

Formulation and Evaluation of pH-Induced Povidone Iodine in Situ Gel for Oral Thrush

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Abstract: Candida species have become a major opportunistic pathogen causing recurrent oral thrush and oropharyngeal candidiasis topical delivery of antimicrobial agents is the most widely accepted approach aimed at prolonging active drug concentrations in the oral cavity. As most antifungal do not possess inherent ability to bind to the oral mucosa, This is best achieved through improved formulations, buccal adhesive gels are successfully used as drug delivery system consider their ability to prolong the drug release, the viscosity of the formulation was found to be in the fluid state at pH (5.8 to 6.0) before administration underwent rapid gelation upon pH 7.4. buccal in situ gels were formulated using different concentration of ion sensitive gellan gum (0.1 to 0.3), sodium alginate (0.1 to 0.5) and pH sensitive carbopol 934 (0.1 to 0.2) along with prolonged release of PVP iodine (2%). formulation were evaluated for gelling capacity, viscosity, Gel strength, Bioadhesive force, spreadability drug content, FTIR and DSC.

Keyword: (PVP-iodine, oral thrush, mucoadhesive gel, gellan gum, carbopol 934, sodium alginate and pH-induced.)

Introduction: candida species have become a major opportunistic pathogen causing recurrent oral thrush and oropharyngeal candidiasis. one third of healthy person with OPC symptoms will have an oral cavity culture that is positive for Candida albicans the most common species for oral candidiasis. this has resulted in frequently high use of expensive antifungal drug like (clotrimazole, amphotericin B, fluconazole, itraconazole, ketoconazole and nystatin) which most of these patients mentality and they look for cheap and best prolonged effective drugs to manage this problem. The medical cost of care using PVP-iodine was very low compared with antifungal care¹. The use of inexpensive topical alternatives, e.g. oil of melaleuca (tea tree oil (TTO)), chlorhexidine (CHX), povidone iodine (PI) and gentian violet (GV), to treat oral candidiasis². Povidone iodinated is a complex of iodine and povidone. it contains not less than 9% and not more than 12% of available iodine from this complex iodine is released for prolonged time, concentration range of PVP-iodine between 3.75 -2500 ppm kill the Candida strain between 10 to 120 seconds^{3,4}. PVP-iodine and their preparation are official in USP, European pharmacopoeia and are recognised as effective broad spectrum biocidal agent the invitro biocidal activity has been studied for years against bacteria, yeast, moulds, viruses, fungi, protozoa, actinomycetes and rickettsia⁵. Compared to other

preparations PVP-iodine exhibits markedly lower oral toxicity. Consequently, the accidental ingestion of PVP iodine solution is much less hazardous than from equal amounts of available iodine solutions. For this reason, PVP-iodine solutions do not require the hazardous, poisonous warning labels on bottle that iodine products must have. Moreover, animal and exposure tests have revealed virtually no skin reactions to PVP-iodine, and only very mild transitory effects on mucous membranes⁶.

Advantage:

- Stable complex
- Film Forming capacity
- Prolonged germicidal action
- Adheres to treated surface
- Water soluble so ease for formulation
- Non irritating to skin and mucous membranes
- It has reduced amount hazard

Candidiasis is an opportunistic infection condition caused by ubiquitous, saprophytic fungi of the genus Candida which includes eight species of fungi, the most common of which is Candida albicans. Oral candidiasis is one of the most common pathological conditions affecting the oral mucosa^{7,8}. In situ gel able to reside in oral cavity for an extended period for more effective candidiasis eradication. PVP iodine constitutes a valuable adjunct to current periodontal therapy because of its broad spectrum of antimicrobial activity, low potential for

developing resistance and few adverse reactions, its antifungal activity against OC has not been studied before⁹.

Material: Gellan gum were gifted by priya multinational (Mumbai), Carbopol 934 and Sodium alginate was procured from Merck Ltd (Mumbai) PVP iodine was a gift sample from Bliss chemicals pharmaceutical India Ltd.Thane,All other chemicals were of research grade.

