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FORMULATION, EVALUATION AND OPTIMIZATION OF OROMUCO-ADHESIVE VITAMIN B12 IN SITU GEL (SPRAY)

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ABSTRACT

Vitamin B12 deficiency is wide spread in all ages, races, economic classes, and both sexes population. It is arguably the most common nutritional deficiency causing injury in the United States. Early diagnosis and treatment is critical to prevent neurologic injury, disability, poor outcomes, and premature death. B12 deficiency causes demyelinating nervous system disease, dementia, psychiatric illness, anemia, vascular occlusions, fall-related trauma, suppressed immune system, and bone marrow failure. The aim of this study was to develop an in situ gel forming oral spray formulation for buccal administration of Vitamin B12. The formulation was developed using poloxamer 407 in the range of 10 % to 14 % and 1 % poloxamer 188 as themo sensitive gelling agents. Gelatin, 1 % to 2 % and 1 % HPMC E5 were used to provide mucoadhesive properties. Nine different formulation were prepared using 3^2 factorial design and evaluated for pH, viscosity, density, gelling strength and in vitro diffusion. The optimization of formulation revealed that the formulation F8 was showing good gelling properties at body temperature and also able to demonstrate good in vitro diffusion across semipermeable membrane.

Keywords: Vitamin B12, in situ gel, oral spray, poloxamer 407, poloxamer 108. poloxamer 188

INTRODUCTION:

Vitamin B12 deficiency was first described in 1849, and it was thought to be fatal until 1926, when a high-vitamin B12 diet of liver was found to slow the disease's progression. Much is now understood about vitamin B12's biochemistry and

metabolism; nonetheless, with the designation of a "sub-clinical" deficient group, typified by serum vitamin B12 values that were earlier deemed adequate, diagnosing its deficiency has become more difficult. Vitamin B12 deficiency was formerly assumed to develop over a long period of time and mainly in devout vegetarians or individuals with pernicious anaemia. More recent research has revealed that subclinical B12 insufficiency, which is most usually caused by malabsorption or dietary inadequacy, has disease implications. Vitamin B12 insufficiency is common in developing nations, especially among the elderly and vegetarians. Long-term effects are unknown, however they could include negative impacts on pregnancy outcomes and ageing **[1]**.

The mucosal linings of the nasal, rectal, vaginal, ocular, and oral cavity, which make up the transmucosal route of drug delivery, provide substantial possibilities benefits over oral route administration for systemic drug delivery. The possibility of bypassing the first-pass effect, avoiding hepatic first pass clearance within the GI tract, and, depending on the treatment, a superior enzymatic flora for drug absorption are only a few of the advantages. The buccal region of the oral cavity is a prominent location for medicine delivery due to its ease of administration. Buccal drug delivery is the process of delivering a desired medication through the buccal mucosal membrane lining of the mouth cavity. Both the mucosal (local effect) and transmucosal (systemic effect) approaches for administering medication are effective. In the first scenario, the goal is to achieve site-specific drug release on the mucosa, whereas in the second scenario, the goal is to achieve drug absorption over the mucosal barrier and into the systemic circulation. Because the medication amount in buccal formulations is typically smaller than in tablets and capsules, toxicity and unwanted side effects are likely to be decreased **[2-4]**.

MATERIALS:

Vitamin B12 (Cyanocobalamin) was purchased from sigma aldrich india., Gelatin, HPMC E5 LV, Poloxamer-407, Poloxamer 188 and Ethanol were procured from Loba chemicals pvt ltd, Bangalore, India. All chemicals used were of analytical grade.

Methodology:

Preparation of oral spray solution of Vitamin B12:

9 different formulations were designed using 32 factorial design. Poloxamer-407 and Poloxamer-188 **(Table 1)** was first dissolved in cold water at room temperature under continuous slow agitation. After the completion of dissolution, then Gelatin and

HPMC was added to the poloxamer solution and then stirred (2 hrs.) & make homogeneous solution. Than drug & ethanol were finally added to the previously prepared mixture and gently stirred. Than mucoadhesive polymer based thermoreversible poloxamer solution was maintained overnight at 4 ◦C prior to use **[5, 6]**.

