Evaluation of in vitro properties of films of saliva substitutes in contact with different surfaces

A comparative study with instruments for measurements of friction and rheologic properties

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An instrument based on friction measurement has been developed to evaluate oral mucosal dryness objectively. The purpose of this study was to compare the friction instrument with instruments measuring in vitro rheologic properties. Measurements were performed against steel and irreversible hydrocolloid after application of different concentrations of aqueous solutions of carboxymethylcellulose and chitosan lactate. The results of the measurements were logical, with inversely proportional values for the friction instrument as compared with values obtained using the instrument measuring rheologic properties; that is, increased viscosity led to decreased friction values. □ *Friction; rheology; saliva substitutes; xerostomia*

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Impairment of the oral mucosal surface protective barrier, such as in xerostomia, increases the risk of soft-tissue infections and functional impairment (soreness and various other complaints from patients) (1–3). To reduce mucosal damage and relieve symptoms, patients may be given a saliva substitute.

Saliva deficiency and efficacy evaluations of saliva substitutes have been the subject of many studies (1, 2, 4–8). The degree of dryness has mainly been evaluated by the use of questionnaires, but objective measurements such as by sialometry, have also been performed. The correlation between sialometry and symptoms of dry mouth seems, however, to be limited (9, 10). It would thus be of interest to develop and evaluate alternative objective methods of assessing dryness. For such measurements, instruments based on some physical principles (electricity, light absorption, friction) have therefore been tested (11–13). Among these instruments, a device based on friction measurements proved to be the most suitable. A computerized version (Probe II) proved to be valid and to have sufficient reliability for assessing oral mucosal dryness (13). In vivo trials have shown the friction instrument to be a useful tool in the efficacy evaluation of different saliva substitutes/stimulants (14–16). It has also proved valuable in the registration of the oral side effects of drugs (17) and assessment of sialoadenitis (18). The friction-measuring device has further proved to be suitable for evaluation of skin surface properties such as the degree of dryness and changes in the surface properties on ointment application (19, 20).

The purpose of this study was to compare the friction instrument with instruments measuring in vitro rheologic properties.

Materials and methods

Saliva substitutes

Aqueous solutions of carboxymethylcellulose (CMC) and chitosan lactate were used in concentrations of 0.0625%, 0.125%, 0.25%, 0.5%, and 1.0%.

Friction measurements

In the in vitro measurements the probe was placed vertically in a stand with the steel measuring plate (lower measuring plate) with a diameter of 6 mm upwards (Fig. 1). The instrument operated with an oscillatory motion of rotation of about 300° and with a frequency of about 0.2 Hz. A steel or an irreversible hydrocolloid surface (Alginoplast) (upper measuring plates) was loaded with 0.1 N in the two series of measurements. The hydrocolloid surface was prepared by mixing one measuring spoon—that is, 8.2 g—of Alginoplast powder with 18 ml water for 30 sec to a homogeneous consistency. The mass was then transferred to an impression in the upper measuring plate. The experiments were carried out at room temperature (19–22°C) and at a room relative humidity of 34–42%. Ten measurements were carried out for each aqueous solution of CMC and for chitosan lactate in con-

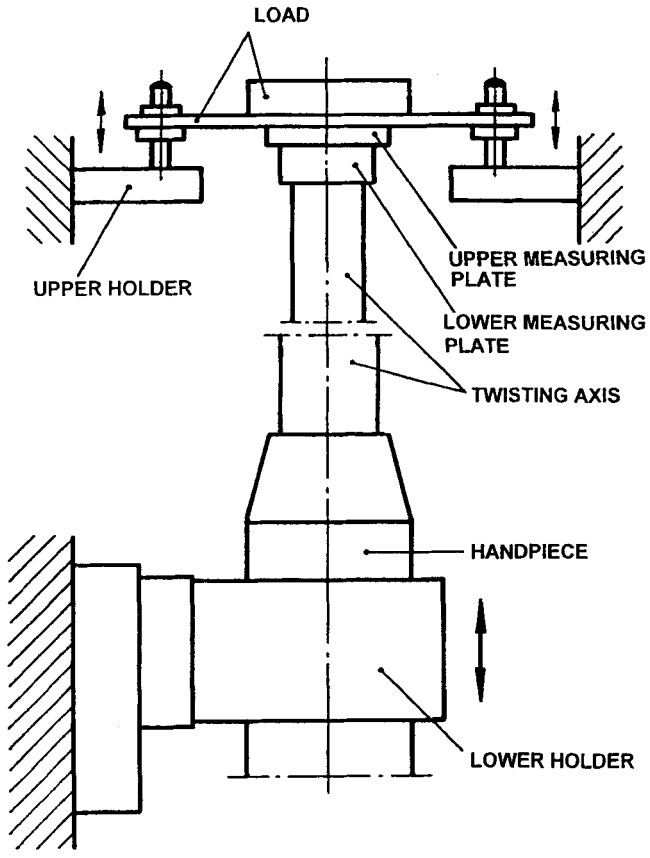

Fig. 1. Experimental set-up for in vitro measurements with the friction device.

