Research article

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OPTIMIZATION AND CHARACTERIZATION OF NATURAL SURFACTANT BASED GLIBENCLAMIDE NANOPARTICLES USING RESPONSE SURFACE METHODOLOGY (BOX-BEHNKEN DESIGN)

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ABSTRACT

In the present study glibenclamide (GB) loaded ethyl cellulose nanoparticles were prepared by solvent evaporation method in the presence of soapnut as a natural emulsifying agent;glutaraldehyde solution was used as a cross-linking agent and dichloromethane as organic solvent. In this work, we present a formulation of these nanoparticle materials by Siverson emulsifier. A 3-factor, 2-level Box-Behnkendesign was used to optimize the process parameters including drug (A), polymer (B) and surfactant (C). Two dependent variables particle size and % of yield were measured asresponses. Mathematical equations and response surface plots were used to relate the dependent andindependent variables. The optimization model predicted particle size of about 259.71 nm and % of yield of 77.64 % with A, B and C levels of 150, 150 and 875 respectively. The observed responseswere in close agreement with the predicted values of the optimized process. The preparednanoparticle was characterized by SEM, Fourier transform infrared spectroscopy, DSC spectra and RPHPLC analysis. Theglibenclamidenanoparticles obtained by natural surfactant (soapnut)exhibited a good size and shape.

Keywords: Soapnut, GB Nanoparticle, HPLC, Box-Behnkendesign

INTRODUCTION:

Solubility is significantmeasure for drug efficacy, independent of route of administration. It also posturesa major challenges for pharmaceutical industries, which are emergent new pharmaceutical products, since 40% of the active substances being recognized,are either insoluble or poorly soluble in aqueous media [1]. A limiting factor for *in-vivo* performance of poorly water soluble drugs, subsequent oral administration, is their resistance to being wetted and being dissolved into the fluid in the gastrointestinal tract. Increasing the dissolution rate of poorly water soluble drugs is thus important for improving bioavailability [2, 3]. The part of solubility enhancement is an endeavor to shift the classification of a drug (II → I) in order to eradicate the problems connected with dissolution-limited compounds. Over the last 10 years, nanoparticle (NP) engineering processes have been developed and stated for enhancement of solubility of poorly aqueous soluble drugs. Current approach, poorly water soluble compounds are formulated as nanometer sized drug particles [4]. According to Muller, NPs are solid colloidal particles size range from 1 to 1000 nm (1 µm). They have the improvement of having an even more surface area, and being categorized, unlike micronized drugs, by enhance in saturation solubility. Glibenclamide is a second-generation sulfonylurea oral hypoglycemic drug used in the management of non-insulin dependent diabetes mellitus. It causes hypoglycemia by stimulating release of insulin from pancreatic β cells and by increasing the sensitivity of peripheral tissue to insulin [5]. It has a record of less bioavailability, which is attributed to poor dissolution [6-8]. Numerous attempts for increasing dissolution and bioavailability of glibenclamide have been made, such as micronization[9], molecular dispersion [10], incorporation of surfactants [11], inclusion complexation with cyclodextrin[12, 13], crystal modification [14,15], glass formation [16,17] and coprecipitation[18]. The solvent evaporation method has been used toformulate biocompatible and biodegradable/nonbiodegradablepolymer micro/nano particles likepoly(lactic-co-glycolic acid), Polylactic acid, ethylcellulose, acrylate polymersetc[19-21].SapindustrifoliatusLinn., a small deciduous tree belongs to the family Sapindaceae which is identified assoapnut in English,Ritha in Bengali and

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Ponnangottai in Tamil[22].It is a native species cultivated in Indo-Gangetic plains, Shivaliks and Sub-Himalayan tracts in Indiaat an altitudes rangesfrom 200m to 1500m [23]. It is one of the most important trees of tropical and subtropical regions of Asia. It is commonly found in the Western Ghats and plains of South India[24].This tree flourishes well in deep clay loamy soil with an annual rainfall of 200 mm. It is a medium sized tree can reaches a height of 25m.The flowers are small greenish white in colour and the fruits are solitary globose appears in the month of July- August. The fruit contains an active principle saponinwhich ranges from 6-10 % of mass weight[25]. Saponin from soapnut is also widely used in the native medicine [26], Pharmaceutical industries [27, 28], used as detergents [29] and used for environmental remediation [30].The objective of this research was to formulate and optimizenanoparticles containing glibenclamide prepared by ethyl cellulose using soapnutto achieve a better size profile suitable for per oral administration with enhanced efficacy than previous glibenclamide delivery.

