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A Study of Mucoadhesive Bond Strength of Buccoadhesive Compacts for Systemic Drug Delivery: *In-vitro/ In-vivo* correlation

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Summary

Compacts prepared from binary combinations of Carbopol® 934 P (CP), Polycarbophil (Noveon® AA1, PC) and Hydroxy propyl cellulose (Klucel®, HPC) and coated on all but one flat surface with Poly Methyl Methacrylate - PMMA (chloroformic solution) were evaluated for mucoadhesive bond strength on a modified mucoadhesive bond strength apparatus using rabbit stomach mucosa (SM) and small intestine mucosa (SIM).

In –vitro mucoadhesion tests indicated that the detachment force increased linearly with concentration of CP/PC in the compacts. Mucoadhesion of the compacts with SIM were higher when compared to SM. The compacts with higher proportions of CP/PC showed longer buccoadhesion time (time the compact remained in contact with the buccal mucosa) than HPC alone in humans. *In-vivo* buccoadhesevity of the coated compacts was studied in healthy human volunteers. An index was used to study the redness and ulceration of the contact buccal mucosa. Compacts with higher proportions of CP/PC showed longer buccoadhesion time than HPC alone. Significant correlation coefficient (r) values ($P<0.01$) were obtained between *in-vitro* fracture strength of the compacts and *in-vivo* buccoadhesion time. Hence, the *in-vitro* mucoadhesive model developed by us provides useful information on the residence time of the compact for systemic drug delivery in the oral cavity, and compacts containing less than 50% of CP/PC were safer to use in humans.

Introduction

Due to its large expanse of smooth, immobile tissue, the buccal mucosa is an ideal surface for the placement of delivery systems such as buccoadhesive patches / films and compacts/ tablets. In addition, the buccal site is less permeable than the sublingual site, a difference that makes the former a more suitable choice than the latter if sustained drug delivery is desired[1-3]. Mucoadhesives provide an intimate contact between a dosage form and the absorbing tissue, which may result in high drug concentration in a local area and hence high drug flux through the absorbing tissue[4].

Numerous techniques for the measurement of bioadhesive bond strength [5-10] are reported in the literature and various model mucous membranes have been used to study the *in-vitro* mucoadhesive bond strength of buccoadhesive dosage forms[11-16].

Intestinal mucosa of pigs[17,18], guinea pigs[19], rats[10], rabbits[20]and gastric mucosa of rabbits[21,22] and pigs[23-26] have been used. Furthermore, it is well established that pH plays an important role in bioadhesion and maximum adhesion is observed for pH 5 to 6[27]. The pH of SIM ranges between 5 to 7, which is similar to the pH of buccal mucosa. Since there is no model tissue earmarked for evaluation of buccoadhesive dosage forms, rabbit stomach mucosa and small intestinal mucosa were used in our studies because of regular availability of albino rabbits of uniform breed from the University's central animal house.

To the best of our knowledge, the study of correlation between a suitable parameter derived from *in-vitro* mucoadhesion and *in-vivo* buccoadhesion of bioadhesive compacts for unidirectional systemic drug delivery in humans is not reported in the literature, except that of Bouckaert et al[28], who have reported their findings on buccoadhesive miconazole tablet for local effect. Hence, the present study was planned to develop an *in-vitro* model to examine the detachment force on carefully maintained rabbit stomach mucosa (SM) and small intestine mucosa (SIM). Various Carbopol 934 P® (CP), Polycarbophil (PC, Noveon AA1®) and Hydroxy propyl cellulose (HPC,

Klucel EF®) compacts were evaluated on the developed model and a correlation of adhesive behaviour from this model with that seen in human buccal applications was obtained.

Experimental

Materials

Carbopol 934 P® (CP), Noveon AA1®, Klucel EF®, Cabosil® were obtained as gift samples from B.F. Goodrich, U.K., B.F.Goodrich, U.S.A., Aqualon, U.K. and Cabot Corp., USA, respectively. PMMA (Aldrich, U.S.A., Molecular weight 1,20000) was obtained commercially. HPC and Talc were used after sieving through #100 and #250 BSS, respectively. All other reagents were of analytical grade. Albino rabbits (Central Animal House, Banaras Hindu University, Varanasi, India) used were of 2.45±0.15 Kg.

Methods

Preparation of placebo oral mucoadhesive compacts:

Weighed quantities of polymer(s), Cabosil®, and talc were gently and uniformly mixed on a vibro mixer. Compaction was done on a Monesty E2 single punch tabletting machine (hardness 10 units, Monsanto hardness tester) using 12 mm punches for the batches BA, BB, BC, BE, BF and BG. The slugs were broken using a mortar and pestle and passed through #30 sieve. The sieved materials were re-compressed using 9.6mm diameter, flat non-beveled punches. Batches BD, BH and BI were directly compressed on a Manesty E2 tablet machine (fitted with 9.6mm diameter, flat non-beveled punches). The compression pressure was controlled to produce compacts of desired hardness. The compacts were coated by spraying 6ml (for one compact) of 0.2% w/v chloroform solution of PMMA (high molecular weight) with di-butyl phthalate (10%w/w of polymer) on all but one flat surface and were dried at room temperature and stored in an air tight container with silica gel bags.

