Current Research in Pharmaceutical Sciences
Available online at www.crpsonline.com

ISSN 2250 – 2688

Received: 08/02/2012
Revised: 15/04/2012
Accepted: 22/05/2012

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Development and Characterization of hydrogel of some synthetic and natural polymers for Treatment of Oral Mucositis

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ABSTRACT

Oral mucositis is a recurrent and potentially severe complications caused by chemotherapy. L-glutamine is considerably reduced the duration and severity of oral mucosa during radiotherapy. Formulation containing L-glutamine reduces this side effect. Mucoadhesive hydrogel formulation can deliver the L-glutamine in the buccal cavity in effective concentration. Hydrogel formulations of L-glutamine were prepared using various concentrations of Carbopol 934 NF, Na CMC, HPMC (K-15) and PEG-400 as base and constant concentration of methyl paraben, glycerin, sodium glycocholate as penetrating enhancer. The prepared hydrogel were evaluated for swelling hydration, matrix erosion, drug release, mucoadhesion and shear stress. The formulation containing Carbopol 934 NF (CP-4) has shown more than 80% drug release in 4 hr, which indicate the sustained release behavior is in the rate controlled manner and which adhere to the buccal cavity for upto 6 hr. Stability study indicate that the hydrogel is stable upto 40^{o}C. The formed hydrogel may well tolerable, comfortable, non-irritant, flexible and protective to wounded surface

Keywords: Oral mucositis, hydrogel, mucoadhesive polymer, buccal drug delivery.

1. INTRODUCTION

Oral mucositis is an inflammatory condition of the oral mucosa leading to atrophy and ulceration, which is a common dose limiting and potentially severe complication of chemotherapy or radiation therapy in cancer patients and which increases threat of infections[1]. Terminally ill patient with cancer and/or AIDS will suffer from some or all of these conditions[2]. Other will tend to suffer from a dry mouth and some degree of infection and so will be likely to have oral symptoms. The major clinical approaches have been provided pain relief with local anesthetics, to moisten and coat the oral mucosa with lubricant, and to locally administered antibiotics or antifungal. A serious problem is that many conventional mouth rinses and antimicrobials contain alcohol or astringent and may have unpleasant taste, which exacerbates the conditions [3,4]. Glutamine is a neutral, non-essential most abundant amino acid, comprising about 60% of the total free amino acid pool [5]. It contains two nitrogen moieties and as such, it may also be one of the most versatile amino acid. Regular supplementation of glutamine (0.57 gm/kg body weight/day) not only heals the injuries but also strengthen the mucosa. Thus it protects GI tract from devastating effects of chemotherapy and radiotherapy and patients don't experience mouth as well as abdominal pain [6]. Swish and swallow with glutamine drink will heal the injury and gives relief from the mouth pain. Oral glutamine might significantly reduce the duration and severity of oral mucositis during radiotherapy and may shorten the duration of Grade 3 subjective mucositis. World Health Organization (WHO) step analgesic medication and body weight change were compared between the two arms. Mean maximum grade of objective oral mucositis was less severe in the glutamine arm (1.6 vs 2.6)[7,8]. The flushing action of saliva also rapidly reduces drug concentration, which should be maintained above the minimum inhibitory concentration throughout the therapy.

There is also need for a nonirritative vehicle that will coat the mucosa to enhance lubrication and provide some degree of occlusion. Bioadhesive polymer appears to be particularly attractive for the development of the drug delivery system to improve intraoral administration and reduce the frequency of application and the amount of drug administered[9-11]. Gels and films may be most suitable for this type of application as they are able to cover a wide area of mucosa for both drug delivery and physical protections[12,13]. The aim of this study was to develop mucoadhesive system that can deliver the drug in the buccal cavity in effective concentration for prophylaxis and/or treatment of oral mucositis. For this purpose hydrogel of L-glutamine with different polymer were prepared alongwith sodium glycocholate to promote the buccal absorption of drugs. The sodium glycocholate (bile salt) act by extraction of membrane protein or lipid, membrane fluidization and reverse micellization in membrane creating aqueous channel[14-16].

2. MATERIALS AND METHODS

Materials

L-Glutamine (as L-Glutamic acid-5-amide) and sodium glycocholate were purchased from HiMedia Lab, Mumbai, Carbopol 934 NF, polyethylene glycol (PEG) 400, hydroxy propyl methyl cellulose (K-15), sodium carboxy methyl cellulose, disodium hydrogen phosphate, dihydrogen potassium phosphate, phosphoric acid were purchased from CDH (P) Ltd, New Delhi. All other chemicals were of analytical grade.