MATERIALS AND METHODS

Materials and method:

Glibenclamidewas obtained as a gift sample from Ranbaxy (M) SdnBhd, (Malaysia).Ethyl cellulosewas purchased from Sigma Aldrich (USA).Soapnutwas purchased fromApex International (India).All otherchemicals and reagents were of analytical grade. HPLC grade waterwas used throughout thestudies.

Method:

The soapnut solution was subjected to saponification value and viscosity studies. The prepared nanoparticles were evaluated by particle sizeand % yield.

Experimental design:

Initially, preliminary experiments (one factor at a time approach) were performed to determine the mainfactors and the appropriate ranges in which the optima lie. The effects of the three factors (drug, polymerand surfactant) on the particle size and % of yield were tested. Throughpreliminary screening the drug, polymerand surfactantwere identified as themost significant variables within the range of 100-200mg, 100-200mg, and 500-1250mg, respectively. Onthe basis of the preliminary trials a 3-factor, 2-level Box-Behnken design was employed to study theeffect of each independent variable on dependent variables (mean particle size and % of yield). Thisdesign is suitable for exploring quadratic response surfaces and constructing second-order polynomialmodels. The design consists of replicated center points and the set of points lying at the midpoint of eachedge of the multidimensional cube that defines the region of interest [31]. The independent factors andthe dependent variables used in the design are listed in Table 1**.** The experiments were conducted as forthe design of experiments and the responses for the dependent variables were entered in Table 2. Theresponse surfaces of the variables inside the experimental domain were analyzed using Stat-Ease Design-Expert software (DX9). Subsequently, three additional confirmation experiments were conducted toverify the validity of the statistical experimental strategies.

Preparation of nanoparticle:

The nanoparticles were prepared by the solvent evaporation method using the composition of ingredients listed in the table 1. There are two phases involves in this preparation which are the oily phase consists of the glibenclamide, ethyl cellulose dichloromethane; the aqueous phase consists of the soapnut solution.

The soapnutsolution was prepared by soaking the amount of soapnutpowder in 100 ml of distilled water for over a night.The soap nut shell absorbs water and releases the saponins which used as a natural surfactant solution. The solution was filtered 4-5 times until get a clear solution.

The oilyphase was added drop wise into the aqueous phase by using the dropper by Silverson emulsifier (removed base plate and emulsor screen) and were continued to stir for 3 hours at 8000 rpm. After 30 minutes 1 ml of glutardialdehyde was added into the solution drop wise. At the end of stirring the drug is encapsulated by the polymer.The nanoparticles were separated by centrifugation at 5000rpm for 30 minutes. The nano formulation was dried at 40±0.5ºC.

In vitro drug release studies:

The *in vitro* release of glibenclamide nanoparticles were performed using (Electrolab tablet dissolution test apparatus) in 900 ml of medium (0.1M hydrochloric acid) for the first 2 h and then in phosphate buffer (pH 7.5) from 3-15 h at 37 ± 0.5 °C and stirring rate of 100 rpm. Samples (5 ml) were collected periodically and replaced with equal volume of fresh dissolution medium on each occasion.The solution was determined by RP HPLC method. RP HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50µL loop volume. The LC

solution version 1.25 was used for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of acetonitrile, 10mm ammonium acetate: acetonitrile (pH 4.5) (10:30, v/v), and detection was made at 240 nm. The mobile phase was prepared daily, filtered through a 0.45µm membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25cm × 4.6mm i.d., 5µ) was used for the separation.

