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FORMULATION AND EVALUATION OF FAST DISSOLVING FILM OF METHYLCOBALAMIN

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ABSTRACT

Over the past decade, there has been increasing interest in using sublingual route of administration. Which directly inject the dug in to the blood stream. So for drugs having less bioavailability it can be helpful to increase absorption. Also it quits the first past metabolism. Therefore drug like methylcobalamine which is having very less bioavailability when given by this route will lead to increase the absorption and at last the bioavailability. Hear by using polymers like PVA and PVP in a combination with penetration enhancer like SLS and PEG 400 as plasticizer we had prepared fast dissolving sublingual film. The result itself shows increase in the absorption (penetration) of methylcobalamine much more than given by the GI tract. Our work demonstrates that the obtained fast dissolving sublingual film of methylcobalamin can be used for the patient.

KEYWORDS: Sublingual film, Methylcobalamine, Plasticizer, Bioavailability.

INTRODUCTION

Introduction of Fast dissolving film

The mucosa of the mouth and esophagus may appear to differ little from the rest of the moist lining of the gastrointestinal tract, with which it is continuous. In fact, with the notable exception of the uterine cervix, this tissue is remarkably different from other mucosa of the body and has more in common with skin, with which it forms a junction at the lips, than with the intestinal mucosa. The soft tissues of the human oral cavity and esophagus are covered everywhere by a stratifying squamous epithelium. In regions subject to mechanical forces associated with mastication (i.e., the gingiva and hard palate) there is a keratinizing epithelium resembling that of the epidermis covering the skin. [2] In these masticatory mucosa, the keratinized epithelium is tightly attached to the underlying tissues by a collagenous connective tissue, or lamina propria. The floor of the mouth, buccal regions, and esophagus, which require flexibility to accommodate chewing, speech, or swallowing of a bolus, are covered with a nonkeratinizing epithelium. The connective tissue of lining mucosae is more elastic and flexible than the connective tissue in the

masticatory mucosa. The dorsum of the tongue is covered by a specialized epithelium, which can be represented as a mosaic of keratinized and nonkeratinized epithelium. This epithelium is attached tightly to the muscle of the tongue. Figure 1 illustrates diagrammatically the distribution of the different types of mucosa within the oral cavity. From measurements made by Collins and Dawes, it can be calculated that the masticatory mucosa represents approximately 25%, the specialized mucosa (dorsum of tongue) approximately 15%, and the lining mucosa approximately 60% of the total surface area of the oral lining. [3,4]

Physicochemical properties of the oral mucosa The oral mucosa presents differently depending on the region of the oral cavity being considered. The masticatory mucosa covers those areas that are involved in mechanical processes, such as mastication or speech, and includes the gingival and hard palate. This masticatory region is stratified and has a keratinized layer on its surface, similar to the structure found at the epidermis, and covers about 25% of the oral cavity. The specialized mucosa covers about 15%, corresponding to the dorsum of the tongue, and is a stratified tissue with keratinized as well as non-keratinized domains.[3,5]

Finally, the lining mucosa covers the remaining 60% of the oral cavity, consisting of the inner cheeks, floor of the mouth, and underside of the tongue. This lining epithelium is stratified and non-keratinized on its surface. The buccal mucosa covers the inner cheeks and is classified as part of the lining mucosa, having approximately 40–50 cell layers resulting in an epithelium 500–600 lm thick. The epithelium is attached to underlying structures by a connective tissue or lamina propia, separated by a basal lamina. These lining mucosa and the lamina propia regions provide mostly mechanical support and no major barrier for penetration of actives.[5,6]