Further, different batches of compacts containing the polymer(s) were prepared by incorporating sodium bicarbonate (SBC) in different concentrations. The compacts were compressed directly and coated as described above.

Compacts were evaluated for various parameters such as uniformity in thickness, weight and hardness. Composition of different batches of placebo mucoadhesive compacts prepared are listed in Table 1.

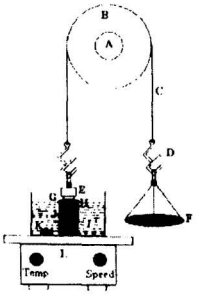
Table 1: Composition and Surface pH of Different batches of placebo and oral mucoadhesive compacts prepared using CP-HPC and PC-HPC.

Batch Code	CP (mg)	PC (mg)	HPC (mg)	Cab-O-Sil® (mg)	Talc (mg)	SBC (mg)
BA	138.6	-	-	0.7	0.7	-
BB	103.95	-	34.65	0.7	0.7	-
BC	69.30	-	69.30	0.7	0.7	-
BD	34.65	-	103.95	0.7	0.7	-
BE	-	138.60	-	0.7	0.7	-
BF	-	103.95	34.65	0.7	0.7	-
BG	-	69.30	69.30	0.7	0.7	-
BH	-	34.65	103.95	0.7	0.7	-
BI	-	-	138.6	0.7	0.7	-
BJ	102.1	-	34.0	0.7	0.7	2.5
BK	100.2	-	33.4	0.7	0.7	5.0
BL	96.45	-	32.15	0.7	0.7	10.0

***In-vitro* mucoadhesive study**

The mucoadhesivity testing apparatus shown in Figure 1 is a modification reported by Mortazavi and Smart[10]. An acrylate tissue mounting stage (5 cm height and 1.7 cm diameter) was attached

Fig. 1: Daigrammatic representation of *In-vitro* mucoadhesive test apparatus



A : Coaxial bearing, **B** : Pulley, **C** : Nylon thread, **D** : Hook, **E** : Device holder, **F** : Pan,
G : Buccoadhesive device, **H** : Mucosal tissue, **I** : Phosphate buffer saline pH 6.6,
J : Mucosal tissue mount, **K** : Magnetic bead, **L** : Magnetic stirrer

to the center of a glass dish (7.5 cm height and 7.5 cm diameter). The dish with tissue mount containing phosphate buffered saline pH 6.6 (PBS) was placed on the magnetic stirrer provided with temperature and speed control. A magnetic bead (6 x 4 mm) was used to agitate (100 rpm) the PBS. An acrylate compact holder of diameter 1.5cm and weighing 2.6g was used. A nylon thread of thickness 0.38mm and length 52cm was placed over the acrylate pulley groove (7.5 cm diameter) such that one end is tied to a pan and the other end to the compact holder.

Over-night fasted (water ad libitum) rabbits were sacrificed and stomach and small intestine were carefully removed. The stomach was cut longitudinally and its inner surface and the small intestine were rinsed with cold saline to remove any loose material. Both the mucosae were stored in cold saline (5-8°C) and used within three days[8]. Saline was selected to store SM and SIM because mucous is hydrophobic in unbuffered saline[29], and it is expected that the nature of mucous does not alter during storage. Small intestine was cut into segments of 3cm length and cut open longitudinally along the mesentry to expose the inner mucosal surface[30].

The tissues (mucosal side out) were mounted securely with the help of silicone rubber band on the tissue mount platform within the dish containing PBS at 37±1°C. The level of PBS in the dish was

maintained in such a way that it just touches the mucosal surface and every care was taken to prevent overhydration of the surface. The compact was fixed on the device holder with cyanoacrylate adhesive. The test compact was placed in contact with the mucosal surface to give a contact area of 0.72cm^2. After 5 minutes, standard weights in increments of 1 g were added on the pan after every 30 seconds. The weight at which detachment took place was noted. This gave the mucoadhesive bond strength of the compact in gramme. The experiment was repeated with fresh compact and fresh mucosa in an identical manner, in triplicate. After every 45 min, 100µl of PBS was added on the mucosal surface during the experiment. Gross observations indicated that adhesive failure occurred at the mucosa-adhesive interface. Zero correction weights for detatchment was determined without compact and mucosal tissue and deducted from the observed test weights.