RESULTS AND DISCUSSION

Optimization of process variables for the nanoparticles:

The most widely used method for formulation of the nanoparticlesis the solvent evaporation method, which usually requires high shear stress. In this work, we reportthe successful result on the formulation of glibenclamidenanoparticles.Throughpreliminaryexperiments the Drug (A), Polymer (B) and Surfactant (C) were identified asthe most significant variables influence the particle size and% yield.Design of experiments (DOE) has been used as a powerful approach to reduce the variation in a processand, ultimately, to produce high % yield withuniform particle size distribution. Among variousdesign approaches, the Box-Behnken design was used to optimize and evaluate main effects, interactioneffects and quadratic effects of the process variables on the particle size and% yield. This design issuitable for exploring quadratic response surfaces and constructing second order polynomial models. Thedesign consists of replicated center points and the set of points lying at the midpoint of each edge of themultidimensional cube. These designs are rotatable (or near rotatable) and require 3 levels of each factor[32].

Seventeen experiments were required for the response surface methodology based on the Box-Behnkendesign. Based on the experimental design, the factor combinations yielded different responses aspresented in Table 2. These results clearly indicate that all the dependent variables are stronglydependent on the selected independent variables as they show a wide variation among the 17 batches.Data were analyzed using Stat-Ease Design-Expert software (DX9)to obtain analysis of variance(ANOVA), regression coefficients and regression equation. Mathematical relationship generated usingmultiple linear regression analysis for the studied variables are expressed as shown in Table 3.

These equations represent the quantitative effect of Drug (A), Polymer (B) andSurfactant (C) and their interaction on Particle size (Y1) and % Yield(Y2). The values of thecoefficient A, B and C are related to the effect of these variables on the responses Y1 and Y2.Coefficients with more than one factor term and those with higher order terms represent interaction termsand quadratic relationship respectively. A positive sign represents a synergistic effect, while a negativesign indicates an antagonistic effect. A backward elimination procedure was adopted to fit the data to thequadratic model. Both the polynomial equations were found to be statistically significant (P <0.01), asdetermined using ANOVA (Table 4 &5), as per the provision of Design Expert software(DX9).

Particle size analysis of glibenclamide nanoparticles was found to be in the range of 205.62 – 285.22 nm as shown in Table 2.The factorial equation for particle size exhibited a good correlation coefficient (1.000) and the Model Fvalueof 85.10 which implies the model is significant. Values of "Prob> F" less than 0.0500 indicatemodel terms are significant. In this case A, B, C and the quadratic term of A^2 aresignificant model terms as shown in Table 4. Results of the equation indicate that the effect of A (Drug) is more significant thanBand C. All the three variables having the negative effect on the particlesize, which means these factors, are inversely proportional to the response. The influence of the main andinteractive effects of independent variables on the practical yield was further elucidated using theperturbation and 3D response surface plots. The individual main effects of A, B and C on particle sizeare as shown in Figure 2. It is found that all the variables are having interactive effects for the responseY1. The 3D response surfaces and the2D contour plots of the response Y1 are shown in Figure 1 and 2 todepict the interactive effects of independent variables on response Y1, one variable was kept constantwhile the other two variables varied in a certain range. The shapes of response surfaces and contour plotsreveal the nature and extent of the interaction between different factors. The interaction between A and Bon particle size at a fixed level of C is shown in Figure 1 & 2. At low levels of A, Y1 reduced from230.31 to205.62nm. Similarly at high levels of A, Y1 reduced from 285.22 to 270.43nm.After generating the polynomial equations relating the dependent and independent variables, the processwas optimized for the responses. Numerical optimization

Table-1: List of Independent variable and Dependent variables in Box-Behnken design

Independent variable	**Levels**				
Variable	Name	Units	Low	Middle	High
A	Drug	mg	100	150	200
B	Polymer	mg	100	150	200
C	Surfactant	mg	500	875	1250
Dependent variable	**Goal**				
Y1	Size	nm		minimize	
Y2	Yield	%		100	