Table 1: formulation chart

Formulation code	Drug (mg)	Poloxamer 407 (% w/w)	Poloxamer 188 (% w/w)	Gelatin (% w/w)	HPMC E5 (% w/w)	Ethanol (% v/v)
F1	50	10	1	1	1.5	10
F2	50	10	1	1.5	1.5	10
F3	50	10	1	2	1.5	10
F4	50	12	1	1	1.5	10
F5	50	12	1	1.5	1.5	10
F6	50	12	1	2	1.5	10
F7	50	14	1	1	1.5	10
F8	50	14	1	1.5	1.5	10
F9	50	14	1	2	1.5	10

Evaluation:

Drug excipients compatibility study:

Drug and excipient chemical and physical compatibility were evaluated using FTIR and DSC methods. The DSC study was performed using DSC-3 & Metter Toledo, U.S Instrument. The thermal traces were obtained by heating from 25 ºC to 300 ºC heating rate of 10 ºC/min under atmospheric condition in open crucibles. Results of FTIR and DSC were analysed to check any possibilities of chemical and/or physical incompatibilities **[7, 8]**.

Determination of pH:

The pH of all 9 formulations were checked using a digital pH meter. The electrode of a calibrated pH meter was dipped inside the oral spray solution for 5 minutes and the pH value was noted once the equilibrium is achieved. All measurements were taken in triplicate and results were noted with standard deviation **[9, 10]**.

Determination of Viscosity:

Viscosity of all 9 formulations were checked using Brookfield LV series viscometer. Viscosity was determined by using 63 number spindle and appropriate quantity of sample. The ambient temperature was kept 25 ºC as per the standard operating procedure of the instrument. All measurements were taken in triplicate and results were noted down with standard deviation. All measurements were taken in triplicate and results were noted with standard deviation **[11, 12]**.

Test for Gelling temperature and gelling time:

Samples from all 9 formulations were taken in plastic tubes and kept in thermostatically controlled water bath. Tubes were shaken

frequently until samples are converted into gel. Complete gelation was confirmed by tilting the test tube upside down, where the gel does not flow out. The gel formation was evaluated visually and gelation time was observed **[13, 14]**.

Measurement Mucoadhesive strength:

The mucoadhesive strength of all 9 formulation was determined by modified weighing balance method. Goat mucosal membrane (thickness 0.05± 0.01 mm) was used as the model membrane for the measurement of mucoadhesive strength. The mucosal membrane was excised by removing the underlying connective tissue. The surface of the mucosal membrane was first blotted with filter paper and then moistened with buffer solution pH 6.8. The weight required to detach the film from the mucosal surface was determined as a measure of mucoadhesive strength. All measurements were taken in triplicate and results were noted down with standard deviation. All measurements were taken in triplicate and results were noted with standard deviation **[15, 16]**.

In-vitro drug diffusion study:

All 9 formulations were tested for the amont of drug diffused from donor compartment to the receptar compartment. Franz diffusion cell was used along with pre activated the semipermeable membrane. 5 ml sample solution containing drug was taken into donor compartment. The receptar compartment was filled with phosphate buffer pH 6.8, and the temperature was maintained at 37 ℃. Samples were taken at every 15 min interval till 120 min and the fresh buffer was added to receptar compartment to maintain the sink condition. Samples collected were subjected to measurement of absorbance at drug's $\boldsymbol{\lambda}$ max using UV spectrophotometer. All measurements were taken in triplicate and results were noted with standard deviation **[17]**.