The fracture strength, σ_b, of the bioadhesive bond, which corresponds to the stress of detachment at the maximum detachment force, F_{max}, was calculated from $\sigma_b = \mathbf{F_{max}/A_\alpha}$,where A_α is the contact area between compact and tissue[31]. The relative adhesive capacity (A_r) of device was determined by dividing the value obtained for the devices (A_x) by the value of the reference (A_t) i.e., pure CP compact[32].

Surface pH Measdurement:

The devices were allowed to swell in closed petridish at 37°C for 2 hours in 0.5 ml of double distilled water (pH 6.0). The little swollen portion of the device was removed and spread on a pH indicator paper to determine the surface pH. After 60 seconds the colour developed was compared with the standard colour scale. All the experiments were performed in triplicate.

***In-vivo* buccoadhesion of placebo compacts in humans:**

The *in-vivo* buccoadhesive time and biocompatibility of the placebo compacts were determined in a double blind cross over study in six healthy human volunteers (5 males and 1 female). The age of the volunteers ranged between 25 and 30 years (27±1.89 years) and their weights between 55 to 79

kg (67.83±8.3 kg). Volunteers agreed to participate in the study after explanation of the experimental protocol and written informed consent was obtained from each volunteer. All subjects were in good health on the basis of medical history and complete physical examination.

Half an hour after a standard breakfast, consisting of 4 slices of bread with butter and (or) jam with 200 ml of tea or coffee or low fat milk, the oral mucoadhesive compact was placed with minimal pressure for 30 seconds, in the buccal sulcus, opposite to the inner canine tooth, after wiping the site with cotton swab. During the experiment the volunteers were allowed to drink water after 30 minutes of administration of the compact. The volunteers took a standard lunch and dinner prepared by the Institute cafeteria at the end of 4 and 12 hours respectively. Both of these consisted of 4 unleavened whole wheat bread, 100 ml of lentil soup, 100 g of plain vegetable curry and 150 g of cooked rice. The food articles contained only small amounts of vegetable oil and spices. The standard lunch and dinner was served daily and was of uniform composition. As such, the type of food consumed is unlikely to alter the flow of saliva and its composition and pH. The subjects were allowed to perform their normal oral activities and instructed not to disturb the device by any means. They were trained to note the retention time of the compact and indicate the acceptability of the composite compact. Indices for pain and irritation of the mucosa, taste alteration, hinderance due to swelling, redness and ulceration after removal of the device were used to describe the side effects of the compacts. Fresh placebo composite compact was placed at each replicate point. A minimum period of 4 days was allowed between replicate applications on the same subject. A score scale of 0, slight:1, moderate: 2 and severe:3 was used to describe the biocompatability and properties of the devices[33,34].

Statistical analysis of data:

Experimental results are expressed as mean ± S.D. Student 't' test was also performed to determine the level of significance. Difference was considered to be stastically significant at $P < 0.05$. The

correlation coefficient observed between *in-vitro* and *in-vivo* parameters were tested for statistical significance by 't' test using the formula $t = r(n-2)^{1/2} / (1-r^2)^{1/2}$ [35], where 'r' is the correlaion coefficient and 'n' is the number of sample points.

Results and Discussion

The prepared placebo CP-HPC, CP-HPC-SBC and PC-HPC compacts showed good uniformity in thickness, weight and hardness. The placebo compacts prepared were 2 ± 0.29 mm in thickness and of average weight 140 ± 1.28 mg. The hardness of the placebo compacts was 10 ± 0.99 kg/cm^2 (Monsanto Hardness tester).

***In-vitro* mucoadhesion**

The studies indicated that the weight required for detachment of mucoadhesive bond increases linearly with concentration of CP/PC in the compacts. With pure HPC (Batch BI), the mucoadhesive layer broke immediately on the application of 2.6 gm initial loading weight. The results show that the mucoadhesiveness decreases in the following order: PC > CP > HPC (Table 2). These results are in agreement with mucoadhesive properties of anionic and nonionic

Table 2: *In-vitro* mucoadhesion, fracture strength and relative percent adhesivity of the prepared CP-HPC and PC-HPC placebo compacts using rabbit stomach mucosa (SM) and rabbit small intestine mucosa (SIM)

