FORMULATION AND EVALUATION OF REPAGLINIDE NANOPARTICULATE GEL

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Abstract:
The aim of the present work is to formulate and evaluate Repaglinide nanoparticulated gel for topical delivery to reduce blood glucose levels by stimulating endogenous insulin production as a result of decreasing the side effects of Repaglinide by administering the drug topically and to increase the bioavailability of Repaglinide by converting in to nanoparticles. Minimizing the drawback of long term use of Repaglinide by converting in to nanoparticles by solvent/Antisolvent precipitation method and converting in to gel. The prepared nanoparticles will be evaluated for various characterization parameter such asParticle Size Analysis,Zeta potential, FTIR study, Differential Scanning Calorimetry (DSC), Surface Morphology (SEM)Further they are converted in to topical gel by using carbopol 934, HPMC. Evaluation parameters of gel Measurement of PH, Drug content, viscosity study, spreadability, extrudability, homogeneity, grittiness, In-vitro diffusion studies, stability. The formulated topical gel containing Repaglinide nanoparticals can be successfully used to increase the bioavailability of drug and to overcome the side effects.

Keywords: Nanoparticles, Gels, Repaglinide Carbopol 934, Hpmc.

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Preparation of Repaglinide Nanoparticles

Repaglinide nanoparticles made by using solvent/antisolvent precipitation technique as an successful technique in preparation of nanodrugs. A definite amount of raw drug of repaglinide was totally dissolved in water miscible solvent. Is opropyl alcohol is taken as solvent. According to USP, Categorizing isopropyl alcohol in Class 3. Class 3 consist of no solvent cause human hazard at levels usually accepted in pharmaceuticals. No long-term toxicity or carcinogenicity studies reported for many of the residual solvents in Class 3. The resultant drug solution is then injected into the water consist of surfactant of SLS and/or TEA as stabilizer under stirring at 10000 rpm. Precipitation of solid drug particles occurred immediately upon mixing. The suspension was centrifuged at 5,300 rpm for 10 min and washed twice with purified water[1]. In technique, introduction of the drug solution to the anti solvent generates high super saturation. This results in fast nucleation rate and produces a large number of nuclei, which reduces the solute mass for subsequent growth. Nanoparticles can thus be obtained provided that the growth of nucleating crystals can be arrested by the stabilizer (surfactant or polymer) through steric or electrostatic mechanism. The effect of SLS and TEA is studied on precipitated repaglinide particle size. The supersaturated drug was injected to separate solutions of SLS and TEA in water with concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 %w/v. The best results were obtained on 0.5 ratio of SLS and TEA, due to both static and electrostatic activity of them. Therefore, SLS/TEA surfactant pair is a crucial way to obtain repaglinide nanoparticles in 43 - 300 nm[2].

Preparation of nanoparticulate gel

Topical gel of repaglinide nanoparticles where prepared by using Carbopol 934,Sodium alginate and HPMC as gelling agent, propylene glycol and water as dispersed phase. Gels where formed by using three different concentration of various gelling agents carbopol934 , Sodium alginate and HPMC by keeping the concentration of repaglinide and other ingredients constant[3].The solvent system is made by dissolving water and propylene glycol 80:20 ratio. The required quantity of Carbopol-934, Sodium alginate and HPMC are taken and mixed into 100ml of solvent system and stirred it for 3 hours to get the uniform solution. The quantity of nanoparticles equivalent to 100mg of repaglinide was weighed and dissolved in to the above Carbopol 934, Sodium alginate and HPMC solution and stirred for 12 hours for uniform distribution[4].