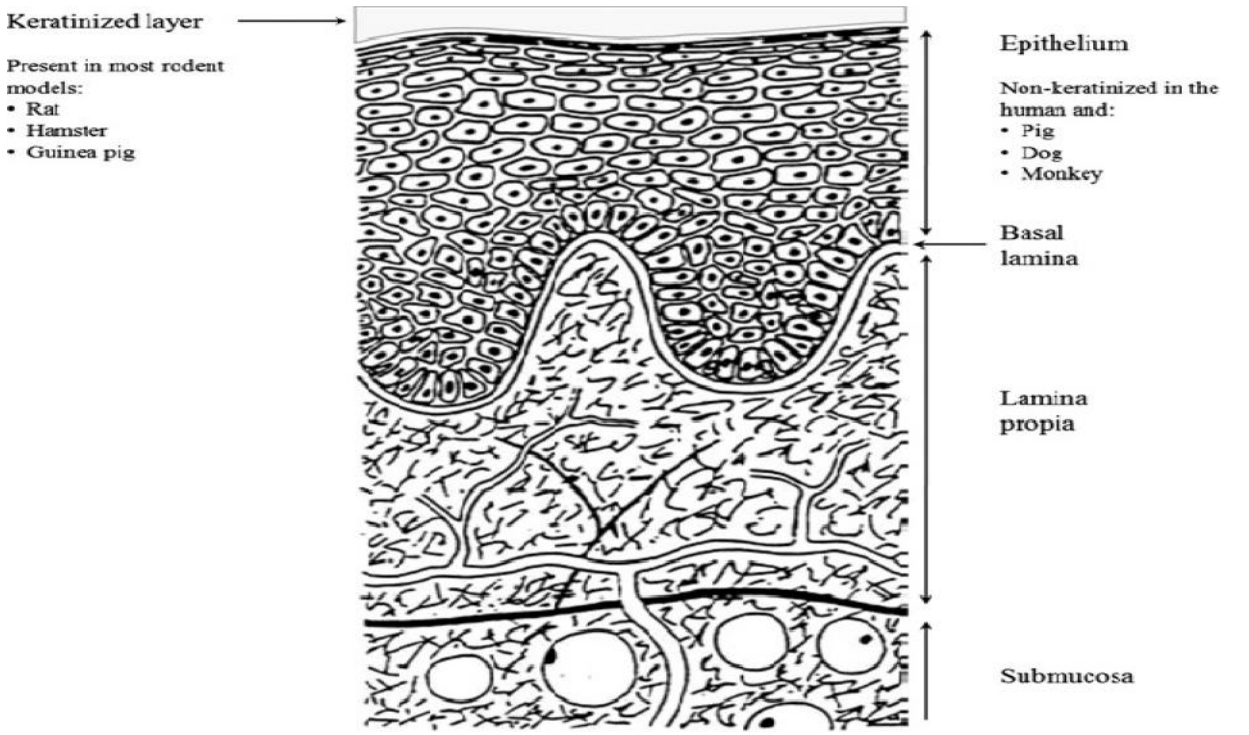


Figure 1: Diagram of a cross section of the buccal mucosa. The keratinized layer is only present in most rodent models while the human has a non-keratinized buccalmucosa.[5]

Methylcobalamine[1][7][8]

It is known that vitaminB12 does more than merely prevent anemia. This water soluble vitamin is necessary for at least eight important areas of whole body health,including:1)energy production,2)production of genetic materials DNA and RNA ,3)nervous system function as it is needed to produce the myelin, the fatty substance that forms a protective sheath around nerves,4)production of acetylcholine, a neurotransmitter that helps with memory and learning,5)brain health, science it may be helpful for some form of depression,6)slowing the cognitive decline that comes with the health(vitamin B12 works with folic acid to control homocysteine levels that dramatically increase the risk of heart disease and stoke) .

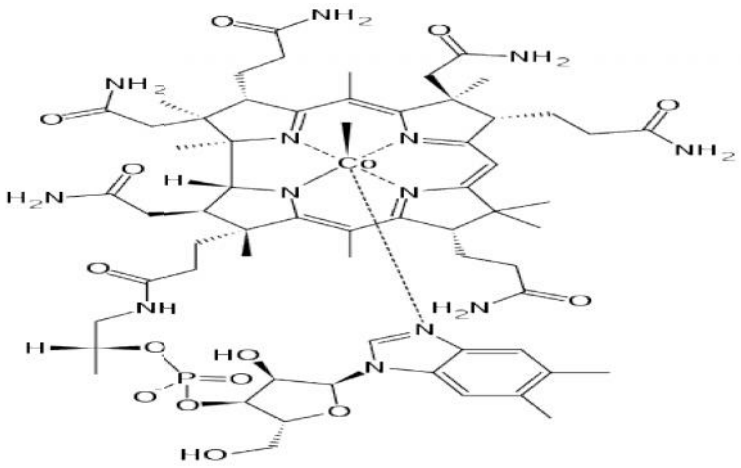


Figure 2: structure of methylcobalamin[7,8]

MATERIALS AND METHODOLOGY

Materials:

Table 1: List of Materials

Sr. No.	Materials	Suppliers / Manufacturer
1.	Methylcobalamin	Purchase from sknrd biotech, gandhinagar
2.	PVA	Sulab,Suvidhan Laboratories, Baroda
3.	PVP	Sulab,Suvidhan Laboratories, Baroda
4	PEG-400	Sulab,Suvidhan Laboratories, Baroda
5	SLS	Sulab,Suvidhan Laboratories, Baroda
6	D-Mannitol	Sulab,Suvidhan Laboratories, Baroda

Preformulation Study:

Colour, odour, taste and appearance:

The drug sample was evaluated for its colour, odour and taste.

Melting point determination:

Melting point of the drug sample was determined by capillary method using melting point apparatus.

Solubility study:

Solubility of the drug sample was determined by saturation equilibrium method. Dissolving the drug in to Water. 5ml water was taken in china dish. Excess quantity of methylcobalamin was added in to the china dish to make supersaturated solution of drug in water. China dish was kept on water bath to evaporate the water. Dry powder was collected and weigh to measure the amount of drug.

Determination of λmax:

Stock solution (1000µg/ml) of methylcobalamin in Phosphate buffer pH 6.8 was prepared. This solution was appropriately diluted with the same solution to obtain stock solution of (100µg/ml). The resultant solution was scanned in the range of 400 nm – 800 nm in UV-Visible spectrophotometer. it showed λ_{max} 522 nm in phosphate buffer pH 6.8 for methylcobalamin.

Determination of calibration curve:

Spectrophotometric analysis of methylcobalamin was carried out on double beam UV-spectrophotometer (UV-1800,shimazdu, japan).

Standard calibration curve of methylcobalamin in phosphate buffer 6.8pH:

methylcobalamin (25 mg) was dissolved in 25 ml water to obtain a stock solution of 1000 µg/ml concentration. From the stock solution 10ml of solution diluted with 100ml to obtain 100µg/ml. This solution (100 µg/ml) was further diluted with water to obtain solution of 10 to 60 µg/ml. Absorbance of each solution was measured at 522 nm using UV-Visible spectrophotometer and water was taken as blank. The standard curve was generated for the entire range from 10 to 60 µg/ml.

Drug – Excipients compatibility study:

Infrared spectra of pure drug, polymer, as well as for combination of drug-polymer were taken by KBr pellet technique and were recorded in the range of 4000 – 400cm^{-1} by using FT-IR Spectrophotometer Shimadzu.

Method of preparation of fast dissolving sublingual film: [9,11]

Solvent Casting Method:

Listed amount of poly vinyl alcohol (PVA) was taken in beaker and dissolved in 10 ml of distilled water and was heated at 60Ċ to get solubilised PVA. In other beaker listed amount of poly vinyl pyrrolidone (PVP), polyethylene glycol-400 (PEG-400), Sodium lauryl sulphate (SLS), D-mannitol were taken and dissolved in 10 ml of distilled water.after cooling PVA solution at room temperature both solution were mixed and mixed well. That solution was poured in petriplate having glycerine layer to prevent adhere film to the surface of plate. Tthis preparation was placed in hot air oven till 24 hr at 50Ċ temperature. After 24 hr film was evaluated out of plate and evaluations were done.