Batch Code	*In-vitro* mucoadhesion (gm) [mean ± SD] (n=3)		Fracture strength (gm) [mean ± SD] (n=3)		Relative %mucoadhesion* (Average of 3 values)	
	SM	SIM	SM	SIM	SM	SIM
BA	51.33 ± 8.33	91.00 ± 9.54	71.29 ± 11.57	126.31± 13.25	100	100
BB	41.67 ± 7.37	59.33 ± 5.03	57.88 ± 10.24	82.40 ± 6.99	81.18	65.20
BC	29.67 ± 3.51	54.33 ± 2.52	41.21 ± 4.88	75.46 ± 3.50	57.80	59.70
BD	25.33 ± 5.03	32.67 ± 6.43	35.18 ± 6.99	45.38 ± 8.93	49.35	35.90
BE	63.00 ± 4.58	114.00 ± 11.5	87.50 ± 6.36	158.33 ± 15.57	122.74	125.27
BF	47.00 ± 10.14	51.66 ± 6.67	65.28 ± 14.08	71.75 ± 9.66	91.56	56.77
BG	33.50 ± 8.74	44.00 ± 10.58	46.53 ± 12.14	61.11 ± 14.69	65.26	48.35
BH	16.00 ± 3.60	13.25 ± 2.06	22.22 ± 5.00	18.40 ± 2.86	31.17	34.25
BI	4.67 ± 0.58	5.00 ± 0.82	6.49 ± 0.81	6.95 ± 1.14	9.10	5.49

* With respect to batch BA

polymers reported by other investigators[10,36,37]. Incorporation of HPC in CP/PC compacts reduced the adhesive force and thus confirming that HPC can be used to dilute CP/PC in a mucoadhesive preparation[38]. This was further confirmed by calculating the percent relative adhesive capacities of CP-HPC and PC-HPC based compacts in comparison with pure CP compact (Table 2). The percent relative adhesive capacity decreased with increasing percent of HPC in CP or PC compacts. Pure PC compact showed 122.74 and 125.27 relative percent adhesive capacity in SM and SIM, respectively, as compared with pure CP compacts.

In general, PC-HPC compacts containing higher proportions of PC showed higher *in-vitro* mucoadhesion when compared with CP-HPC compacts (Table 2). This is apparently due to less cross-linking of polyacrylic acid chain in PC and thus dehydration of the contact mucous and swollen rubbery matrix takes place at relatively higher rate than CP compact[36]. The ability of a polymer to take up water from mucous is a primary determinant of mucoadhesive potential of a polymer[10,17,39].It was also observed that mucoadhesion of the compacts with SIM was higher in almost all the cases studied when compared to SM. This may be due to the less ionized mucin molecules in stomach mucous, which holds less water at stomach pH. The pH of the mucin of SIM is reported to be between 5 and 7 and hence, mucin molecules are completely ionised and hold nearly 40 times its weight of water[7,40,41]. Hence compacts are hydrated more quickly with SIM and faster hydration occurs over the 5 minutes contact time. Similar results, where the degree of hydration of the polymer depended upon the state of ionisation of the mucosal membrane, have been reported in the literature[38,27]. Although electrostatic repulsion exists between anionic groups of CP/PC and the mucin terminal carboxylate groups at higher pH (5-7) , the force of bioadhesion of CP/PC can be explained on the basis of molecular shape dynamics of polyelectrolytes described by Katchalsky[42] and by Hassan and Galo[7]. As mentioned above the adhesive force is related to the amount of water taken up by the polymer as it hydrates in contact with the mucosa. However, in this

study the available water in the mucosa was limited and thus overhydration to form mucilage did not apparently occur.

***In-vivo* buccoadhesion of composite compacts in humans:**

The mean scores of redness and ulceration after removal of the compacts from human buccal mucosa for the various treatments are given in Table 3. The buccoadhesive formulations were readily retained on the human buccal mucosa. No redness or ulceration was experienced by any of the subjects with compact BI, whereas the compacts BA, BB, BE and BF (75% or more of CP/PC) caused severe redness with very slight ulceration. The high concentration of carboxyl groups in a dry compact of CP/PC generated a low surface pH on moistening, and pH values of between 2 and 3 have been detected[43]. This low surface pH would be expected to damage the contacting mucosal surface[44,45]. However, the slight local reaction (redness or ulceration) of contact mucosa after removal of the compact disappeared within 24 to 48 hours in all the cases studied, possibly due to the fast regeneration of the oral mucosal tissue[1].

The surface pH of the CP-HPC and PC-HPC based compacts varied between 3.00 ± 0.39 and 3.67 ± 0.29, respectively, while the compacts containing SBC showed surface pH values of 4.00 ± 0.00, 4.83 ± 0.29 and 6.17 ± 0.29 for batches BJ, BK and BL, respectively. The increase in the surface pH observed in cases of SBC containing batches may be due to neutralization of carboxylic acid groups of CP by SBC. Since CP resins are hydrophilic and pH sensitive polymers because of the presence of carboxylic acid functional groups[43], the addition of basic materials tends to form the corresponding salt of CP. Moreover, divalent metal ions form water insoluble complexes, while monovalent metal ions form water soluble complexes with carboxylic acid groups of CP.[43, 46] The increase in the surface pH was directly proportional to the amount of SBC incorporated.

