ISSN: 2347-1956

Annals of
Pharma Research

Volume 11 Issue 10 (2023) pp.801-810

Open Access

Research Article

Evaluation of Formulated Insulin Bioadhesive Gel by Using Different Polymers

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Abstract

Aim & objectives: Evaluations of any prepared dosage form is important step in confirm the prepare formulation is correct or not. Bioadhesive nasal gel are evaluated like appearance, pH, viscosity, in-vitro drug release study, gelling temperature, mucoadhesive strength. **Methods:** The wavelength of insulin is detected in 400-700nm by Lowry method and standard calibration curve of insulin is detected by Lowry method and plotted the graph. The formulations are prepared by using different polymers. The 7 formulations are prepared. The prepared gels are evaluated like appearance by visual analysis of its smoothness. pH is evaluated by using digital pH meter. Viscosity is estimated by Brookfield viscometer. Drug content and in-vitro drug release is estimated by using photometric colorimetric method. **Result and Discussion:** The insulin wavelength in colorimetric range is found out and it is 510nm. By using this wavelength the standard calibration curve graph is plotted and slope and intercept is detected by graph. The slope value is 1.450 and intercept is value is 0.047 detected by graph. pH value of all formulation is ranges from 5.5 to 6.5, Gelation Temperature lies between 65.7to 75.2^{0}C, Viscosity estimated and ranges from 8.5-17.8%, Measurement of Gel strength value is 0.28 to 2.39 sec, percentage of drug in formulations 95-98%, Drug absorption is estimated by In-vitro Analysis by membrane less method and at 7hrs value ranges 90.5-94% . **Conclusion**: The formulations are prepared are carried out the different evaluation tests and results are satisfied.

Key words: Evaluation, Calibration Curve, In-vitro drug release, wavelength.

INTRODUCTION

In the past few years, an increasing number of in situ gel forming systems have been investigated and many patents for their use in various biomedical applications including drug delivery have been reported. The in situ gel forming system has the advantages like

Ease of administration.
Reduction of taste impact (in nasal delivery)
Reduction of irritation (in nasal delivery)
Improved patient compliance.
Accuracy of dosing as leakage of drug is prevented.
Prolonged residence time.
Improved bioavailability.
Sustained and controlled drug delivery. [Prajapati, N. B. *et al*., 2013].

The pH of the formulation and nasal surface, can affect a drug's permeation. To avoid nasal irritation, the pH of the nasal formulation should be adjusted to 5.5–6.5 because lysozyme is found in nasal secretions, which is responsible for destroying certain bacteria at acidic pH. Under alkaline conditions, lysozyme is inactivated and the tissue is susceptible to microbial infection. In addition to avoiding irritation, it results in obtaining efficient drug permeation and prevents the growth of bacteria. Concentration gradient plays very important role in the absorption / permeation process of drug through the nasal membrane due to nasal mucosal damage. The drug distribution in the nasal cavity is one of the important factors, which affect the efficiency of nasal absorption. The mode of drug administration could effect the distribution of drug in nasal cavity, which in turn will determine the absorption efficiency of a drug. The absorption and bioavailability of the nasal dosage forms mainly depends on the site of disposition. A higher viscosity of the formulation increases contact time between the drug and the nasal mucosa thereby increasing the time for permeation. At the same time, highly viscous formulations interfere with the normal functions like ciliary beating or mu-

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DOI- https://doi.org/10.21746/apr.2023.11.10.1

cociliary clearance and thus alter the permeability of drugs [Alagusundara, M. *et al*., 2010].

MATERIALS AND METHOD

Determination of λ max

In quartz cuvetts take a blank solution and correct the base line in the range of 450-700nm. Take the sample solution in the cuvett and scan in the range of 450-700nm [https://cdn.juniata.edu].

Preparation of standard calibration curve

Insulin (30mg/100mL) solution was added in 11 test tubes as 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml, respectively in each tube. Distilled water upto 1.0 ml volume 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0 ml was added in each test tube. Then reagent I was added (0.5 ml) and the solutions were mixed well and incubated for 15 min at room temperature. The reagent II solution was added (5 ml) to each tube and incubated for 30 min. The spectrophotometric absorbance of each standard sample was taken at 510nm to establish the standard curve after plotting protein concentration against absorbance [Azhar, *et al*., 2013].