RESULTS AND DISCUSSION:

Drug excipients compatibility study:

On comparison with the pure drug IR spectra **(Figure: 1)**, it was observed that all characteristic peaks of functional groups present in the chemical structure of Vitamin B12 are also visible in IR spectra **(Figure 2)** of physical mixture of drug and all other excipients. Thus, there is no significant chemical reaction between the drug and other excipients and no signs of chemical incompatibility. The formulation prepared thus will remain stable for a sufficiently long period.

The reported melting point of Vitamin B12 is greater than 300 ℃ and in a DSC thermogram of pure drug a broad peak was observed in the range of 304-306 ℃. This melting point temperature range remained unaffected in a DSC thermogram of a

physical mixture of drug and other excipients. Thus it can be concluded that there are no signs of any physical incompatibility and the prepared formulation may remain stable for a sufficiently long time **(Table 3)**.

Determination of pH:

The pH of formulations was 7.00 ± 0.14 to 7.41 ± 0.09. The lowest pH of 7.00 ± 0.14 was of formulation F3 and the highest of 7.41 ± 0.09 was of formulation F1. The pH of all formulations was found to be neutral and will not produce any mucosal irritation upon application.

Determination of Viscosity:

The viscosity of formulations was found to be in the range of 10 ± 3 mPa.s to 35 ± 5 mPa.s. The lowest viscosity was found in formulation F1 and the highest viscosity was found in formulation F9. The viscosity value indicates that the amount of polymer directly correlates with the overall viscosity of the liquid oral spray solutions.

Test for Gelling temperature and gelling time:

The gelling temperature for formulations F1 to F4 was found to be more than 50 ℃. For the remaining formulations, the gelling temperature was in the range of 33 ℃ to 44 ℃. Formulations F8 and F9 have the gelling temperature of 36-39 ℃ and 33-35 ℃, close to the actual body temperature.

Measurement Mucoadhesive strength:

The mucoadhesive strength was found to be in the range of 25 ± 1 to 49 ± 2. The lowest mucoadhesive strength was 25 ± 1 gm/cm2 for the formulation F1 and the highest 49 ± 2 gm/cm2 for the formulation F9. The amount of mucoadhesive polymer has a direct correlation with the mucoadhesive strength.

In-vitro drug diffusion study:

The % drug diffusion was found to be in the range of 80 ± 0.64 to 90.81 ± 0.10 after 120 mins. The maximum % drug diffusion of 90.81 ± 0.10 was observed in formulation F9, whereas the lowest, 80 ± 0.64 was observed in formulation F1. it can be concluded from the result that as the amount of mucoadhesive polymer increases, due to increasing mucoadhesive strength and the retention time at the site of application the % drug diffusion increases.

Optimization:

The formulation chart was prepared using design expert software by applying 32 factorial design. Two formulation factors, amount of mucoadhesive polymer and poloxamer 477 were selected and three responses, viscosity, Gelling time and % drug diffuse were selected for optimization. The regression model analysis gave R^2, adjusted R^2 and predicted R^2 value for all three resonances close to 1 that indicating that the selected model is significant. The higher F value and low P value indicate that

the null hypothesis is not true and the selected model is significant **(Table 4)**.