Some subjects noted that care was required in removing the device from oral mucosa to prevent subsequent mucosal lesions. When CP and PC content was less than 50% in the compacts (Batches

Table 3: Biocompatability and *in-vivo* buccoadhesion parameters of prepared CP-HPC and PC-HPC placebo buccoadhesive compacts in human volunteers

Batch Code	*In-vivo* Buccoadhesion Parameters Scores* (Mean ± SD) (n=6)							
	Irritation of the mucosa	Swelling hindrance	Taste alteration	Pain of the mucosa	Redness after removal of device	Ulceration after removal of device	Reason for detachment	Buccoadhesion time (hr)
BA	0.67 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.45 ± 0.18	3.00 ± 0.00	0.92 ± 0.38	Brushing	22.00 ± 0.50
BB	0.20 ± 0.19	0.16 ± 0.41	0.00 ± 0.00	0.50 ± 0.22	3.00 ± 0.00	0.67 ± 0.41	Brushing	21.67 ± 2.08
BC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.50 ± 0.32	0.00 ± 0.00	Brushing & Eating	17.88 ± 6.25
BD	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.17 ± 0.26	0.00 ± 0.00	Brushing & Eating	15.63 ± 3.64
BE	0.49 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.22	3.00 ± 0.00	1.08 ± 0.20	Brushing	22.00 ± 1.83
BF	0.48 ± 0.11	0.17 ± 0.40	0.00 ± 0.00	0.42 ± 0.30	3.00 ± 0.00	0.75 ± 0.27	Brushing	19.00 ± 4.50
BG	0.17 ± 0.40	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.09	1.58 ± 0.28	0.00 ± 0.00	Brushing & Eating	17.00 ± 5.20
BH	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.14	0.92 ± 0.20	0.00 ± 0.00	Brushing & Eating	12.00 ± 0.82
BI	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Came of its own	3.50 ± 1.06

BC, BD, BG and BH), no to very slight redness was observed in the subjects after removal of the compact. A possible reason could be an increased surface pH of the compact due to the diluting effect of HPC and lesser adhesion time[44].

The *in-vitro* mucoadhesion results are reflected in the *in-vivo* buccoadhesion time i.e., the compacts with larger proportions of CP and PC showed higher buccoadhesion time (Table 3). The mean adhesion times for compacts BA, BB and BC were 22±0.5, 21.67±2.08, and 17.88±6.25 hours, respectively. No significant difference could be observed between compacts BA, BB and BC ($p > 0.05$), whereas compact BA showed significantly higher buccoadhesion time than compact BD ($p < 0.05$). No significant difference was observed in the buccoadhesion times of PC/HPC based compacts BE, BF and BG (22±1.83, 19±4.50 and 17±5.20 hr., respectively; $p > 0.05$), whereas formulation BE showed significantly higher buccoadhesion time than compact BH ($p < 0.01$). Batches containing higher proportions of HPC (50% or less) reduced, but not significantly, the overall buccoadhesion times of CP/PC compacts ($p > 0.05$). This may be atteributed to a variation in the buccoadhesion time between subjects[17].

Bouckaert et al[28] reported that placebo tablets without backing layer consisting of 95% thermally modified starch and 5% of Carbopol® 907, Carbopol® 910 and Carbopol® 934 showed bioadhesion times of 814±1.07, 791±1.44 and 782±1.91 min., respectively, in human volunteers. In our study, placebo compacts remained in adhesion to the buccal mucosa longer than reported by Bouckaert et al[28] due to intactness of the PMMA coat and higher CP and PC content. None of the subjects in the study complained of taste alteration or increase in salivary viscosity, suggesting that the PMMA coat was intact throughout the study. These composite systems developed in this study may ensure unidirectional drug release, protect the device from being overhydrated by saliva and thereby increase the buccal residence time of the compact.