Determination of melting point

Take a fine capillary of length 5-6cm. seal its one end by inserting the end of the capillary tube horizontally to small steady Bunsen flame for a few seconds, rotating the capillary meanwhile. Take a small quantity of the drug in a porous plate and powder it with a spatula and then to form a column at the bottom of the tube (2.5-3.5 mm height), when packed down closely as possible by moderate tapping on solid surface. The capillary tube was placed in a melting point apparatus and the range of temperature when the drug just starts melting and till it completely melted was noted [https://davjalandhar.com].

Appearance:

All formulations were inspected visually for clarity in sol and gel form under black and white background [Verma, *et al*., 2016].

Determination of pH

Weighed 50 gm of each gel in a beaker and measured it by using the digital pH meter. pH of the *In Situ* nasal gel formulations should be between 5.5 to 6.5 suitable for nasal delivery. Before determination of pH the pH meter is calibrated by using standard buffer solution 4.1 and 6.8. The 3 readings is note down [Mishra, S. S. *et al*., 2018].

Viscosity Studies:

The rheological studies were carried out using Brookfield viscometer. The gel formulation s under study was placed in sample holder
and the 64no spindle selected was lowered perpendicular into the sample. The spindle was rotated at 100 rpm to determinations of formulation viscosity [Verma, *et al*., 2016].

Determination of gelation temperature:

The gelation may be defined as that the temperature at which the liquid phase makes a transition to gel. The liquid formulation is kept in a test tube, immersed in a water bath. The samples shall be examined for gelation, which is said to have occurred when the thermometer meniscus would no longer move upon tilting through 90°C. The gel melting temperature is a critical temperature when the gel starts flowing upon tilting through 90°C shall be recorded. Gel Formation is indicated by a lack of movement of meniscus on tilting the tube [Kashid, *et al*, 2016].

Determination of Gel strength:

Take a 5ml of sample in a 10ml of measuring cylinder and iron ball of 6mm diameter and 1.045g weight is drop in on surface of gel. The 5cm distance travelled by ball for specific period of time was measured [Godbole, M. D. *et al*., 2014].

Drug content:

1ml of test solution is taken in test tube. Then reagent I was added (0.5 ml) and the solutions were mixed well and incubated for 15 min at room temperature. The reagent II solution was added (5 ml) to each tube and incubated for 30 min. The spectrophotometric absorbance of each standard sample was taken at 510nm. The drug content is estimated by using standard calibration curve [youtube.com].

In Vitro Drug Release:

A membrane less dissolution method was used for in vitro. The Insulin gels were transferred into the test tubes and placed in a 37 degree C water bath. Then 2ml of the Phosphate buffer Solution of pH 7.4, pre- equilibrated at the ex-

perimental temperature was layered over the surface of the gel. At predetermined sampling times, the supernatant was completely removed and replaced with fresh solution 1to maintain sink conditions. After centrifugation the samples were analyzed for insulin concentration by Lowry method at 510 nm against blank gels tested with the same method [Galgatte, U. C. *et al*., 2014].

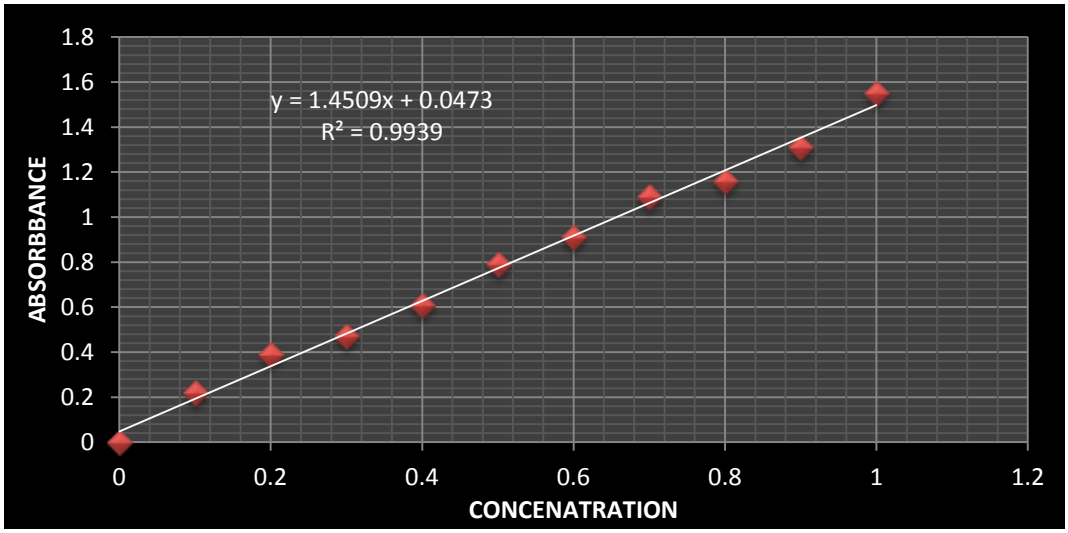
RESULTS AND DISCUSSION

Evaluation Parameters of Nasal Mucoadhesive Gel

Preparation of standard calibration curve

Absorbance values of Insulin.

