Journal of Pharmaceutical Research Vol. 13, No. 3, July - September 2014 : 74-79

BOX-BEHNKEN DESIGNED NANOPARTICLES-IN-MICROPARTICLES SYSTEM (NiMS) FOR FORMULATING MOUTH DISSOLVING TABLETS OF ACETAZOLAMIDE

Garg Preeti [1], Pandey Parijat[1], Rewri Praveen[2], Marwaha Rakesh Kumar [1] and DurejaHarish[1,*]
[1]Department of Pharmaceutical Sciences, M.D.University, Rohtak, India - 124 001, Haryana, India.
[2]Sankara Netharalya, 18 College Road Nungambakkam, Chennai - 600 006, India.

Received on: 15.04.2014 Revised: 17.07.2014 Accepted: 23.07.2014

ABSTRACT

The aim of the present work was to prepare acetazolamide (ACZ) loaded nanoparticle - in- microparticle system (NiMS) using ionotropic-gelated chitosan for formulating orally disintegrating tablets (ODTs). The objective behind the study was to investigate the effect of - concentration of drug (X_1), concentration of chitosan (X_2) and volume of sodium tripolyphosphate (NaTPP) (X_3) on the encapsulation efficiency of ACZ from NiMS using Box-Behnken Design. The freeze dried optimized NiMS were further formulated into ODTs using direct compression method. The delivery of ACZ by oral route can be an effective way for the treatment of paediatric glaucoma. The encapsulation efficiency and loading capacity of NiMS-10 was highest at high levels of chitosan concentration and low levels of volume of NaTPP. The encapsulation efficiency and loading capacity of NiMS were lowest at mid levels of volume of NaTPP, which shows that, the proper ratio of chitosan: NaTPP should be present in the formulation for cross linking. A drug entrapment efficiency of about 98.53 ± 3.36% (w/w), loading capacity of 99.62 ± 5.77% (w/w) and particle size of 593 nm were achieved for the optimized batch of nanoparticles. Analysis of Variance (ANOVA) was applied on the encapsulation efficiency of NiMS to study the fitting and the significance of the model. The mathematical model developed in the present study can be further utilized to design NiMS with desired encapsulation efficiency. The batch NiMS-10 showed 96.2% drug release after three hours in phosphate buffer (pH 7.4). Infrared analysis of NiMS-10 showed no interaction between drug and polymer during the formulation process. The particles of NiMS-10 were found spherical in shape. The formulated ODTs have a disintegration time of 35 seconds. The hardness, friability and weight variation of ODTs were found to be within pharmacopoeial limits. *In vitro* release study revealed that the cumulative percent drug release was found to be 97.8 ± 2.27% in 60 min and the release data followed the first order kinetics. The NiMS approach has been successfully applied for the preparation of ODT of ACZ. The developed ODT can be advantageous for the treatment of glaucoma in paediatric patients.

Keywords: *Box-Behnken design; Chitosan; Acetazolamide ; Ionic gelation method; Sodium tripolyphosphate; Orally disintegrating tablets.*

INTRODUCTION

During the past decade, the FDT (fast dissolving tablet) technology, which makes tablets dissolve or disintegrate in the mouth without additional water intake, has drawn a great deal of attention. The technology is also referred to as fast disintegrating tablet, fast dispersing tablet, mouth dissolving tablet (MDT), rapid melt tablet, quick disintegrating tablet, and orally disintegrating tablet (ODT)[1,2]. The FDT formulation is defined by the Food and Drug Administration (FDA) as "*a solid dosage form containing medicinal substances which disintegrates rapidly, usually within a matter of seconds, when placed upon the tongue*"[3].

Unfortunately, the quicker disintegrating dosage forms usually have an unpleasant taste, local stimulation, and other side effects caused by short, intense exposure to high concentrations of the active agent. Microencapsulation is a new technique that could be used to control the drug release rate, protect it from premature destruction, and avoid the unpleasant taste by entrapping the active drug into a micro carrier [4]. Spherically shaped and surface-distorted microparticles (embedded with the drug) could lessen the unwanted effects of ODTs by controlling the drug release rate. Recently, a novel nanoparticles-in-microparticles system (NiMS) has been developed using a double emulsion technique and evaluated for drug and gene delivery in specific regions of the gastrointestinal tract [5-6]. The combination of microparticles and nanoparticles called NiMS offers the possibility of dual or multiple functionalities within a formulation, e.g. multiple release profiles and/or site specificity, *in vivo* protection, imaging capabilities and embolisation[7].