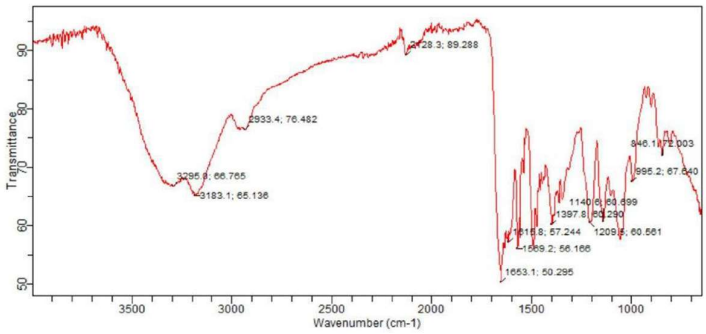

Figure 1: IR spectra of Vitamin B12

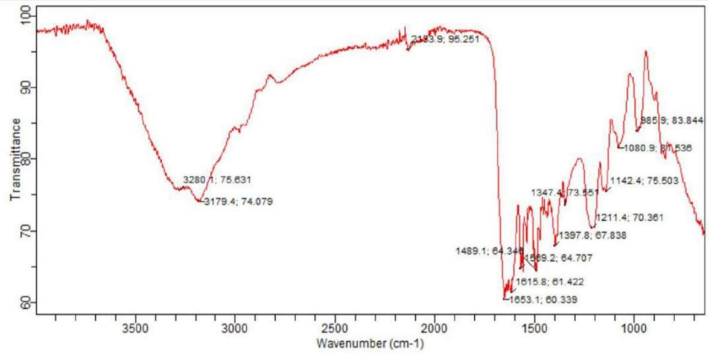

Figure 2: IR spectra of physical mixture of Vitamin B12 and other excipients

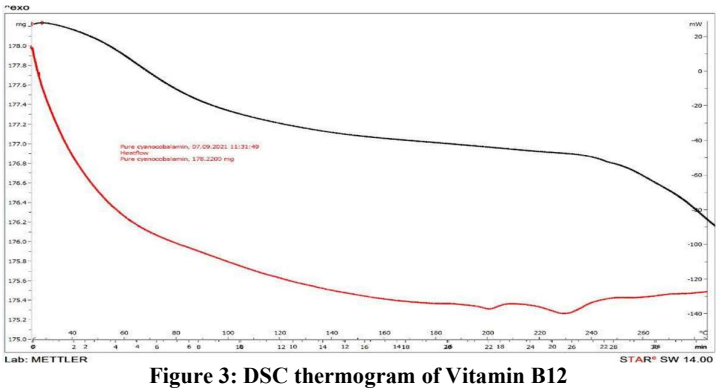

Figure 3: DSC thermogram of Vitamin B12

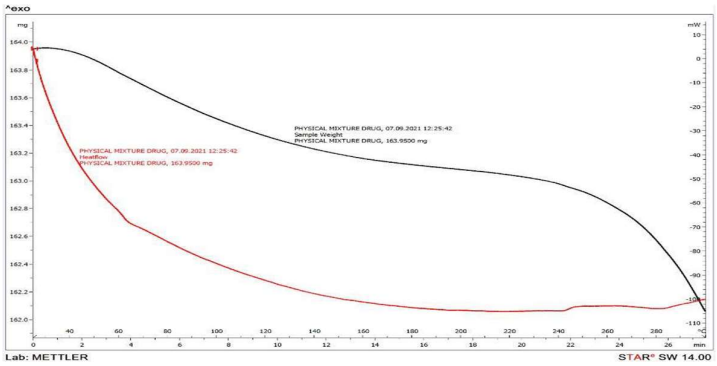

Figure 4: DSC thermogram of Vitamin B12 and other excipients

Table 2: Evaluation parameters of in situ gel forming Vitamin B12 oral spray

Formulation code	pH (Mean± SD)	Viscosity (mPa.s)	Gelling Temperature (°C)	Gelling Time (sec)	Mucoadhesive strength (gm/cm2)
F1	7.41 ± 0.09	10 ± 3	>50 °C	50 ± 4	25 ±1
F2	7.11 ± 0.14	10 ± 4	>50 °C	52 ± 5	28 ±2
F3	7.00 ± 0.14	15 ± 4	>50 °C	52 ± 3	31±1
F4	7.22 ±0.14	15 ± 3	>50 °C	48 ± 4	38 ±2