Sr. No	Concentration	Absorbance
1	0	0
2	0.1	0.22
3	0.2	0.39
4	0.3	0.47
5	0.4	0.61
6	0.5	0.79
7	0.6	0.91
8	0.7	1.09
9	0.8	1.16
10	0.9	1.31
11	1	1.55



Statistical data for calibration curve.

Serial no.	Parameters	Value
1	max(nm)	510
2	Slope	1.450
3	Constant	0.047
4	R2	0.993

Solubility:

Soluble in dilute acetic acid or hydrochloric acid at pH 2-3, but insoluble in water at physiological pH. But dissolves relatively rapidly in plasma.

Melting Point:

The melting Point of Insulin was found to be $233^{O}C$.

Ph Value of the Formulations

Sr.No	Formulation	Reading 1	Reading 2	Reading 3	Average
1	F1	5.7	5.6	5.8	5.7
2	F2	5.3	5.7	5.5	5.5
3	F3	5.8	5.7	6.0	5.8
4	F4	5.9	6.1	6.2	6.0
5	F5	6.3	6.6	6.6	6.5
6	F6	5.1	5.4	5.9	5.5
7	F7	5.7	5.8	5.5	5.7

Viscosity of the Formulations

Sr.No	Formulation	Reading 1	Reading 2	Reading 3	Average
1	F1	10%(600)	10.2%(612)	10%(600)	10%(604)
2	F2	8%(504)	9.2%(552)	8.5%(520)	8.5%(525)
3	F3	16%(1002)	15.3%(918)	15.7%(918)	15.6%(946)
4	F4	16.8%(1130)	19.1%(1146)	17.5%(1142)	17.8%(1139)
5	F5	15.3%(918)	11.7%(702)	12.4%(800)	13.1%(806)
6	F6	11.2%(672)	12%(720)	11.2%(672)	11.4%(688)
7	F7	14.4%(864)	12.7%(850)	14.4%(864)	13.8%(859)

Gelation Temperature of the Formulations (^{0}c)

Sr.No	Formulation	Reading 1	Reading 2	Reading 3	Average
1	F1	71.4	71.2	71.7	71.4
2	F2	75.2	74.9	75.5	75.2
3	F3	70.8	71.3	71.1	71.0
4	F4	71.2	71.5	71.0	71.2
5	F5	66.5	66.8	66.2	66.5
6	F6	70.9	70.4	71.5	70.9
7	F7	65.8	65.9	65.4	65.7

Drug Content of the Formulations

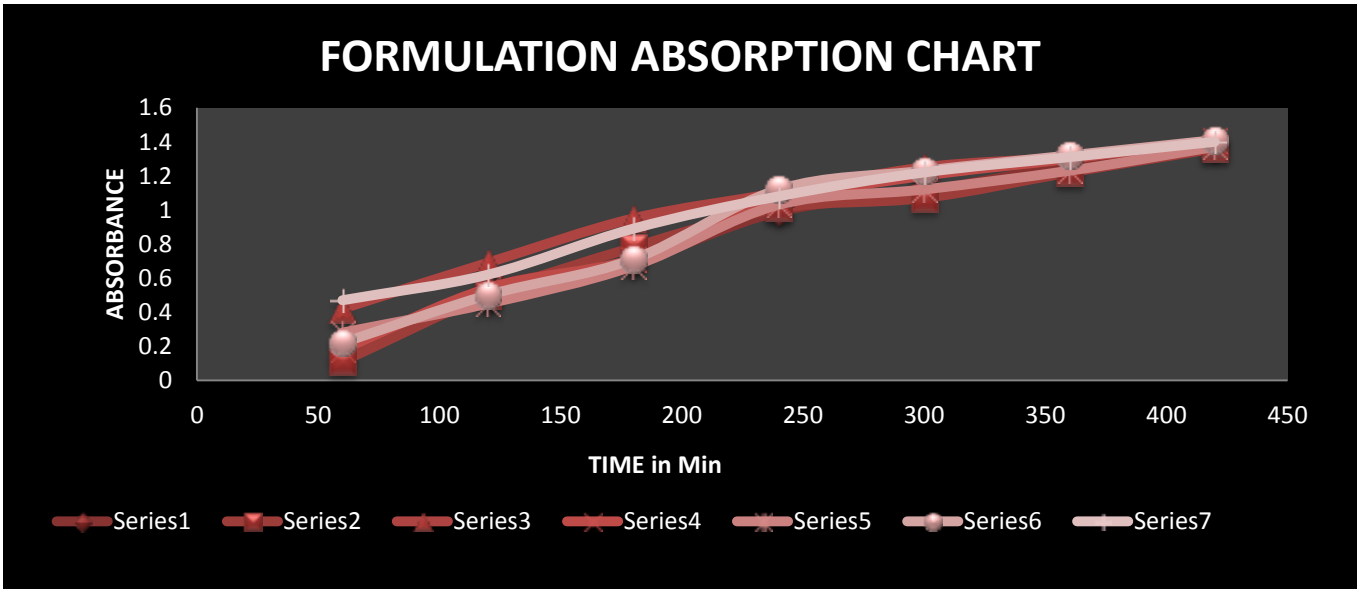
Sr.No	Formulation	Reading 1	Reading 2	Reading 3	Average
1	F1	1.43(95%)	1.48(98%)	1.43(95%)	1.44(96%)
2	F2	1.42(95%)	1.45(97%)	1.44(96%)	1.43(95%)
3	F3	1.45(97%)	1.50(100%)	1.47(98%)	1.47(98%)
4	F4	1.42((95%)	1.42(95%)	1.45(97%)	1.43(95%)
5	F5	1.44(96%)	1.48(98%)	1.47(98%)	1.46(97%)
6	F6	1.43(95%)	1.44(96%)	1.47(98%)	1.44(96%)
7	F7	1.46(97%)	1.48(98%)	1.42(95%)	1.45(97%)