Table 1: Formulation of nanogel

Ingredients	Formulation code									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Repaglinide nanoparticles	100mg	100mg	100mg	100mg	100mg	100mg	100mg	100mg	100mg	
Carbopol-934	0.5g	1g	1.5g	-	-	-	-	-	-	
Sodium alginate	-	-	-	0.5g	1g	1.5g	-	-	-	-
HPMC	-	-	-	-	-	-	0.5g	1g	1.5g	
Carbopol-934,Sodium alginate & HPMC	-	-	-	-	-	-	-	-	-	1.5 g
Propylene glycol	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml
Water	90ml	90ml	90ml	90ml	90ml	90ml	90ml	90ml	90ml	90ml

Preformulation study:

Preformulation study's are one of the important process of optimizing the formulation of novel dosage form. Science preformulation affect drug performance and efficacious, safe and stable dosage form.Various preformulation studies which were carried out are discussed in the following sections

Identification Tests

Determination of Melting Point

Melting point determination of the drug sample has been done by open capillary method. Thedrug which has been taken in the glass capillary in which one end was sealed by flame. Inthis method capillary tube was sealed with gentle heating from one end. Then the small quantity of repaglinide was filled in to the small capillary. Capillary was tied in such a way that the tube containing repaglinide was dipped in to the oil phase in such a way that the sealed part of the capillary containing repaglinide was dipped into the oil. Gently the oil bath was heated. As soon as the powder starts melting, the heating was stopped and the temperature was noted down[5]

Compatibility Studies by IR-Spectroscopy

Compatibility is one of the most important factor which affect the stability of the product. FT-IR spectroscopy used to ensure compatibility between polymer and drug . The FT-IR spectra of the drug with polymers were compared with the standard FT-IR spectrum of the pure drug. For determining the compatibility of drug with polymers, IR spectra of pure repaglinide, pure polymer like carbopol-934,HPMC,physical mixture of drug and polymer was taken[6]

Differential Scanning Calorimetry (DSC)
Differential scanning calorimetry (DSC) was performed on DSC 131 SETARAM. The samples equilibrated at 20ºC for 30 min. Indium standard was utilized to adjust the DSC temperature and enthalpy scale. The powder samples were hermetically sealed in aluminum pans and heated at a continuous rate of 3ºC/min, over a temperature series of 20– 170 ºC. Inert atmosphere was conserved by purging nitrogen at the flow rate of 15.8 ml/min, linear velocity 35 cm/sec and pressure 24.7 kPa.

Evaluation parameters of gel
a) Measurement of P^H
P^H is measured by digital P^H meterEg- 1g of gel mixed in 100 ml distilled water and kept for 2 hrs. Measurement of P^H in triplicate and calculate average value.

b) Drug content
1g of gel dissolved in 100 ml of appropriate solvent stoke solution has been filled. The prepared aliqouers of various concentration by using suitable dilution and absorbance is measured. To calculated drug content linear regression analysis of calibration curve can be used[7].

c) Viscosity study
Brook field viscometer is utilized for its examination turn the gels at 0.3, 0.6 and 1.5 Rpm. Relating dial perusing are been noted at each speed. Consistency was gotten by dial perusing X factor given the brook field viscometer inventories

d) Spreadability
It demonstrate the degree of territory to which gel promptly spreads on application to the skin or influenced part. The therapeutic potency is depended on spreading value.The time in sec taken by two slides to sneak off from gel which is set in the middle of the slides towards the heading of certain lead is spreadability less time taken better spreadability. It tends to be determined by utilizing the equation[8]
Spreadability [s] = M x L/T
Where
M= weight tied to upper slide
L= length of glass slides
T= time taken to separate the slides.

e) Extrud ability studies
Formulations are packed in collapsible tubes before set inside the container. This is determined in terms of weight in gm required to extrude a 0.5 cm ribbon at gel in 10 second.

f)Homogeneity
Visual examination is done to find homogeneity fill gel in a container and tested for appearance and existence of any aggregated[9]

g) Grittiness
Light microscope is used formulations were evaluated microscopically to find if there is presence of any visible particulate matter

h) In-Vitro diffusion studies
It is carried out by Franz diffusion cell, for studying dissolution release of gel through a cellophane membrane. 0.5 of gel taken in cellophane membrane. Diffusion studies carried out at 37±1ºc using 250ml P^H buffer(P^H 7.4) as the dissolution medium[10,11]

Stability Studies:
Stability of a drug has been defined as the stability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications throughout its shelf life
As per ICH guidelines Stability studies were carried out at 30 ºC / 60% RH, 40 ºC / 75% RH for the selected formulations for 60 days and evaluated for their drug content and in vitro dissolution study[12].