Method of Preparation

This method was accepted with slight modification as proposed by Silva S. Gutter 2003, weighed amount of polymer as per "Table (1)" (Carbopol 934 NF, NaCMC, PEG 400, HPMC K-15) methyl paraben (0.1%, preservatives), sodium glycocholate (5%, penetration enhancer) and glycerin (5%, hydrating agent) were placed in the beaker and L-glutamine (drug) was dissolved in 10 ml of water with stirring. The final volume was made up to 100 ml with distilled water[17].

Evaluation of Hydrogel

The prepared hydrogels were evaluated for swelling hydration, matrix erosion, drug release, mucoadhesion, shear stress and stability, which is prerequisite for the mucoadhesive buccal drug delivery. The drug content was determined spectrophotometrically with PBS pH 6.75.

Percentage hydration

Swelling studies of hydrogel was done by placing the formulations in a known amount of PBS (10 ml) and allowed to swell. The initial weight of the gel was compared with the swollen weight. This allows for a transient analysis of the swelling of hydrogel. Weight of the gel was taken after every ten minutes. The PBS was absorbed into the gel thus increasing the weight of the gel with time until equilibrium swelling is reached. While weighing the gel, care was taken to remove the excess PBS on the surface the hydrogel were wiped off from the surface by using filter paper, so that only the weight of the PBS incorporated into the hydrogel was considered. The following equation was used to determine the swelling of the hydrogel and the obtained results are given in Table 2[18]

$$\text{Formula} = \frac{W2 - W1}{W1} \times 100$$

W 1= Initial weight.
W 2 = After PBS treatment

Table No 1.Composition of various hydrogel formulation of L-Glutamine

Formulation	Carbopol 934 NF %	Na CMC %	HPMC K-15 %	PEG 400 %
CP 1	0.25	-	-	-
CP 2	0.50	-	-	-
CP 3	0.75	-	-	-
CP 4	1.0	-	-	-
CP 5	1.25	-	-	-
CP 6	-	1	-	-
CP 7	-	2	-	-
CP 8	-	3	-	-
CP 9	-	4	-	-
CP 10	-	5	-	-
CP 11	-	-	5	-
CP 12	-	-	10	-
CP 13	-	-	15	-
CP 14	-	-	20	-
CP 15	-	-	25	-
CP 16	-	-	-	7.5
CP 17	-	-	-	15
CP 18	-	-	-	22.5
CP 19	-	-	-	30
CP 20	-	-	-	37.5

Matrix erosion

In case of matrix erosion, the swollen hydrogel were dried at 60^0C and kept in desiccators for stipulated time and after drying it was weighed (W3). The results are given in Table 3 and Fig 2.

$$\text{Formula} = \frac{W1 - W3}{W1} \times 100$$

W 3 – Weight of gel after drying 24 hr at 60^0C.

***Ex vivo* mucoadhesive time**

The *ex vivo* mucoadhesive time was determined after application of film mucosa. The goat mucosa was fixed on internal side of the beaker. Hydrogel was divided in portion of 4 cm^2 and wetted with 50 µl of simulated saliva fluid and pasted to the goat buccal tissue by applying a light force with the finger tip for 20 sec. The beaker was filled with 800 ml simulated saliva fluid kept at 37^0C with 150 rpm stirring rate and film adhesion was monitored during 8 hr. The results obtained are given in Table 4[19].

Table No 2: Percentage hydration of different formulation

S. No	Time Interval (min.)	% Hydration (CP 4)	% Hydration (CP 9)	% Hydration (CP 13)	% Hydration (CP18)
1	10	90.12	88.75	84.7	80.12
2	20	92. 89	89.26	86.2	81.17
3	30	94. 33	91.30	86.88	82.7
4	60	95.38	92.77	87.9	84.2
5	90	96.1	92.98	88.81	85.12
6	120	96.78	93.78	89.97	86.78

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Table No 3: Matrix erosion of different formulation

S. No	Time interval in min	Erosion study of the formulations			
		% Erosion (CP 4)	% Erosion (CP 9)	% Erosion (CP13)	% Erosion (CP18)
1	10	80.12	82.2	83.78	84.4
2	20	81.22	82.98	84.1	85.22
3	30	81.98	83.1	85.33	86.88
4	60	82.2	83.8	86.91	88.16
5	90	83.12	88.91	87.26	89.12
6	120	84.88	86.99	89.12	91.96