Gel Strengh of the Formulations (SEC)

SrNo	Formulation	Reading 1	Reading 2	Reading 3	Average
1	F1	1.23	1.18	1.25	1.22
2	F2	0.66	0.83	0.59	0.69
3	F3	0.28	0.33	0.24	0.28
4	F4	1.32	1.37	1.42	1.37
5	F5	2.35	2.44	2.39	2.39
6	F6	1.62	1.65	1.57	1.61
7	F7	0.99	1.04	0.91	0.98

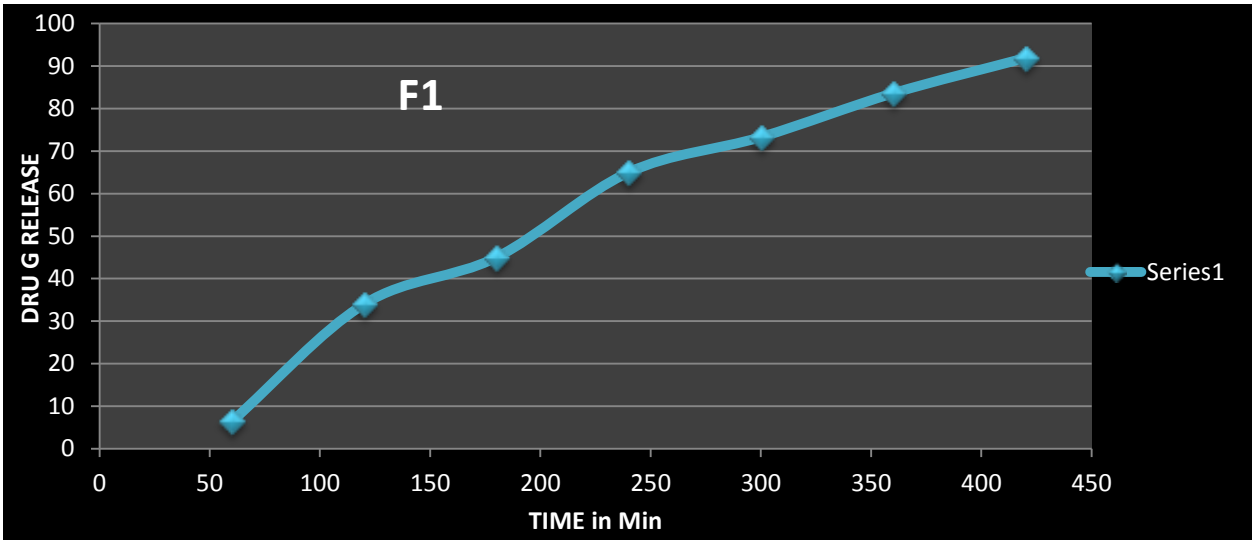
In Vitro Cummulative Drug Release Absorbance of the Formulations

