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NANOPARTICLE, A SMART NANOSYSTEM FOR THE THERAPY OF BREAST CANCER-DESIGN DEVELOPMENT AND CHARACTERIZATION

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Abstract

Background and Objective: The current research was focused on development and characterization of Anastrazole nanoparticle for breast cancer therapy. Pectin was used as polymer for the preparation of nanoparticle depending upon solubility and compatibility. The optimized ratio of drug: polymer: surfactant was selected depending upon the release studies. The in-vitro study shows higher uptake of drug from nanoparticle and enhancement in the penetration of the drug.

Method: Anastrazole nanoparticle was prepared by precipitation method and optimized by design expert. Particle size, entrapment efficiency were selected as critical quality attribute.

Results: Optimized nanoparticle exhibited particle size of 94.41 nm, entrapment efficiency of 62.23. Anastrazole nanoparticle has shown 19.73 % release at 1 hr and 71.10% release at 8 hr.

Keywords: Anastrazole, Nanoparticle, Pectin.

Introduction: The term 'Nanotechnology' was derived by Professor Norio Taniguchi, Tokyo Science University in 1974; it was meant to describe the precision of manufacture of materials with nanometers sizes.

Nanomedicine is an emerging of nanotechnology, which uses small sizes particles that are more than 10 million times smaller than the human body. Due to this, nanomedicine presents today many revolutionary opportunities in the fight against all types of cancer, neurodegenerative disorders and other diseases[1-3].

Nanoparticles are defined as particulate dispersions or solid particles with a size in the ranging from 10-1000nm.The drug containing nanoparticle is dissolved, entrapped, encapsulated or attached to a nanoparticle

matrix. Nanoparticle at the systemic level, include longer circulation of half-lives and also improved pharmacokinetics and reduced side effects of the drugs[4-5].

Cancer is a category of ailments in which a group of cells creates unrestrained growth, incursion, and sometimes metastasis (spread to other positions in the body via lymph or blood). These three malignant characteristics of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasize[6]. Breast cancer is the leading diagnosed cancer and the most common cause of cancer-related death in women[7]. Breast carcinoma is the most commonly occurring carcinoma comprising almost one-third of all malignancies in females both in the developed and the developing world (WHO 2014)[8].

Breast cancers can start from different parts of the breast. Most breast cancers begin in the ducts that carry milk to the nipple (ductal cancers). Some start in the glands that make breast milk (lobular cancers). There are also other types of breast cancer that are less common[9]. A malignant cancer can spread to other parts of the body. It accounts for 16% of all female cancers and 22.9% of invasive cancers in women[10].

Anastrozole for breast cancer

The trial suggested that anastrozole is the preferred medical therapy for postmenopausal women with localized breast cancer, which is estrogen receptor (ER) positive. Anastrozole lowers estrogen levels in postmenopausal women, which may slow the growth of certain types of breast tumors that need estrogen to grow in the body. Aromatase inhibitor inactivates the production of estrogen from androgens, by suppressing aromatase enzyme activity. The breast cancer patients treated with aromatase inhibitors show, less level of estrogen secretion in the tumor cells[11].

Materials and Methods

Anastrazole was obtained from AstraZeneca as gift sample. Polymer pectin, Tween 80, Sodium acetate, PEG400, Acetone and sodium chloride was obtained from Yarrow Chemical products Mumbai.

FT- IR Studies

FTIR studies between Anastrazole and the excipients were carried out to find interactions among the drug and polymers. Peaks of pure drug and combination with the polymer were obtained and were further checked for

compatibility between them. FTIR spectra of pure drug and the drug-polymer mixture were obtained in KBR pellets using IR- affinity-I Shimadzu Auto 00518 spectrometer.

Differential Scanning Colorimetry[12-13]

The thermo gram of pure Anastrazole drug and physical mixture with polymers and pure drug was obtained. 10 mg of sample was sealed in aluminum micropan and introduced in analytical system (DSC-50 Tokyo, Japan).

Preparation of Nanoparticles

- Anastrazole nanoparticles were prepared by polymer dispersion method. Pectin was dispersed in purified water containing tween 80 and sodium acetate using magnetic stirrer at ambient temperature for 5 hrs. at 150 rpm. Anastrazole was dissolved in a non-aqueous phase acetone: polyethylene glycol 400 in the ratio of 1:1. Non aqueous phase was added slowly at the rate of 2.5 ml/ min using syringe during homogenization using a high pressure homogenizer at 20000.0 rpm for 20 min. sodium chloride added at the end of homogenization.

  Anastrazole was dissolved in a non-aqueous phase acetone: polyethylene glycol 400 in the ratio of 1:1. Non aqueous phase was added slowly at the rate of 2.5 ml/ min using syringe during homogenization using a high pressure homogenizer at 20000.0 rpm for 20 min. sodium chloride added at the end of homogenization.

Characterization and Evaluation of Anastrazole Nanoparticle Particle size analysis and zeta potential:

The size of nanoparticles was determined by dynamic light scattering (Nano ZS 3600, Malvern Instruments, Malvern, UK), with varying duration greater than 20 s. The dispersant used was water having RI (1.33), viscosity (0.8872 cP). Disposable sizing cuvette was used for determination.