Acetazolamide (ACZ) is a carbonic anhydrase (CA) inhibitor. In the eye CA inhibition decreases the flow of bicarbonate, sodium, and water into the posterior chamber. Suppression of this reaction in the ciliary process reduces the production of aqueous humor by enzyme inhibition. Thus intraocular pressure in both normal and glaucomatous eyes is reduced[8]. Presently,

***Correspondence :** email: harishdureja@gmail.com Mob: +91 9416357995

ACZ is delivered to paediatric patients by ophthalmic route. The delivery of ACZ by oral route can be an effective way for the treatment for pediatric glaucoma, but ophthalmologists may have concerns that it retards weight gain in children and may choose surgical management rather than non-surgical management with oral ACZ, however, no published evidence is available to evaluate this effect of ACZ [9]. The delivery of drug by oral route is much more acceptable as compared to ophthalmic route mainly for paediatric patients. Since no marketed ODT formulation is available for paediatric patients, therefore, it is desirable to design and develop an oral delivery system for ACZ which can be easily administered to children. Chitosan is a biodegradable and biocompatible polymer and has an excellent potential for the development of nano-particulate drug delivery and has the ability to control the release of drug[10]. Experimental designs are useful in developing a formulation requiring less experimentation and providing estimates of the relative significance of different variables. Box-Behnken design (BBD) is useful in optimizing pharmaceutical formulations, for example, the BBD has been success-fully used in development of ACZ microspheres[11].

The aim of the present study is to prepare nanoparticle-in-microparticle system (NiMS) of ACZ using BBD. The objective behind the work was to study the effects of concentration of drug, concentration of chitosan and volume of NaTPP on the encapsulation efficiency of ACZ. Further, the optimized NiMS system was formulated into ODTs.

MATERIALS AND METHODS

Materials

Chitosan (95% deacetylated) has a molecular weight of 40–80 kDa, and was supplied by Boi Gen Extracts, Bangalore. Sodium tripolyphosphate (NaTPP) and talc were purchased from Central Drug House**,** New Delhi. Acetazolamide was obtained from Kaizen Pharmaceuticals, Chandigarh. Sodium starch glycolate (Glaxo SmithKline, Gurgaon), microcrystalline cellulose, mannitol (Loba Cheme Pvt Ltd, Mumbai), magnesium stearate (Shah Scientific, Mumbai) and lactose (Sisco Research Laboratories) were also procured. All other chemicals were of reagent grade.

Methods

Preparation of Acetazolamide loaded Chitosan Nanoparticles-in-Microparticles (NiMS)

For the preparation of NiMS, ionic gelation method[12] was used in the present study. In this method, chitosan (0.5 to 1% w/v) was dissolved in 1% v/v glacial acetic acid. The drug (100 to 300 mg) was added to the solution of chitosan. The volume of chitosan solution in each formulation was kept constant (35 ml). The solution of NaTPP (0.5%w/v) was prepared in distilled water. The solution of NaTPP was added into solution of chitosan at 1 drop/sec. with continuous stirring. A suspension of nanoparticles was formed due to ionic interaction between two oppositely charged ions. The suspension was then purified by centrifugation (20000 rpm, 30 min and at 25ºC) (Remi Compufuge, CPR-30, India). The supernatant was analyzed by HPLC for amount of free drug and solid content was freeze dried (-80ºC) (Allied Frost, Macflow Engg., India) to obtain a free flowing dry powder.

Optimization of NiMS using Box-Behnken Design

Box-Behnken design (BBD) was utilized to study the effects of three factors on the encapsulation efficiency of NiMS for ACZ. The three factors were concentration of drug (X_1), concentration of chitosan (X_2) and volume of NaTPP (X_3). The three factors at three levels lead to the thirteen formulations as per BBD (NIMS 1-NIMS 13) are shown in Table 1.

