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Tanned leather: a good model for determining hydrogels bioadhesion

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Abstract

The main goal of this work was to study determined factors which influence the adhesion capacity of hydrogels obtained from Carbopol. The adhesive capacity was assessed using a tensile tester measuring adhesive work as the main parameter. In preliminary studies, different parameters were studied to determine their influence on adhesion work (applied force, hydration volume and contact time). To achieve this, Carbopol 940 was used as in previous studies and it showed intermediate adhesion work. It was observed that a decrease in hydration volume and an increase in contact time gave rise to an increase in adhesion work. Thus, from preliminary data, it would be interesting to test the existent relationship between-among adhesion values when different substrates were used (a semisynthetic one and a biological one). The assays was made in different hydration conditions. When experiments were performed using low hydration of the system, correlations showed to be exponential whereas when hydration was increased adhesion work values in both substrates were similar and, therefore, the correlation was virtually linear.

Keywords: Bioadhesion; Carbopol; Applied force; Contact time; Hydration volume; Adhesion work

1. Introduction

During the past few years, increasing interest has arised in the use of bioadhesive formulations for the development of drug release systems. The main reason for this is the possibility of placing bioadhesive pharmaceuticals in specific regions of the organism, thus increasing the residence time

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and ensuring an optimal contact between the dosage form and the absorption site (Gandhi and Robinson, 1988; Gupta et al., 1992; Smart, 1993).

An important aspect is the search for adequate substances, either synthetic or natural, that can be used in pharmaceutical technology. Polymers derived from acrylic acid have frequently been used for this purpose as they are described as possessing appropriate bioadhesive properties (Smart et al., 1984; Lejoyeux et al., 1989; Anlar et al., 1993; Jabbari et al., 1993).

Many different techniques and methods have been developed to measure bioadhesion, among them one should pay special attention on those based on viscosity measurements (Mortazavi et al., 1992; Caramella et al., 1993) and those that measure directly the force or work required to separate two substrates that are in intimate contact (Lejoyeux et al., 1989; Jiménez-Castellanos et al., 1993; Mortazavi and Smart, 1994; Tsutsumi et al., 1994). In these type of assays, there exist very many variables which can affect results; thus, it is interesting to conduct a preliminary study to establish the effect of the different variables and parameters. The majority of in vitro studies concerning the assessment of the bioadhesive capacity were carried out using mucosa of different natures as well as synthetic substrates; as a result, discrepancies may arise among results obtained from different authors (Saettone et al., 1989; Bouckaert and Remon, 1993; Jiménez-Castellanos et al., 1994).

A high number of experiments would needed in order to conduct a thorough study of the bioadhesive capacity of different polymers and/or formulations and, in turn, an unnecessary high number of animals would be sacrificed for this purpose. Thus, synthetic or semisynthetic substrates were chosen as they were highly advantageous from the accessibility, handling and economic point of view. The main goal of this work was to carry out a comparative study of different polymers and formulations with bioadhesive capacity.

This was achieved by assessing the variables which influence adhesion values; further, the differences that arised when a semisynthetic or a biological substrate were used to measure adhesion were evaluated.

2. Materials and methods

2.1. Materials

The adhesive capacity of various polymers derived from acrylic acid has been studied: Carbopol 941 (CP 941) of nominal molecular weight 1 250 000, Carbopol 934 (CP 934) of molecular weight 3 000 000 and Carbopol 940 (CP 940) of molecular weight 4 000 000 (Goodrich) were supplied by J Escuder. Sodium Carboxymethyl Cellulose (CMCNa) of medium viscosity 400–800 cps was obtained from SIGMA. Hydroxypropylmethylcellulose HPMC), methocel K100M was supplied by J. Escuder.

Goat tanned leather and bovine sublingual mucosa were used as substrates.

2.2. Methods

2.2.1. Substrate preparation

Samples of sublingual mucosa were obtained from the local slaughter house immediately after the animal had been sacrificed.

The mucosa was carefully separated from the muscular tissue and it was kept in the freezer. Before using, samples were defrosted in an isotonic solution (ClNa 0.9%) at room temperature. Tannned leather was used as supplied.

2.2.2. Sample preparation

Two hundred milligrams of the assaying material were compressed in 13 mm disks in a hydraulic press applying a force of 7500 kg for 2 min. In order to achieve a greater range of adhesion values, some of the polymers, particularly CP 491 and CP 940, were crosslinked, as it has been shown that crosslinking influences adhesion properties. Crosslinking was achieved by applying heat (Blanco Fuente et al., 1993).