Compatability studies:
The FTIR spectroscopic studies were carried out for standard repaglinide, carbopol 934, HPMC and mixture of repaglinide- carbopol 934, repaglinide-HPMC and repaglinide- carbopol 934-HPMC by KBr pellet technique using FTIR spectrophotometer. The FTIR spectrum of standard is compaired with that of mixture and found that there is no interference.

Results and discussion

Table 2:Intensity bands of repaglinide & formulation

Formulation code	C-H stretching	COOH stretching	CH_3 Bending	C-O stretching
Pure drug	2955.40	1720.73	1416.96	1268.42
Formulation	2956.08	1718.74	1420.21	1266.99

Major peaks of FT-IR Spectra

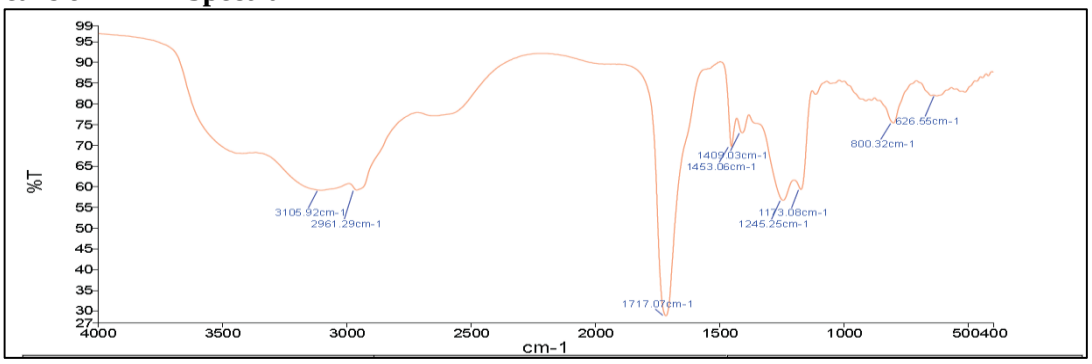


Figure 1: FTIR spectrum for carbopol 934

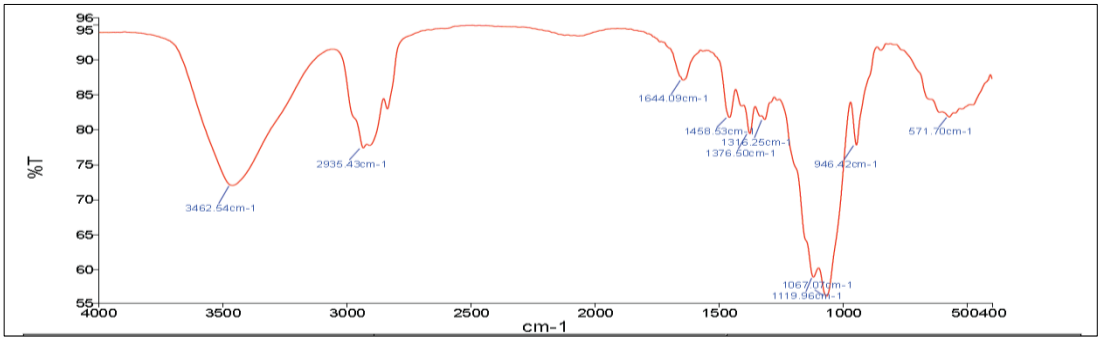


Figure2:FTIR spectrum for HPMC

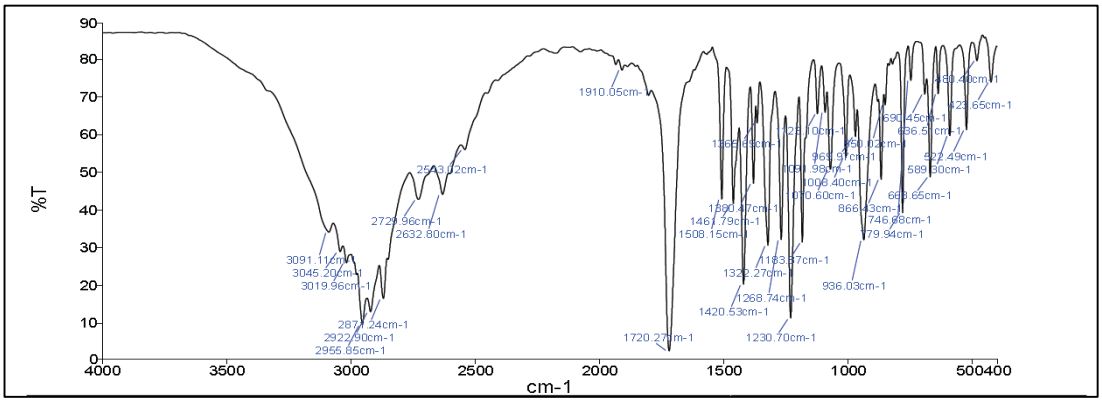


Figure3:FTIR spectrum of repaglinide

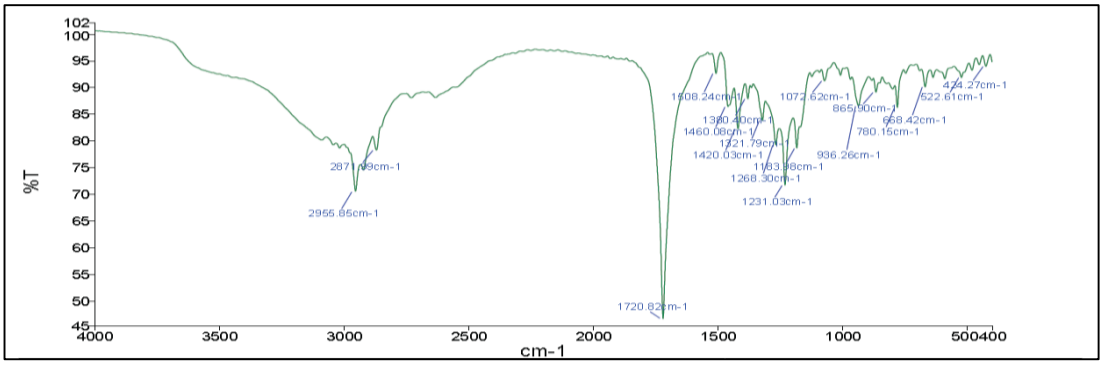


Figure4:FTIR spectrum of carbopol 934+Drug

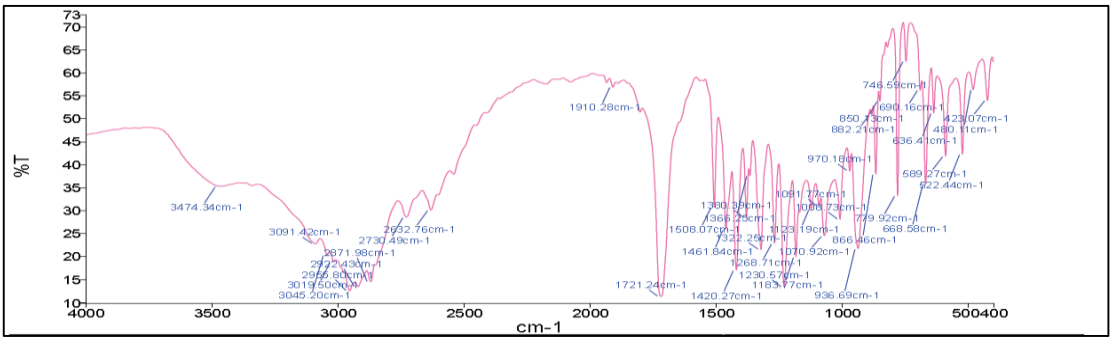


Figure5:FTIR spectrum of HPMC+Drug

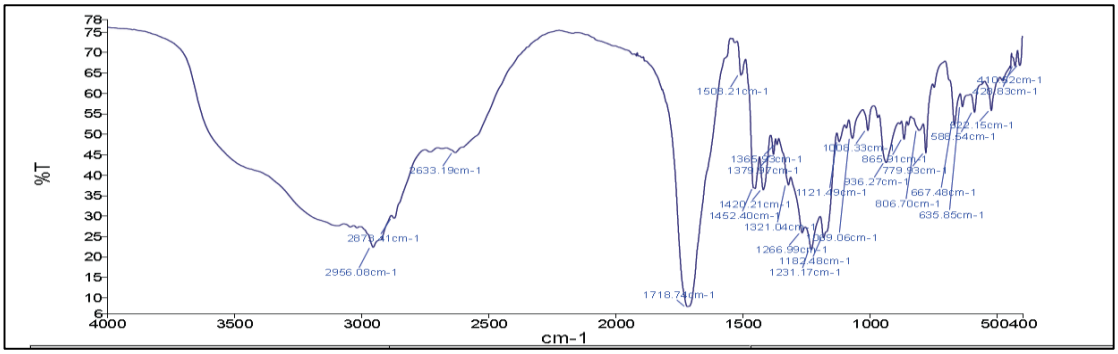


Figure6:FTIR spectrum of carbopol 934+HPMC+Drug

Differentialscanning calorimetry:

Thermal behaviour of repaglinide & formulation was studied using DSC to observe the effect of polymer on repaglinide