Percentage yield:

The obtained solid lipid nanoparticles were weighed. % yield was calculated by using following formula.

$$\% \text{ yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Determination of Entrapment efficiency (EE):

An amount of nanoparticle equivalent to 5 mg of Anastrazole was accurately weighed and then added to 10 mL of ethanol. The solutions were kept shaking for 1 hr.

Then the solutions were centrifuged at 15000 rpm for 30 min. The supernatant was collected and the drug content was estimated by UV Spectrophotometer at 231 nm.

$$EE = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

In vitro Drug Release

The invitro release profile of anastrazole loaded pectin was studied using franz diffusion cell in Hcl 1.2 as the dissolution media. During the study, 10ml of aliquots were removed at predetermined time intervals 1-2hrs from the dissolution medium and replaced with fresh media.

The amount of anastrazole released in the dissolution media was determined by uv-visible spectroscopy at 230nm. After 2 hours the buffer was changed with PH 6.8 and again amount of anatrazole released was determined as mentioned above.

Stability Studies:

Long term testing; 25°C ±2°C/60 % RH ±5 for 3 months.

Accelerated Testing: 40°C ± 2°C/75 % RH 5% for 3 months

Procedure:

The selected formulation were stored at 40°C/75% RH inNewtronic temperature/Humidity control chamber QLH-2004, and at room temperature and humidity for a period of 3 months.

The sample was withdrawn every month. The drug content and entrapment efficiency was analysed at 231 nm.

Discussion

The present study was carried out to formulate and evaluate nanoparticle formed by precipitation method. Hence it was necessary to select polymer with good compatibility.

Pre-formulation studies

Standard graph of Anastrazole in ethanol solution:

The λmax of Anastrazole with etanol was found to be 231 nm. The method has reproducibility and precision. The correlation coefficient for the standard curve was found to be 0.999 in the concentration range of 2-10 µg/mL.

The regression equation was 0.0269x - 0.012

Drug excipients interaction studies by Fourier Transform Infrared Spectroscopy:

The FT-IR was performed for drug, polymer and mixture of drug and polymer. The spectrum obtained from FT infrared spectroscopy studies at wavelength from 4000cm-1 - 400cm-1 are shown in fig.no:6-8 and the characteristic peaks obtained are shown in the table 2. Characteristic peaks in the region of 2234.60cm-1, 1605.44cm-1, were found to be observed in physical mixture which was identical to that of the pure drug; this confirmed the intactness of the drug in the physical mixture.

DSC analysis:

Figure no.4 illustrates thermograms for pure anastrazole, pectin and physical mixture of pure drug and polymer in the ratio of 1:1. For the pure anastrazole sharp endothermic peak at 84.62°C was observed. Bulk pectin show melting endotherm at 92.68°C and183.39°C. slight shift in peak position for both. Anastrazole and pectin was observed in physical mixture indicating that there is no interaction between anastrazole and pectin.

Formulation of nanoparticles:

As pectin is compatible with the drug it was selected for preparation of nanoparticles. Many preparation method was used in trials to prepare nanoparticle using pectin as polymer. But nanoprecipitation method was used for further proceedings as nanoparticles formed by this method was likely to give reproducible result.

Characterization and evaluation of Nanoparticle

% Encapsulation efficiency:

The encapsulation efficiencies for formulation were found to be between 64.77 and 94.44. A close look of the results has revealed that the encapsulation efficiency increased with increase of lipid amount and concentration

of surfactant. However, the concentration of surfactant affects much less on the %EE as compare to the effect of lipid amount.

Particle size analysis: The particle sizes of the formulations were found between 94.3 nm.

In-vitro release:

In vitro drug release study:

The in-vitro release of Anastrazole nanoparticle from the prepared nanoparticles formulation was studied in acid buffer of 1.2 for 8hours. The results are presented in the fig no. 7 as the concentration of polymer increased, the drug release also decreased proportionally. This may be because pectin retards release to more extent. The release of the drug at 8th h ranged from 58.148 to 76.669 .The highest release of the drug took from the formulation F7 .The result has shown that anastrazole released the drug immediately while all pectin formulation controlled the release of drug for longer period of time for 8 hr. pectin permits a protection for therapeutic agent from the hostile conditions of the upper gastrointestinal tract and release the entrapped agent specifically in the stomach through degradation of the glycosidic pectin. Controlled drug delivery systems follow several mechanisms and it is still ambiguous. Here it is followed either purely diffusion or erosion controlled.

Drug release at 1hr

The amount of anastrazole released from nanoparticles in 1hr ranges from 10.58% to 25.69%. The 1 hr release of drug from particles was mainly dependent on polymer concentration, as the concentration of pectin increased the drug release decreased.