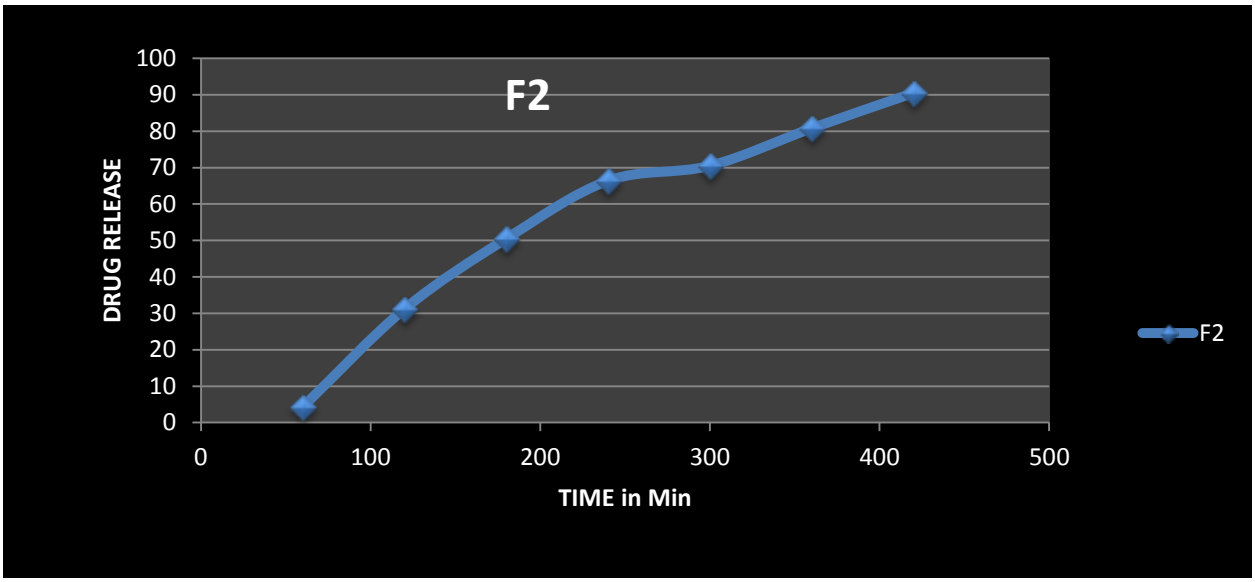
Sr.No	TIME (min)	FORMULATTIONS ABSORBANCE						
		F1	F2	F3	F4	F5	F6	F7
1	60	0.14	0.11	0.42	0.18	0.28	0.22	0.47
2	120	0.54	0.5	0.69	0.54	0.45	0.5	0.62
3	180	0.7	0.78	0.95	0.71	0.67	0.71	0.89
4	240	0.99	1.01	1.1	1.05	1.03	1.12	1.08
5	300	1.11	1.07	1.25	1.2	1.12	1.23	1.22
6	360	1.26	1.22	1.31	1.3	1.23	1.32	1.31
7	420	1.38	1.36	1.39	1.41	1.37	1.41	1.4

