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ROLE OF PROPOLIS IN AUGMENTING THE BUCCAL MUCOADHESION- AN EXPERIMENT BASED REPORT

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ABSTRACT

***Plan**: The present research has been undertaken with the aim to develop an oral mucoadhessive gel for gingivitis and periodontitis, evaluation of its physicochemical characteristics.*

***Preface**: Gingivitis and periodontitis require prolonged medication which is challenging with the nature and anatomy of the oral cavity. Here a sincere attempt was done to enhance the adhesion and contact time of a developed oral adhesive medicament by virtue of its protective layer itself should prevent abrasions, thus aiding in healing.*

***Methodology**: In the contemporary work, effect of propolis, vitamin C and vitamin E on mucoadhessive nature of oral adhesive dosage form intended for the treatment of gingivitis were investigated. Oral mucoadhessive preparation was prepared by preparation of emulsion system and incorporation into gel to form an emulgel. This emulgel was compared for its physiochemical characterization with gel in the presence and absence of propolis extract. The work was further enhanced by considering a combination of vitamin C and E incorporated gel.*

***Outcome**: From the study it was concluded that excellent mucoadhesion resulted by addition of propolis, vitamin C, vitamin E. Results clearly indicated propolis because of mucoadhesion can augment the contact time of the medicament with that of the oral cavity. Freeze thaw cycles of stability Performa indicated the propolis-emulgel combination was stable for 8 cycles and there was no globule size alteration, means no agglomeration tendency.*

1. INTRODUCTION

While bio adhesion refers to adhesion of two surfaces of which at least one surface should be of biological in nature, mucoadhesion specifies the biological surface to be mucosal membrane. Several mucoadhessive sties have been investigated in the past decade out of which buccal adhesion have gained tremendous attention for many drug delivery systems, perhaps due to the ease of administration, scope of removing the medicament if necessary and a perfect non-invasive route[1].

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Several dosage forms have surfaced including buccal gels, films and tablets. Gels by its virtue of flexibility in administration on the irregular buccal surface is capable of maintaining the surface contacts and thus meeting the patient compliance. Whatever the formulation be, the adhesive property is the contribution of the polymer which holds the API (active pharmaceutical ingredient) for a prolonged time at the site of administration. These polymers include cellulose derivatives (methylcellulose, ethyl cellulose, hydroxy-ethyl cellulose, hydroxyl propyl cellulose, hydroxylpropyl methylcellulose, sodium carboxy methylcellulose, poly (acrylic acid) polymers (carbomers, polycarbophil), poly (hydroxyethyl methylacrylate), poly (ethylene oxide), poly (vinyl pyrrolidone), poly (vinyl alcohol). The present study focus on the role of water and fat soluble vitamins in muco adhesion of propolis incorporated oral gel. A technical report was prepared after preparing several formulations which include emulsion, gel and emulgel with and without *propolis* extract. Several characterization and evaluation were conducted which include FTIR, size analysis, Color, Homogeneity, Consistency, pH, mucoadhessive test, rheology and Freeze thaw stability test and reported.

Gingivitis and the need for a protective lining

Gingivitis and periodontitis contributes maximum out of all periodontal diseases. While gingivitis refers to an inflammatory condition of the gingiva, periodontitis result in loss of periodontal tissue from the tooth. The former is reversible while in the latter case regeneration is not predictably achieved[2]. Ranney *et al* in his publication points out that in pre pubertial children gingivitis rarely progress into periodontitis because it dominates at lymphocytes level rather than plasma[3]. Although there is no correlation evidence of different bacterial species and clinical features, bacteria as ethological agents are widely accepted. Dysfunctional PMN chemotaxis and B-cell hyper responsiveness to polyclonal activation attributed to *T cell* regulatory defect is observed in adolescents with severe periodontal destruction[4]. During this condition mouth ulcers and halitosis is also observed and since the affected regions are poly dispersed within the buccal cavity, the most preferred dosage form will be a semisolid.