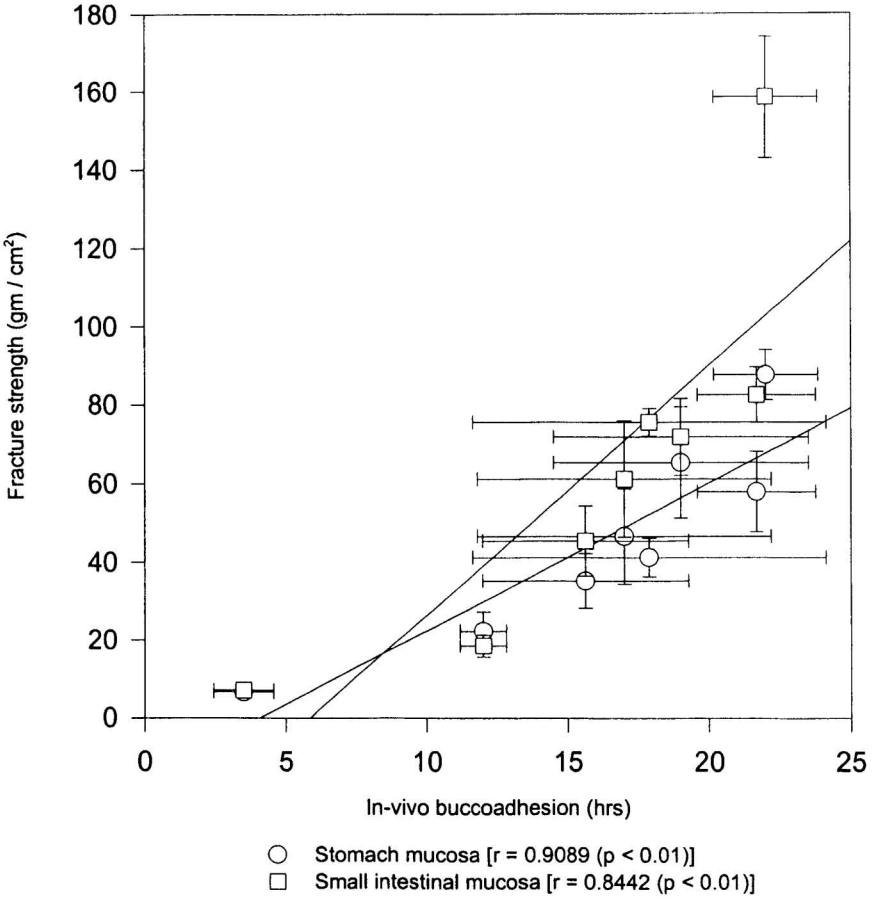
Because of the above mentioned reasons and negligible salivary turnover at the contact surface[47], the buccoadhesive device should be tested *in-vitro* without immersing the mucosa and compact contact surface in PBS. Mortazavi and Smart[10] and several other investigators[24,48] conducted their experiments under complete immersed conditions. When the mucosa and compact contact surface is submerged in the medium, slippery mucilage is formed at relatively higher rate because of overhydration than actually occurring in the oral cavity for buccoadhesive dosage form for systemic use and hence, this would result in wrong evaluation of mucoadhesive compacts or films used for systemic delivery.

Correlation between *in-vitro* mucoadhesion and *in-vivo* buccoadhesion in humans:

Two different applied tensile stress were used by Mortazavi and Smart[10] for evaluating different polymers of their interest and reported that the mucoadhesive joint broke immediately on the application of 0.0846 N loading force for HPC compacts. However, they observed the HPC compact to remain adhesive to the mucosal surface for a longer period (24 hours) when subjected to a loading force of 0.0358N. Literature reports do not mention HPC to be a potential bio-adhesive polymer[20,49]. Hence, in our opinion all bio-adhesive dosage forms may not be amenable to these two applied tensile stresses and therefore modification of Mortazavi and Smart's mucoadhesive apparatus was needed to differentiate between mucoadhesive bond strength of different buccoadhesive compacts used for systemic drug delivery. In our study, HPC compact (Batch BI) showed the least buccal residence time and *in-vitro* mucoadhesivity among the tested compacts, and the modified experimental protocol, described here by us, shows linearity in the observed results.

The *in-vitro* fracture strength of compacts with SIM or SM was correlated with *in-vivo* human bio-adhesion time. The calculated fracture strength (σ_b) values are presented in Table 2. The results indicate that a correlation exists between the *in-vitro* and *in-vivo* mucoadhesivity (Figure-2).

Fig.2 : Correlation between fracture strength and in-vivo buccoadhesion of CP/PC and HPC compacts



Furthermore, significant regression coefficient was found, when *in-vitro* σ_b values obtained with SIM ($r=0.8442$; $p<0.01$) or SM ($r=0.9089$; $p<0.01$) were correlated with *in-vivo* bioadhesion time. Bouckaert et al[28] have reported that their *in-vitro* results did not correlate well with in-vivo data, whereas our results showed a reasonable degree of correlation. This difference may not be

attributable to a single reason, rather a host of factors may have contributed to it. First, the slow release tablets of Bouckaert et al[28] eroded completely in the buccal cavity, whereas our compacts remained intact during test in humans. The maximum adhesion time of 13.5 hours is reported by Bouckaert et al[28], possibly due to their formulations containing 95% of thermally modified maize starch (DDWM). Apparently, the high proportion of DDWM caused a higher degree of hydration of the tablet, resulting in over hydration and formation of slippery mucilage at higher rate, and consequent loss in mucoadhesion. Second, the coating of our compacts with PMMA prevented over hydration and consequently longer mucoadhesion times were observed. Third, Bouckaert et al[50] had conducted their in-vitro mucoadhesion tests under complete immersed condition; whereas the state of hydration of the human buccal cavity is very different, in that the amount of saliva is far too less in comparison to the volume of buffer solution used in their in-vitro test. Thus, since mucoadhesion is very much affected by the state of hydration of the device/muscosa, a probably strong reason for lack of correlation in the work of Bouckaert et al[28] is their test conditions. The role of an optimum amount of water for bioadhesion is reported.[32, 40,48,51,52] When the mucous is overhydrated, it remains as a separate gel phase instead of dispersing[53], resulting in lower mobility of glycoprotein chains. Interpenetration of the polymer chains and mucus to a sufficient depth is essential for mucoadhesion[54], it implies that lower chain mobility will affect mucoadhesion adversely[55]. Thus the difference in the hydration of the device / mucosa is an important determinant in studies involving in-vitro - in-vivo correlations. In this study care was taken to keep the mucosa hydrated by periodical addition of 100 µl of PBS. This may not have been an ideal solution to the simulation of the actual in-vivo conditions, but it may be expected to overcome the twin problems of overhydration (loss of mucoadhesion) and "dry" conditions, resulting in the bioadhesive compact absorbing all the available moisture from the mucosa and the consequent adhesion due to more of cappilary action than other "true" bioadhesion mechanisms. No literature information is available