Table 3: Comparison of FT-IR spectra of Drug & other excipients

Time (min)	% Drug diffusion (Mean± SD)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
15	17.98 ± 0.91	17.87 ± 0.76	19.78 ± 0.60	21.15 ± 0.25	21.05 ± 1.26	19.17 ± 0.44	17.84 ± 0.65	19.03 ± 0.60	21.87 ± 0.55
30	41.44 ± 0.60	40.65 ± 0.54	41.48 ± 1.10	42.56 ± 0.62	42.09 ± 1.92	39.82 ± 0.25	41.73 ± 0.78	39.17 ± 0.63	42.52 ± 0.44
45	57.87 ± 0.12	60.97 ± 0.39	56.58 ± 0.41	57.12 ± 0.50	55.35 ± 3.84	53.19 ± 0.11	52.40 ± 0.45	56.72 ± 0.81	56.40 ± 2.12
60	70.67 ± 0.25	70.45 ± 0.38	68.94 ± 0.55	67.50 ± 1.28	65.62 ± 2.64	66.45 ± 0.50	63.71 ± 0.60	63.06 ± 2.04	62.09 ± 0.69
75	81.87 ± 0.31	79.89 ± 0.39	79.17 ± 0.60	77.69 ± 1.43	76.83 ± 1.19	78.23 ± 0.12	73.12 ± 0.51	73.23 ± 1.48	71.64 ± 0.83
90	87.96 ± 0.50	86.56 ± 0.17	85.19 ± 0.50	83.57 ± 0.82	83.78 ± 0.66	83.21 ± 0.44	81.15 ± 0.17	78.77 ± 0.44	78.20 ± 0.82
105	90.05 ± 0.22	89.33 ± 0.51	87.32 ± 0.53	86.13 ± 0.25	86.20 ±0.23	84.50 ± 0.69	82.59 ± 0.22	81.73 ± 0.60	80.00 ± 0.19
120	90.81 ± 0.10	90.05 ± 0.57	88.83 ± 0.45	87.78 ± 0.37	87.14 ± 0.50	85.73 ± 0.60	82.95 ± 0.23	82.99 ± 0.60	80.64 ± 0.41

Table 4: Optimization summary

No.	Functional Groups	Observed Wavelength(cm-1)	Standard Wavelength(cm-1)
1	C = C Alkenes	1636.3	1620-1680
2	C = O Carbonyl group	1653.1	1650-1780
3	C - H Alkanes group	2879.4	2850-2950
4	N - H group	3181.3	3180-3350
5	O - H Alcohol group	3352.7	3200-3600

Table 5: Results of regression analysis for responses

		Factor 1	Factor 2	Response 1	Response 2	Response 3
Std	Run	A:POLOXAMER 407	B:GELATIN	VISCOSITY	GELLING TIME	% DRUG DIFFUSED
1	3	10	1	10	50	90.81
4	2	10	1.5	10	52	90.27

7	8	10	2	15	52	88.97
2	5	12	1	15	48	87.56
5	6	12	1.5	20	49	86.59
8	1	12	2	25	48	85.83
3	7	14	1	30	47	83.13
6	4	14	1.5	30	48	83.02
9	9	14	2	35	45	80.64

Viscosity	
R^2	0.9677
Adjusted R^2	0.9484
Predicted R^2	0.8054
Adeq Precision	18.9737
F-Value	50
P- Value	0.0004
Gelling time	
R^2	0.8824
Adjusted R^2	0.8118
Predicted R^2	0.6864
Adeq Precision	10.113
F-Value	12.50
P- Value	0.0093
% drug diffused	
R^2	0.984
Adjusted R^2	0.9744
Predicted R^2	0.8972
Adeq Precision	26.1927
F-Value	102.57
P- Value	< 0.0001