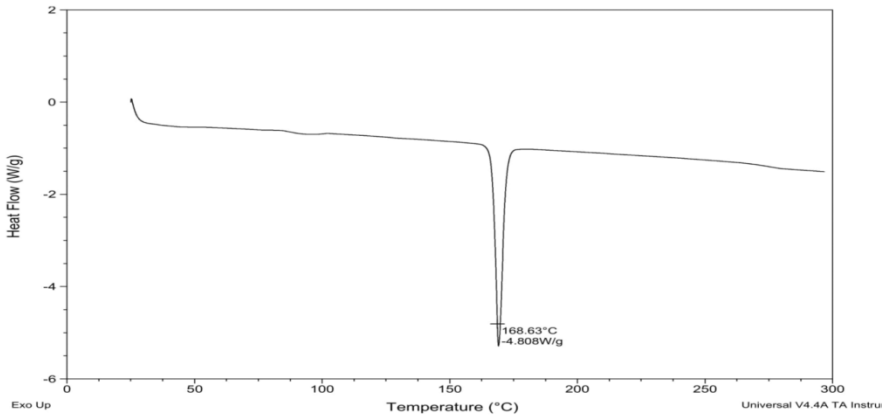


Fig 7:DSC of repaglinide

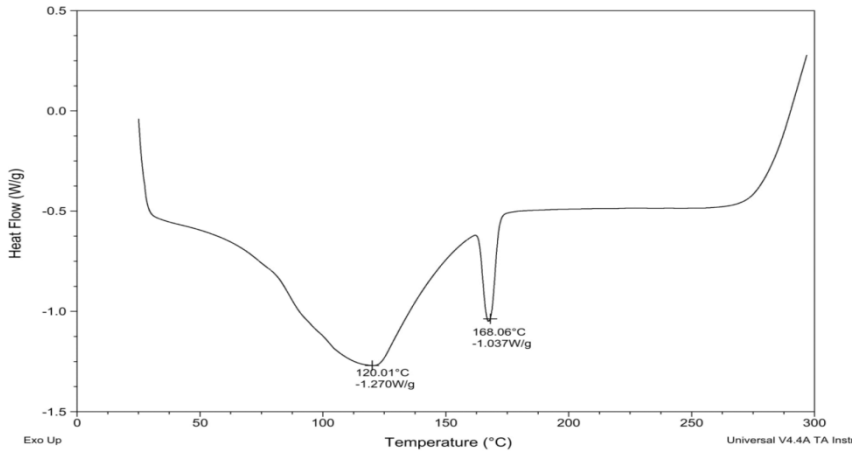


Fig 8:DSC of repaglinide + Physical mixture

Characterization of Nanoparticles:

Measurement of particle size of Nanoparticles

The particle sizes of nanoparticles were measured using microphotograph of 100 particle size range from 160 to 405 nm for various batches.

Table 3 : Particle size of Nanoformulation

S.NO	FORMULATION	PARTICLE SIZE
1.	F1	405
2.	F2	320
3.	F3	311
4.	F4	215
5.	F5	187
6	F6	185
7	F7	160
8	F8	330
9	F9	275
10	F10	350