Drug release at 8hr

The amount of anastrazole released from nanoparticle in 8 hr ranges from 58.14% to 76.33% . it shows that concentration of tween 80 does affected the release at 8 hr. as the concentration of tween 80 increases the release rate also increases. Entrapment efficiency. The values for entrapment efficiency are shown in table n.4 the entrapment efficiency was found to be in the range between 56.452 to 70.3214%. it shows that entrapment efficiency increase with the increase in rpm. The formulation F8 has highest entrapment while formulation F4 has the lowest entrapment.

Kinetics of drug release:

The drug release data were fitted into the different models like zero order, first order, Higuchi equation and Korsmeyer-Peppas (table n. 12 to 19. The result have shown very close and above 0.9 R2 for first order. It indicates that the drug release is diectly proportional to amount of drug remained to be released. The results have also shown very close and above 0.9 R2 values for higuchi and korsmeyer peppas model. The n values lie between 0.596 - 0.919 which indicates non fickian diffusion and therefore suggested that the release mechanism is by both diffusion and relaxion.

Anova

The results of ANOVA demonstrate that the model was significant for all dependent variables. Regression analysis was carried out to determine the regression coefficients. All the independent variables (Factors) were found to be significant for all R1, R2, and R3 response variables. The linear model was found to be significant for all responses. So above result indicate that all the four factors (polymer, Tween80 and RPM) play an important role in the formulation of nanoparticles of Anastrazole.

Optimization:

In the numerical optimization techniques, the desirability approach was used to generate the optimum settings for the formulation. For the optimized formulation, the particles size was kept at the targeted value, the drug release at 1st hr was kept at minimum, and the drug release at 8th hr was kept in minimum. The encapsulation efficiency was also kept at maximum. The optimized formulation was prepared according to predicted model and evaluated for responses.

All the selected parameter shows p-value less than 0.05, which indicates that all models term are significant. The p-value was found to be 0.0038 for 1 hr and 0.018 8hr release.

Stability study:

The optimized formulation was subjected to accelerated stability study for period of 12 weeks. Physical ability was analyzed by appearance, and chemical stability was by the change in the drug content. Resilts are reported in the table 13. the formulation was stable for 3 months period in accelerated stability study. The drug content of nanoparticle formulation did not vary to a large extent.

Table 1: Factorial design for formulation (using design expert).

Run	Pectin	Tween 80	Rpm
1	0.2	4	10000
2	1	1	10000
3	0.2	4	20000
4	1	4	10000
5	1	4	20000
6	0.2	1	10000
7	1	1	20000
8	0.2	1	20000

Table no: 2.Interpretation for FT-IR spectra of Anatrazole and Pectin.

Name of pure drug	**Standard value of drug (cm^{-1})**	**Observed value with Pectin (cm^{-1})**
Anastrazole	3500-3200	3101.13
	3000-2850	2983.79
	2210-2260	2234.60
	1335-1250	1272

Table no:3. Data showing evaluation of % encapsulation efficiency.

Formulation code	**% Encapsulation efficiency**
F1	64.77
F2	78.55
F3	77.89
F4	71.12

F5	80.26
F6	85.53
F7	90.02
F8	94.44

***n-Vitro* drug release studies:**

Table no:2. Data showing *in-vitro* drug release for formulations F1-F8.

Time	F1	F2	F3	F4	F5	F6	F7	F8
1	20.4603	10.584	23.06	11.318	23.084	17.42	25.698	17.323
2	26.773	17.881	28.696	18.623	30.614	25.773	32.359	25.149
3	32.727	23.406	35.644	24.466	36.618	31.288	40.921	32.664
4	40.201	30.091	42.958	31.561	43.519	36.489	48.707	40.657
5	47.546	38.261	48.695	39.575	50.51	43.138	55.897	46.136
6	54.116	45.413	56.613	47.298	59.525	52.152	61.745	53.806
7	60.829	52.502	61.368	53.569	65.741	60.519	68.678	62.161
8	66.443	60.633	68.821	58.148	71.489	68.505	76.669	72.103

Table no: 3 Data showing comparison of kinetics for formulations F1-F8.

Formulation Code	Zero order	First order	Higuchi model	Korsmeyer-Peppas equation
F1	Y=6.7104x+13.44 R^2= 0.999	Y=0.054x+1.9768 R^2=0.9873	Y=25.904x+9.1625 R^2=0.9798	Y=0.5835x+1.2734 R^2=0.9802
F2	Y=7.1147x+2.8306 R^2=0.9981	Y=-0.0497x+2.0231 R^2=0.9773	Y=24.605x+20.087 R^2=0.531	Y=0.8387X+1.0006 R^2=0.9934
F3	Y=7.1496x+9.7379 R^2=0.9928	Y=-0.0574x+2.0028 R^2=0.9578	Y=27.392x-13.924 R^2=0.9594	Y=0.6461x+1.213 R^2=0.9784
F4	Y=6.8938x+4.5476 R^2=0.9969	Y=-0.0479x+2.011	Y=26.663x-18.775 R^2=0.9815	Y=0.8064x+1.0329 R^2=0.9951