Table 1: *Formulation and characterization of acetazolamide loaded NiMS*

Batch No.	X_1 (mg)	X_2 (%w/v)	X_3 (ml)	Encapsulation Efficiency (%)±S.D	Loading Capacity (%)±S.D	Mean Particle Diameter (nm)±S.D
NiMS-1	-1(100)	-1(0.5)	0(10)	92.12 ± 1.50	76.09 ± 1.02	460±4.87
NiMS-2	+1(300)	-1(0.5)	0(10)	96.70 ± 2.81	95.60 ± 4.61	1929±6.39
NIMS-3	-1(100)	+1(1.0)	0(10)	91.80 ± 1.420	86.12 ±1.87	485±3.92
NIMS-4	+1(300)	+1(1.0)	0(10)	93.40 ± 1.87	92.64 ± 3.75	2169±7.11
NIMS-5	-1(100)	0(0.75)	-1(8)	91.80 ± 1.420	83.65 ±1.15	656±4.02
NIMS-6	+1(300)	0(0.75)	-1(8)	90.00 ± 0.89	81.00 ± 0.393	618±3.21
NIMS-7	-1(100)	0(0.75)	+1(12)	82.04 ± 1.39	30.02 ± 14.3	994±1.18
NIMS-8	+1(300)	0(0.75)	+1(12)	95.40 ± 2.45	42.00 ± 10.8	890±4.94
NIMS-9	0(200)	+1(1.0)	+1(12)	97.60 ± 3.09	84.17 ± 1.56	996±3.98
NIMS-10	0(200)	+1(1.0)	-1(8)	98.53 ± 3.36	99.62 ± 5.77	593±2.94
NIMS-11	0(200)	-1(0.5)	+1(12)	96.85 ± 2.87	86.70 ± 2.04	733±2.24
NIMS-12	0(200)	-1(0.5)	-1(8)	96.41 ± 2.74	85.10 ± 1.57	767±1.31
NIMS-13	0(200)	0(0.75)	0(10)	97.91 ± 3.18	92.70 ± 3.77	907±3.99

X_1, Concentration of drug; X_2 concentration of chitosan; X_3 volume of NaTPP. The value in the brackets besides the transformed values of factor X_1, X_2 and X_3, represent real values

Formulation of NiMS based Acetazolamide ODTs

The optimized batch of nanoparticles was utilized to produce ODTs. The nanoparticles equivalent to 20 mg of ACZ were blended along with the other excipients (super disintegrates, diluents, sweetener and lubricant) and processed to tabletting (having a diameter of 1.2 cm, concave faced). The formula for ODTs of ACZ is shown in Table 2.

Table 2 : *Formula for ODT of acetazolamide*

Ingredients	Amount (mg)/tablet
Nanoparticles equivalent to 20 mg of drug	76
Sodium Starch Glycolate	70
Microcrystalline Cellulose	20
Mannitol20Lactose	10
Talc	2
Magnesium Stearate	2

Physicochemical characterization of NiMS

Particle Size and Size Distribution

Particle size determination was carried out by means of laser diffractometry, using the Zetasizer Instrument (Malvern, UK) equipped with the Hydro dispersing unit. The freeze dried NiMS were dispersed in filtered water prior to analysis. The values were measured as an average of triplicate experiments.

Drug encapsulation efficiency and loading capacity[13]

The ACZ encapsulation efficiency and loading capacity was determined directly using freeze dried NiMS. These were determined by analysis of the samples in suspension after centrifugation at 20,000×g for 30 minutes. The extracted ACZ in the supernatant after ultracentrifugation of the NiMS suspension was

determined using the high-performance liquid chromatography (HPLC) method[14]. The amount of drug entrapped was calculated as the difference between the total amount used and the amount found in the supernatant. Each sample was assayed in triplicate.

The HPLC analysis was carried out using the Agilent Technologies 1200 series, Germany [Quaternary pump, Eclipse XDB-C18 column (4.6 mm×150mm), UV detector (dual wavelength)]. The mobile phase was acetonitrile: methanol: water (3:2:95). The flow rate was 1.5 ml/min at 25°C, and the wavelength was set at 265 nm[13]. The drug loading capacity (LC) of the nano-particles and the encapsulation efficiency (EE) were calculated according to the following equations:

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Totaldrug-Freedrug}}{\text{Totaldrug}} \text{ X100}$$

$$\text{Loading Capacity (\%)} = \frac{\text{Totaldrug-Freedrug}}{\text{NanoparticlesWeight}} \text{X100}$$

Drug release from the NiMS *in vitro*[15]

The formulation (NiMS) equivalent to 20 mg of drug, was placed in a dialysis membrane 110 (specification: Av. Flat width-32.34 mm, Av. Diameter-21.5 mm, Capacity-3.63 ml/cm), which was tied from both the sides with a thread. This dialysis membrane was then immersed in a beaker containing 900 ml of phosphate buffer (pH 7.4). Aliquots of dissolution fluid (10 ml) were withdrawn at specified time intervals and the beaker was fresh buffer was replenished with equal volume of fresh buffer. The sample withdrawn was estimated by HPLC analysis. Similarly, *in vitro* release studies were also carried out using the 0.1N HCl as medium.