OPTIMIZATION BY FACTORIAL DESIGN:

VARIABLE	Code
Conc^{n} of SLS	X1
Ratio Of PVP to PVA	X2
Conc^{n} of peg400	x3

Table no 2: variable with code

Batch no	-1	0	+1
X1	0%	2%	4%
X2	1 : 1	1 : 3	1 : 6
x3	20%	30%	40%

Table no 3: variable code with concentration

B1	-1	-1	-1
B2	-1	-1	0
B3	-1	-1	+1
B4	-1	0	-1
B5	-1	0	0
B6	-1	0	+1
B7	-1	+1	-1
B8	-1	+1	0
B9	-1	+1	+1
B10	0	-1	-1
B11	0	-1	0
B12	0	-1	+1
B13	0	0	-1
B14	0	0	0
B15	0	0	+1
B16	0	+1	-1
B17	0	+1	0
B18	0	+1	+1
B19	+1	-1	-1
B20	+1	-1	0
B21	+1	-1	+1
B22	+1	0	-1
B23	+1	0	0
B24	+1	0	+1
B25	+1	+1	-1
B26	+1	+1	0
B27	+1	+1	+1

Table no 4: Code for batch of films:

EVALUATION OF SUBLINGUAL FILMS:[9,10]

physicochemical parameters[9]

The average weight each of 10 samples of each formulation was determined. The thickness of each of sample was measured using micrometer screw gauge at five locations, and the mean thicknesses were calculated. The folding endurance was determined by repeatedly folding one film at the same place till it broke or folded up to 300 times which is considered satisfactory to reveal good film properties. The number of times the film could be folded at the same place without breaking gives the value of the folding endurance. The surface pH of films was determined to investigate the possible side effect because of change in pH *in vivo*, since an acidic or alkaline pH may cause irritation to buccal mucosa. The film to be tested was placed in a Petri dish and was moistened with 0.5 mL of distilled water and kept for 30 s. The pH was noted after bringing the electrode of the pH meter in contact with the surface of the formulation and allowing equilibrating for 1 min. The average of 10 determinations for each of the formulation was taken. The results for all the films are shown in Table. The film formulations were also subjected to IR spectral studies to determine compatibility between drug and other components in the films.

Measurement of swelling index

The studies for swelling index of the film were conducted in simulated salivary fluid of pH 6.75. The film sample (surface area 4 cm2) was weighed and placed in a preweighed stainless steel wire sieve of approximately 800-µm mesh. The mesh containing the film sample was submerged into 50 mL of simulated salivary medium contained in a mortar. At definite time interval (30 s), the stainless steel mesh was removed, excess moisture removed by carefully wiping with absorbent tissue and reweighed. Increase in weight of the film was determined at each time interval until a constant weight was observed. The degree of swelling was calculated using the formula

$$SI=w_t-w_o/w_o$$

where SI is the swelling index, *W*t is the weight of the film at time '*t*', and *W*o is the weight of film at $t = 0$.

Tensile strength measurement

This mechanical property was evaluated using instrument were both end of film were griped with clamps. One clamp was joined with stable end and another was threaded with plate on which different weight for measurement of film strength can be placed. The force and elongation were measured when the film broke. Results from film samples, which broke at and not between the

clamps, were not included in the calculations. Measurements were run in triplicate for each film. Two mechanical properties namely, tensile strength (TS) and percentage elongation were computed for the evaluation of the film. TS is the maximum stress applied to a point at which the film specimen breaks and can be computed from the applied load at rupture as a mean of three measurements and cross-sectional area of fractured film from the following equation.

Tensile strength =Force at break/Initial cross-sectional area of the sample (mm2)

Percentage elongation can be obtained by following

equation:

%Elongation at break = Increase in length/Original length × 100

Uniformity of drug content

This parameter was determined by dissolving one film of dimension 2 cm × 2 cm containing 4 mg of ondansetron hydrochloride by homogenization in 100 mL of stimulated saliva of pH 6.8 for 30 min with continuous shaking. From this, 10 mL was diluted to 50 mL with simulated salivary fluid. The absorbance was measured at 522 nm using an UV spectrometer. The experiments were carried out in triplicate for the films of all formulations and average values were recorded and given in.

In vitro dissolution studies

Dissolution profile of fast dissolving films of ondansetron hydrochloride was carried out using USP type II (paddle apparatus) with 300 mL of simulated salivary fluid (pH 6.8) as dissolution medium maintained at 37 ± 0.5 °C. Medium was stirred at 100 rpm. Samples were withdrawn at every 30 s interval, replacing the same amount with the fresh medium. Absorbance was determined by UV spectrophotometer at 522 nm. [13]

Ex vivo permeation studies through porcine oral mucosa

Permeation studies were carried using the diffusion cell of internal diameter of 2.5 cm. Porcine oral mucosa was used as the model membrane. The buccal pouch of the freshly killed pig was procured from the local slaughter house. The buccal mucosa was excised and trimmed evenly from the sides and then washed in isotonic phosphate buffer of pH 6.6 and used immediately. The membrane was stabilized before mounting to remove the soluble components. The mucosa was mounted between the donor and receptor compartments. The receptor compartment was filled with 200 mL of isotonic phosphate buffer of pH 6.8 which was maintained at 37 °C ± 0.2 °C and the hydrodynamics were maintained by stirring with a magnetic bead at 50 rpm. One film of dimensions 2 cm × 2 cm and previously weighed was placed in intimate contact with the mucosal surface of the membrane that was previously moistened with a few drops of simulated

saliva. The donor compartment was filled with 1 mL of simulated saliva of pH 6.8. Samples were withdrawn at suitable interval, replacing the same amount with the fresh medium. The percentage of drug permeated was determined by measuring the absorbance in a UV–Visible spectrophotometer at522 nm.