		R^2=0.9914		
F5	Y=7.026x+16.021 R^2=0.9981	Y=-0.0618x+1.974 R^2=0.981	Y=27.113x-7.6257 R^2=0.9783	Y=0.5545x+1.381 R^2=0.98
F6	Y=7.1496x+9.7379 R^2=0.9928	Y=-0.0574x+2.0028 R^2=0.9578	Y=27.392x-13.924 R^2=0.9594	Y=0.6461x+1.213 R^2=0.9784
F7	Y=7.2387x+18.76 $R^2$0.9983	Y=-0.0693x+1.9713 R^2=0.9763	Y=0.5343x+1.3773 $R^2$0.9873	Y=28.031x-5.7999 R^2=0.9853
F8	Y=4.5884x+9.6024 $R^{2=}$0.9967	Y=-0.0633x+2.0114 R^2=0.958	Y=29.214x-15.794 R^2=0.9722	Y=0.6774x+1.2111 R^2=0.9892

Table no. 4: Response 1: 1 hour release.

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	207.19	3	69.06	28.02	0.0038	Significant
A-pectin	160.74	1	160.74	65.22	0.0013	
B-tween 80	4.57	1	4.57	1.85	0.2451	
C-rpm	41.89	1	41.89	17.00	0.0146	
Residual	9.86	4	2.46			
Cor Total	217.05	7				

Table no5: 22 Coefficients in Terms of Coded Factors.

Factor	**Coefficient Estimate**	**df**
Intercept	18.64	1
A-pectin	-4.48	1
B-tween 80	0.7555	1
C-rpm	2.29	1

ANOVA for selected factorial model

Table no.6: Response 2: 8 hour release.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	232.29	3	77.43	11.74	0.0188	Significant
A-pectin	72.19	1	72.19	10.95	0.0297	
B-tween 80	9.40	1	9.40	1.42	0.2986	
C-rpm	150.70	1	150.70	22.85	0.0088	
Residual	26.38	4	6.60			
Cor Total	258.68	7				

Table no.7: Coefficients in Terms of Coded Factors.

Factor	Coefficient Estimate	df
Intercept	67.85	1
A-pectin	-3.00	1
B-tween 80	1.08	1
C-rpm	4.34	1

Table no8: Response 3: Entrapment efficiency.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	219.62	3	73.21	11.22	0.0204	significant
A-pectin	52.16	1	52.16	8.00	0.0474	
B-tween 80	1.59	1	1.59	0.2437	0.6474	
C-rpm	165.87	1	165.87	25.43	0.0073	
Residual	26.09	4	6.52			
Cor Total	245.71	7				

Table no.9: Coefficients in Terms of Coded Factors.

Factor	Coefficient Estimate	df
Intercept	61.88	1
A-pectin	-2.55	1
B-tween 80	0.4458	1
C-rpm	4.55	1

Table no: 10. Data showing *in vitro* drug release for formulation F9

Time (hr)	% Cumulative drug release
0	0
1	19.73684
2	26.32675
3	34.17719
4	42.32939
5	51.13553
6	57.17763
7	64.56447
8	71.10965

Table no. 11 Comparison of Kinetics for optimized formulation F9.

Formulation Code	Zero Order	First Order	Higuchi Model	Korsemeyer - Peppas
F9	y = 7.4834x + 12.44 R^2 = 0.9982	y = 0.0634x + 1.9957 R^2 = 0.9857	y = 28.954x – 13.195 R^2 = 0.9835	y = 0.6373x + 1.2586 R^2 = 0.9857

Table no: 12 Comparison between the predicted and experimented values of the optimized formulation.

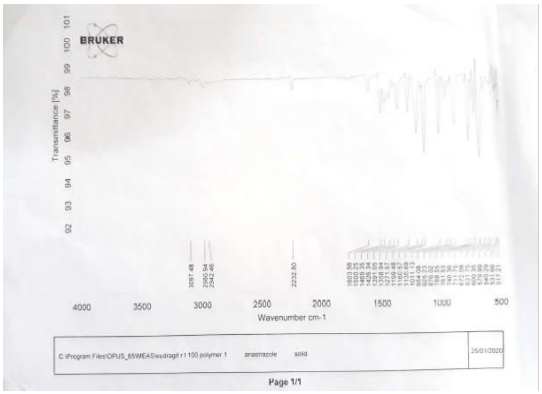
Optimized formula(F9)	Dependable variables		
	Drug release		Entrapment Efficiency
	At 1 hr	At 8 hr	
Predicted	19.846	71.24	65.093
	19.73	71.109	62.23

Stability study

Table no: 13. Physicochemical properties of optimized formulation (After Stability).

Time	Drug content (%)	E.E (%)	Appearance
Intial	95.84	91.21	Yellowish
After 3 month	94.45	91.01	No change

Fourier transform infra-red (FT-IR) studies:



Figno.1: FTIR Spectra of Anastrazole

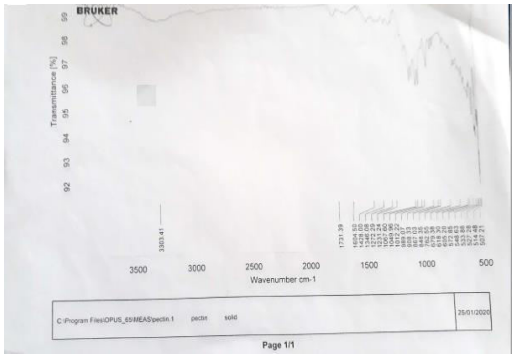


Fig no.2: FTIR Spectra of Pectin.