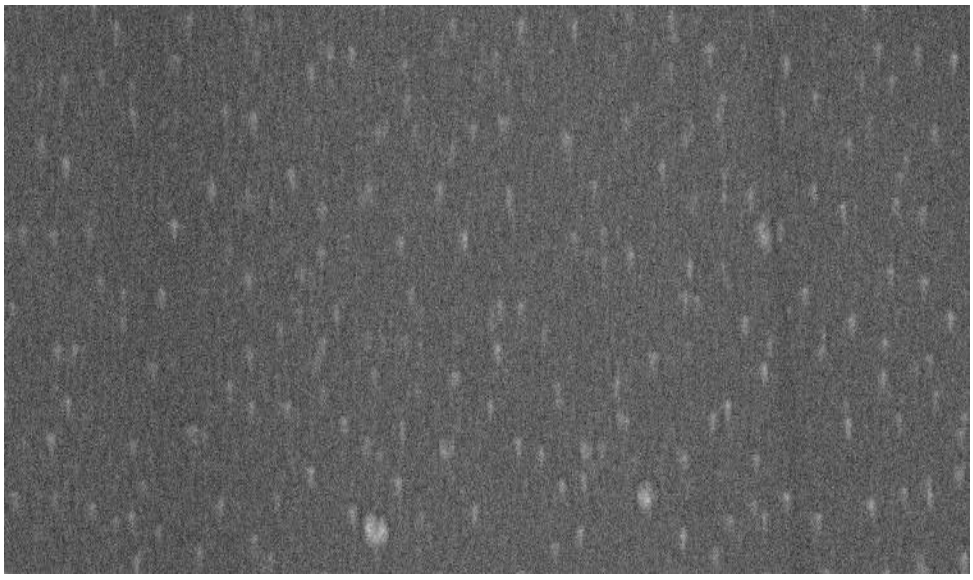
Zeta potential:

Table 4: Zeta potential of Nanoformulation

S.NO	FORMULATION	ZETA POTENTIAL(mv)
1.	F1	17.0
2.	F2	17.3
3.	F3	18.0
4.	F4	18.8
5.	F5	20.0
6	F6	12.5
7	F7	11.9
8	F8	15.7
9	F9	16.4
10	F10	18.2

Scanning electron microscope (SEM):

SEM analysis of the prepared formulation was carried out to understand the morphology of Nanoparticles. In the SEM images indicates that the nanoparticles were discrete, uniform and spherical with a smooth surface. Hence the images show that proper expected shape has been achieved.