***In vitro* release**

A standard USP dissolution basket apparatus was employed to evaluate drug release. Weighed amount of hydrogel was placed in a basket after 2 min, the vessels was filled with phosphate buffer pH 6.75 and maintained at 37^0C while stirring at 50 rpm for 8 hr. 10 ml sample was collected after each predetermined time intervals and replaced with an equal volume of phosphate buffer pH 6.75. The concentration of L-Glutamine into the hydrogel was determined by UV spectrophotometer and the results are given in Table 5[20-22] and Fig 3.

pH of the hydrogel

The hydrogels were allowed to swell by keeping them in contact with 0.5 ml of distilled water (pH 6.5±0.5) for 1 hr at room temperature and pH was recorded by bringing electrode in contact with the surface of the hydrogel, allowing it to equilibrate for 1 min of the hydrogel. The pH of the formulations were determined with the help of pH meter and the pH of the formulations CP 4, CP 9, CP 13 and CP 18 was 3.2, 6.7, 6.2 and 5.8 respectively[23] Table 6.

Table No 4: Mucoadhesion time of different formulations

S. No.	Formulations	Mucoadhesive time (Hr)
1	CP 4	6.15
2	CP 9	6.35
3	CP 13	5.24
4	CP 18	6. 25

Shear stress measurements

An instruments designed in house with slight modification as per Rao and Charry 1998[24,25]. It consist with two smooth glass plates measuring 4" x 4" is fixed on the wooden small table and another plate is free to move with the help of thread attached to pan. The thread is passed down through a pulley measuring 1. The upper sliding plate is having attachment to hold biological membrane firmly without wrinkle.

The shear stress was measured with the help of the above mentioned instrument. The porcine buccal mucosa received by local slaughter house measuring 4"x4" kept in Kreb's solution and prepared and used within 3 hr. The porcine buccal mucosa were fixed on the sliding plates and 0.5 gm hydrogel spreaded on the mucosa uniformly and placed on the fixed plates. The weight was

Table No 5: Cumulative percentage release of different formulations

Time (min.)	CP 4	CP 9	CP13	CP18
0	0	0	0	0
30	12	9	8	8
60	24	16	15	15
90	36	20	25	24
120	45	35	33	30
150	56	42	39	36
180	65	50	45	42
210	78	56	55	48
240	85	61	69	56
270	88	70	73	60

recorded on condition when upper plate started sliding. The process was repeated for each optimized formulations, where initial weight was counter weight (36.25 gm) and weight of hydrogel (0.5 gm) and final weight was added weight in gm when plate started sliding the comparative shear stress recording are shown in the "Table 7