Table-2: Factorial design of glibenclamide nanoparticle formulations

	Factor 1	**Factor 2**	**Factor 3**	**Response 1**	**Response 2**
Run	**A:Drug**	**B:Polymer**	**C:Surfactant**	**Size**	**yield**
	mg	**mg**	**mg**	**nm**	**%**
1	200	100	875	270.43	82.4
2	200	150	1250	268.11	83.7
3	100	200	875	218.42	66.2
4	150	150	875	259.16	77.1
5	150	150	875	259.75	77.3
6	150	100	500	262.19	76.8
7	150	150	875	259.89	77.5
8	150	200	1250	264.13	78.2
9	150	200	500	269.28	78.8
10	100	100	875	209.67	58.3
11	150	150	875	259.77	78.2
12	150	100	1250	250.16	76.6
13	150	150	875	259.99	78.1
14	100	150	1250	205.62	58.6
15	200	150	500	282.11	84.1
16	100	150	500	230.31	60.8
17	200	200	875	285.22	85.2

Table-3: Regression equation for the response

Response	**Regression equation**
Y1	$259.71 + 30.23A + 5.58 B -6.98 C +1.51 AB +2.67AC +1.72 BC -14.34 A^2 +0.56 B^2 +17C^2$
Y2	$77.64 + 11.44 A +1.79B -0.42 C -1.28AB +0.45AC -0.100BC -5.21 A^2 +0.59B^2 -0.63C^2$

Table-4: ANOVA results of the quadratic model for the response particle size (Y1)

Source variations	Sum of Squares	DF	Mean Square	F Value	p-value Prob> F	R^2

Model	8866.41	9	985.16	85.10	< 0.0001	0.9909
A-Drug	7311.43	1	7311.43	631.59	< 0.0001	
B-Polymer	248.65	1	248.65	21.48	0.0024	
C-Surfactant	390.18	1	390.18	33.71	0.0007	
AB	9.12	1	9.12	0.79	0.4042	
AC	28.57	1	28.57	2.47	0.1602	
BC	11.83	1	11.83	1.02	0.3457	
A^2	865.80	1	865.80	74.79	< 0.0001	
B^2	1.33	1	1.33	0.12	0.7443	
C^2	5.72	1	5.72	0.49	0.5049	
Residual	81.03	7	11.58			
Lack of Fit	80.62	3	26.87	256.85	< 0.0001	

Table-5: ANOVA results of the quadratic model for the response % of yield (Y2)

Source variations	Sum of Squares	DF	Mean Square	F Value	p-value Prob> F	R^2
Model	1198.85	9	133.21	83.48	< 0.0001	0.9908
A-Drug	1046.53	1	1046.53	655.87	< 0.0001	
B-Polymer	25.56	1	25.56	16.02	0.0052	
C-Surfactant	1.44	1	1.44	0.91	0.3730	
AB	6.50	1	6.50	4.08	0.0833	
AC	0.81	1	0.81	0.51	0.4992	
BC	0.040	1	0.040	0.025	0.8787	
A^2	114.18	1	114.18	71.56	< 0.0001	
B^2	1.48	1	1.48	0.93	0.3679	
C^2	1.68	1	1.68	1.06	0.3384	
Residual	11.17	7	1.60			
Lack of Fit	10.22	3	3.41	14.31	0.0132	

Table-6: Optimized values obtained by the constraints applies on Y1 and Y2

Independent variables	**Values**	**Predicted values**		**Batch**	**Observed values**	
		P. Size (Y1)	**P. Yield (Y2)**		**P. Yield (Y2)**	**P. Size (Y1)**
Drug	150	259.712	77.64	GB4	77.3	259.18
Polymer	150			GB5	77.2	259.79
Surfactant	875			GB7	77.4	259.87

using the desirability approach was employedto locate the optimal settings of the process variables to obtain the desired responses. Optimizedconditions were obtained by setting constraints on the dependent and independent variables.