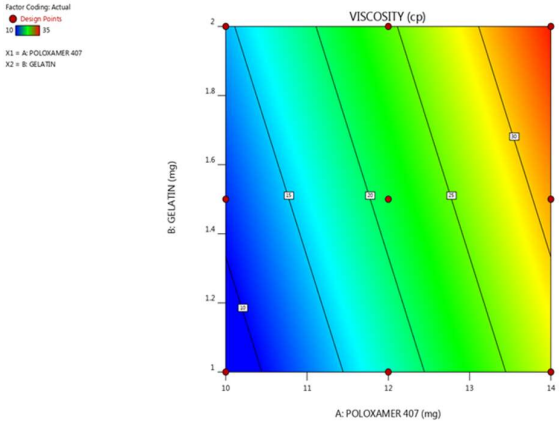

Figure 5: 2D contour plot of effect of formulation variables on Viscosity of in situ gel formulation

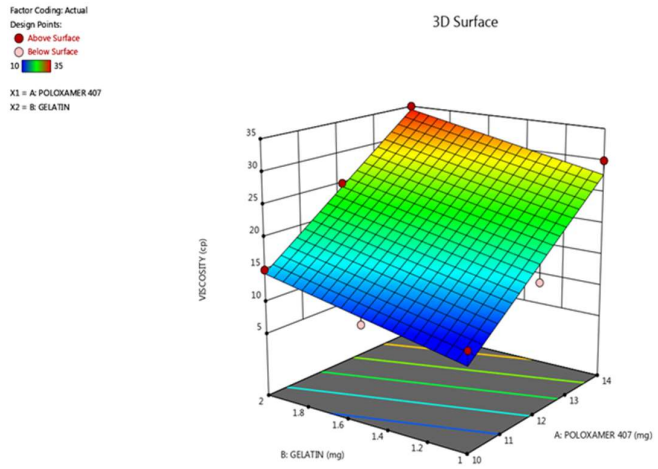

Figure 6: 3D surface plot of effect of formulation variables on Viscosity of in situ gel formulation

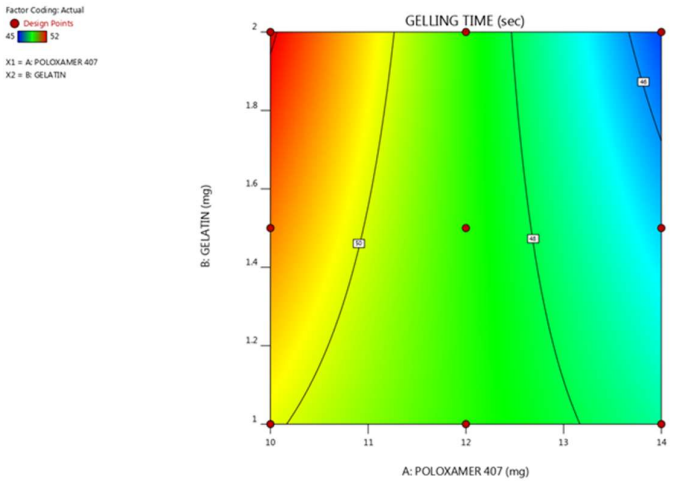

Figure 7: 2D contour plot of effect of formulation variables on Gelling time of in situ gel formulation

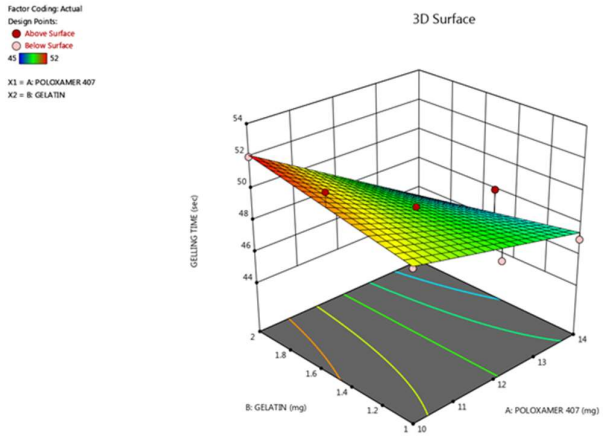

Figure 8: 3D surface plot of effect of formulation variables on Gelling time of in situ gel formulation