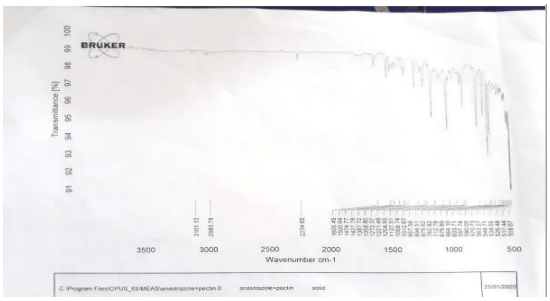


Fig no.3: FTIR Spectra of Anastrazole+Pectin

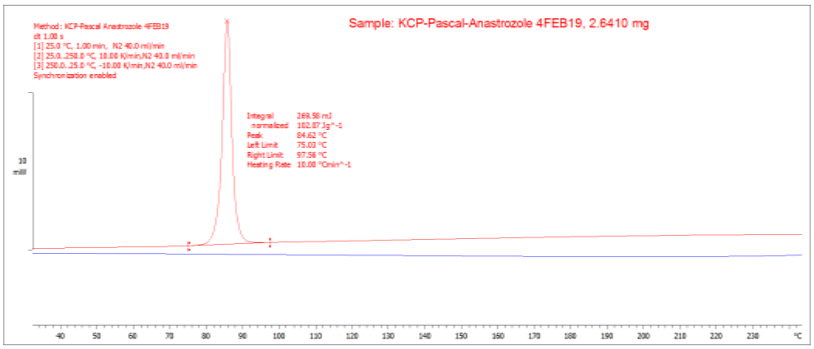
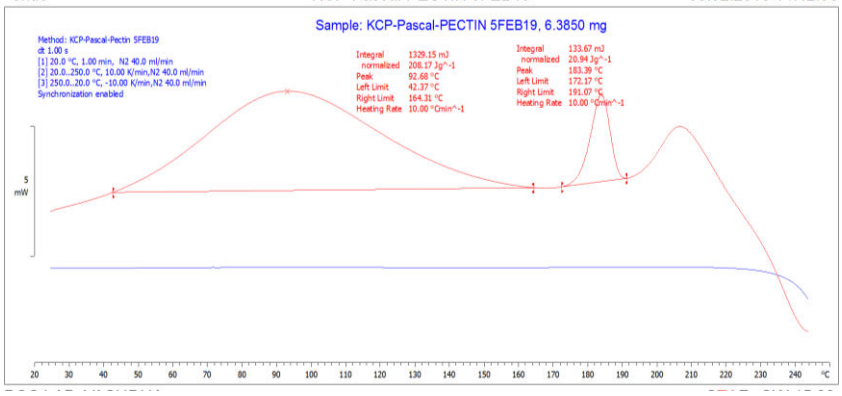


Fig no.4: DSC of Anastrazole.



Figno.5: DSC of Pectin.

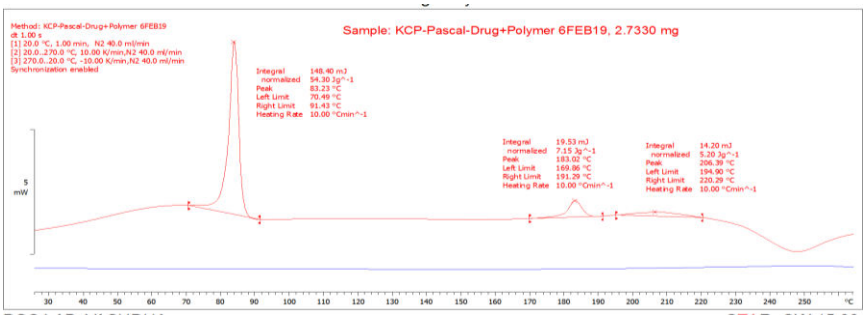


Fig no.6: DSC of anastrazole+Pectin.