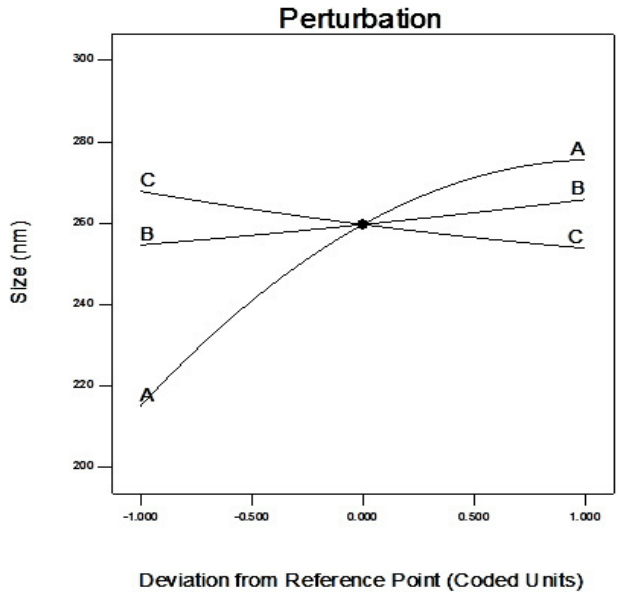

Figure-1: Perturbation plot showing the main effect of drug (A),polymer (B) and surfactant (C) on particle size (Y1)

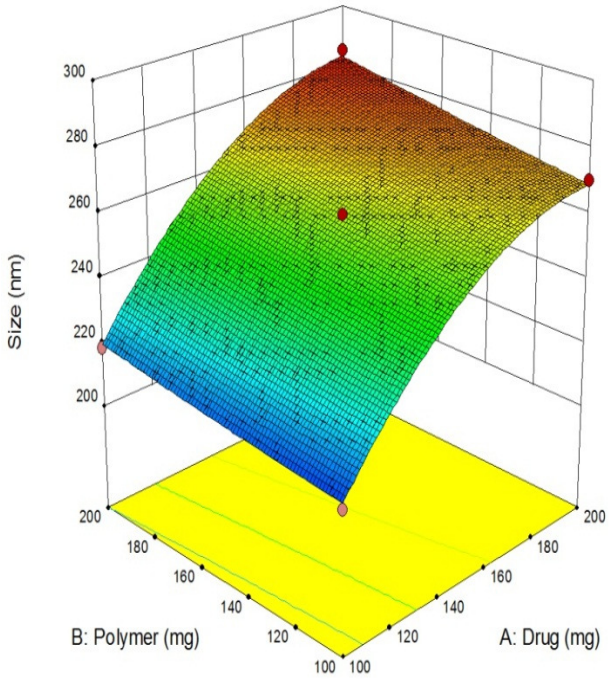

Figure-2: Response surface plot presenting the interactionbetween the drug and polymer affecting the particle size at constant surfactant concentration.

The mathematical model generated for % (Y2) was found to be significant with F-value of 83.48 ($p < 0.0001$) and R^2value of 0.9908. The independent variables A, B, C and the quadratic termof A^2have significant effects on the % yield, since the P-values less than 0.05represent the significant model terms as shown in Table 5. Results of the equation indicate that the effectof A is more significant than B and C. The influence of the main and interactive effects of independentvariables on the % yield was further elucidated using the perturbation and 3D response surface plots. The perturbation plot (Figure 3) showing the main effects of A, B and C on the percentage yield(Y2) of glibenclamide nanoparticles. This figure clearly shows that A has the main and the major effect on Y2 followedby B which has a moderate effect on Y2 followed by C which has a little effect on Y2. The relationshipbetween the dependent and independent variables was further elucidated using response surface plots.Figure 4 shows the interactive effect of A and B on the practical yield (Y2) at fixed level of C. At lowlevels of A (Drug), Y2 increases from 58.3% to 66.2%. Similarly, at high levels of A, Y2 increases from 82.4% to 85.2%.