on the relative roughness of the porcine gingiva and rabbit gastric and intestinal mucosa, but their degree of roughness could be a significant aspect of bioadhesion[55] since the bioadhesive material has to penetrate the crevices of the biophase. In the absence of this information it is rather difficult to conclude which *in vitro* model tissue is best to predict the *in vivo* attachment in humans.

Conclusions

Determination of mucoadhesive bond strength is important in the development of buccoadhesive drug delivery systems for quantitative comparison of different bioadhesive dosage forms and allows for quality control testing.

A significant correlation coefficient (r) value between *in-vitro* fracture strength and *in-vivo* bioadhesion was obtained when SIM or SM was employed as model mucous membranes. Therefore, the *in-vitro* adhesion characteristics seem to be well related to the *in-vivo* adhesion measurements. Hence, the modified *in-vitro* method developed by us seems to provide information on the residence time of the compact in the oral cavity. In our opinion SIM can be an ideal model mucous membrane for the development of oral muccoadhesive devices, as the pHs of buccal and small intestinal mucosa are similar, even though significant correlation coefficient values (r) were obtained for both SIM and SM. The advantages of SIM are the large number of samples that could be obtained from a single animal and the thickness and sturdiness of the mucosal layer when compared to SM.

The surface pHs of the compacts showed a linear increase with increase in the concentration of SBC. However, the findings of Leussen et al[48], who have reported the formation of water soluble complex with carboxylic acid groups of CP could be crucial in determining the time of adhesion of the compacts to the human buccal mucosa. Neutralization of the carboxylic acid groups of CP with electrolytes like calcium carbonate and disodium hydrogen phosphate and the in-vitro

mucoadhesion and the in-vivo buccoadhesion studies of the compacts containing the electrolytes are in progress.

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References

1. Harris, D, Robinson, J.R. (1992), J. Pharm. Sci. 81: 1.
2. Garren,K.W, Repta,A.J. (1988), Int. J. Pharm. 48: 189.
3. Gu, J.M., Robinson, J.R., Leung, S.H.S. (1988), CRC Crit. Rev. Ther. Drug Carrier Syst. 5 (1): 21.
4. Lee,Y., Chien, Y.W.(1995), J. Controlled Rel. 37: 251.
5. Park, K., Robinson, J.R. (1984), Int. J. Pharm. 19: 107.
6. Park, K. (1989), Int. J. Pharm. 53: 209.
7. Hassan, E.E. Gallo, J.M. (1990), Pharm.Res. 7: 491.
8. Dyik,K., Graffner, C. (1992), Acta Pharm. Nord. 4: 79.
9. Jabbari, E., Wisniewski, N., Peppas, N.A. (1993), J. Controlled Rel. 26: 99.
10. Mortazavi, S.A., Smart, J.D. (1994), J. Controlled Rel. 31: 207.
11. Ishida, M., Machida, Y., Nambu, N., Nagai, T. (1981), Chem. Pharm. Bull. 29: 810.
12. Bodde, H.E., DeVries, M.E., Junginger, H.E. (1990), J. Controlled Rel. 13: 225.
13. Smart, J.D. (1991), Int. J. Pharm. 73: 69.
14. Save,T., Venkitachalam, P. (1994), Drug Dev. Ind. Pharm. 20: 3005.

15. Anlar, S., Capan, Y., Guven,O., Gogus,A., Dalkara, T., Hincal, A.A. (1994), Pharm. Res. 11: 231.

16. Gupta, A., Garg, S., Khar, R.K. (1993), Ind. Drugs 30: 152.

17. Smart, J.D., Kellaway, I.W., Worthington, H.T.C. (1984), J. Pharm. Pharmacol. 36: 295.

18. Schnurrer, J., Lehr, C.M. (1996), Int. J. Pharm. 141: 251.

19. Sam, A.P., Heuij, J.T.M.V., Tukker, J.J. (1992), Int. J. Pharm. 79: 97.

20. Cvetkovic, N., Nesic, M., Moracic, V., Rosic, M. (1997), Pharmazie 52: 536.

21. Saettone, M.F., Giannaccini, B., Torracca, M.T., Burgalassi, S. (1987), Eur. Conf. of Biopharm. Pharmacokinetic Proc. Vol. I: 413.