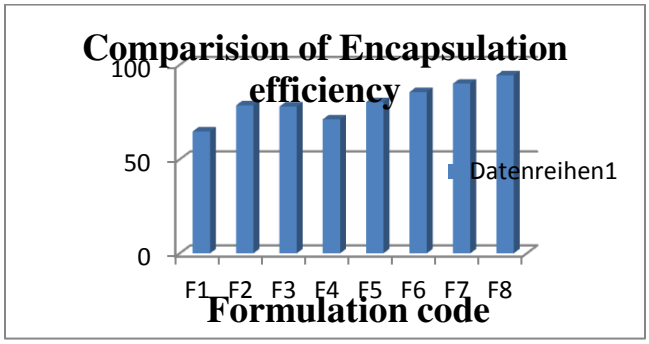
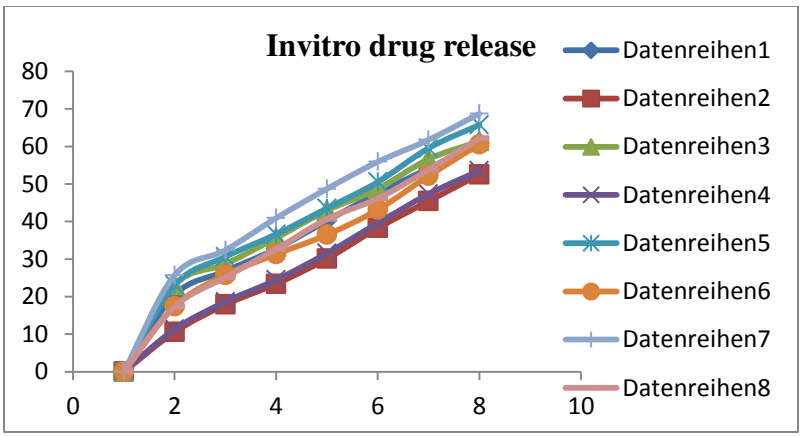


Fig no.7: Comparison of % EE of formulations F1-F8.



Figno.8: Comparison of invitro drug from F1 to F8.

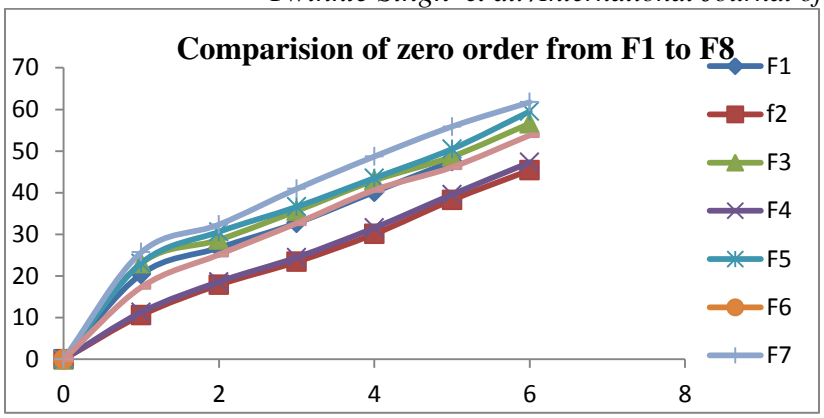


Fig no.9: Comparison of Zero order drug release F1-F8.

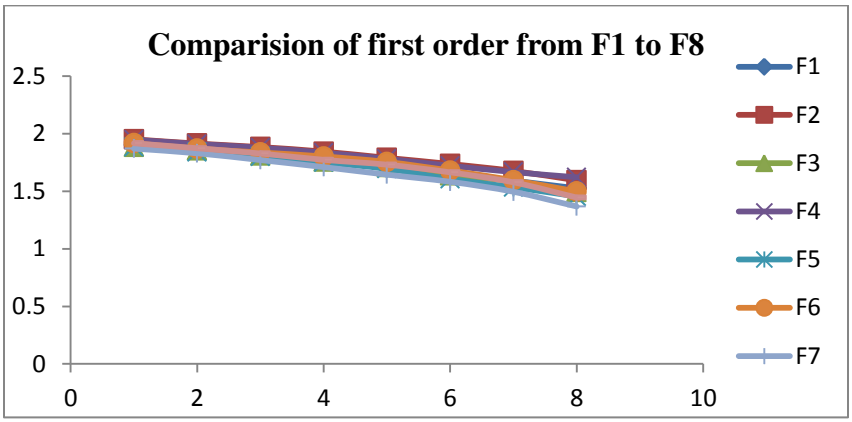


Fig no.10: Comparison of first order of drug release F1-F8.

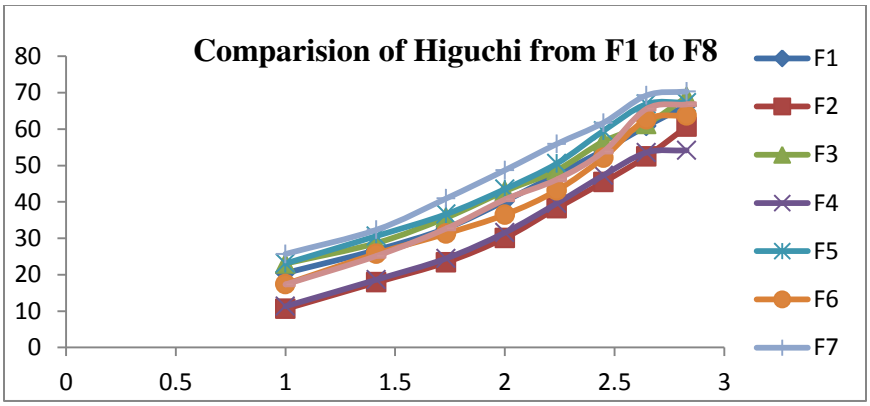


Fig no.11: Comparison of Higuchi from F1 to F8.