Transmission electron microscopy (TEM)[16]

The morphology and surface appearance of the prepared NiMS were studied by transmission electron microscopy. An aqueous solution (1 mg/ml) of the lyophilized chitosan nanoparticles was placed on a TEM grid surface with a filter paper (Whatman No.1). One drop of 1% Uranyl acetate was added to the surface of the carbon coated grid. After one min of incubation, excess fluid was removed and the grid surface was air dried at room temperature. It was then loaded into the transmission electron microscope attached to a Gatan SC 1000 CCD camera.

Fourier Transform Infrared (FTIR) Spectrophotometry[17]

The FTIR spectra were obtained using the potassium bromide disc method for acetazolamide and drug loaded NiMS-12 on FT-IR-Alpha, Bluker, Germany. The samples were scanned at a range of 400-4000 cm^{-1}. The infrared spectra were acquired to draw information on drug-excipient compatibility.

Evaluation of ODTs[18]

***In vitro* disintegration time**

The disaggregation test for ODTs was performed at 37 ±0.5°C using a disintegration time apparatus (Excel Enterprises, Kolkata) using phosphate buffer as a dispersion medium. The disintegration time is the time required to transform a tablet immersed in water into small fragments with vertical vibrations at a rate of 30 vibrations per minute. The disintegration time of the six formulations was recorded.

***In vitro* dissolution study**

The release rate of ACZ from immediate release tablets was determined using USP dissolution testing apparatus 2 (Paddle method) (Lab India DS 8000, India). The dissolution test was performed using 900 ml of 0.1N HCl at 37±0.5ºC and 100 rpm. Sample (10 ml) withdrawn at various predetermined intervals was replenished with equal amount of fresh medium. The sample was analysed by HPLC. The results are expressed as a percentage of released drugs as a function of time.

Tablet Friability

Twenty tablets of formulation were weighed and subjected to abrasion by employing a Roche Friabilator at 25 rev/min. The tablets were then weighed and compared with their initial weights and percentage friability was obtained. Determination was made in triplicate.

% Friability = [(W1-W2)100]/W1

Where, W1= Weight of tablets before test (Initial Weight) W2 = Weight of tablets after test (Final Weight).

Tablet Hardness

Monsanto Hardness Tester was used to determine the crushing strength. Six tablets were randomly selected from formulation and the pressure at which each tablet was crushed was recorded. The determinations were made in triplicate.

Weight Variation Test

Twenty tablets were weighed individually selected at random, were weighed individually and the average weight was calculated.

RESULTS AND DISCUSSION

TPP is a non-toxic and multivalent anion that can form cross linkages with the positively charged amino groups of CS involving ionic interactions. With the drop wise addition of TPP, the nanoencapsulation was processed with the electrostatic attraction. Meanwhile, with the incorporation of ion TPP to CS solution, the opalescence indicated the formation process of nanoparticles[19]. A total of 13 formulations of NiMS were formulated using BBD for optimization of 3 factors- concentration of drug (X_1), concentration of chitosan (X_2) and volume of NaTPP (X_3) at three levels. Analysis of particle size, encapsulation efficiency and loading capacity was carried out for all the formulations and is shown in Table 1. The optimized batch (NiMS-10) has the high encapsulation efficiency and loading capacity of 98.53% and 99.62%, respectively with an average particle size of 593 nm. The encapsulation efficiency and loading capacity of NiMS-10 was high at high levels of chitosan concentration and low levels of volume of NaTPP, which shows that the proper ratio of chitosan: NaTPP should be present in the formulation for cross linking. BBD was