Preparation and Evaluation of topical gel containing repaglinide nanoparticles

Based on the result of the particle size and SEM images the best formulation F5 was selected and further converted in to topical gel. Topical gels where prepared by using nanoparticles containing equivalent to 100mg repaglinide, carbopol 934, HPMC, propylene glycol and water

Measurement of pH

The pH values of formulated gel ranges from 6.4-7.1.Which is suitable for applying to skin and minimise irritation.

Drug Content

The drug content estimated for formulated gel. The drug content showed that the drug was distributed uniformly throughout the gel

Percentage Yield and Viscosity

Percentage yield of topical gel containing repaglinide was in range of 95.43-98.87%. It was found that the percentage yield of F10formulation has larger % yield than other formulations. Generally consistency of formulation relies upon on the ratio of solid fraction to liquid fraction which produces gel structure.

Spreadability

It is measured as a important factor that shows gelcharacter which comes out from tube. For all the formulations spreadability test is carried of. With respect to increase in the polymer concentration spreadability of the gel formulation decreases

Homogenecity

Visual examination is done to ensure the syneresis and colour of the prepared gel. The gel must be clear and transparent. The formulated gel are found to be good homogeneity without any lumps and syneresis.

Table 5:pH, Spreadability and Drug content of Formulations (F1-F5)

Formulation code	pH	Spredability (gm.cm^2)	Drug content %
F1	6.5	11.00	95.32± 0.012
F2	6.6	11.08	96.32± 0.012
F3	6.8	11.75	97.43± 0.024
F4	6.9	10.25	96.01 ± 0.018
F5	7.1	10.75	98.67 ± 0.021
F6	6.5	11.07	94.13±0.212
F7	6.6	10.95	95.75±0.154
F8	6.4	11.35	97.89±0.013
F9	6.7	11.56	99.54±0.021
F10	7.0	10.85	97.45±0.012

Table 6: Percentage Yield and Viscosity of different formulations (F1-F10)

Formulation code	Viscosity(centipoises)	Percentage yields %
F1	4502	96.80
F2	4321	95.90
F3	3321	97.87
F4	3741	97.01
F5	2431	98.10
F6	3642	97.20
F7	4523	96.54
F8	4321	95.43
F9	4123	96.56
F10	3632	98.43

In vitro diffusion

Diffusion cell are used to find drug release profile of repaglinide nanoparticulated topical gel formulations**.** the graphical representation is shown in Fig .7 &8.The percentage drug release of all formulations after 4.5 hours using carbopol 934,HPMC,and sodium alginate combination was found to be 83.80 %(F1), 78.96% (F2), 85.70%(F3) and 75.20% (F4), 89.98% (F5) ,F6 (91.03%), F7(89.51%),F8(85.92%)F9(85.21) & F10(87.21) respectively. The most important factors in the drug release is the type of polymer and the concentration of polymer

Table 7: Comparative Dissolution Study of Different Formulations

S.NO	TIME	%DRUG RELEASE				
		F1	F2	F3	F4	F5
1.	**30**	11.45	9.54	10.15	8.90	10.96
2.	**60**	22.79	17.07	21.50	14.06	25.56
3.	**90**	35.41	23.91	35.01	24.55	38.12
4.	**120**	47.33	35.44	49.02	38.23	52.23
5.	**150**	64.21	49.71	61.25	49.10	64.45
6.	**180**	70.39	59.23	68.09	55.18	75.07
7.	**210**	75.06	69.11	73.99	64.10	80.02
8.	**240**	80.05	73.86	80.52	72.24	84.14
9.	**270**	83.80	78.96	85.70	75.20	89.98