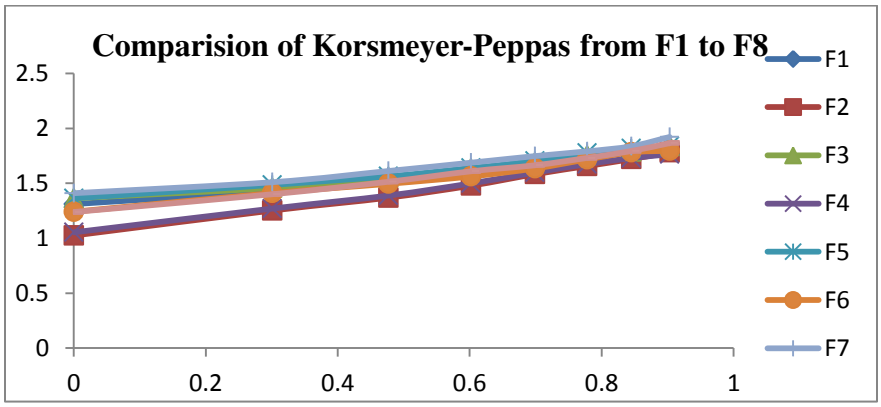


Fig no.12: Comparison of Korsmeyer-Peppas from F1 to F8.

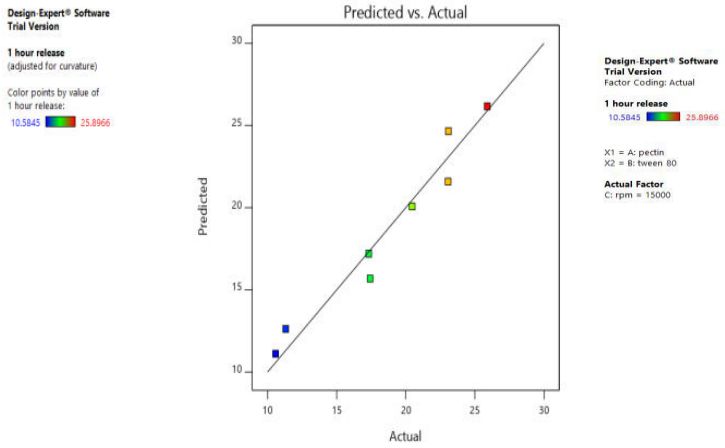


Fig no.13: Predicted vs Actual for 1hr release.

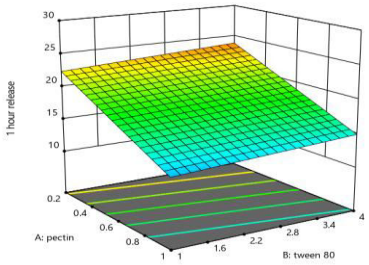


Fig no.14: 3-D graph of pectin and Tween 80 at 1 hr.

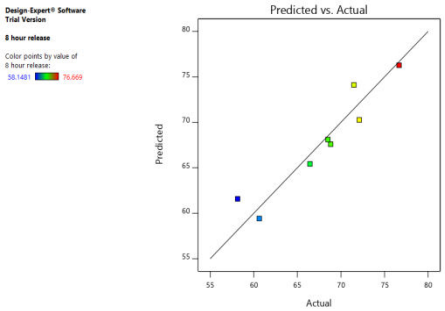


Fig no.15: Predicted vs Actual for 8hr release.

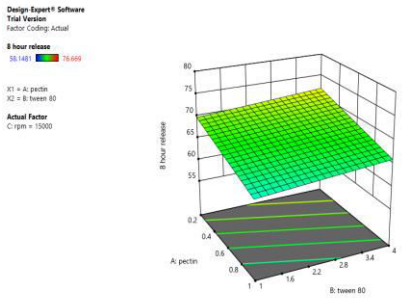
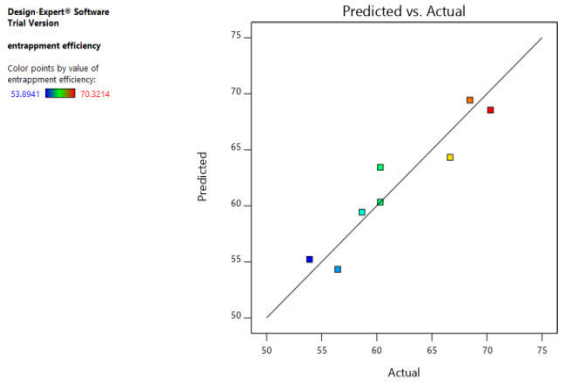
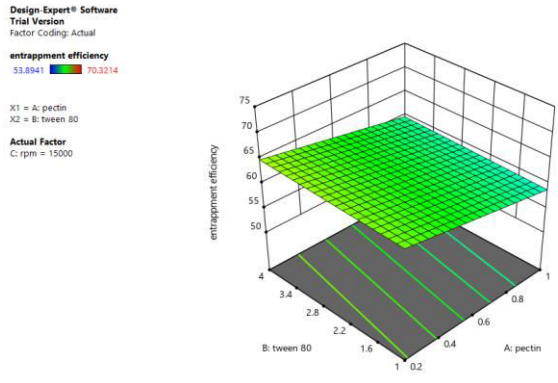


Fig no.16: 3-D graph showing effect of pectin and tween 80 concentration 8 hour drug release (R2).