Role of hydrophilic ascorbic acid (vitamin C) and liphophilic tocopherol (vitamin E) as anti-oxidant and dermal cell replenish respectively are well established[5,6]. Propolis, ‘bee glue’ is a resinous material collected by bees from exudates and buds of plants, then mixed with wax and bee enzymes and is found to have good bioadhessive character. There is neither information about propolis nor carboxymethylcellulose used as a mucoadhessive polymer as far as the current issue[7].

Here we try to investigate the mucoadhessive nature of natrium-carboxymethylcellulose incorporated with nanoemulsion loaded with vitamin C and E in combination with propolis. To understand the mechanism of mucoadhesion it is wise to know about the biochemical nature of mucous membrane. Mucous membrane (mucose) are the moist biological surface lining various body cavities like oral, nasal, vaginal, gastrointestinal etc. structurally they are connective tissues (lamina propria), above which is an epithelial layer which is moistened by the presence of mucus, as a gel or luminal soluble or suspended form[8,9]. It consists of mucin glycoproteins, lipids, inorganic salts and water (95%). This biochemical nature renders as an ideal mucoadhesion. The epithelia may be single layered as in the case of GIT and bronchi or multi-layered as in oesophagus, cornea and vagina[10, 11].

Theories of mucoadhesion

There are as many as hundred and fifty scientific papers each year getting published exclusively on mucoadhesion. Many theories have surfaced depending on the need and nature of the polymer or formulation. Basically they are comfortably classified into chemical and physical[13]. Various theories and its explanatory notes are tabulated in table 1.

Table 1: Theories of mucoadhesion and nature of adhesion

Type	*Theory*	*Adhesive nature*
Chemical	Electronic	Mucoadhessive and biological materials possess opposing electrical charges leading to double electric layer at the interphase.
	Adsorption	*Van der Waals* and H- bonds, electrostatic attraction or hydrophobic interactions.
	Wetting	Liquid systems which present affinity to the surface depending on the contact angle which is inversely proportional to spreadability and adhesion.
	Diffusion	Penetration of polymer chains depends on the diffusion coefficient, flexibility and nature of the mucoadhessive chains, mobility and contact time.
	Fracture	Analyses the force required to separate two surfaces after adhesion is established
Physical	Mechanical	Irregular surface provides more surface interactive area depending on intrinsic nature of the polymer. This is related to molecular weight, concentration and chain flexibility. For linear polymers, mucoadhesion increases with molecular weight, but the same relationship does not hold for non-linear polymers.

For a mucoadhessive polymer to perform its adhesive property it must be in hydrated form, only then the chains will be detangled and free to diffuse into the mucosa and interact to form bonding. Basically mucoadhesion takes place in two stages- contact stage and consolidation stage. When the former is a period of wetting between the interfacial surface, later is the physicochemical interactions which strengthens and prolongs adhesion.

In the current work effect of propolis, vitamin C on mucoadhessive nature of oral adhesive dosage form intended for the treatment of gingivitis were investigated. Oral mucoadhessive preparation was prepared by preparation of emulsion system and incorporation into gel to form an emulgel. This emulgel was compared for its physiochemical characterization with gel in the presence and absence of propolis extract. The work was further enhanced by considering a combination of vitamin C and E incorporated gel.

2. MATERIALS AND METHODS

Preformulation studies: FTIR- Propolis Extract alone and in combination with excipients mixed in geometrical mixing was subjected to FTIR.

Methods: The samples were subjected to IR Spectral analysis by using FTIR analysis (Thermo Nicolet Nexus 670 IR Spectrometer), detector-DTGS KBr Beam splitter KBr, Source IR.