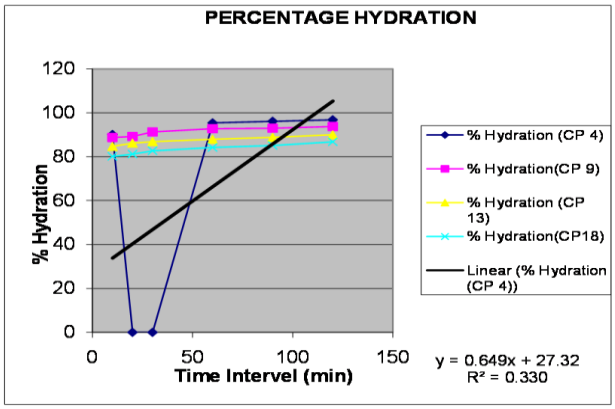

Figure 1 : Percentage hydration of different formulation

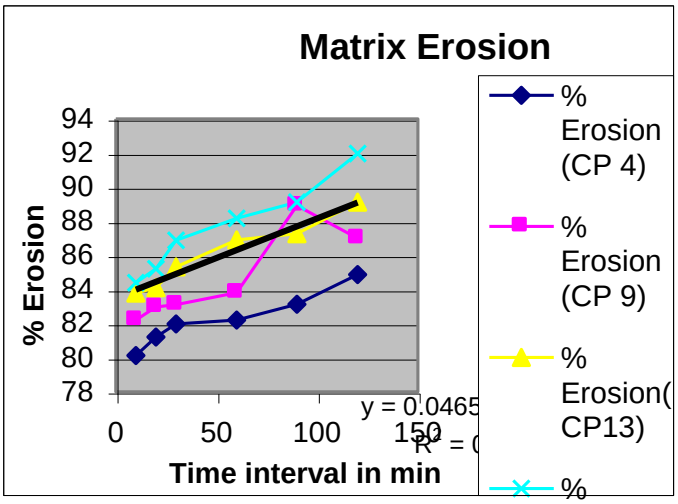

Figure 2 : Matrix erosion of different formulation

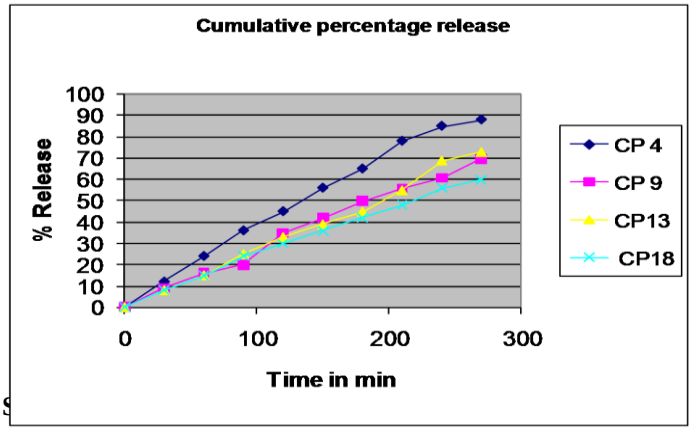

Figure 3: Cumulative percentage release of different formulations

The stability study was performed on the optimized formulation. The prepared hydrogel was subjected to stability studies in screw caped glass tubes at three different temperatures (4, 25, and 40^{0}C) and the temperature was evaluated periodically for (10 days) for percentage drug content after 10 days, sample from formulation was taken and now the samples were placed with 10 ml PBS 6.75. The samples were assayed for drug concentration and the amount was calculated. The obtained results are given in "Table (8)"[26,27].

3. RESULTS AND DISCUSSION

L-glutamine containing oral mucoadhesive hydrogel formulations were prepared and evaluated. The formulation containing carbopol 934 NF as a polymer have used in the range of 0.25% to 1.25% in CP 1 and CP 2 and which contain 0.25 and 0.50% hydrogel were not formed after constant stirring so it was rejected. The formulation CP 3 containing 0.75% of polymer gave slight viscous gel but it was not consistent after stirring so it was not taken for further study. The formulation CP 4, which contain 1.0% of polymer have shown the consistency and the hydrogel was formed but it was highly viscous and semi-solid, so it was rejected. Formulations from CP 1 to CP 5 containing carbopol 934 in concentration range between 0.25% to 1.25%. Hydrogel were formed in the range of 1.0% of carbopol 934 NF (formulation CP 4) and it was selected for further studies.

Formulation containing NaCMC as gelling agent have used the reported range between 1 to 5%. In formulations CP 6 and CP 7, the concentrations 1% to 2% were used, but hydrogel or gel was not formed after constant stirring so both were not selected. In formulation CP 8, the slurry was formed but it was not gel like so it was rejected and formulation CP 9 containing 4% as a gelling agent, it produced gel so it was selected and for further studies.

Formulation CP 11 to CP 15 containing HPMC as a polymer (5% to 25%) was used and observed that CP 11 and CP 12 containing 5% and 10% of polymer respectively. Here the gel was not formed as concentration has been increased, the gel was formed at the concentration of 15% of polymer (CP 13) and it was consistent after constant stirring. The formulation containing 20% and 25% of polymer, the viscous and semisolid agglomerates were formed, so both were rejected. Among the formulations, CP 13 was suitable and was selected.

The formulations containing PEG as a gelling agent in the range of 7.5% to 37.5% (CP 16 to CP 20), the formulation CP 16 and CP 17 did not give gel so it was rejected and in the formulation CP 18, gel has formed and the formulation CP 19 gave gel but it was less transparent and less consistent as compared to CP 18, as concentration increased, the semisolid mass was formed which was not taken for further study. So here the formulation code CP 18 was selected for studies.

Therefore among the 20 formulations, four formulations were selected for further studies, which are CP 4, CP 9, CP 13 and CP 18 respectively.

Swelling hydration and matrix erosion studies

All the hydrogel were hydrated very quickly, reaching 80% swelling hydration after just few minutes. The percentage hydration studies was carried out in the time interval between 10 min to 120 min. in PBS pH 6.75 and the matrix erosion studies in swollen hydrogel, which were dried at 60^0C for 10 min to 120 min and kept in desiccators, after drying the weight was recorded. The obtained results are presented in "Table (2) and (3)".