Figno.17: Predicted vs. Actual for Entappment release.



Figno.18: 3-D graph showing effect of RPM on entrapment efficiency (R3)

Results

		Size (d.nm...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 94.41	Peak 1:	552.5	76.2	283.7
PdI: 1.000	Peak 2:	15.32	22.5	3.871
Intercept: 0.962	Peak 3:	5207	1.4	471.4
Result quality Refer to quality report				

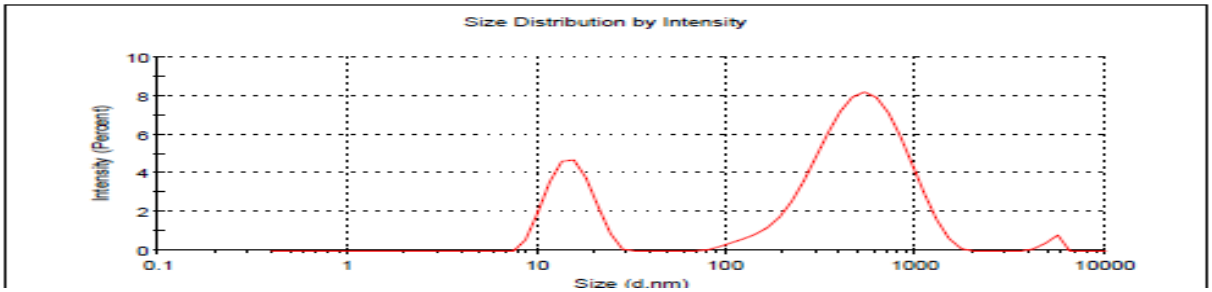


Fig no. 19 Particle size.

***In-vitro* drug release of formulation F9**

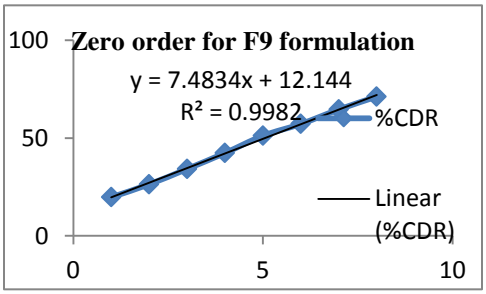


Fig no.20: Zero order for F9 formulation.

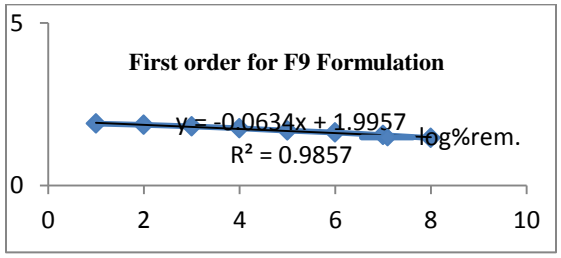


Fig no.21: First order for F9 formulation.

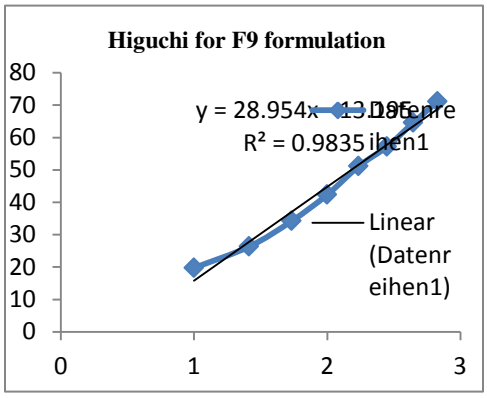


Fig no.22 Higuchi order for F9 formulation.

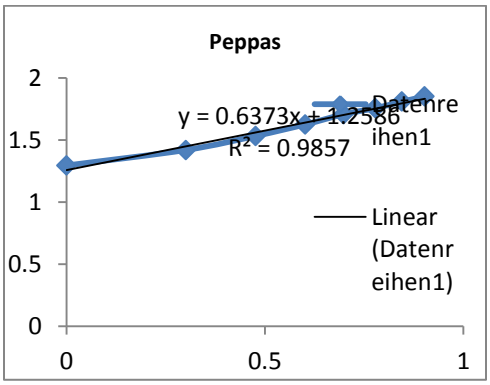


Fig no.23 Kors-meyer Peppas for F9.

Conclusion

The present study demonstrated the feasibility anastrazole polymeric nanoparticle using factorial design. The formulation release property and its invitro kinetic model were illustrated. The understanding effect of the input variable on the selected response has been proven from 3D graph. We conclude that the preparation method as

well as the optimization technique used for this study is promising for anatrazole polymeric nanoparticle preparation.

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