used to study the effect of amount of drug, concentration of chitosan and volume of NaTPP (ml) on the encapsulation efficiency. The real values of factors were transformed to facilitate orthogonality of results and each calculation. To determine the magnitude of contribution of different factors towards encapsulation efficiency multiple linear regression analysis was performed using Design Expect Software version 8.0.1.0. The effect of coefficients on encapsulation efficiency is shown in Figure 1. The 3D surface of and contour plot of encapsulation efficiency are shown in Figures 2 and 3, respectively. The model, developed from multiple linear regressions, to estimate encapsulation efficiency (Y) can be represented mathematically as:

$$Y = +97.91 + 2.2X_1 - 0.3X_2 - 0.90X_3 - 0.75X_1X_2 + 3.79X_1X_3 - 0.34X_2X_3 - 5.97X_1^2 + 1.57X_2^2 - 2.1X_3^2$$

Where, X_1= Amount of Drug (mg); X_2= Concentration of chitosan (%w/v); X_3= Volume of NaTPP (ml)

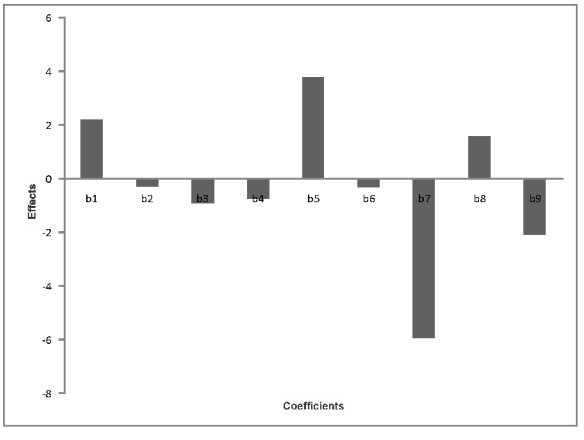

***Fig. 1**: Effect Plot of coefficient of encapsulation efficiency (b1, b2, b3=coefficients of main effects; b4, b5, b6=coefficients of interaction terms; b7, b8, b9=coefficients of interaction terms).*

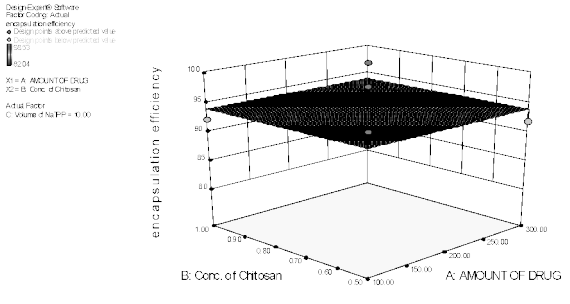

***Fig. 2:** 3D Surface of encapsulation efficiency as a function of formulation variables.*

ANOVA was applied on the encapsulation efficiency to study the fitting and significance of model in Table 3. F-test was carried out to compare the regression mean square with residual mean square the ratio F = 13.22 shows regression to be significant.

The *in vitro* drug release study using batch NiMS-10 showed that the release was higher after three hours i.e. 96.2% in phosphate buffer (pH 7.4) and 63% in 0.1 N

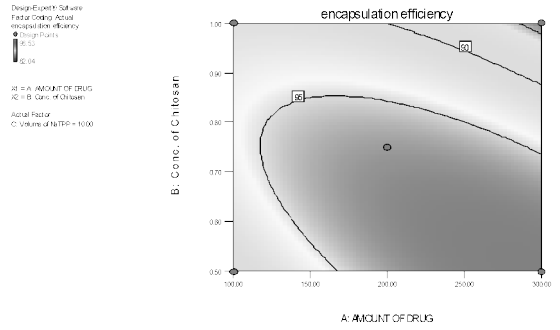

***Fig. 3:** Contour plot of encapsulation efficiency as a function of formulation variables.*

HCl. Therefore, NiMS-10 was further used for the preparation of ODTs.

The infrared spectrum of NiMS-10 (Figure 4) exhibited no significant difference in the characteristic peaks when compared to the spectrum of pure ACZ, indicating no interaction between the drug and polymer. The results suggested no incompatibility during the encapsulation process.