Ex vivo mucoadhesion time and drug release

The ex vivo mucoadhesive time was performed by application of the film on freshly cut porcine buccal mucosa. The porcine tissues were fixed on the internal side of a beaker with cyano acrylate glue. The film was wetted with 50 µL of simulated saliva fluid and was pasted to the porcine buccal tissue by applying a light force with a fingertip for 20 s. The beaker was filled with 200 mL simulated saliva fluid and kept at 37 °C. After 2 min, a 50- rpm stirring rate was applied to simulate the buccal cavity environment and during the test, the time taken for the film to completely erode or detach from the mucosa was observed as the ex *vivo* mucoadhesion time. A 5-mL sample was withdrawn at every 30-s time interval, replacing the same amount with fresh medium. After filtration, the amount of drug in the withdrawn samples was determined by UV spectrophotometer at 522 nm.

Stability studies

The film formulations were also subjected to stability studies by storing them for 8 weeks under environmental conditions such as room temperature of 27 ± 2 °C/65% RH, oven temperature of 40 ± 2 °C/75% RH and in the refrigerator at 4–8 °C. At the end of the period, drug content, swelling index, surface pH, and release profiles were determined.

RESULT AND DISCUSSION

PREFORMULATION

Colour, Odour, Taste and Appearance:

Sr.No.	Parameter	observations
1	Colour	red
2	Odour	Odourless
3	Appearance	Crystalline powder

Table 5: Colour, Odour and Appearance of methylcobalamin

Determination of Solubility study:

Solvent	Solubility	Terms
Water	5 mg/ml	Freely soluble

Table 6: solubility in water

Melting point:

Reported Melting Point	Observed Melting Point
>300^{0}C	300 - 302^{0}C

Table 7: Melting point of methylcobalamin

Determination of λmax:

Drug	λmax (nm)
cyclophosphamide	522

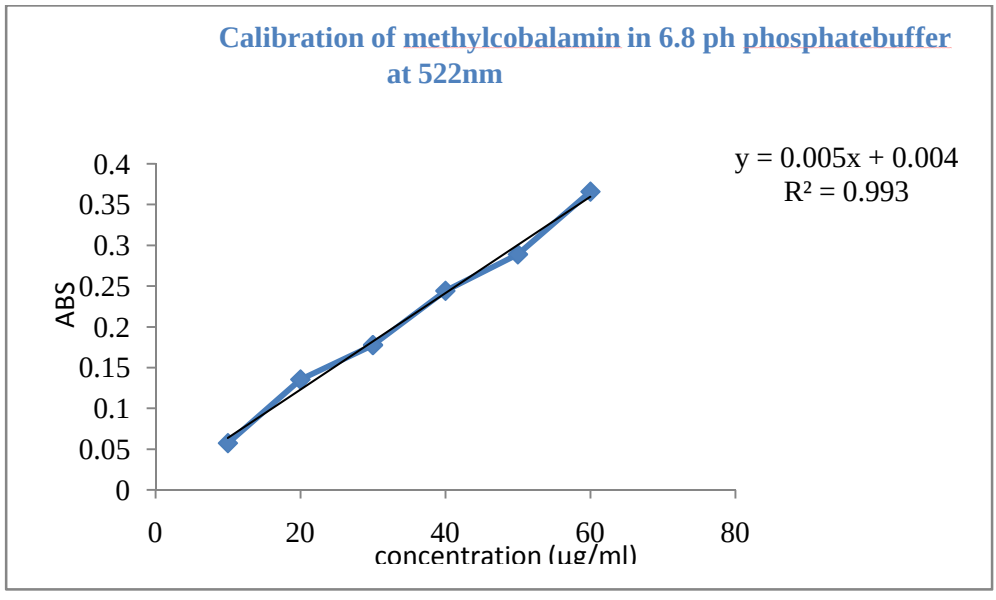
Table 8 : λmax (nm) of methylcobalamin

Calibration curve:

Sr no.	Concentration (µg/ml)	Absorbance			Mean ± SD*
		1	2	3	
1	**10**	0.056	0.058	0.058	0.057 ±0.001
2	**20**	0.135	0.137	0.134	0.135 ±0.001
3	**30**	0.176	0.178	0.179	0.177 ±0.001
4	**40**	0.244	0.246	0.243	0.244 ±0.001
5	**50**	0.287	0.289	0.291	0.289 ±0.002
6	**60**	0.365	0.367	0.366	0.366 ±0.001

n=3

Table 9: Calibration curve of methylcobalamin in phosphate buffer pH 6.8 at λmax 522 nm



n=3

Figure 3 : methylcobalamin calibration curve in phosphate buffer 6.8 pH at λmax 522 nm

Drug Polymer compatibility Study

FT-IR studies:

Plain drug methylcobalamin:

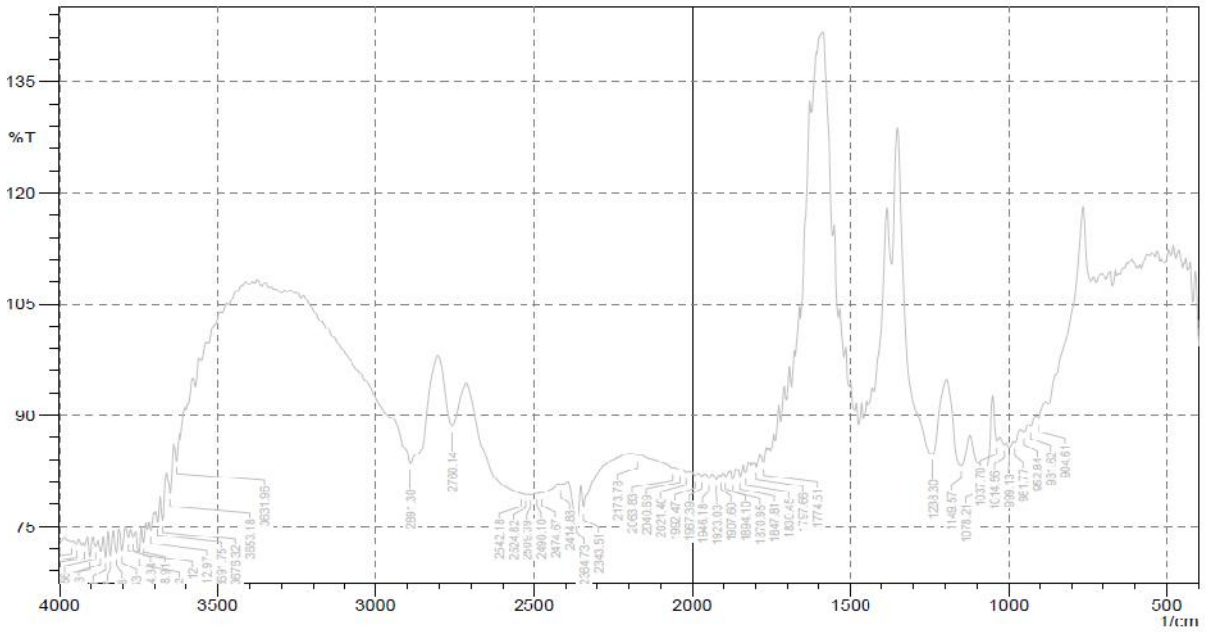


Figure 4: FT-IR Spectrum of pure drug in range 4000 to 400 cm $^{-1}$.

Pure drug +PVA

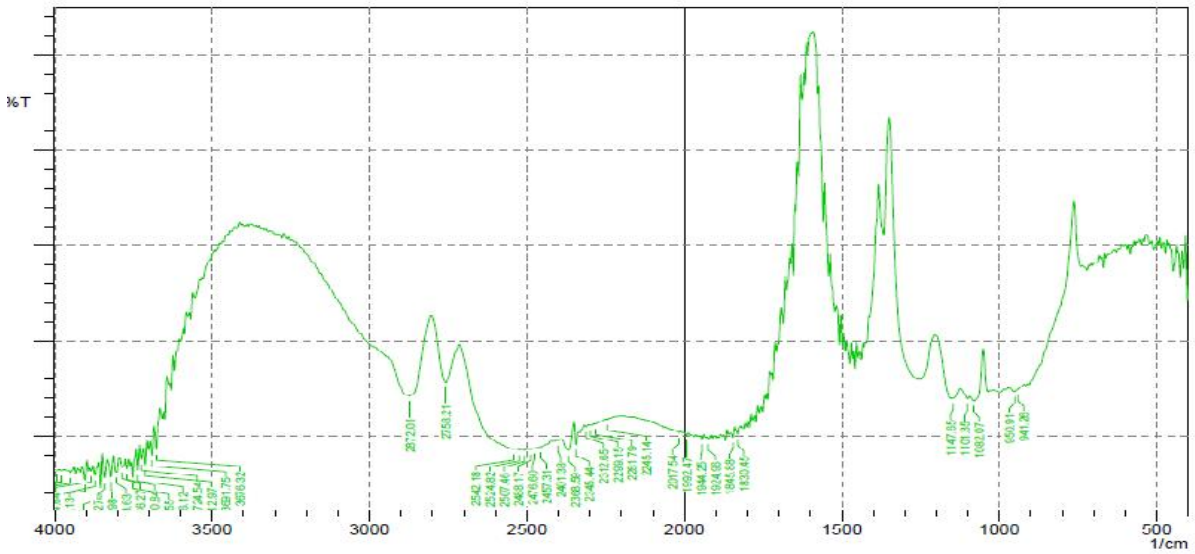


Figure 5: FT-IR Spectrum of pure polymer PVA in range 4000 to 400 cm^{-1}.