2.2.3. Adhesion studies

Adhesive capacity was determined by measuring the maximum detachement force and the adhesion work using a tensile tester (Lloyd Instruments LR5K). The tablet was stuck to the upper support and the substrate to the lower support by using a cyanocrylate adhesive. Upon

Table 1
Values of adhesion work

Water (μl)	Applied force (N)	Time (min)	Adhesion work (mJ)	
			Mean*	Standard deviation
25	0.25	5	1.0247	0.2465
		10	2.7792	0.3341
		15	11.7946	4.2770
	0.50	5	2.1940	0.4500
		10	10.8026	3.8535
		15	16.0554	4.8433
	0.75	5	2.3743	0.5422
		10	11.3015	1.6402
		15	19.6410	4.2821
50	0.25	5	0.2690	0.2163
		10	1.3916	0.3988
		15	7.1852	0.6164
	0.50	5	0.4575	0.1911
		10	3.0859	1.3429
		15	4.9207	2.1827
	0.75	5	0.3794	0.2301
		10	2.6545	1.1535
		15	9.4867	2.7764
75	0.25	5	0.0646	0.0324
		10	0.2580	0.0178
		15	1.3292	0.2384
	0.50	5	0.2183	0.0383
		10	0.5092	0.0944
		15	1.1525	0.3221
	0.75	5	0.4431	0.0126
		10	0.6380	0.2918
		15	1.6988	0.5463
25.00	0.75	1	0.2658	0.0414
		2.5	2.7073	0.8826
		20	32.8600	6.2562
		30	37.4400	4.0700
	1	15	17.9740	2.8350

* $n = 3$.

contact of the tablet-substrate, a defined force was applied during certain time. This was proceeded by an extension phase at a defined rate until total separation of the components was achieved.

During this experiment the force (N) versus elongation was registered. The adhesion work was calculated (mJ) as the area below the obtained graph.

The modules of Young were obtained from the initial slope rates of force-elongation (Ponchel et al., 1987).

3. Results and discussion

The parameters that influence the adhesion work values were studied by a factorial design 3^3 using 3 replicates.

The studied variables were the following:

(1) Added hydration water: 25, 50 and 75 μl.
(2) Applied force during the compression phase: 0.25, 0.50 and 0.75 N.
(3) Time during which the force was applied: 5, 10 and 15 min.

During the experiment, traction rate was kept constant at 5 mm/min.

As there would be a large amount of assays if this study was performed in each obtained formulation, tablets made of CP 940 were selected as, in previous studies, it showed intermediate adhesion values. Moreover, only tanned leather was used as too much sublingual mucosa would have been required.

Table 1 shows the adhesion work values obtained in the different conditions studied.

The multifactorial analysis showed that all factors bore influence upon the obtained values for $\alpha < 0.01$.

All values were subjected to multiple sequential regression from which the following equation was obtained (r2 = 0.8663, $\alpha < 0.01$):

$$W = -8.8308 + 1.7302\,t + 10.399\,F - 0.0021\,V^2 - 0.0257\,tV + 0.5779\,tF - 0.2235\,VF$$

where:

W = adhesion work
t = contact time (min)
F = applied force (N)
V = added water volume (μl)

The results were plotted in Fig. 1. as response surfaces. It can be observed that when there was a short contact time, low adhesion values were registered; moreover, hydration volume and applied force hardly influenced results. This was to be expected as a minimum time is required to achieve hydration and, therefore, polymer chain interpenetration into the leather porous structure.

However, as contact time increased between the polymer and the substrate, adhesion values also started to increase. On the other hand, as contact time increased so did the influence of other parameters such as applied force and hydration volume.

In order to determine the optimum values, a method based on the maximum slope was used. The study involved changing every parameter (force and contact time) with the exception of water values below 25 μl because problems arose regarding appropriate surface distribution of the hydrogel. It was observed that, at short intervals of time, the adhesion work values were low. When the contact time was increased from 5 to 20 min, a lineal increase in the adhesion work values was seen which was more or less constant after 20 min (Fig. 2).