The hydrogen formulation containing carbopol (CP 4) have shown good hydration properties and it swell more than 80% in first few minutes and more than 90% swelling in first 20 min, it have not shown considerable difference in swelling after 60 min. It shows swelling upto 96.78% after 120 min in PBS (pH 6.75).

Formulation CP 9 containing NaCMC as gelling agents have shown hydration range in between 88.75 to 93.78% which can be considered as good but it is less than the hydrogel containing carbopol. Formulation CP 9 exhibit swelling more than 85% in first 10 min, and it swell upto 93.78% at 120 min, which is not significant when the time gradually increases up to 120 min. Formulation CP 13 containing HPMC, which shows hydration between 84 to 89.97%. It swells more than 82% in first 10 min of study but the swelling not increased in proportion with time and its hydration ranges between 84% to 89.97%, which is significantly less than carbopol and NaCMC polymers. Formulation CP 18 containing PEG has shown hydration between 80% to 86.78%. In first 10 minutes of swelling, it have shown 80% and increased upto 86.78% in 120 min of studies.

As far as matrix erosion studies concerned, among the formulation optimized for studies, CP 4 have shown erosion between 80.12% to 84.88%, which is between 10 min to 120 min. Erosion increases as time increase which is deleterious effects on hydrogel formulations.

Formulation CP 9 shows erosion between 82.20% to 86.99% which is more as compared to CP 4 in first 10 min it was more than 80% and gradually erosion increased upto 86.99%. CP 13 has shown erosion between 83.78% to 89.12% and CP 19 has shown erosion between 84.40% to 91.96% which is more among all the formulations. It is highlighted that swelling properties are probably more important when gel integrity is evaluated. But it has shown that the swelling properties of hydrogel containing Carbopol polymer is more as compared to other polymer used.

After polymerization, the hydrophilic gel is brought in contact with water, the network expands. The thermodynamically driven swelling forces is counterbalanced by the retractive forces of the cross linked structure. Two forces become equal at same point and equibilium is reached. When drug are loaded into these hydrogel, as water is absorbed into the matrix chain relaxation occurs and the drug molecule are released through the spaces or

channel within the hydrogel network. The pseudo-hydrogel swell infinitely and the components molecule dissolve for the surface of the matrix. Drug release would occur through the spaces or channel with the network as well as through the dissolution and or the disintegration of the matrix.

In fact, as already discussed, Carbopol (CP 4) have increased swelling capacity with respect to NaCMC and other polymers. So among these four optimized formulations, CP 4 has shown high percentage hydration and less erosion.

Mucoadhesion time of hydrogel formulations

Hydrogel mucoadhesion time varies from 5.24 hr to 6.35 hr. The highest mucoadhesion time has shown by formulation CP 9 is 6.35 hr and lowest mucoadhesion was shown by formulation CP 13, which is 5.24 hr and formulation CP 4 and CP 18 have shown mucoadhesion time 6.15 hr and 6.28 hr respectively.

This difference in mucoadhesion time is depends upon several factors that affect the effectiveness of such formulation. First of all, the utility of NaCMC favors mucoadhesion and the outward diffusion of the drug from matrix. Moreover, the NaCMC due to its solubility in water, result effective polymer as mucoadhesive polymer.

The mucoadhesion time of formulation CP 4, CP 9 and CP 18 give mucoadhesion time 6.15, 6.35 and 6.28 respectively, which is close and higher than 6 hr. Among all three formulations, CP 13 has shown less mucoadhesion time.

In fact, when using carbopol, mucoadhesion time always resulted high, because the polymer although manifesting decisively higher swelling. Bodde *et al* investigated the relationship between structure and adhesion for mucoadhesive polymers. Their study was based on an assumption that bioadhesion should posses two properties first optimal polarity to make sure that the polymer is wetted by the mucus and second optimal fluidity to allow for the mutual adsorption and interpenetration of polymer and mucus to take place[28].

Mucoadhesion time of hydrogel was studied and observed that all selected formulation are remain adhered with mucosa for 5 hr. Mucoadhesion time properties show that formulation may meet our requirement for releasing drug in the buccal cavity for extended period of time and show the positive effect against mucositis.