pure drug+PVP

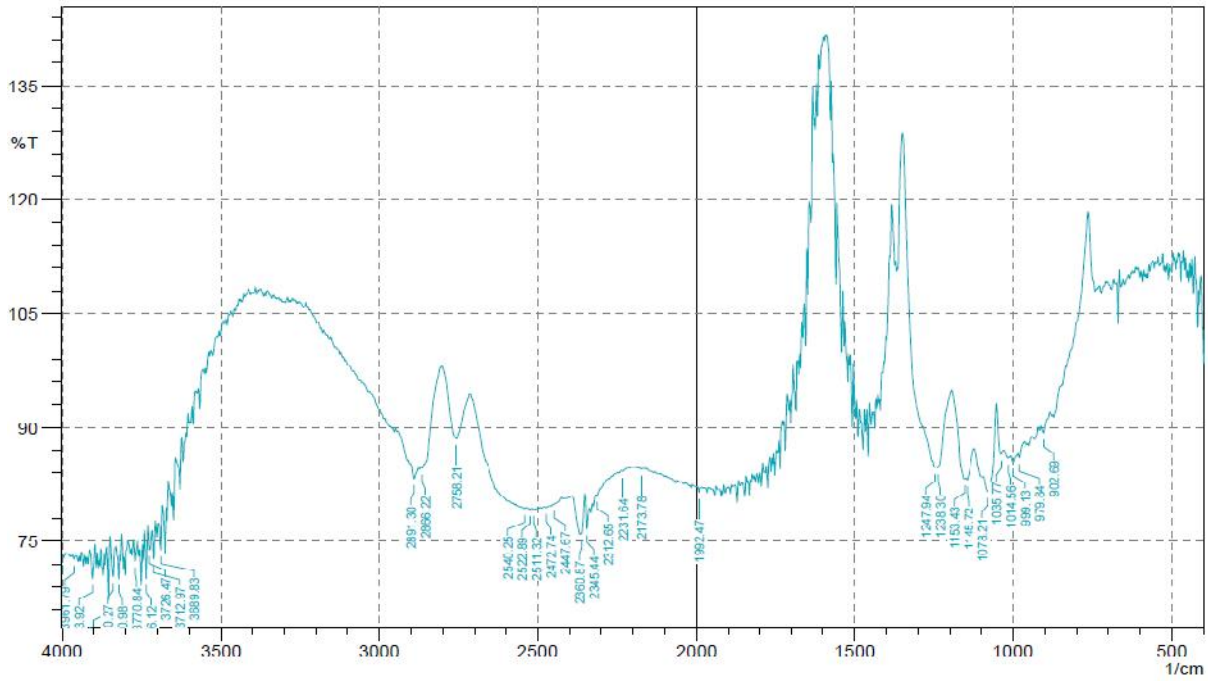


Figure 6 : FT-IR Spectrum of pure polymer PVP in range 4000 to 400 cm^{-1}.

Pure drug+ SLS

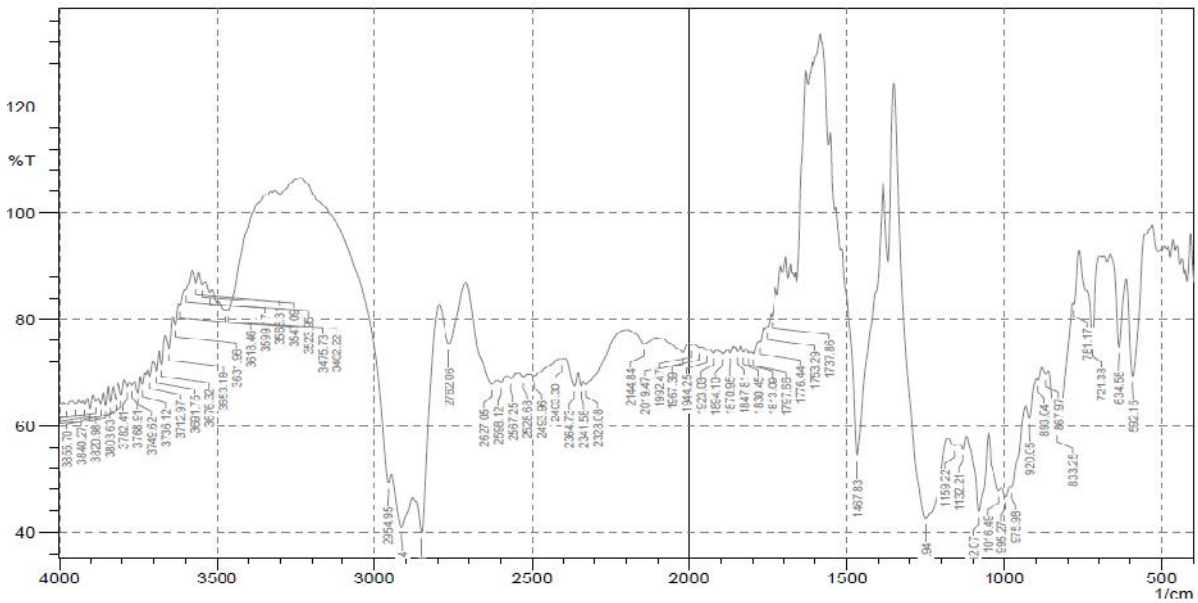


Figure 7: FT-IR Spectrum of drug+SLS in range 4000 to 400 cm^{-1}.

Pure drug +d-mannitol:

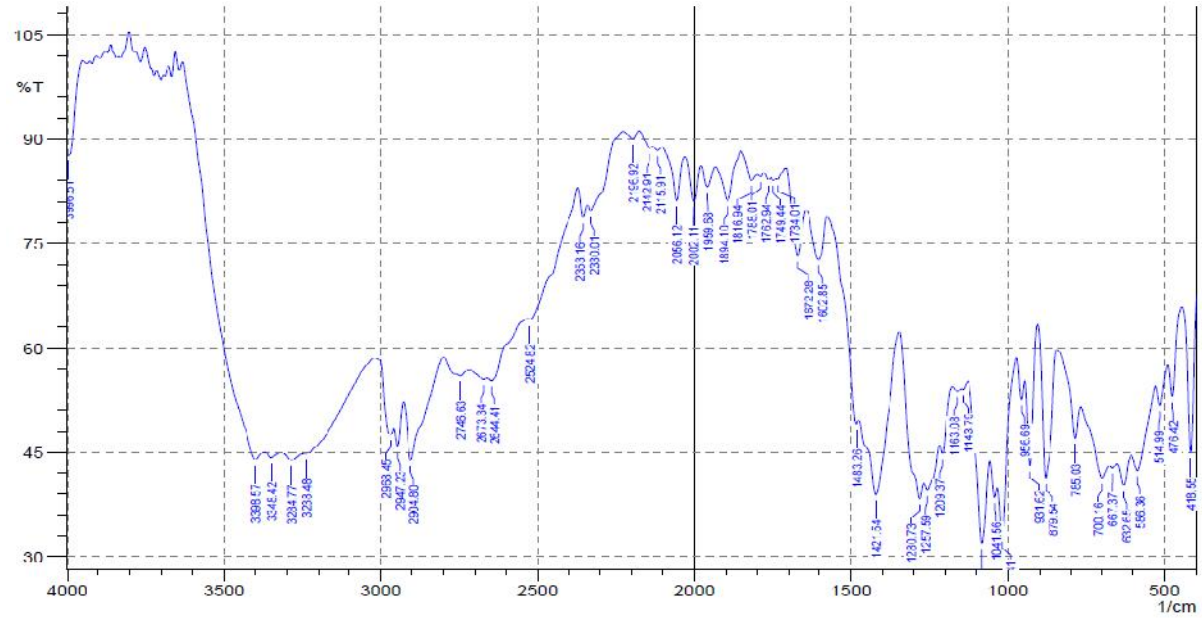


Figure 8: FT-IR Spectrum of drug+d-mannitol in range 4000 to 400 cm^{-1}.