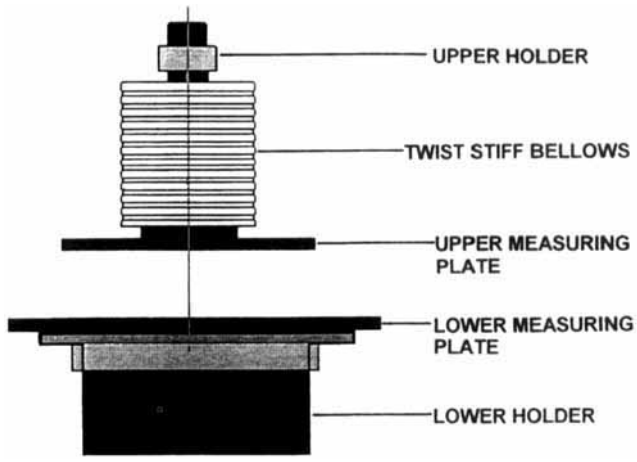

Fig. 2. Experimental set-up for in vitro measurements with the VOR 2.

centrations of 0.0625%, 0.125%, 0.25%, 0.5%, and 1.0%. For the measuring procedure, one droplet of saliva substitute was applied to the steel measuring plate of the friction instrument, which was then brought in contact with the steel or hydrocolloid surface at the moment of measurement (Fig. 1). Between each measuring procedure, the surfaces—that is, the steel measuring plate of the friction instrument and the tested surfaces of steel or hydrocolloid—were cleansed with doubly distilled water at room temperature. Between the measurements a moist compress was applied to the hydrocolloid surface to minimize dehydration.

Rheologic measurements

Two instruments were used, the Bohlin VOR (Bohlin Rheology (VOR 1)) and a specially designed VOR adapter (VOR 2). The viscosity of CMC and chitosan lactate was measured with the VOR 1 at concentrations of 0.0625%, 0.125%, 0.25%, 0.5%, and 1.0%, using Couette geometry (C25 Bohlin Rheology). The VOR 2 is an adapter for the VOR 1 which was constructed to evaluate the influence of a normal force applied to the upper measuring surface in a plate-to-plate geometry (Fig. 2). The diameter of the upper steel measuring plate was 15 mm. The instrument operated with an oscillatory motion of rotation of about 2° and with a frequency of 0.4 Hz. The elastic and viscous components and the resulting complex shear stress were determined. The measurements with the VOR 2 were carried out with CMC and chitosan lactate in the same concentrations as the measurement with the friction instrument and the VOR 1. When the VOR 2 was used, the measurements were performed between a hydrocolloid surface and a steel measuring probe with a load of 0.13 N, which is close to the 0.1 N load used in measurements with the friction probe. For the measuring procedure, three droplets of saliva substitutes were applied to the hydrocolloid surface (lower measuring plate), which was then brought in contact with the upper steel measuring plate at the moment of measurement (Fig. 2). Between each measuring procedure the surfaces—the upper steel measuring plate and the hydrocolloid surface—were cleansed with doubly distilled water at room temperature. Between the measurements a moist compress was applied to the hydrocolloid surface to minimize dehydration.

To evaluate the surface energy properties of the irreversible hydrocolloid surface, contact angle measurements were performed with equipment previously described (21). The following test liquids (surface tension) were applied with a freshly flamed platinum wire to the hydrocolloid surface: propylene carbonate (40.5 mN/m), *S*-tetrabromoethane (49.7 mN/m), methylene iodide (50.8 mN/m), thiodiglycol (54.0 mN/m), and glycerol (63.4 mN/m). Two series of measurements were performed on two different hydrocolloid plates. For each test liquid, contact angle measurements were performed on each side of the profile of the applied droplet and again after a second droplet of the same liquid was placed on top of the first, thus creating a

drop with a larger volume. Each new liquid was placed on a different spot on the hydrocolloid surface. Contact angles were measured at the visual establishment of mechanical equilibria. For each liquid a mean value was calculated, from which the cosine of the contact angle was determined. The cosine of the average contact angles and the surface tension of each liquid was plotted in accordance with Zisman (22).

No statistical evaluation was performed between the different saliva substitutes and concentrations, because the purpose of this study was to compare the friction instrument with instruments measuring in vitro rheologic properties.