Method: Different formulation were prepared with varies ratio of (gellan gum: carbopol 934),(gellan gum:sodium alginate),(sodium alginate:carbopol 934),(gellan gum:sodium alginate:carbopol 934).many experiments were conducted varying the concentration of those polymer in order to identify the optimum

concentration required for polymer solution.

Step 1: Required quantity of gellan gum, sodium alginate, carbopol 934 was kept overnight for swelling.

Step 2.Required volume of deionised water was added to the mixture along with propyl paraben.

Step 3.The polymer solutions were mixed with magnetic stir, until uniform solution obtained.

Step 4.An appropriate amount of PVP iodine was added to this polymer solution with continuous stirring until uniform solution obtained.

Step 5. Finally a small amount of Triethanolamine was added to adjust pH.

Table 1: Composition of formulation code I

s. n o	Ingredients (w/w)%	I ₁	I ₂	I ₃	I ₄	I ₅	I ₆	I ₇	I ₈	I ₉	I ₁₀	I ₁₁	I ₁₂	I ₁₃
1	PVP- iodine	2	2	2	2	2	2	2	2	2	2	2	2	2
2	Gellan gum (a)	0.1	0.2	0.3	0.1	0.2	0.3	--	--	--	0.1	0.2	0.3	0.3
3	Sod.algi-nate (b)	--	--	--	0.2	0.3	0.4	0.1	0.2	0.3	0.4	0.5	0.3	0.5
4	Carbopol-(c)	0.2	0.2	0.2	--	--	--	0.2	0.2	0.2	0.2	0.2	0.2	0.2
5	Propyl paraben	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001
6	Triethan-ol amine	q.s	q.s	q.s	q.s									
7	Deionize-d water	q.s	q.s	q.s	q.s									
	Gelling capacity	**	**	**	*	*	*	--	--	--	***	***	***	***

** Gelation immediately, remains for after few hours (stiff gel); * Gelation immediately, remains few hours (moderate gel); -- No gelation; *** Gelation immediately, remains for extended period of time (stiff gel)

Evaluation of in situ gel:

Determination of pH: The PH of the gel was determined using a calibrated pH meter. The readings were taken for average of 3 samples.

Gelling Capacity: The gelling capacity of the formed gel was determined visual inspection and the different grades were allotted as per the gel integrity, weight and rate of formation of gel with respect to time.

Viscosity Studies: The rheological studies were carried out using Brookfield programmable DVII+ Model pro II type (USA). The viscosity of in situ gel and the solution were determined at different angular velocities(0,10,20,30,40....to 100 rpm) average of two reading were used to calculate the viscosity. Evaluation was conducted in triplicate.

Spreadability: For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability (S)

$$S = ML/T$$

Where, M = weight tide to upper slide.

L = length moved on the glass slide.

T = time taken.

Measurement of Gel Strength⁷: A sample of 50 Gms of gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at 37⁰c. The apparatus for measuring gel strength (weigh or apparatus as shown in figure 1, weighing 27 gm) was allowed to penetrate in buccal gel. The gels strength, which means the viscosity of the gels at physiological temperature, was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel.

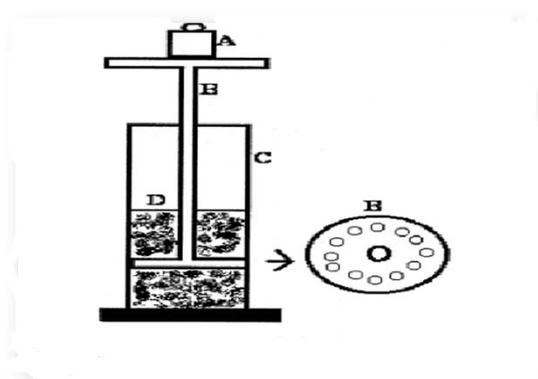


Figure 1.diagrammatic representation of apparatus used for finding gel strength.

Determination of mucoadhesive Force ⁸.