***In vitro* drug release studies**

In vitro release studies shown in first 30 min of the formulations CP 4, CP 9, CP 13 and CP 18 have shown 12, 9, 8 and 8% respectively of drug release on subsequent 60 min. The formulation CP 4 and CP 9 have shown highest 24% and 16% of drug release respectively which is more than of CP 13 and CP 18. At 90 min, the CP 4 has shown 36% of drug release and 28% shown by CP 9, CP 13 and CP 18. At 150 min, the drug release shown by the CP 4 was 45% and CP 9 has shown 42% of drug release and CP 13 have shown 39% of drug release. At 270 min, the drug release by CP 4 and CP 13 were found 88% and 73% respectively and CP 9 and CP 18 have shown 70% and 60% respectively. So it is revealed that the highest drug release shown by CP 4 *i.e.* 88% and lowest was shown by CP 18, which is 60%. CP 13 and CP 9 showed the intermediate release, which is 73% and 70%.

It is observed that the hydrogel containing carbopol have shown considerable release profile. Hydrogel containing NaCMC and HPMC have shown no significant difference in release. From this study, it is concluded that the L-glutamine hydrogel (containing carbopol) were developed which are suitable for buccal drug delivery.

Hydrogel released drug in two steps a hydrogel-drug system that relies on the consecutive action of two trigger mechanism to release a drug has the potential to target specific sites within the body. The triggers are a stimulus that converts the gel into a solution and an enzyme that cleaves the hydrogelator drug link. Raising the temperature lowering the pH causes lower molecular weight gelator molecule to come out of gel fibers and go into the solutions. Only when which are in solution can be molecule be cleaved by an enzyme and the drug be released. The combination of a low-molecular weight gelator gel sensitive to both its environment and enzyme is unique[29,30].

The physiochemical properties of the hydrogel network as well as the selection of the drug loading method will determine the mechanism by which the loaded drug is released from the crosslinked matrix. In *in-situ* loading drug or drug-polymer conjugates are mixed with polymer precursor solution and hydrogel network formation and drug encapsulation are accomplished simultaneously. In this system, the release of drug can be controlled by diffusion, hydrogel swelling, reversible-drug polymer interaction and degradation of labile covalent bonds[30].

Shear stress measurements

For the bioadhesive system, the shear stress is the exclusive and important property to be evaluated. This shear stress measurement test measures the strength of different mucoadhesive polymer and the effect of amount of polymer in the formulation as the force needed to detach it. The result shown by CP 4 (11.20) and CP 9 (11.50) was considerably high. Whereas the CP 13 (10.15)

and CP 18 (8.65) have not shown good shear stress properties as compare to CP 4 and CP 9.

Stability studies

The result shown by CP 4 was better among all optimized formulation so it was selected for stability studies. It has been found that formulation placed at 4-8^{0}C shows very less degradation after 10 days that is 1.9% as compare to standard, while formulation placed at 40^{o}C shows 4.3% degradation at room temperature and the formulation shows no degradation in 3 days but after 7 days and 10 days it shows decrease in concentration i.e. 2.01% and 2.8% respectively. At 40^{o}C or more it is observed that the concentration was decrease the formulation are more stable at lower temperature but more degradable at high temperature.

4. CONCLUSION

From the study it is concluded that the hydrogel are biocompatible and flexible material, among the optimized formulations, CP 4 have shown good results. The swelling studies of the formulation reveals that the formulations can swell easily and soften the surface of hydrogel and may releases drug in oral cavity. The *in vitro* release studies shows that the formulation can release the drug in buccal cavity for more than 80% in 4 hr, which indicate sustained release behavior of the hydrogel, which release the drug in the rate controlled manner. From the mucoadhesion time studies it can be accomplished that the formulation can adhere to the buccal cavity for prolonged time and release the drug in the buccal cavity up to 6 hr. The pH determination shows biocompatibility of the system. From the stability studies, it can be concluded that the hydrogel formulation is stable up to 40^{o}C.

The main benefit of this formulation is that it hold dose, which is adequate for therapeutic effect as it is positioned directly on the site of mucositis. Moreover this hydrogel is very tolerable and comfy because it is non-irritant and may be chosen over other adhesive dosage form in terms of flexibility, capability and protective to wounded or inflamed surfaces.

5. ACKNOWLEDGEMENT

The authors are very much grateful to Prof. S. C. Dwivedi, Dean, Research, from Suresh Gyan Vihar University, Jaipur, for their valuable suggestions and motivation.

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