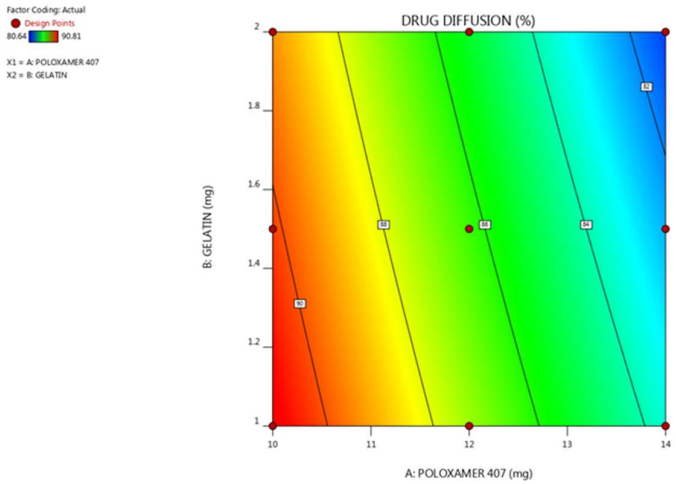

Figure 9: 2D contour plot of effect of formulation variables on % Drug Diffusion of in situ gel formulation

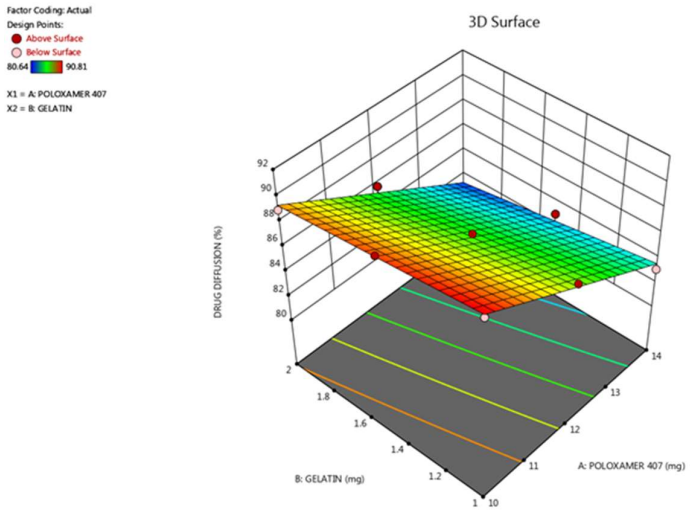

Figure 10: 3D surface plot of effect of formulation variables on % Drug Diffusion of in situ gel formulation

CONCLUSION:

Insitu gel forming oral spray of vitamin B12 was prepared using poloxamer 407 and 188 as in situ gel forming polymer along with HPMC E5 and Gelatin as mucoadhesive polymers. Total 9 formulations were prepared using 32 factorial design and evaluated for various in vitro evaluation parameters like gelling time, pH, viscosity, gelling temperature, mucoadhesive strength and in vitro drug diffusion. The formulation F8 was selected as best formulation as it got converted into gel at body temperature and showed good mucoadhesive strength. Moreover the formulation F8 also showed good drug diffusion properties after 120 min. Further the optimization study showed that there is a direct correlation between the formulation factors, amount of mucoadhesive polymer gelatin and in situ gel forming polymer poloxamer 407, with selected responses i.e.

mucoadhesive strength, gelling time and % drug diffused. It can be concluded that as the amount of poloxamer 407 increases the gelling time decrease and as the amount of mucoadhesive polymer, gelatin, increases the mucoadhesive strength also increases. In case of % drug diffusion it can be concluded that as the total amount of polymer increases, the overall viscosity increases and that leads to a minor decrease in % drug diffuision.

REFERENCES:

[1] Mohamad, Soad & Sarhan, Hatem & Abdelkader, Hamdy & Mansour, Heba. (2017). Vitamin B12–Loaded Buccoadhesive Films as a Noninvasive Supplement in Vitamin B12 Deficiency: In Vitro Evaluation and In Vivo Comparative Study With Intramuscular Injection. Journal of Pharmaceutical Sciences. 2017.03.040.