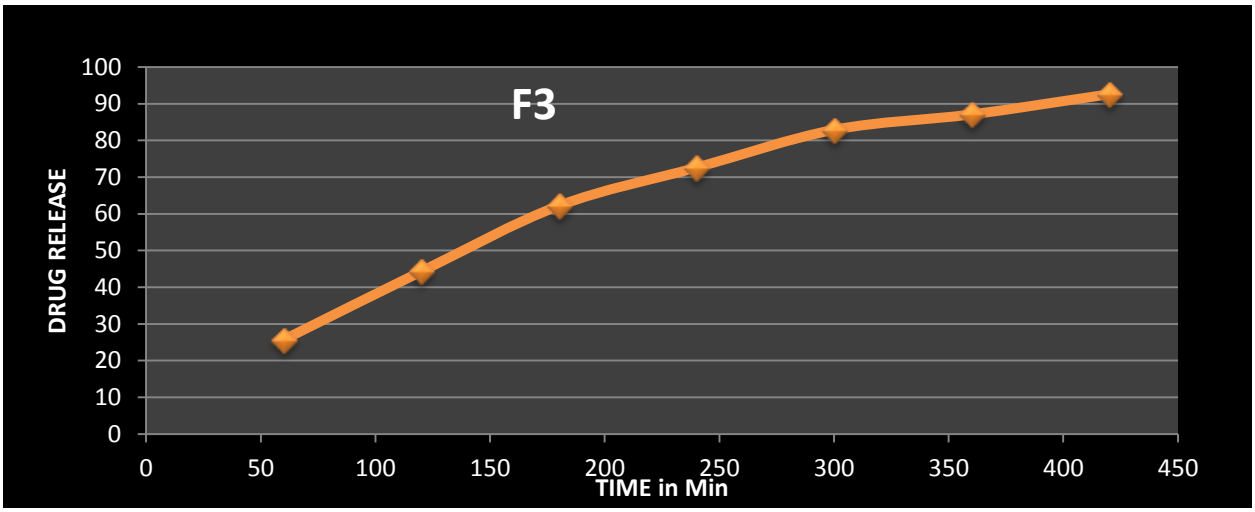


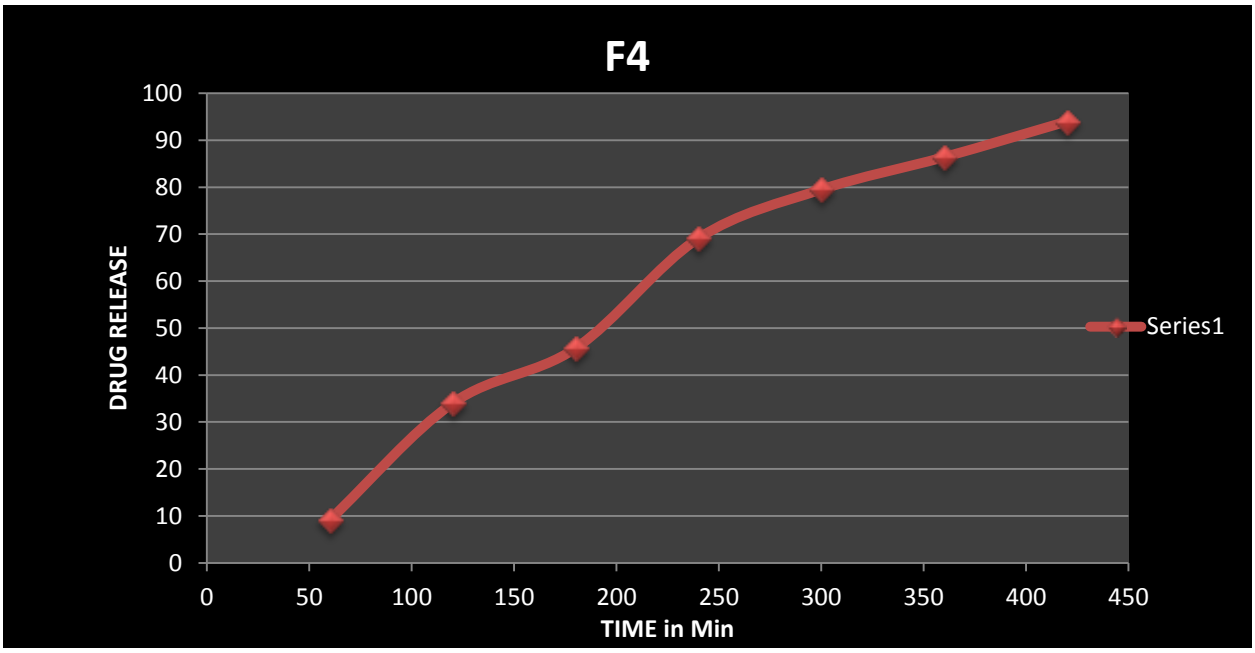
In Vitro Cummulativve Drug Release Study of the Formulations