***Table 3:** ANOVA of regression (% encapsulation efficiency)*

	Degree of Freedom	Sum of Square	Mean Square	F Value	F-Significance
Regression	9	238.41	26.49		
Residual	3	6.01	2.0	13.22	0.0284*
Total	12	244.42			

*P <0.05%

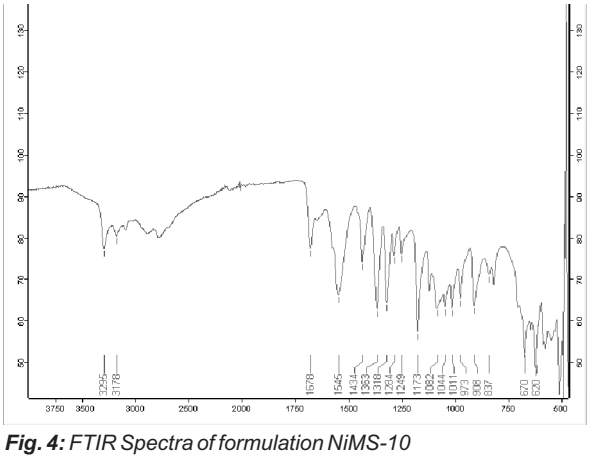

***Fig. 4:** FTIR Spectra of formulation NiMS-10*

As observed from the transmission electron microscopic analysis (Leo 0430, Leica Cambridge Ltd., and Cambridge, UK) (Figure 5), the ACZ -loaded NiMS was found to be almost spherical in shape.

ODTs of NiMS (batch NIMS-10) were prepared by direct compression method. The tablets were evaluated for weight variation, hardness, drug content, friability, disintegration and *in vitro* dissolution rate. It was found that all the evaluation tests of tablets were within the specified range as per Indian Pharmacopoeia (2013). The results are shown in Table 4.The disintegration time of the drug was found to be 35 ± 2 seconds.

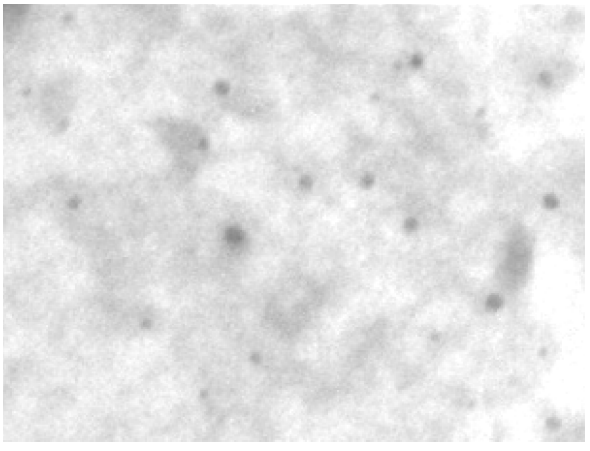

***Fig. 5**: TEM of Formulation (NiMS-10).*

***Table 4:** Evaluation results of tablets*

Parameter	Results
Weight Variation	198± 3.07 mg
Hardness	4.7±.54 kg/cm^2
Friability	0.6 ± 0.05%
Drug Content	94% ± 0.35%
Disintegration Test	35 ± 2.0 sec

It has been found that drug released was 97.8 ± 2.27% after 60 minutes by *in vitro* drug release studies. *In vitro* drug release at different time intervals is shown in Figure 6.

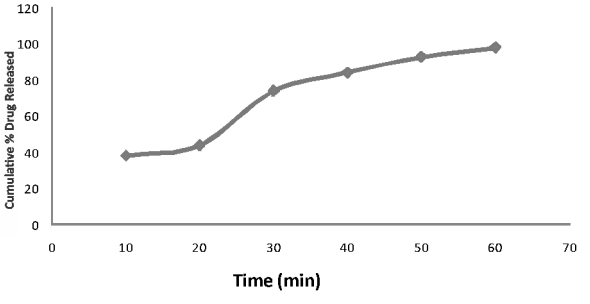

***Fig. 6:** In Vitro cumulative percent Release of acetazolamide from orally disintegrating tablets*

In order to understand the mechanism and kinetics of drug release from ODT, the in vitro release data was fitted to various kinetic equations. Higher correlation (0.994) was observed in first-order, indicating the first order release of ACZ from ODT.