2.1. Preparation of emulsion system

Weighed 5 g PEG and added 7.5 ml of liquid paraffin, added 0.5 ml of tween 20 and 1 ml of span 20. The contents were stress mixed by a three blade propeller stirrer (Remi motors, Mumbai) at 200 rpm and ethylene glycol 10 ml was added. Stirring was continued for another ½ h and the emulsion was made up to the required volume by double distilled water[14,15]. Various evaluations were performed such as size analysis, Colour, Homogeneity, Consistency, pH (Digital pH meter). Results are comprehended in table no 3.

2.2. Preparation of gel, emulgel (emulsion + gel), gel + propolis extract, emulgel + propolis

2.2.1. Preparation of gel

Different concentrations of Na CMC (1%, 2.5%, 5%) was added into 100 ml vitamin C solution in water and mechanically stirred. Into this added 1 ml of triethanolamine and stirred to form a homogenous gel. Gel excluding vitamin C also was prepared[16].

Table 2: formulation variables for gel preparation

Form. Code	*Vitamin C solution 1(ml)*	*Na CMC (g)*	*Triethanolamine (ml)*
G0	-	2.5	1
G 1	100	1	1
G 2	100	2.5	1
G3	100	5	1

2.2.2. Preparation of emulgel

Optimised emulsion (E 2) was incorporated into the optimised gel (G2) to prepare emulgel. For this, the emulsion and the gel were mixed in 1:1 ratio with gentle stirring by a 3 blade propeller stirrer at low rpm to form emulgel[17].

2.2.3. Preparation of gel + propolis extract

Fusion method was performed by mixing gel and 2% propolis extract. The gel was levigated on a clean white slab and incorporated propolis extract in geometrical ratios to obtain homogenous distribution. Propolis extract was hydrated before incorporation. The product was kept in cool dry environment until further analysis.

2.2.4. Preparation of emulgel + propolis extract

As described above, fusion method was performed by mixing gel and 2% propolis extract. The gel was levigated on a clean white slab and incorporated propolis extract in geometrical ratios to obtain homogenous distribution. Propolis extract was hydrated before incorporation. One more extra step was considered by adding vitamin E (1:1 with vitamin C) to the formulation. The products was kept in cool dry environment until further analysis.

Table 3: Comprehensive report of emulsion system gel, emulgel (emulsion + gel), gel + propolis extract, emulgel + propolis

Sample code	*Average globule size (µm)*	*pH*	*Colour*	*Optical physical nature*	*Consistency (gm.cm.sec^{-1})*	*Mucoadhesion (min)*	*Rheology Spindle no. 64 (spindle speed/ cps)*	
							rpm	*cps*
E	102.05	5.6 ±1.2	Off white	Translucent	2.66±0.31	1.7±1.99	10	160
							50	97
G0	-	7.5 ±0.55	Colourless	Translucent	2.59±2.21	1.5±1.53	10	397
							50	110
G1	-	6.5 ±1.01	Colourless	Translucent	2.80±0.2	2.4±1.70	10	400
							50	112
G2	-	6.9 ±0.59	Colourless	Clear and transparent	3.09±1.2	3.5±1.82	10	400
							50	113
G3	-	7.0 ±2.5	Colourless	Clear and transparent	3.32±1.92	3.7±2.54	10	401
							50	113
EG (E + G2)	98.88	6.5 ±0.8	Colourless	Clear and transparent	5.21±1.43	4.2±1.3	10	413
							50	155
G2 + P	-	6.8 ±1.01	Pale golden yellow	Almost opaque	7.11±0.63	5.1±1.01	10	411
							50	150
EG + P	95.29	6.7 ±1.60	Pale golden yellow	Almost opaque	7.82±1.01	6.3±3.01	10	420
							50	160
EG_{EC}+ P	97.29	6.9±1.99	Pale golden yellow	Almost opaque	7.95±0.09	6.5±0.93	10	409
							50	151

Note: E = emulsion, G = gel, EG = emulgel, G + P = Gel + propolis, EG + P = emulgel + propolis, EG_{EC} + P = *emulgel incorporated vitamin C and E (values expressed as mean ± SD, n =3)*