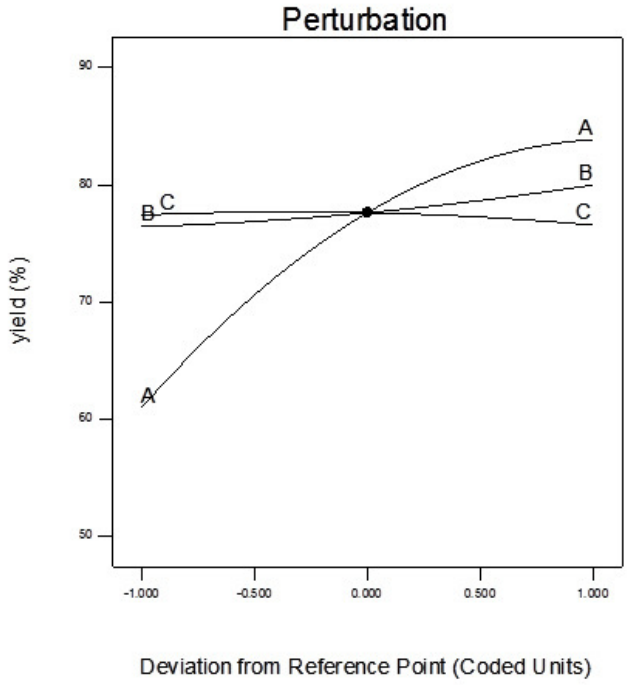

Figure-3: Perturbation plot showing the main effect of drug (A), polymer (B) and surfactant (C) on percentage of yield (Y2)

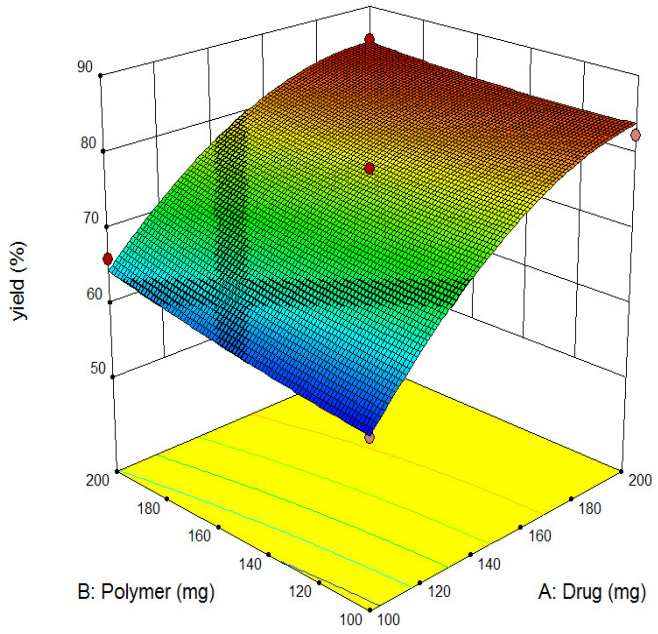

Figure-4: Response surface plot presenting the interaction betweenthe polymer and drug affecting the percentage of yield at constant surfactant concentration.

GB4, GB5 and GB7 batches code ofglibenclamide nanoparticles were prepared according to these optimized levels. Observed responses were in close agreement with the predicted values of the optimized process, thereby demonstrating the feasibility Table 6.

The percentage of drug release from nanoparticles over the period of 15 h for formulation GB4, GB5 and GB7 was found to be 78.2%, 83.3% and 86.6% respectively as shown in Figure 5 and 6.