CONCLUSION

The ACZ loaded NiMS were successfully formulated by applying ionotropic- gelated chitosan and further formulated into ODTs using direct compression technique. The mathematical model developed in the present study can be used to formulate NiMS of ACZ with desired encapsulation efficiency. The ODT developed using NiMS disintegrate quickly i.e. within 35 seconds. Therefore, the ODT developed in the present study would be advantageous in case of pediatrics for the treatment of glaucoma. Formulations containing NiMS represent an example of quickly dispersible releasing tablets that are effective alternative to traditional tablets.

REFERENCES

1. Bogner RH and Wilkosz MF. New dosage convenience for patients: fast dissolving tablets.US Pharmacist. 2002; 27: 34-43.
2. Olmez SS and Vural I. Advantages and quality control of orally disintegrating tablets. FABAD J. Pharm. Sci. 2009; 34:167-72.
3. Fu Y, Yang S, Jeong SH, Kimura S and Park K. Orally fast disintegrating tablets: developments, technologies, taste-masking and clinical studies. Critical Reviews in Therap Drug Carr Syst. 2004; 21(6): 433–75.
4. Li FQ, Yan C, Bi J, Lv WL, Ji RR and Chen X. A novel spray-dried nanoparticles-in-microparticles system for formulating scopolamine hydrobromide into orally disintegrating tablets. Int J Nanomedicine.2011; 6: 897–904.
5. Bhavsar MD and Mansoor MA. Development of novel biodegradable polymeric nanoparticles-in-microsphere formulation for local plasmid DNA delivery in the gastrointestinal tract. AAPS Pharm Sci Tech. 2008; 9(1): 288-94.
6. Bhavsar MD, Tiwari SB and Amiji MM. Formulation optimization for the nanoparticles-in-microsphere hybrid oral delivery system using factorial design. J. Control. Rel. 2006; 110(2): 422–30.
7. Lee Y-S, Johnson PJ, Robbins PT, Bridson RH. Production of nanoparticles-in-microparticles by a double emulsion method: A comprehensive study. Eur J Pharm Biopharm. 2013; 83(2): 168-73.
8. Granero GE, Longhi MR, Becker C, Junginger HE, Kopp S, Midha KK, et al. Biowaiver monographs for immediate release solid oral dosage forms: Acetazolamide. J Pharm Sci. 2008; 97(9): 3691-99.
9. Sharan S, Dupuis A, Hebert D and Levin AV. The effect of oral acetazolamide on weight gain in children. Can J Ophthalmol. 2010; 45: 41–5.
10. Agnihotri AS, Mallikarjuna NN and Aminabhavi TM. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J. Control. Rel. 2004; 100: 5-28.
11. Abdallah MH, Box-Behnken design for development and optimization of acetazolamide microspheres. Int J Pharm Sci Res. 2014; 5(4): 1228-39.
12. Reis PC, Neufeld RJ, Ribeiro AJ and Veiga F. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomedicine: Nanotech, Biol Med.2006; 2: 8-21.
13. Elzoghby AO, Samy WM and Elgindy NA. Novel spray-dried genipin- crosslinked casein nanoparticles for prolonged release of a alfuzosin hydrochloride. Pharm Res. 2012; 30: 512-22.

14. Mora MJ, Longhi MR and Granero GE. Development and validation of a method for determining acetazolamide in rat intestinal fluid by HPLC. Available at "http://www.fbioyf.unr.edu.ar/ricifa/Resumenes2010%5CControl%20de%20calidad/Mora.pdf".
15. Singh N, Gagoria J, Arora SC, Khatkar A and Saraf A. Development and characterization of poly (ε-caprolactone) microspheres containing acetazolamide. Int J Pharm Sci Res. 2011; 2(8): 2080-90.
16. Kommanaboyina B. and Rhodes CT. Trends in stability testing with emphasis on stability during distribution and storage. Drug Dev Ind Pharm. 1999; 25(7): 857-68.
17. Pirooznia N, Hasannia S, Lotfi AS and Ghanei M. Encapsulation of Alpha-1 antitrypsin in PLGA nanoparticles: In Vitro characterization as an effective aerosol formulation in pulmonary diseases. J Nano biotech. 2012; 10(20): 1-15
18. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare. The Indian Pharmacopoeia Commission, Ghaziabad, India. 2014; 985-90.
19. Wang JJ, Zeng ZW, Xiao RZ, Xie T, Zhou GL, Zhan XR, et al. Recent advances of chitosan nanoparticle drug carriers. Int J Nanomedicine. 2011; 6: 765-774.