⁹:The mucoadhesive force of all the

optimized batches was determined as follows, a section of mucosa was cut from the chicken cheek portion and instantly fixed with mucosal side out onto each glass vial using rubber band. The vial with chicken cheek mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan. Oral gel was added onto the buccal mucosa of first vial. . Before applying the gel, 150μL of simulated saliva solution (2.38 g Na₂HPO₄, 0.19 g KH₂PO₄ and 8 g NaCL in 1000 ml of distilled water adjusted to pH 7.4) was evenly spread on the surface of the test membrane. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then weight was kept rising in the pan until vials get detached. Mucoadhesive force was the minimum weight required to detach two vials. The cheek mucosa was changed for each measurement.

$$\text{Detachment stress (dynes/cm}^2\text{)} = mg/A$$

Where m is the weight added to the balance in grams; g is the acceleration due to gravity taken as 980 cm/s²; and A is the area of tissue exposed. All the above mentioned experiments were carried out in triplicates.

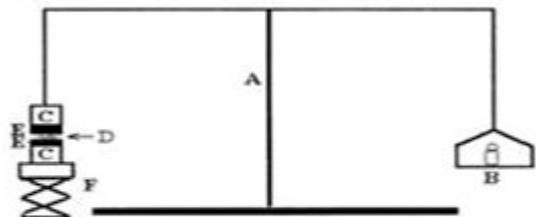


fig.2.bioadhesive force measuring device, (A)modified balance,(B)weights,(C)glass vial, (D)gellan based gel,(E)chicken cheek mucous membrane,(F)height adjustable pan.

Available iodine contents: Transfer 1.0 gm of PVP iodine in situ gel system into a round bottom stoppered iodine flask containing 150 ml of water and stir for 1 hour. Add 0.1 ml of diluted acetic acid and titrate against 0.1M sodium thiosulphate using starch solution as indicator towards the end¹⁰.

1ml of 0.1M sodium thiosulphate is equivalent to 12.69 mg of available iodine.
 $\text{Titre volume} \times \text{Molarity factor of sodium thiosulphate} \times \text{equivalent factor} \times 100$

Weight taken in (gm) sample
 = % gm of available iodine

Diffusion across the chicken cheek mucosa: Buccal cavity of Isolation of chicken cheek mucosa from the chicken – the cheek of a healthy chicken was obtained from the local slaughter house. It was cleaned and the mucosa was re-moved from the buccal cavity. The mucosa was stored in normal saline with few drops of gentamycin sulphate injection, to avoid bacterial growth. After the removals of blood from the mucosal surface it becomes ready for use.

Diffusion Medium: The diffusion medium used was phosphate buffer pH 7.4. Assembly of diffusion cell-For in – vitro diffusion studies the oral diffusion cell was designed as per the dimension given. The diffusion cells were placed on the magnetic stirrers. The outlet of the reservoir maintained at $37 \pm 0.5^{\circ}\text{C}$ and was connected to water jacket of diffusion cell using rubber latex tubes. The receptor compartment was filled with fluid. Then the prepared chicken cheek mucosa was mounted on the cell carefully so as to avoid the entrapment of air bubble under the mucosa. Intimate contact of mucosa was ensured with receptor fluid by placing it tightly with clamp. The speed of the sitting was kept content throughout the experiment. With the help of micropipette 10 ml of sample was withdrawn at a time intervals of one hour from sampling port of receptor compartment and same volume was the replaced with receptor fluid solution in order to maintain sink condition. The samples were withdrawn and drug content was carried out as per the above procedure.

Results and Discussion:

- Polymer code: (a) Gellan gum
 (b) Sodium alginate
 (c) Carbopol 934

Table 2: Physicochemical properties of PVP iodine solution

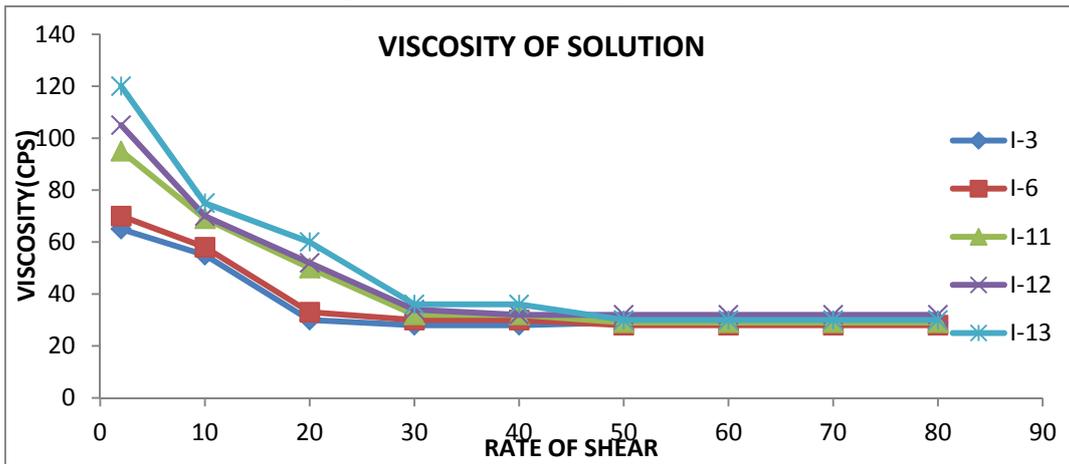
Physicochemical properties	I ₃	I ₆	I ₁₁	I ₁₂	I ₁₃
Visual appearance	+	+	+	+	+
pH	6.0	6.0	6.0	5.9	6.0
content uniformity % (w/w)	98.5	98.2	98.6	98.5	98.5

Viscosity: The viscosity of gels of various formulations was determined at various shear rates. As the shear increased the viscosity of gel decreased and also the increase in gellan gum ratio to about caused increase of viscosity in solution. The formulation I₁₃ having the maximum concentration of gellan gum and sodium alginate, Carbopol 934 showed maximum viscosity in solution forms as well as in gel Form. It is also predictable that the formulation I₁₃ having higher concentration of polymer were poor candidate for in situ formulation.

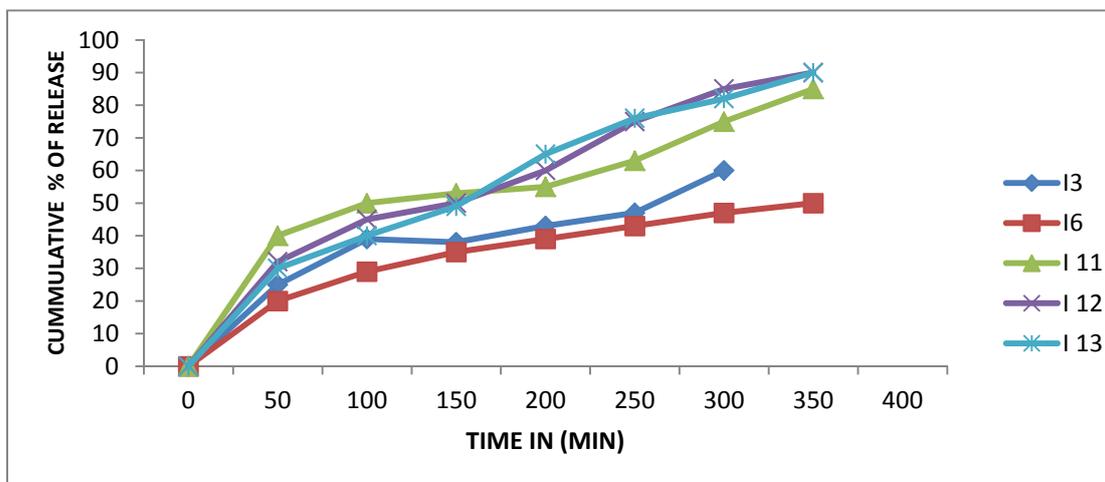
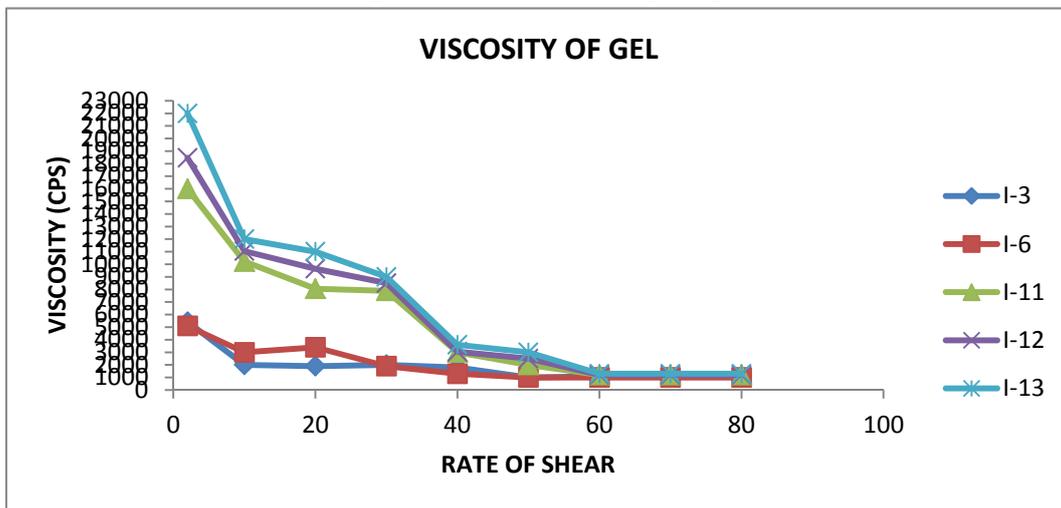
The effect of pH: All the optimized formulations were fluid state at pH (5.8 to 6.0) before administration underwent rapid gelation upon pH 7.4 after administration. Easily neutralized by the buffering action of the salivary fluid pH 7.4. All three polymer were utilized without compromising the gelation capacity and rheological properties of the delivery system may be achieved by the addition of polymer (a: c, a: b, a: b: c) except (b: c), It was also found that combination of (b:c) could not show better sol to gel conversion at stimulated saliva pH.