[2] Gilhotra, R. M., Ikram, M., Srivastava, S., & Gilhotra, N. (2014). A clinical perspective on mucoadhesive buccal drug delivery systems. Journal of Biomedical Research, 28(2), 81–97.

[3] Viralkumar F P, Fang L and Marc B B, Advances in oral transmucosal drug delivery. J. Controlled Release. 2011, 153, 106-116.

[4] Nishan N B, Sandeep C A, Vikrant P W, Pande S D and Tapar D D, A Review on Buccal Drug Delivery System. Int. J. Pharm. & Pharm. Sci. Res. 2013, 3(1), 35-40.

[5] Vidyasagar N, Mallikarjunarao K, Gnanaprakash K, Divya A, Sowjanya A and Gobinath M, "A review on buccal drug delivery system." J. Pharm. Res. Dev. 2012, 1(2), 29-35.

[6] Tsuneji N and Ryogi K, "Buccal/Gingival drug delivery systems." J. Controlled Release, 1987, 6, 353-360.

[7] Raghavendra Rao N G, Shravani B and Mettu S R, "Overview on Buccal Drug Delivery Systems." J. Pharm. Sci. & Res. 2013, 5(4), 80-88.

[8] Rajesh B G and Joseph R R, "Oral cavity as a site for bioadhesive drug delivery," Adv. Drug Del. Rev. 1994, 13, 43-74.

[9] Yajaman S, Ketousetuo K and Bandyopadhyay A K, "Buccal bioadhesive drug delivery - A promising option for orally less efficient drugs." J. Controlled Release. 2006, 114, 15- 40.

[10] Shaffi K and Satheesh M, "Mucoadhesive drug delivery: mechanism and methods of

evaluation." Int. J. Pharm. Bio. Sci. 2011, 2(1), 1-10.

[11] Nazema P, Patel A, Bhavesh A, Noolvi MN. Role of Plant Based Natural Polymers in Development of Mucoadhesive Drug Delivery Systems. Inventi Rapid: Novel Excipients 2016;(3):108-15.

[12] Adil P, Shah D, Desai TR, Noolvi MN. Mucoadhesive Buccal Drug Delivery System: A Review. Inventi Rapid: NDDS 2015;2015(3):1–8.

[13] Adil P, Shah D, Desai TR, Noolvi MN. Development and Evaluation of Chitosan and Aloe Vera Gel Mucilage Interpolymer Complex-Based Mucoadhesive Buccal Films of Tramadol Hydrochloride. Asian J Pharm. 2016;9(5):843-49.

[14] Patel A, Shah D, Desai TR, Noolvi MN. Mucoadhesive Buccal films based on chitosan and Carboxymethylated Feronia Limonia fruit pulp mucilage Interpolymer complex for delivery of opioid analgesics. Asian J Pharm. 2016;10(2):137-143.

[15] Patel A, Patel N, Shah D. In vitro and In vivo Evaluation of Chitosan/ Karaya Gum Interpolymer Complex Based Mucoadhesive Buccal Films of Tramadol HCl. Asian J Pharm. 2017;11(4, Suppl.): 818-29.

[16] Xu, x., Shen, Y., Wang, W., Sun, C., Li, C., Xiong, Y., & Tu, J. Preparation and in vitro characterization of thermosensitive and mucoadhesive hydrogels for nasal delivery of phenylephrine hydrochloride. European journal of pharmaceutics and biopharmaceutics, (2014). 88(3), 998–1004.

[17] Sandri G, Cristina Bonferoni M, Ferrari F, Rossi S, Del Fante C, Perotti c *et al*. An in situ gelling buccal spray containing platelet lysate for the treatment of oral mucositis. Current drug discovery technologies. 2011;8(3):277-285.