Results

Friction measurements

Measurements with CMC and chitosan against the steel plate resulted in steadily decreasing friction values with increasing concentrations (Fig. 3). Chitosan showed higher mean friction values for almost all concentrations (range, 0.86–5.00 arbitrary units) than CMC (range, 0.47–3.84 AU). Further, measurements with CMC and chitosan against the hydrocolloid sheet resulted in considerably higher mean friction values for all concentrations for chitosan (range, 4.79–15.11 AU) and for CMC (range, 2.62–7.13 AU) (Fig. 3) compared with the values obtained when measuring against the steel plate. In addition, steadily decreasing friction values were also seen with increasing concentrations when measuring against the hydrocolloid sheet, and consistently higher values for chitosan than for CMC at all concentrations. For all series of friction measurements only small standard deviations (SD) were registered (range, 0.04–1.01 AU).

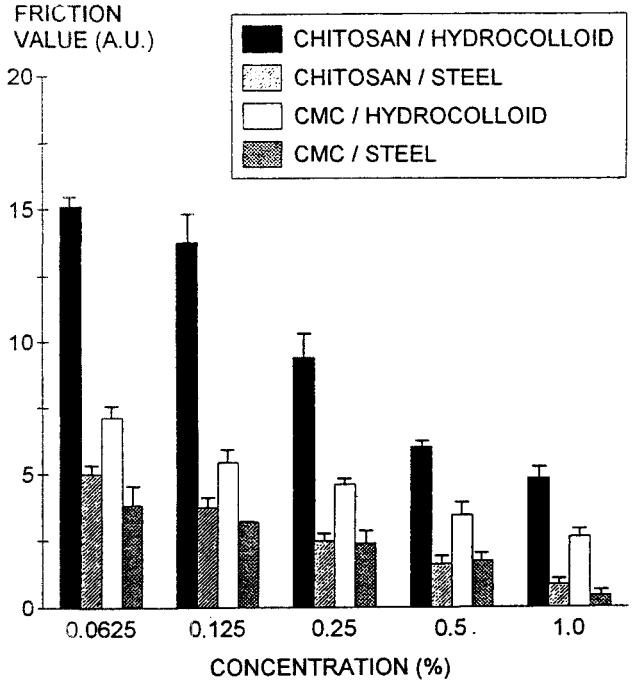

Fig. 3. Mean friction values in arbitrary units (A.U.) ± SD of 10 measurements with chitosan lactate and carboxymethylcellulose (CMC), respectively, against the steel plate and the hydrocolloid sheet.

Table 1. The viscosity (mPas) of carboxymethylcellulose (CMC) and chitosan lactate measured with the Bohlin VOR

Concentration (%)	CMC	Chitosan lactate
0.0625	4.15	2.0
0.125	5.61	3.4
0.25	8.64	7.1
0.5	15.5	15.0
1.0	49.0	45.0

Rheologic measurements

When viscosity was measured with the VOR 1, increasing values were registered with increasing concentrations of CMC and chitosan lactate (Table 1).

When measuring with the VOR 2, the complex shear stress showed increasing values with increasing concentrations of CMC up to 0.5% (Fig. 4). However, a decreased value was obtained at concentrations above 0.5%. The values for chitosan decreased after 0.25% and showed steadily lower values as compared with CMC.

Contact angle measurements

When the cosine of the average contact angles in the Zisman plot from the two series of measurements were compared, duplicate samples showed good agreement for propylene carbonate, *S*-tetrabromoethane, and methylene iodide, whereas a considerable scatter was observed for thiodiglycol and glycerol (Fig. 5). The critical surface tension for wetting could be estimated to be approximately 35 mN/m.

Discussion

The series of measurements with CMC and chitosan lactate performed with the friction instrument showed a covariance with the VOR 1—that is, with inversely proportional values for the friction instrument as compared with values obtained with the VOR 1. When the obtained values were compared, consistently decreasing friction values and increasing viscosity values were seen with increasing concentrations of CMC and chitosan lactate. This indicates that high viscosity is probably