Gelling Capacity: The two main prerequisites of an in situ gelling system are viscosity and gelling capacity. All the optimized formulation shown good gelling capacity. The formulation I₃ exhibited good gelation immediately, remains for after few hours. In comparison with I₃ the formulation I₆ showed (moderate) gelling capacity remains, few hours and the in situ system I₇, I₉, I₁₀ could not showed any gelation. In comparison with I₃ and I₆ the formulation I₁₁, I₁₂, I₁₃ found to be good

Graph-(a) shows viscosity of solution



Graph-(b) shows viscosity of gel



Graph-(c) Shows the In Vitro Release Profile of PVP Iodine Gel

gelling capacity remain for extended period of time.

Gel Strength: -Gel strength of formulation I₃ was found to be more as compared to formulation I₆. All the

formulation (I₁₁,I₁₂,I₁₃) exhibited good gel strength. This may be due to increase concentration of Gellan gum and Sodium alginate and carbopol934 polymers in the formulation

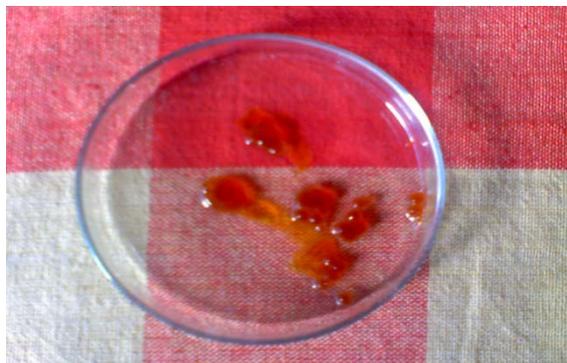


Figure 1.Photography showing the appearance of in situ gel formed in simulated artificial Saliva fluid pH 7.4.

Bioadhesive Force: - The mucoadhesive force is an important physicochemical parameters for prolonging buccal retention time and there by better therapeutic effects. Detachment stress of I₃ was found

to more in comparison with I₆ formulation. The formulation (I₁₁,I₁₂,I₁₃) showed more mucoadhesive force than I₃ and I₆. This may be due to increased concentration of Gellan gum and Sodium alginate make along with carbophol 934 in the formulation.