DRUG + PEG400

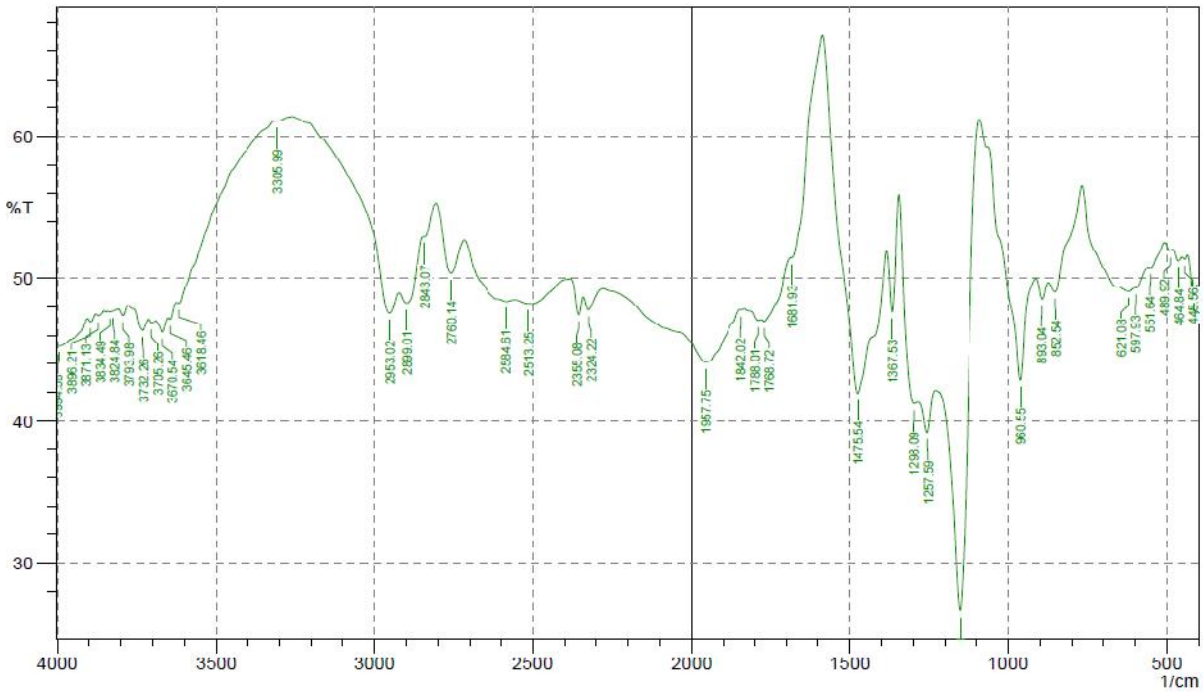


Figure 9: FT-IR Spectrum of drug+PEG-400 in range 4000 to 400 cm^{-1}

EVALUATIONS OF FORMULATION

Sr no.	variable	Batch		
		8	**17**	**26**
1	Tensile strength (gm)	128.01±0.3	128.01±0.2	128.10±0.3
2	Folding endurance	>300	>300	>300
3	Thickness (mm)	0.55±0.02	0.55±0.01	0.56±0.01
4	pH (solution)	7.37±0.2	7.39±0.2	7.39±0.4
5	Solubility (in salivery solution)	Freely soluble	Freely soluble	Freely soluble
6	Drug content (per 4×4cm^2 area)	99.96%±0.1	99.96%±0.17	99.98%±0.14
7	%elongation	5%±0.04	5%±0.03	5%±0.03
8	Disintegration time (sec)	29.40±0.03	29.39±0.03	29.40±0.02
9	Weight variation (per 4×4cm^2 area in %)	0.010±0.01	0.010±0.02	0.01±0.02

Table 10: Data of tensile strength, folding endurance, thickness, pH, solubility, drug content, %elongation, disintegration time, weight variation.

In-vitro Diffusion Study:

Time (sec)	%CDR		
	B8	**B17**	**B26**
0	0	0	0
30	18.98±0.020	19.12±0.04	19.22±0.027
60	28.63±0.04	29.42±0.037	29.86±0.020
90	39.41±0.055	40.21±0.024	40.37±0.014
120	54.16±0.037	54.27±0.027	54.35±0.041
150	80.14±0.050	80.96±0.014	81.28±0.024
180	93.40±0.027	93.47±0.020	94.88±0.021
210	97.25±0.017	98.37±0.020	98.63±0.030

Table 11: % Cumulative Release Data of B8,B17 and B26 batches.

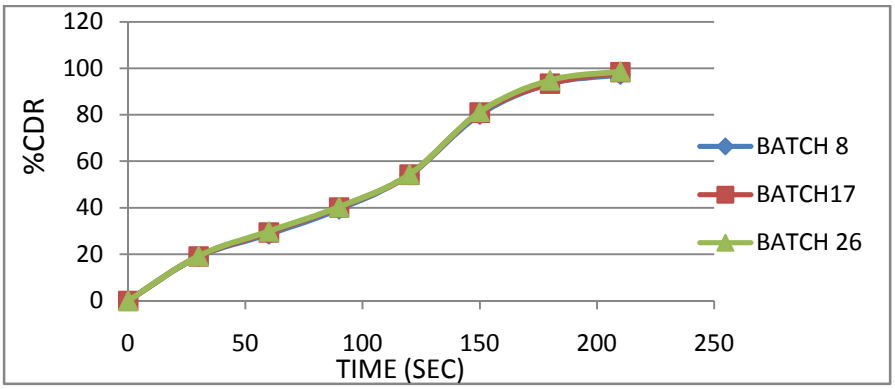


Figure 10- In vitro dissolution profile of B8, B17, B26

TIME (MIN)	%DRUG PERMITED		
	B8	B16	B26
0	0	0	0
1	5.98±0.010	7.15±0.017	8.13±0.031
2	16.18±0.018	18.22±0.026	20.12±0.042
3	26.52±0.021	30.22±0.021	33.48±0.021
4	45.20±0.012	52.84±0.014	57.29±0.030
5	64.40±0.024	70.51±0.019	73.92±0.03
6	70.20±0.017	78.52±0.018	83.18±0.032
7	80.27±0.032	92.40±0.011	97.94±0.020