22. Saettone, M.F., Chetoni, P., Torracca, M.T., Burgalassi, S., Giannaccini, B. (1989), Int. J. Pharm. 51: 203.

23. Sanzgiri, Y.D., Topp, E.M., Benedetti, L., Stella, V.J. (1994), Int. J. Pharm., 107: 91.

24. Tobyn, M.J., Johnson, J.R., Dettmar, P.W. (1995), Eur. J. Pharm. Biopharm. 41:235.

25. Tobyn, M.J., Johnson, J.R. and Dettmar, P.W. (1996a), Eur. J. Pharm. Biopharm., 42: 56.

26. Tobyn, M.J., Johnson, J.R. and Dettmar, P.W. (1996b), Eur. J. Pharm. Biopharm., 42: 331.

27. Ch'ng, H.S., Park, H., Kelly, P. Robinson, J.R. (1985), J. Pharm. Sci. 74: 399.

28. Bouckaert, S., Lefebvre, R.A., Remon, J.P. (1993), Pharm. Res., 10: 853.

29. Peppas., N.A., Sahlin, J.J. (1996), Biomaterials 17: 1553.

30. Mortazavi, S.A., Smart, J.D. (1995), Int. J. Pharm. 116: 223.

31. Martini,L., Attwood, D., Collett, J.H. D'Emanuele, A. (1995), Int. J. Pharm. 113: 223.

32. Robert, C., Buri, P., Peppas, N.A. (1988), Acta Pharm. Technol. 34: 95.

33. Collins, A.E., Deasy, P.B. (1990), J. Pharm. Sci. 79: 116.

34. Nakane, S., Kakumoto,M., Yukimatsu,K., Chien, Y.W. (1996), Pharm. Dev. Tech. 1: 251.

35. Kapur, J.N. and Saxena, H.C., Mathematical statistics, 14th edition, S. Chand and Company Limited, New Delhi, 491 (1989).

36. Anlar, S., Capan, Y. and Hincal, A.A., *Pharmazie*, 48, 285 (1993).

37. Preda, M., Vladut, C., Leucuta, S.E. (1995), Farmicia-Bucharest. 43: 51.

38. Rillosi, M., Buckton, G. (1995), Pharm. Res. 12: 669.

39. Mortazavi, S.A., Carpenter, B.G., Smart, J.D. (1992), Int. J. Pharm. 83: 221.

40. Gandhi, R.B. Robinson, J.R. (1988), Ind. J. Pharm. Sci. 50: 145.

41. Gandhi, R.B. Robinson, J.R. (1994), Adv.Drug Del. Rev. 13: 43.

42. Katchalsky, A. (1964), Biophys. J. 4: 9.

43. Technical Literature on Caebopols and Polycarbophils., B.F.Goodrich, USA.

44. Bottenberg, P., Cleymaet, F., DeMuynck, C., Remon, J.P., Coomans, D., Michotte, Y., Slop, D. (1991), J. Pharm. Pharmacol. 43: 457.

45. Taylan, B., Capan, Y., Guven,O., Kes, S., Hincal, A.A. (1996), J. Controlled Rel. 38: 11.

46. Luessen, H.I., De-Leeuw, B.J., Lehr, C.M., Junginger, H.E., et al. (1996), Eur. J. Pharm. Sci., 4: 117.

47. Rathbone, M.J., Drummond, B.K., Tucker, I.G. (1994), Adv. Drug Del. Rev. 13: 1.

48. Lejoyeux, F., Ponchel, G., Wouessidjewe, D., Peppas, N.A., Duchene, D. (1989), Drug Dev. Ind. Pharm. 15: 2037.

49. Lehr, C.M., Bouwstra, J.A., Schacht, E.H., Junginger, H.E. (1992), Int. J. Pharm. 78: 43.

50. Bouckaert, S., Remon, J.P. (1993), J. Pharm. Pharmacol., 45 : 504.

51. Chen,J.L., Cye, G.N. (1970) in Adhesion in Biological systems, Manly, R.S. (Ed.), Academic press, New York, p 163.

52. Neolman, I.G., Smales, F.C. (1995), 16 : 617.

53. King, M., Gilboa, A., Meyer, F.A., Silberg, A. (1974), Amer. Rev. Resp. Dis. 110: 740

54. Peppas, N.A., Buri, P. (1985), J. Control Rel. 2 : 257.

55. Mikos, A.G., Peppas, N.A. (1986), STP Pharma, 2: 705.

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