In vitro Release Studies: The in vitro dissolution profile of PVP iodine from in situ gels containing different composition of (a: c, a: b, a: b: c) .The formulation I₃ and I₆ containing the two polymer ratio (0.3:0.2) ,(0.3:0.4).which showed the release profile up to 5 hours with 60% release and 6 hours with 50%.the in situ system were not found to be ideal formulations for prolong retention on buccal mucous membrane.In comparison, the gels containing(a: b: c) Polymers in situ system(I₁₁,I₁₂,I₁₃) were showed 89%,90% and 90% of release profile within 6 hours.

Table 3: Characteristics of optimized gel

formulation	pH	Viscosity (cps)	Content uniformity(%w/w)	Mucoadhesive force(dynes/cm ²)	Gel strength(sec)	Spreadability gms/sec
I ₃	6.0	5420	96.4	17726	97	26.7
I ₆	6.0	6200	96.7	14668	74	24.0
I ₁₁	5.9	16000	98.2	15449	108	26.3
I ₁₂	6.0	18460	98.5	18654	115	26.9
I ₁₃	5.9	22000	98.5	18680	121	30.5

Table 4: FTIR- Spectra

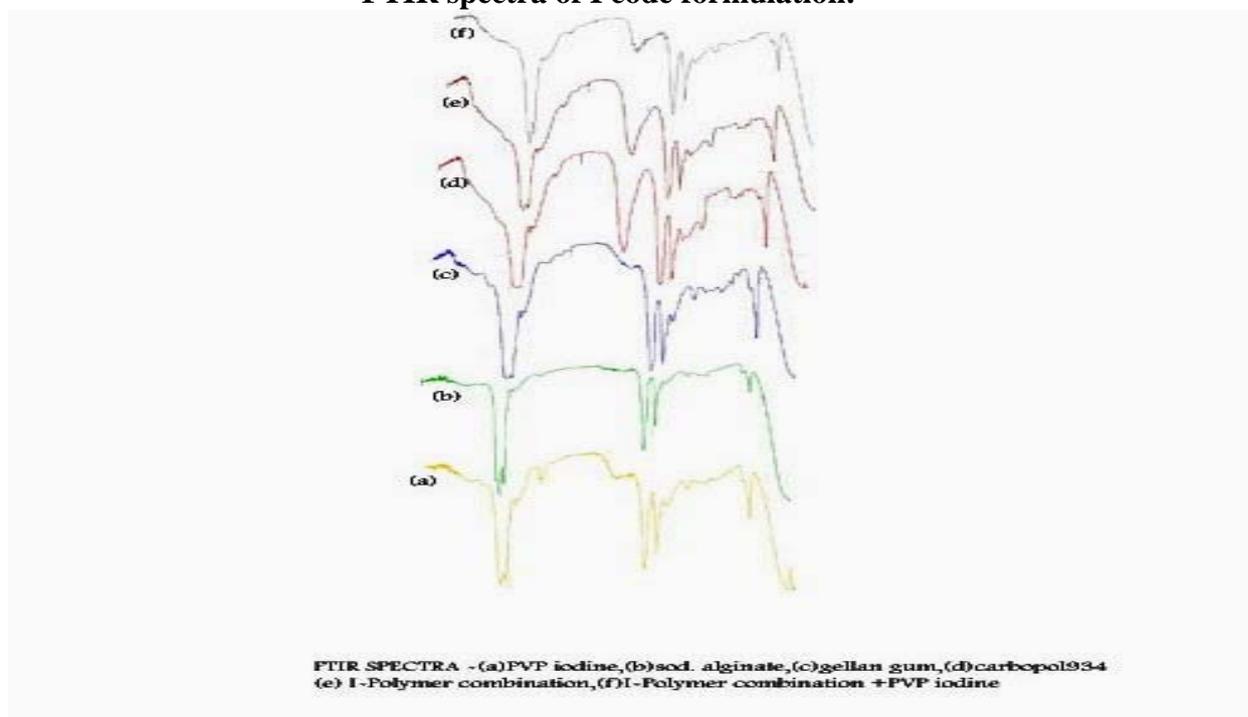
Compound	C-H stretching	Carboxylate amine stretching	Polymeric Associated	OH Bending	Free OH
Gellan gum + Sod.alginate + Carbopol 934 *	2960	1720	3520	1100	3650
Gellan gum + Sod.aginate + Carbopol 934 + PVP Iodine	2960	1460	1380	720	3650