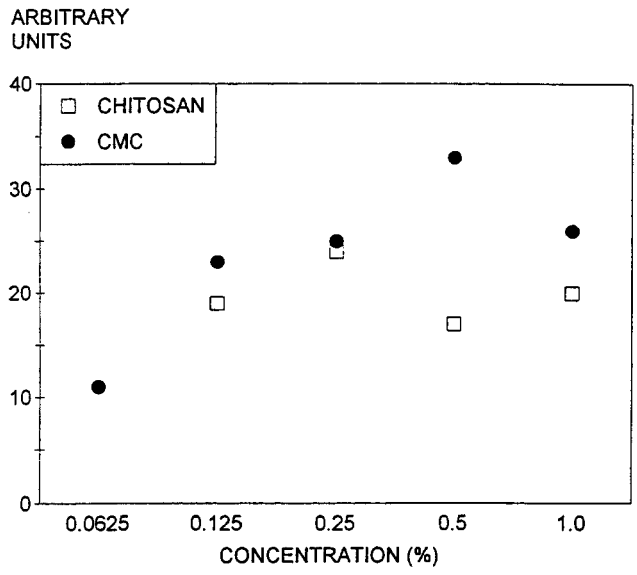

Fig. 4. VOR 2 measurements with carboxymethylcellulose (CMC) and chitosan lactate. Complex shear stress in arbitrary units.

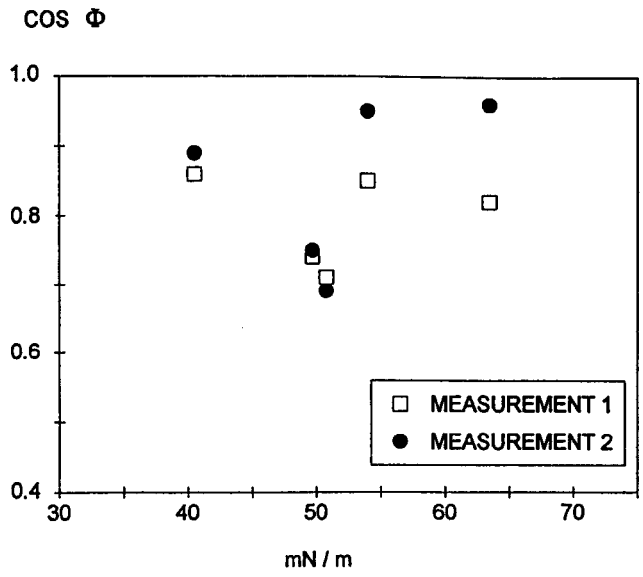

Fig. 5. Relationship between the surface tension and the cosines of the average contact angles between the different liquids and hydrocolloid surfaces. Liquids from left to right with surface tension values: propylene carbonate 40.5 mN/m), *S*-tetrabromethane (49.7 mN/m), methylene iodide (50.8 mN/m), thiodiglycol (54.0 mN/m), and glycerol (63.4 mN/m).

an important positive lubricating factor. The values for CMC and chitosan lactate obtained when measuring with the VOR 2 increased with increasing concentrations up to a certain level. Thereafter, lower values were registered at higher concentrations. This may be due to an increase in the film thickness with increased viscosity of the liquid. Thus, at the higher concentrations of CMC and chitosan lactate, the film between the plates is probably thicker, giving a lower shear rate and thus a decrease in the complex shear stress.

When the friction values for CMC and chitosan lactate were compared, chitosan showed considerably higher values when measuring against both steel and hydrocolloid. This could be explained by the lower viscosity for chitosan, at least at the lower concentrations (Table 1). Interestingly, chitosan in a clinical trial has shown a considerably shorter duration of clinical effect than CMC and some other saliva substitutes (16).

Further, the friction values showed a small SD, which demonstrates a good precision of measurements with the instrument. Small SD has also been shown with in vivo measurements (18).

When the steel and the hydrocolloid surfaces were compared, it is obvious that the hydrocolloid surface is more similar to the oral mucosa, which can be described as a hydrophilic and elastic surface. Contact angle measurements against the two hydrocolloid surfaces showed that the surfaces are very sensitive to dehydration, which is reflected by the different levels of the cosine of the average contact angles for thiodiglycol and glycerol, which are polar liquids. This was taken into consideration in the in vitro measurements, in which the hydrocolloid surface was protected by a moist compress between the measurements.

When we measured with the friction instrument against the steel and the hydrocolloid surfaces, the friction values in arbitrary units were almost within the same range as the in vivo measurements (18). There is obviously an agreement in the relationship between in vivo and in vitro measurements with regard to the magnitude of the friction values, which indicates that the chosen in vitro model is relevant in the sense that it may reflect important properties of the oral mucosa. Further, no statistical calculations were performed between measured values of the different saliva substitutes, since the only aim of this study was to compare the correspondence between the friction instrument and the instruments measuring in vitro rheologic properties.

It is thus obvious that not only quantitative but also qualitative properties of saliva substitutes and probably also of saliva are of decisive importance for mucosal lubrication. With the friction instrument it seems possible to register these qualitative properties in a manner that cannot be done by sialometric measurements. The friction instrument may, among other potential uses, be useful in efficacy evaluation of different saliva substitutes/stimulants and for registration of oral mucosal dryness caused by drugs and salivary gland diseases (13–18).

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