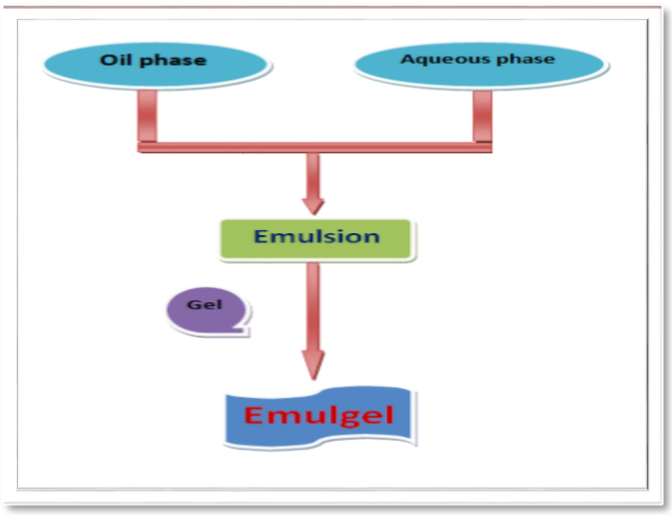

Figure1: Flow chart representing preparation of emulgel

Characterization of emulsion system, gel, emulgel, gel + propolis, emulgel + propolis
Report of Fourier-transfer-infrared spectral studies:

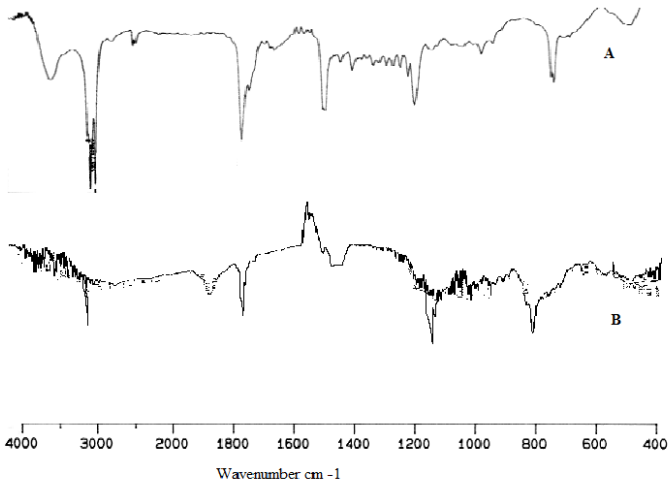

Figure 2: Fourier-transfer-infrared spectra of propolis alone (A) propolis + excipients

Table 4: FTIR principal peaks obtained and corresponding interpretation

Principle peaks frequency observed in IR spectrum (cm^{-1})	*Assignments*
1173.4	C-C
1736.3	C=O (aldehydes)
2918.6	C-H (aliphatic)
3131.1	C-H (aromatic)

Globule size was determined by optical microscopy at 10 X by using an eye piece micrometer. The eye piece micrometer was standardized by a stage micrometer. The pH of the formulations were determined by a digital pH meter (pH Meter LI 120, Elico Ltd, Hyderabad). Colour as well as Optical physical nature were observed visually and reported.

2.3. Rheological studies

Rheology was performed by Brookfield viscometer (cup and bob model) model no. LO-DVE-8549000. Spindle no. 64 at 50 rpm and 35±2°rate of shear was obtained as cps and tabulated in table no. 3[18, 19].

2.4. Mucoadhessive characterization

The *in vitro* residence time of gel, emulgel, gel + propolis, emulgel + propolis was determined using IP disintegration apparatus. The disintegration medium was 800 ml of pH 6.75 simulated saliva solution maintained at 35±2° (human oral temperature) [20].