PVP Iodine *	2960 C-H Stretchin-g Ar.	1460 C-C Multiple bond stretching (Aromatic)	1380 C=O Stretching Ar.Tertiary	720 Adjacent hydrogen	
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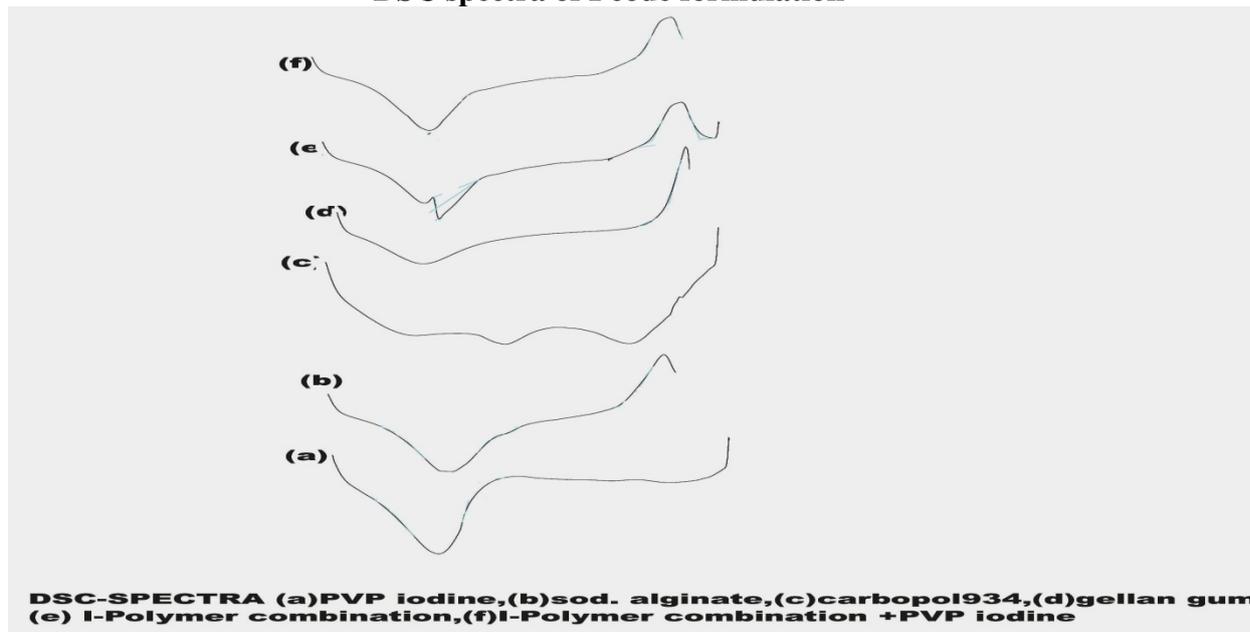
PVP Iodine and Gellan gum + Sodium alginate + Carbopol 934 bonds were analysed and It is found that the bonds of PVP Iodine and polymer mixture were not disturbed so PVP-Iodine can be formulated with the mentioned above polymer to given the same activity of PVP-Iodine like other formulation.

DSC Spectra: PVP iodine endothermic peak at 106.8, sod. Alginate peak at 119.29 and 257.73, gellan gum peak at 266.56, I-combination of polymers peak at 114.14 and 273.09, I-combination of polymers and PVP-iodine peak at 106.7

FTIR spectra of I code formulation:



DSC spectra of I code formulation



Conclusion: The present work was carried out to develop a Novel in situ gel combination with PVP iodine. The methodology adopted for preparation of in situ gel solution was very simple and cost effective. It is newer approach to improve easy buccal instillation residence time and prolong drug release. From the study conducted, the following conclusion were drawn by varying the polymers ratio (a: c), (a: b) and (a: b: c).The in situ systems were offered the increase residence time, which may result in high concentration in local area and faster recovery from the OPC symptoms as compared to conventional PVP iodine preparation.

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