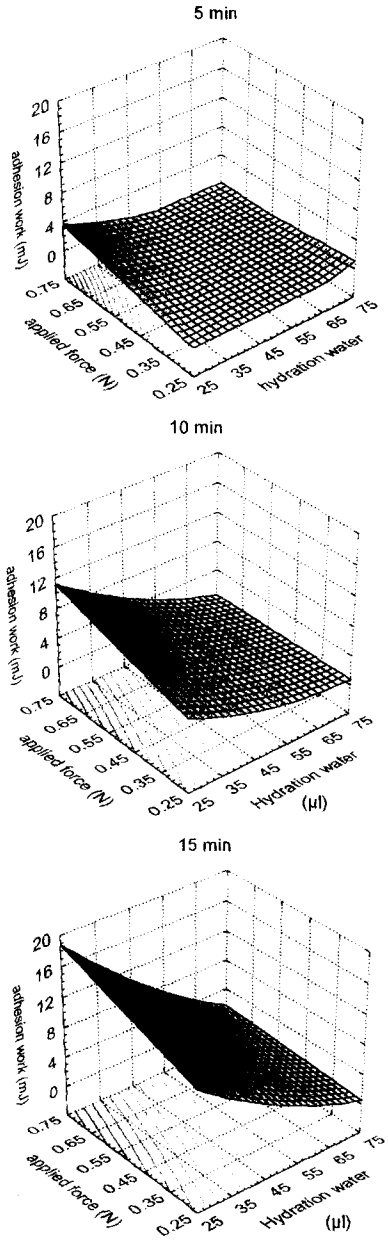

Fig. 1. Response surfaces of the adhesion work as a function of the different parameters used.

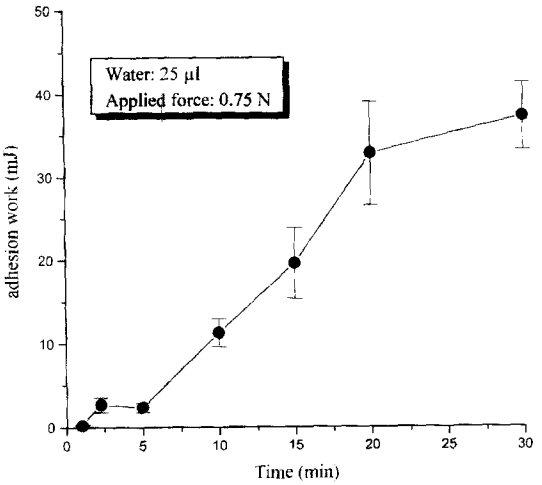

Fig. 2. Adhesion work as a function of contact time.

With respect to the applied force during the compression period, the variance analysis did not reflect any significant differences, although adhesion work values tended to increase as the applied force increased (Fig. 3). In order to achieve interpenetration or diffusion of polymeric chains, a minimum force is required: once this is achieved, diffusion will only depend on time, thus an increase in time gave rise to a deeper chain penetration into the substratum (Leung, 1987).

On the other hand, it is interesting to point out that the highest adhesion values were obtained

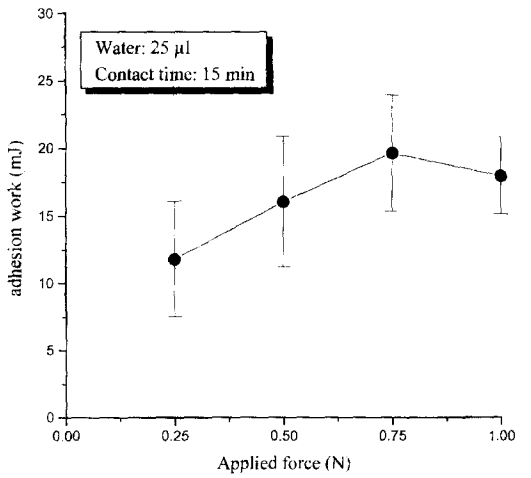

Fig. 3. Adhesion work as a function of applied force.

with low hydration grades (25 µl). The role which hydration plays on these type of polymers bioadhesion is well known. The water or hydration liquid leads to hydrogel formation and facilitates the mobility of the polymeric chains, thus these can diffuse and form bioadhesive unions much more easily (Chen and Cyr, 1970; Smart et al., 1984). However, an excessive degree of hydration may modify the mechanical resistance of the hydrogel. Moreover, it must be taken into account that hydration implies a swelling of the formulation and, therefore, a decrease of the density of adhesion promoter groups in the adhesive interface. The optimum hydration results from a compromise between the mechanical resistance and the mobility of the polymeric chains (Lejoyeux, 1991).

From the studied values, it can be concluded that for this type of polymers the optimum conditions to achieve appropriate adhesion work values could be: a compression force of 0.5 N during a period of 20 min and adding a volume of 25 µl. However, it should be stressed that ideal conditions will largely depend on the structure of the material to be used. For example, it has been demonstrated that the degree of hydration has a more significant influence in these polymers than in others such as CMCNa or HPMC.