The goat oral mucosa used as mucoadhessive layer and was prepared by cutting 4cm goat cheek mucosa and de-fated using acetone. Then the segments of goat oral mucosa, each of 4 cm length, were glued to the surface of a glass slab, which was then kept in 1000ml beaker containing 600ml of 6.75 pH ethanol-water and was attached in the apparatus and allowed to move up and down. The formulations were brought into contact with the mucosal membrane. The time required for complete erosion or detachment of the formulation from the mucosal surface was recorded (n = 3). The reports are shown in table no3.

2.5. Performing 'Freeze Thaw' method

As a part of stability studies, Freeze Thaw method was performed for emulgel + propolis by storing at 4°C ± 1 for 24 h and then at controlled RT (27°C ±1) for 8 cycles. After each cycle physical appearance, crystal growth, pH, and consistency were observed by a consistency tester[21].

3. RESULTS AND DISCUSSION

Formulations emulsion, gel, emulgel and in combination with propolis were prepared separately.

Table 5. Results of Freeze thaw method for 8 consecutive cycles

Freeze thaw cycle	*Average particle size (μ)*	*Consistency ($g.cm.s^{-1}$)*	*pH*	*Crystal growth*
1	94.05	7.92±2.99	6.6±2.99	Nil
2	95.88	8.02±1.81	6.7±1.19	Nil
3	96.13	7.80±1.55	6.6±2.09	Nil
4	94.39	7.82±2.01	6.8±1.19	Nil
5	95.29	7.52±2.89	6.6±1.55	Nil
6	94.07	7.92±2.11	6.±2.70	Nil
7	96.60	7.82±2.74	6.6±0.90	Nil
8	96.81	7.93±1.42	6.8±2.60	Nil

Results of FTIR suggested all ingredients used in the present study were compatible and there was no pharmaceutical incompatibility. The pH of emulsion was low and thus was acidic in nature, and gel without vitamin C was found to be slightly alkaline. But all other formulations were found between the ranges of 6.5 to 7.0. As size of the globules decreased, consistency as well as mucoadhessive nature increased, thus directly proportional. This is because of the enhanced effective surface area by micronization. But it was evident that without propolis, the formulations either emulsion, gel, or emulgel had a slipping tendency rather than erosion nature from the mucosal surface.

Hypothetically it could be said that as the temperature increased to 35º C, there could be internal entanglement of the polymer chains which facilitated a covalent bond with that of the mucosal membrane. Thus, mucoadhesion could be augmented primarily by the presence of propolis, there was also an effect of vitamin C, may be because of the lower surface charge and enhance the bonding of propolis with that of the mucosa. Results clearly indicated propolis because of mucoadhesion can augment the contact time of the medicament with that of the oral cavity. We propose, enhancement of mucoadhession when Vitamin E was added in the formulation, was because of its lyphophilic nature must exhibited a synergic effect probably because of hydrophilic- liphophillic nature which is complying with the cellular bilayer is also excellent for healing the tissue scar. Freeze thaw cycles of stability Performa indicated the propolis-emulgel combination was stable for 8 cycles and there was no globule size alteration, means no agglomeration tendency. Further, the consistency remained the same, stating that during the shelf life if stored as recommended, patients can achieve the same spreadability of the formulation on the mucosal surface[22].

Lack of crystal growth proved there was no nuclei formation and was free from grittiness. The pH during the cycles were not changed significantly which indicates that the formulation complies with the skin pH. Also it is suggested that the area may be properly wet before applying the emulgel for better results. This could be achieved either by rinsing the mouth with water at optimum room temperature or even with the self-saliva within the mouth. Mucoadhesion enhanced with the addition of the vitamins in spite of reasonable increase in the globule size.

4. CONCLUSION

The optimized emulgel has proven to be effective as a mucoadhessive device in the treatment of gingivitis and periodontitis by virtue of its mucoadhessive character and antioxidant activity. In torto, excellent mucoadhesion resulted by addition of propolis, vitamin C, vitamin E.

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