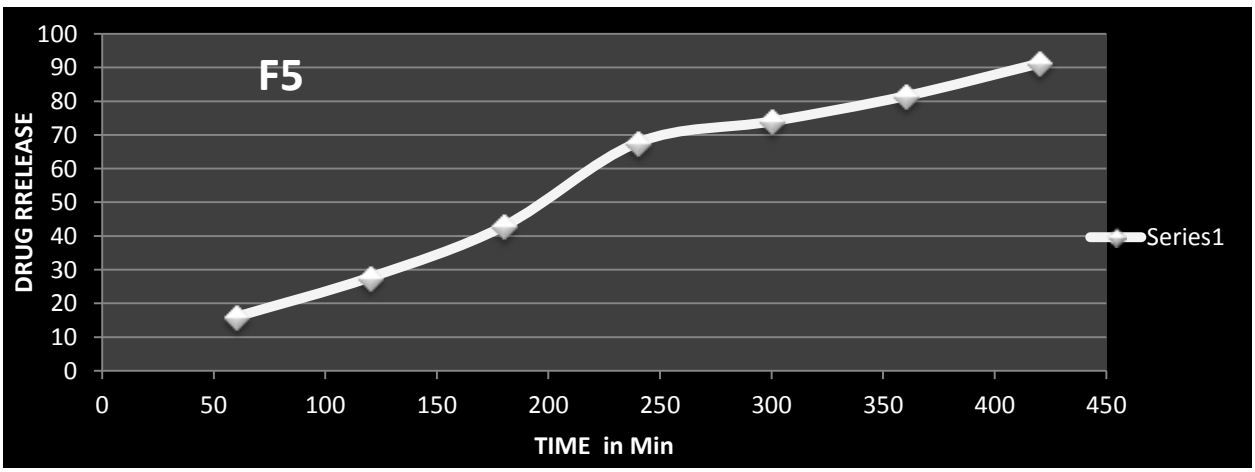
Sr.No	TIME(min)	FORMULATTIONS DRUG RELEASE PROFILE (%)						
		F1	F2	F3	F4	F5	F6	F7
1	60	6.41	4.32	25.7	9.17	16	11.9	29.1
2	120	34	31.2	44.3	34	27.7	31.2	39.5
3	180	45	50.5	62.2	45.7	42.9	45.7	58.1
4	240	65	66.4	72.6	69.1	67.7	74	71.2
5	300	73.3	70.5	82.9	79.5	74	81.5	80.8
6	360	83.6	80.8	87.1	86.4	81.5	87.7	87.1
7	420	91.9	90.5	92.6	94	91.2	94	93.3