Once the preliminary study of selecting the optimal adhesion conditions was concluded, it seemed very interesting to ascertain the relationship between the adhesion values and different substrates: a semisynthetic and a biological one. The assays were conducted using various formulations that potentially presented different adhesive capacities.

Two different methods were used for this purpose, although named as dry and humid medium by some authors (Lejoyeux et al., 1988), will be referred to in this study as assays where water limitation exists (Fig. 4a) and assays where aqueous limitation does not exist (Fig. 4b).

When the first method was used, tanned dried leather and sublingual mucosa were used as substrates; 25 µl of water were added to the formulation and were homogeneously distributed on the surface. As it can be observed from Fig. 5, all adhesion work values obtained when using sublin-

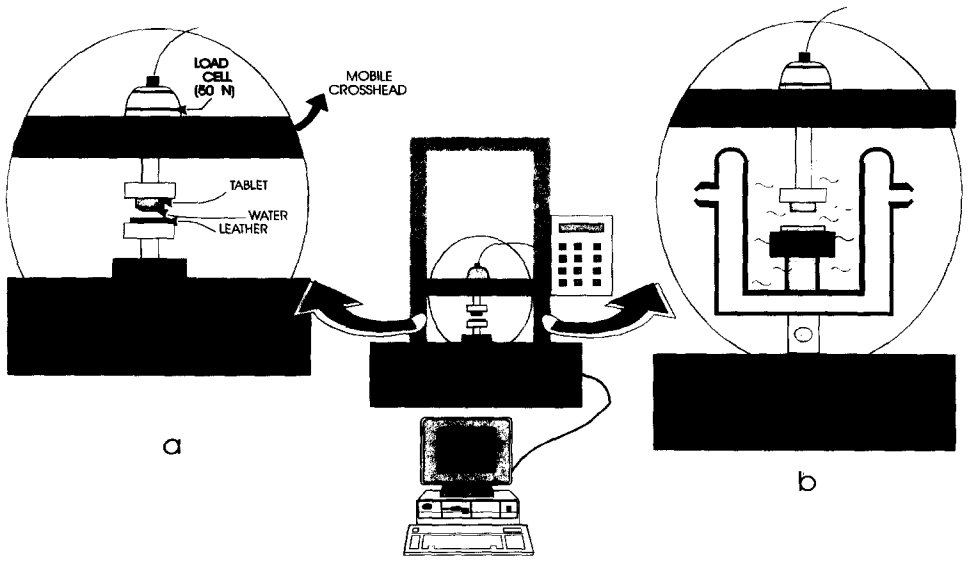

Fig. 4. Outline of the tension equipment used. (a) Water limitation. (b) No water limitation

gual mucosa were much lower than those obtained with tanned leather. We have previously described that hydration affects adhesion and, in the case of polymers like Carbopol, a small increase in the degree of hydration gives rise to a decrease in adhesion. On the other hand, these polymers exhibit a high ability to uptake water, thus in assays performed on sublingual mucosa (itself being hydrated), the tablet was able to seize water; this plus the 25 μl of water added to the tablet made it sufficient to decrease adhesion values. It can also be observed that these differences were lower in the cases of HPMC and CMCNa.

A practically exponential correlation was obtained between the adhesion work in leather and mucosa (Fig. 5a). The Young modules were calculated for all systems in both substrates (Fig. 5b), which show differences obtained from both substrates. This means that the systems present different elasticity, which explain the differences obtained between the adhesion work on leather and mucosa.

As the sublingual mucosa is more hydrated than leather, a new assay was designed in order to have the two substrates presenting similar hydration conditions. This was achieved by previously hydrating the leather with saline solution. It can be observed in Fig. 6a how, in this manner, we obtained a exponential correlation but the adhesion work values were more similar. When the modules of Young were compared it was seen that there existed less differences among them, thus two conclusions could be drawn: first, the elasticity in both substrates was similar and, second, the behaviour of the tanned leather resembled that of the sublingual mucosa.

In order to minimise further the variations among substrates, a system which did not present any water limitation was designed which can be seen in Fig. 4b. From this model, an appropriate lineal correlation was obtained between the adhesion work values of both substrates, as it can be seen in Fig. 7a. From the slope values, nearly = 1, it could be concluded that when these conditions were applied the values obtained were approximately the same for both substrates. It should be stressed that some formulations used showed such low adhesion values that it was impossible to record them (HPMC, CMCNa). The analysis drawn from the Young modules (Fig. 7b) reconfirms the hypothesis of the elasticity properties being similar when both systems had similar hydration conditions.