Table 12: Ex-vivo Diffusion Study of B8, B17, B26

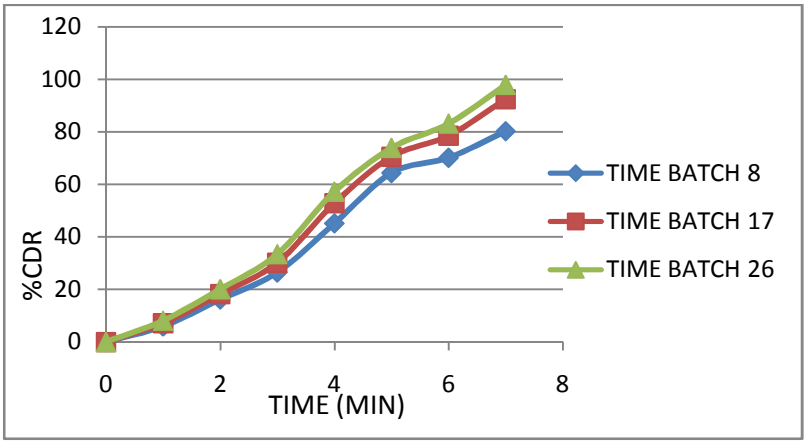


Figure 11- In vitro diffusion profile of B8, B17, B26

Stability studies

Physical properties and %drug content.

Time (days)	2-8^{0}C temperature and 45% relative humidity		25-30 ^{0}C temperature and 60% relative humidity		45-50^{0}C temperature and 75% relative humidity	
	Physical properties	%Drug content	Physical properties	%Drug content	Physical properties	%Drug content
0	No change	99.98±0.04	No change	99.98±0.02	No change	99.98±0.02
15	No change	99.97±0.02	No change	99.96±0.01	Hard	99.96±0.01
30	No change	99.97±0.01	No change	99.96±0.04	Harder	99.96±0.04
45	No change	99.96±0.02	No change	99.96±0.01	Brittle	99.96±0.05

Table 13: Physical properties and %drug content

%cumulative drug release

Time (sec)	2-8^{0}C temperature and 45% relative humidity			
	Days			
	0	15	30	45
0	0	0	0	0
30	19.20±0.02	19.18±0.01	19.15±0.03	19.01±0.02
60	29.86±0.02	29.80±0.04	29.78±0.01	29.70±0.04
90	40.32±0.01	40.28±0.02	40.24±0.02	40.20±0.01
120	54.32±0.04	54.28±0.03	54.24±0.01	54.19±0.01
150	81.21±0.02	81.18±0.03	81.12±0.02	81.07±0.01
180	94.84±0.02	94.75±0.02	94.70±0.01	94.64±0.02
210	98.60±0.03	98.58±0.01	98.50±0.02	98.30±0.01

Table 14: 2-8^{0}C temperature and 45% relative humidity

Time (sec)	**25-30 ^{0}C temperature and 60% relative humidity**			
	Days			
	0	15	30	45
0	0	0	0	0
30	19.22±0.01	19.20±0.01	19.12±0.01	19.00±0.01
60	29.84±0.02	29.82±0.02	29.72±0.02	29.64±0.03
90	40.33±0.02	40.24±0.04	40.22±0.01	40.18±0.01
120	54.30±0.03	54.22±0.03	54.28±0.01	54.14±0.02
150	81.24±0.01	81.14±0.03	81.17±0.03	81.00±0.01
180	94.85±0.02	94.72±0.02	94.64±0.01	94.62±0.04
210	98.62±0.04	98.50±0.01	98.47±0.02	98.28±0.01

Table 15: 25-30 ^{0}C temperature and 60% relative humidity

Time (sec)	**45-50^{0}C temperature and 75% relative humidity**			
	Days			
	0	15	30	45
0	0	0	0	0
30	19.20±0.02	10.17±0.01	9.12±0.01	7.00±0.01
60	29.82±0.01	22.80±0.02	20.72±0.02	14.64±0.03
90	40.32±0.03	32.27±0.04	30.22±0.01	20.18±0.01
120	54.30±0.02	47.24±0.03	45.28±0.01	32.14±0.02
150	81.24±0.02	74.17±0.03	70.17±0.03	47.00±0.01
180	94.85±0.01	82.84±0.02	78.64±0.01	54.62±0.04
210	98.62±0.03	87.70±0.01	82.47±0.02	62.28±0.01

Table 16: 45-50^{0}C temperature and 75% relative humidity

CONCLUSION

The main objective of the study was to formulate and evaluate fast dissolving films containing methylcobalamin. The fast dissolving film can be easily formulated by solvent casting method by using polymers such as PVP, PVA and PEG-400 in different ratio with sweetener like d-mannitol and penetration enhancer like SLS. Compatibility of methylcobalamin with polymer and other was proved by FT-IR studies. It was observed that the physical characteristics like uniformity of weight, thickness, folding endurance, surface pH, and uniformity of drug content of the entire film sample showed satisfactory results. Also the penetration of methylcobalamin increases with concentration of SLS. B26 batched film gives all satisfactory results. So it was considered as a best formulation apart from all other formulations.

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