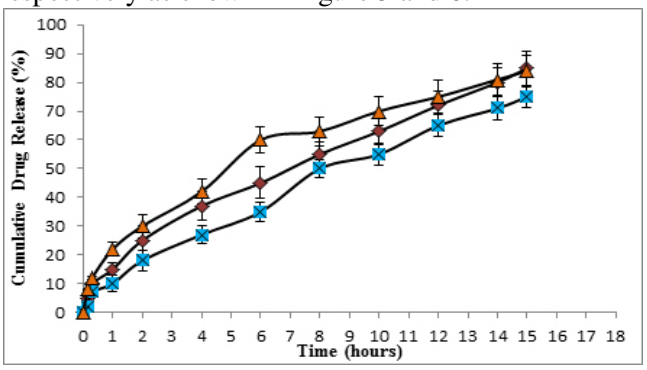

Figure-5:Showing the drug release of glibenclamide nanoparticle

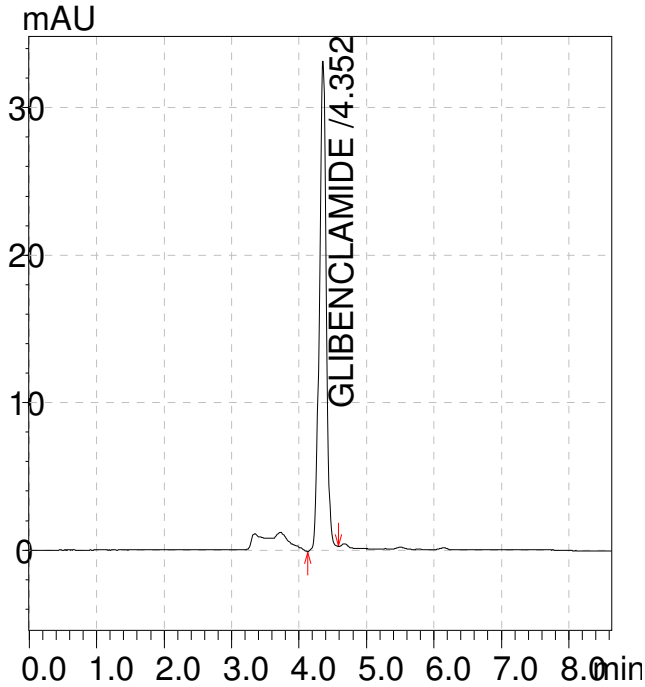

Figure-6: Typical chromatogram of Glibenclamide

The FTIR spectral analysis of glibenclamide, pure drug showed that, the principal peaks were observed at wavenumbers of 3368.02, 3315.70, 3118.64, 2988.42, 2931.18,2853.42, 2493.53, 2247.97, 2015.96, 1914.70, 1710.69,1453.78, 1342.83, 1247.29, 1154.29, 1122.56, 1093.29, 1033.43, 905.66, 883.08, 839.86, 819.97, 756.65, 685.69, 609.59 and 572.97 (unit in cm^{-1}).The spectra of pure ethyl cellulose showed the peaks at wavenumber of 3924.67, 3789.78, 3476.48, 2974.44, 2610.60, 2363.01, 2151.85, 1983.50, 1738.19, 1640.19, 1108.59, and 663.26 (unit in cm^{-1}).

The spectra of physical mixture of glibenclamide with ethyl cellulose showed the peaks at wavenumbers of 3480.90,3368.42, 3315.98, 3118.77, 2975.00, 2931.05, 2855.44,2613.17, 2493.40, 2249.54, 1915.05, 1800.51, 1715.17,1618.07, 1523.95, 1342.62, 1277.32, 1246.30, 1158.59,906.14, 883.02,840.45,820.58,757.36, 685.86, 610.16, and 573.82 (unit in cm^{-1}).The FTIR studies of physical mixture of drug and polymer does not show any significant changes. Thus, these results indicate that there is no interaction between drug and selected polymer (Figure7).