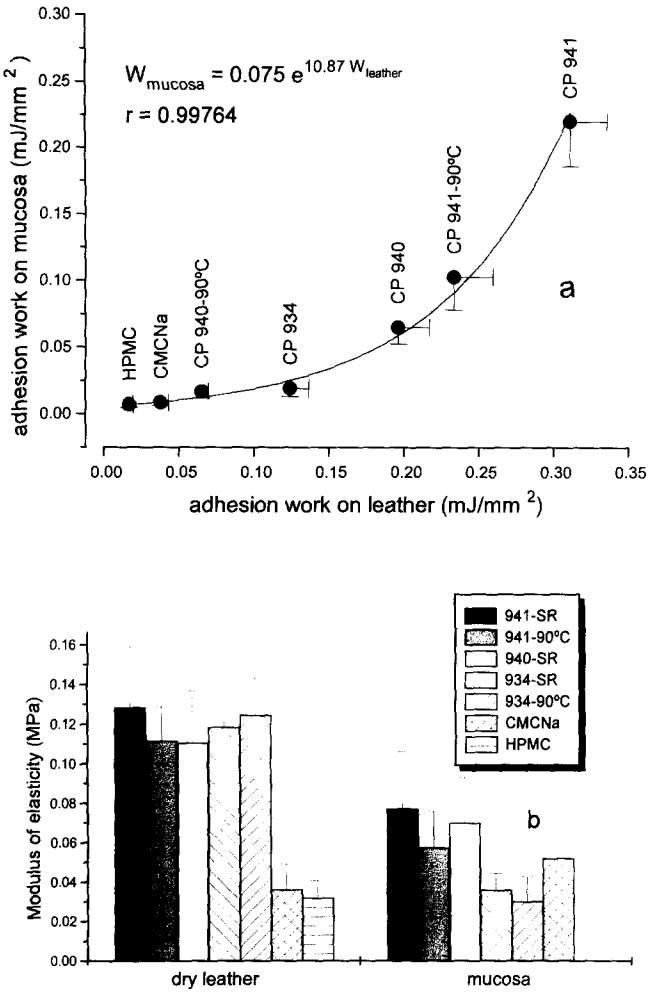

Fig. 5. (a) Correlation between adhesion work in dry leather and sublingual mucosa. (b) Elasticity modules among the formulations in (a).

The effect of crosslinking and molecular weight on the adhesion properties of these polymers was obvious in every experiment. Carbopol 941 (molecular weight 1 250 000) shows great adhesion properties which decrease in higher molecular weight polymers (CP 934 and CP 940) (Saettone et al., 1989; Lejoyeux et al., 1989, Anlar et al., 1993). Crosslinking gave rise to a considerable decrease in these systems adhesion due to an increase in the rigidity of the polymeric chains, thus decreasing chain diffusion into the substrate (Park, 1986).

The low adhesion values obtained for cellulose derivatives (HPMC and CMCNa) were to be expected as the optimum conditions chosen to assay Carbopols may be very different to those required for cellulose. The adhesion capacity of cellulose when different conditions are used has been demonstrated elsewhere (Mortazavi and Smart, 1995).

It can be concluded that when the bioadhesive properties of a compound are studied by in vitro methods based on tension measurements, one should carefully consider the importance of the