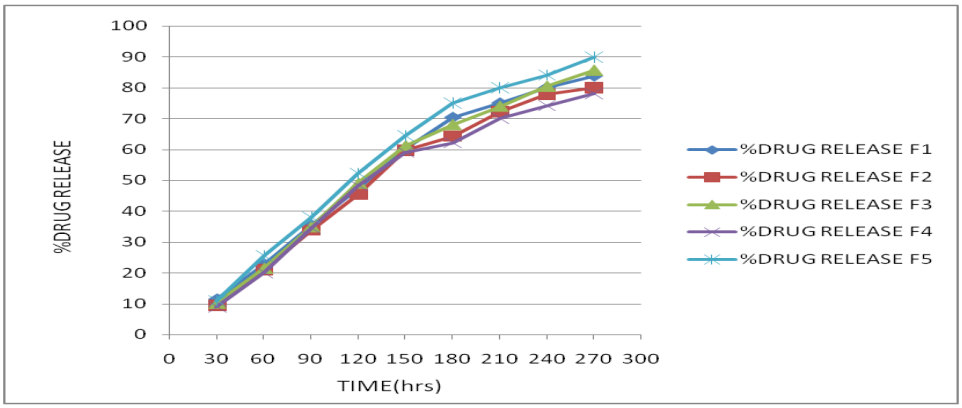


Figure 9: %Comparative Dissolution Study of Different Formulations (F1-F5)

Table 8: Comparative in vitro drug release of different formulations

S. No.	Time(mts)	% Drug Release				
		F6	F7	F8	F9	F10
1	30	4.34	3.24	3.12	3.56	4.67
2	60	8.64	8.15	7.32	6.64	9.54
3	90	17.25	15.38	16.38	14.32	13.78
4	120	27.13	26.43	25.05	24.11	25.76
5	150	39.06	38.06	37.62	34.84	35.21
6	180	56.52	54.43	57.06	51.92	55.67
7	210	67.25	64.79	63.96	65.29	66.32
8	240	82.85	78.29	75.09	73.55	76.14
9	270	91.03	89.51	85.92	83.21	87.25

Stability studies:

Stability studies of the prepared formulations were carried out by storing the optimized formulation F9 at 30 ± 2ºC& 60 ± 5% RH and 40 ± 2ºC& 75 ± 5% RH for 60 days. Two parameters namely percentage Drug content and *in-vitro* release studies were carried out. It was observed that there was no change in drug content and invitro drug release profile even after storage at 30 ± 2ºC& 60 ± 5% RH and 40 ± 2ºC& 75 ± 5% RH for 60 days.

Table 9: Invitro drug release studies After Storing At Different Temperatures (F9)

S.No	Time(hrs)	0 Days	Percentage(%) drug release			
			30 days		60 days	
			$30^0\pm2^0$ C	$40^0\pm2^0$ C	$30^0\pm2^0$ C	$40^0\pm2^0$ C
1	30	4.34	3.24	3.12	3.56	4.67
2	60	6.64	6.54	6.51	6.54	5.96
3	90	14.32	14.2	14.15	13.8	13.7
4	120	24.11	24.05	23.9	23.75	23.58
5	150	34.84	34.73	34.65	34.22	33.8
6	180	51.92	51.85	51.72	51.58	51.45
7	210	65.29	65.21	65.05	64.96	64.8
8	240	73.55	73.5	73.38	73.3	73.24
9	270	83.21	83.15	83.08	82.95	82.9

Table 10: Drug Content After Storing At Different Temperatures (F9)

S.No	Drug content	Drug content			
		$30^0 \pm 2^0$ C		$40^0 \pm 2^0$ C	
		30 days	60 days	30 days	60 days
1	99.544± 0.2	99.38±0.12	99.35±0.08	99.26±0.01	99.20±0.06

CONCLUSION:

The main objective of the present study was to develop Repaglinide nanoparticulated gel for topical delivery to reduce blood glucose levels by stimulating endogenous insulin production as a result of to increase the bioavailability of Repaglinide by converting in to Nanoparticles. Formulations were prepared and evaluated for physical parameters, drug content, Spread ability Viscosity and diffusion studies. All the parameters are within the limits. Based on the observation it can be concluded that the formulated gel containing Repaglinide can be successfully used to increase the bioavailability of drug and to overcome the side effects of drug**.**

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