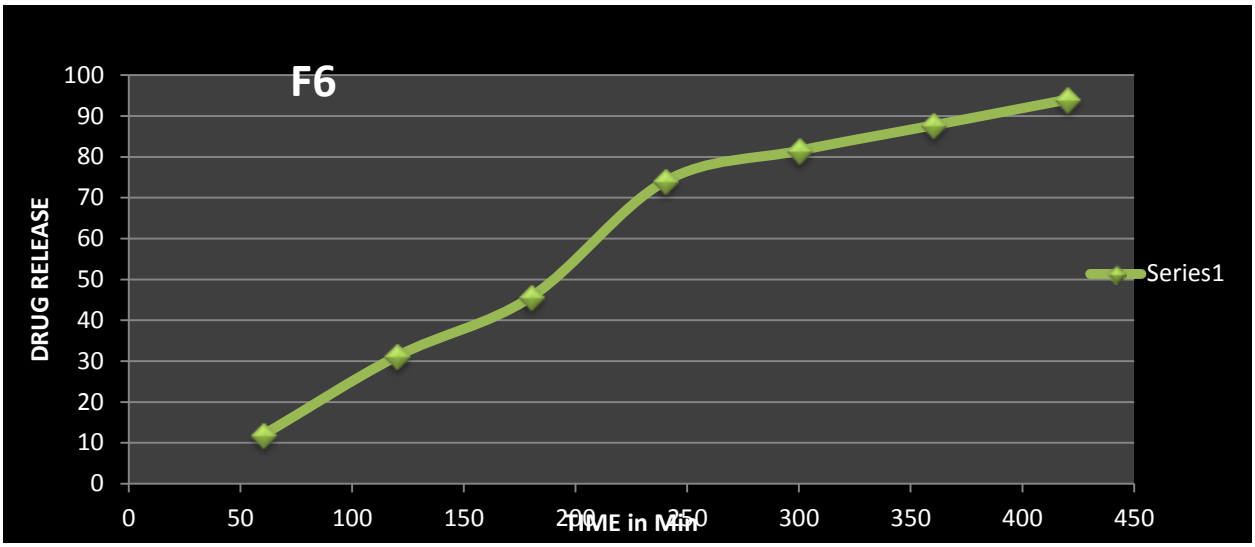


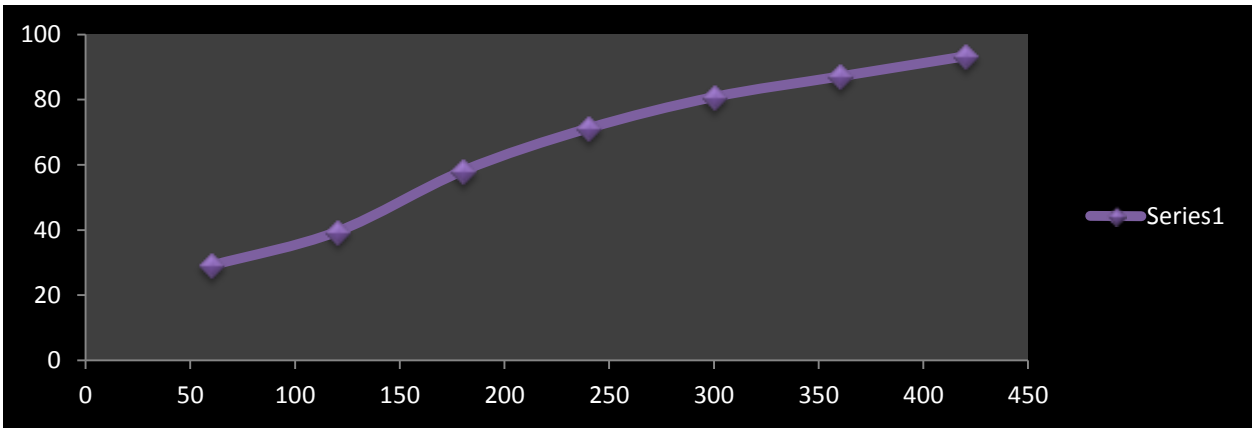



F3
DRUG RELEASE
TIME in Min
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
250
300
350
400
450


F4
DRUG RELEASE
TIME in Min
Series1
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
250
300
350
400
450


F5
DRUG RRELEASE
TIME in Min
Series1
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
250
300
350
400
450





DISCUSSION

Insulin is polypeptide and it has a molecular weight o about 6000. It contains 51 amino acids arranged in two chains. It is soluble in water and dilute solution of mineral acids and insoluble in alcohol. Chloroform and ether. It is admistered through the subcutaneous route because the insulin is destroy in stomach when come contact with hydrochloric acid. Bioadhesive insulin gel is drug delivery system is needed for Insulin to enhance clinical efficiency and patient compliance. The gels are prepared by cold method technique by using the different polymers carried out the analytical method and there results are described below.

ANALYTICAL METHOD

Determination of λ max

λ max of Insulin was determined as per the method described in the methodology section. The λ max of Insulin was found to be 510.0nm.

Preparation of standard calibration curve

The standard calibration curve of Insulin is carried out by Calorimetric spectroscopic method. This is performed to know the purity of sample and find out the slope value of sample which is helpful for further study. It is conducted at **510.0nm**. The slope of calibration curve **1.450**, constant is **0.047** and r2 is **0.993**.

Physical appearance:

The physical property of insulin substance is white in colour and it is in the form of white crystalline powder.

Solubility:

It is soluble in water and dilute solution of mineral acids and insoluble in alcohol, Chloroform and ether.

The formulations are prepared and they are evaluated of different insulin nasal bioadhesive gel test is carried out and value as follows.

Sr. No.	Formulation	pH Value	Viscosity Value	Gelation Temperature	Drug Content	Gel Strength	In- Vitro Drug Release
1	F1	5.7	10%(604)	71.4	1.44(96%)	1.22	91.9
2	F2	5.5	8.5%(525)	75.2	1.43(95%)	0.69	90.5
3	F3	5.8	15.6%(946)	71.0	1.47(98%)	0.28	92.6
4	F4	6.0	17.8%(1139)	71.2	1.43(95%)	1.37	94
5	F5	6.5	13.1%(806)	66.5	1.46(97%)	2.39	91.2
6	F6	5.5	11.4%(688)	70.9	1.44(96%)	1.61	94
7	F7	5.7	13.8%(859)	65.7	1.45(97%)	0.98	93.3

CONCULSION

From the obtained results it can be concluded that Bioadhesive Nasal gel drug delivery Systems offer a simple and practical approach to achieve increased nasal residence time and to modify drug release profiles essential for controlled, site specific and localized drug action.

Melting point, solubility and standard calibration curve results of drugs indicate the purity of drug.

The drug content was well within the Pharmacopoeia limits indicate uniform distribution of drug within the bioadhesive nasal gel.

The drug – polymer ratio, viscosity grades of HPMC, carbopol, lecithin, starch, gelatin and sodium alginate were found to influence the release of drug and bioadhesive nasal gel characteristics from the prepared bioadhesive Insulin nasal gel.

The bioadhesive Insulin nasal gel (F3) showed satisfactory results with (F5) showed good gel strength value, F7 is have less mucoadhesive temperature, Drug content(F3) and control drug release of(F4 &F7) showed excellent result upto 7 hrs.

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Source of interest- Nil
Conflict of Interest- Nil

Cite this article
Kulkarni, S.R. and Rajasekaran, S. "Evaluation of Formulated Insulin Bioadhesive Gel by Using Different Polymers." *Annals of pharma research* 11.12 (2023). Pp 801-810.