effect on the EE and MDT when compared to Zn ions alone. Increasing the curing time significantly increased the MDT but had a non significant effect on %EE. Gellan gum failed alone to give the desired response. Preparation of the candidate formula at pH 11.5 and reinforcement using ethyl cellulose and coating with Eudragit RS100 significantly increased the %EE up to 90% and sustained the drug release for more than 8 hours. It could be concluded that a promising controlled release beads of the water soluble drug TZ, was successfully designed. The candidate formulation might be a promising drug delivery system for the treatment of muscle pain.

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