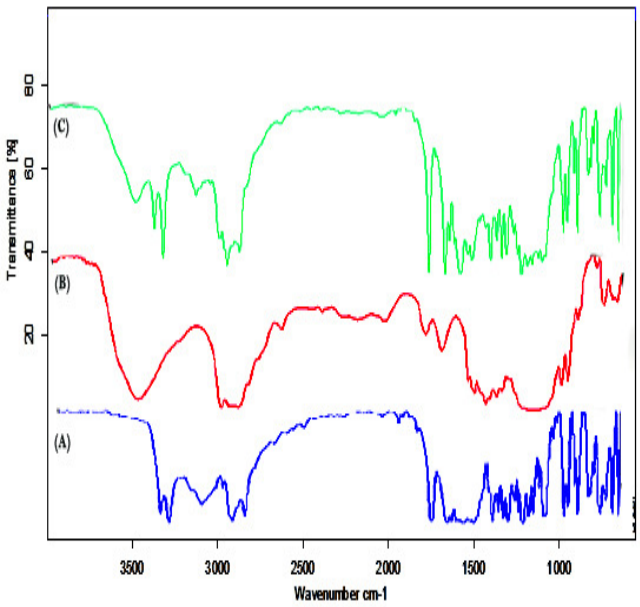

Figure-7: FTIR Spectra of (A) glibenclamide (B) EC (C) glibenclamide + EC

The DSC spectral analysis of glibenclamide, pure drug showed the endothermic peak at 167.80 °C. The DSC spectral analysis of physical mixture of glibenclamide with ethyl cellulose showed the endothermic peak at 171.09 °C. Therefore, there is no significance change in the endothermic peak for physical mixture of glibenclamide with ethyl cellulose when compared with the pure drug and polymer. This indicates the pure drug and ethyl cellulose are compatible (Figure8).

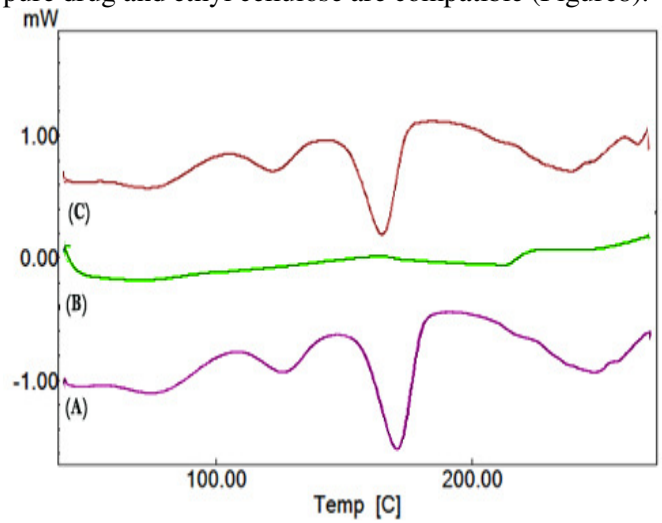

Figure-8: DSC Spectra of (A) glibenclamide (B) EC (C) glibenclamide + EC

This particle size was best suit for oral delivery of glibenclamide nanoparticles. The morphology of prepared nanoparticles was uniform and spherical shape, which can facilitate the penetration of drug via orally (Figure9).

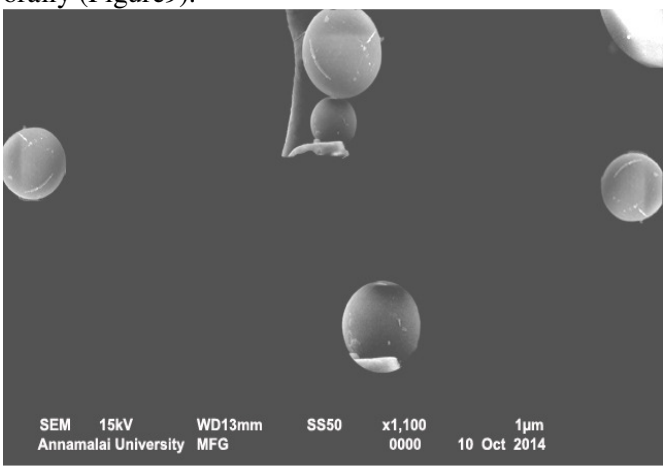

Figure -9: SEM photography of glibenclamide nanoparticles

CONCLUSION

The present research work proposed a novel nanoparticulate formulation technique using soapnutextract. They possessed good % of yield and uniform particle size distribution. The release profile was a prolonged, therefore prepared formulation follows sustained release pattern which could be effective in the management of non-insulin dependent diabetes mellitus. The developed nanoparticulate system could reduce dose frequency, decrease side effects, and improve patient compliance.

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