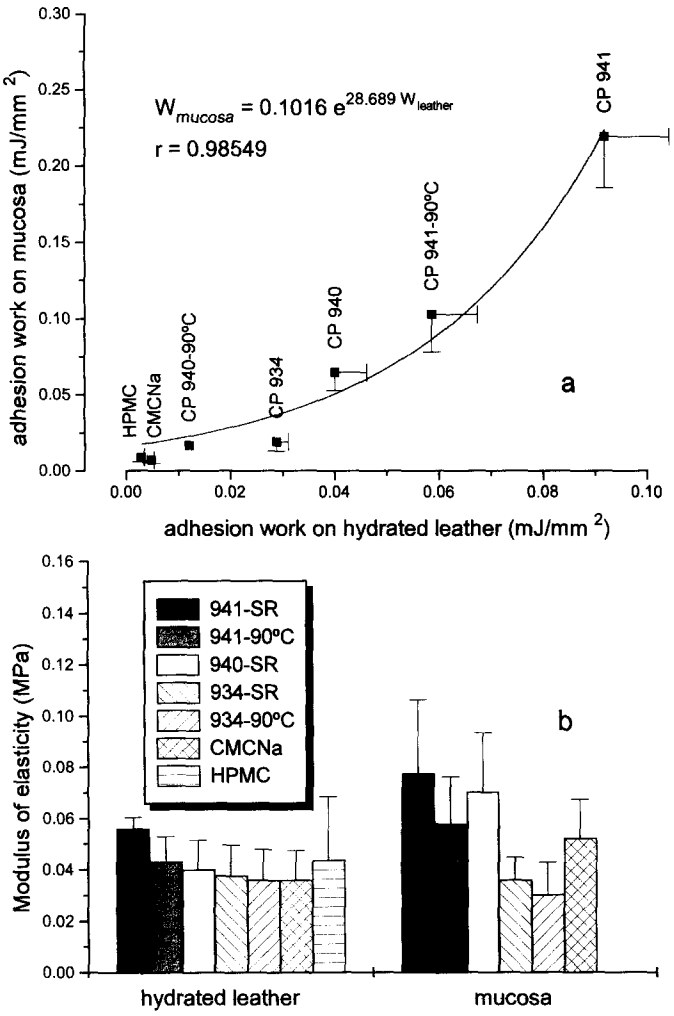

Fig. 6. (a) Correlation among the adhesion work in humidified leather and sublingual mucosa. (b) Elasticity modules among the formulations in (a).

different parameters tested in this study. Again, it should be stressed that optimum conditions can differ greatly from compound to compound and it is very difficult to adequately classify the adhesive capacity of different materials.

Finally, an adequate correlation between assays performed on a semisynthetic substance (tanned leather) and those performed on bovine sublingual mucosa was obtained. Therefore, large numbers of experiments using different formulations can be performed using tanned leather, material which is more readily available and easier to handle than mucosa.

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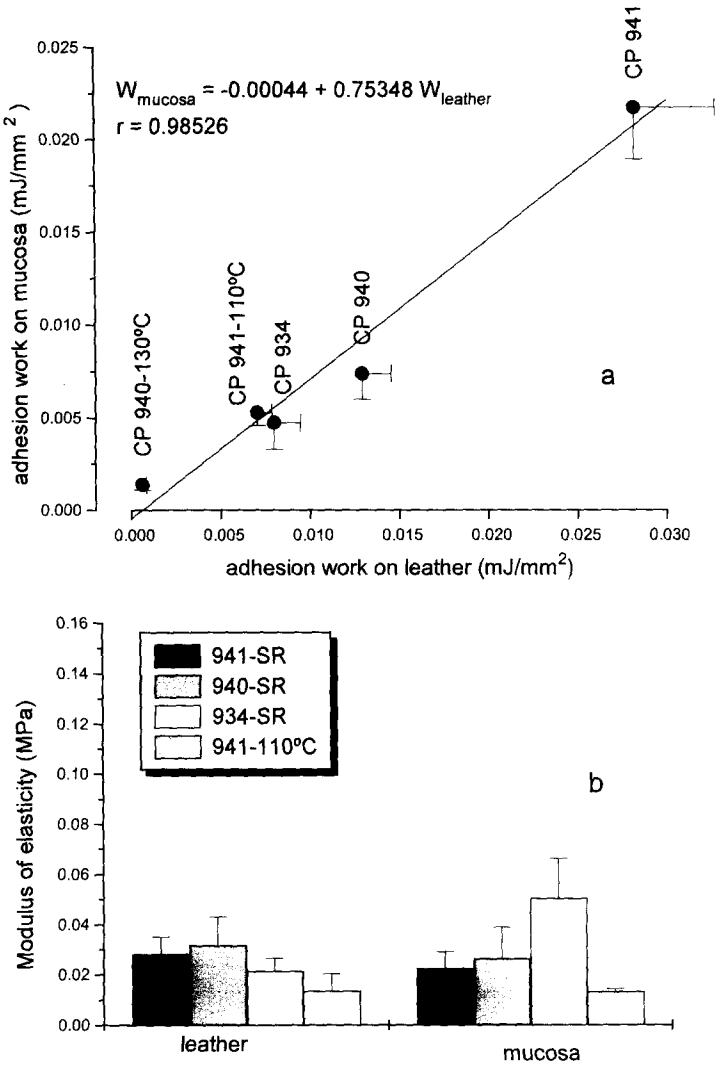

Fig. 7. (a) Correlation among the adhesion work on leather and sublingual mucosa when there was no water limitation. (b) Elasticity modules among the formulations in (a).

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