ARTIFICIAL SALIVA SUBSTITUTES EVALUATION: THE ROLE OF SOME CHEMICAL-PHYSICAL PROPERTIES

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ABSTRACT

To evaluate some chemical-physical properties of artificial saliva substitutes easily available on the E.U. market, such as viscosity, pH, buffering capacity, superficial tension, density and spinnbarkeit and compare the results with human natural saliva bibliographic data.

All the saliva substitute products were purchased on the net using University students as customer models. Viscosity and density measurement were performed using a falling ball type viscometer (Anton Paar® - Lovis 200 ME); Buffering capacity was determined according to method described in literature. Viscosity values ranging from a min. value of 1.682 mPa.s to a maximum value of 21.727 mPa.s, CMC based products have the highest viscosity values. Four products (Saliveze®, Xerotin®, Saliva Orthana® and Xerostom®) have a pH value higher than 6.00. Buffering capacity values ranging from a min. value of 0.909 mmol H+/L to 3.33 mmol H+/L reveal broad results between the samples. Form our data and literature review lower values of viscosity and surface tension can be ascribed as the most important features that obtain patient compliance in artificial saliva substitutes. Nevertheless, products selection had to be based on market availability and personal preference.

INTRODUCTION

Human saliva is responsible for many different functions such as the maintenance of moist oral mucosa, removal of microorganisms and lubrication during speaking, mastication and swallowing. An extensive review has recently been conducted by Dawes et al.1, they summarized all the known functions of saliva. Buffering ability, for example, is fundamental to protect oral mucosae and the teeth from acid insults, furthermore the presence of antibacterial, antifungal and antiviral agents modulates oral microbial flora.

The complexity of the system is easily noticeable; it consists of water (more than 99%), glycoproteins (mucins), antimicrobial substances, proteins and a large variety of electrolytes. Most common proteins present in saliva are α-amylase, maltase, serum albumin, mucins and immunoglobulins. Saliva is produced from major salivary glands, including parotid glands, submandibular and sublingual glands, and from minor glands that can be found in the lower lip, tongue, palate, cheeks and pharynx.3 At rest, without any stimulation, saliva is constantly produced and this phenomenon is denominated basal secretion. Being always the presence of a saliva film that covers, moisturizes and lubricates oral tissues. Exogenous and pharmacological stimulations can induce an increase in the salivary flow. Daily salivary production in a healthy subject is around 1 L, whereas the salivary flow rate (FR) has been considered in 2013, data about stimulated whole saliva (UWS) from a very select sample of healthy young adult were collected. Values of UWS/FR ranged from 0.164 to 1.656 mL/min (percentile 25 = 0.400 mL/min, percentile 50 = 0.643 mL/min, percentile 75 = 0.832 mL/min) and they were normally distributed (p < 0.05)4. Understanding daily production saliva is as fundamental to know its biophysical properties as viscosity: where values alteration has been published an interesting study about saliva substituents considering apparent viscosities of three different types of saliva substitutes with those of human whole saliva. One product was based upon carboxymethyl cellulose (CMC) or polyethyleneoxide (PEO). Mucin based products seem to show very good rheological properties, which makes them useful for protection against desiccation and environmental insult, lubrication and, moreover, they show to have anti-microbial effect5. Previous works on artificial substitute properties comparison have already been published, but still it is not possible to find a study conducted on a relative high number of products and focused on determination of multiple characteristics. For example, in Preetha et al. work attention was focused on viscosity and surface tension detection on three commercial products, still other properties were not taken into account6. Another interesting work, but not representative of all products that are currently commercially available, has been conducted by Vissink et al.7, they compared apparent viscosities of three different types of saliva substitutes with those of human whole saliva. One product was based upon carboxymethyl cellulose, one was mucin containing and the last one, a solution of polyethyleneoxide. Hatton et al. gathered together five different CMC based products and one mucin based saliva substitute and tested their viscosity at different shear rate. Christersson et al. published an interesting study about saliva substituents considering more properties, such as viscosity, pH, surface tension and adsorption to surfaces8. This study was conducted over three different products based upon CMC, mucin and linsed oil.

In the present study, we considered a heterogeneous group of artificial saliva substitutes based on their easy availability on the market. Our attention has been directed toward determining a set of chemical-
physiological properties: viscosity, pH, buffering capacity, superficial tension and density. Moreover, spinbarkeit has been considered to increase the rheological characterizations.

**MATERIALS AND METHODS**

**Materials**

All the saliva substitute products were purchased on the net, to five University Students of “University of Estem Piedmont” was asked to spend, each one, 2 hours to web-search focused on artificial saliva products. Seven products were found, to note that no student has been able to find all seven products (max four products), three different artificial salivas based on carbomylmethylcellulose as rheological modifier component were obtained: Glandosane®, Saliveze® and Xeroton®. Biotene® is Carbomer® and hydroxyethylcellulose based. Saliva Orthana® and Saliva Natura® have been designated as mucin-based saliva substitutes. While Xerostom® is a complex mixture of natural Oils. For each product, three different samples were purchased and analysed. HCl 37% w/w was obtained from Sigma-Aldrich® and de-ionized water was used for all the experimental purposes.

**Viscosity and density measurement**

Data were collected using a falling ball type viscometer (Anton Paar®-Lovis 200 ME); all tests were conducted at room temperature (20°C) and at 36°C. The pipe inclination angle was set either at 80° either at 70° depending on the measured viscosity. The density of each sample was tested at room temperature (20°C) and at 36°C, using a vibrational densitometer coupled with the viscometer (Anton Paar®- DMA 4000 M). All experiments were conducted in triplicate.

**pH and buffering capacity determination**

pH analyses were carried at room temperature using a pH-meter (Mettler Toledo® - Five Easy). Buffering capacity was studied with the same instrument, according to the procedure described by Gittings et al. Here summarized: from each product, a 12 mL sample was taken and pH was analyzed, this measurement was repeated three times. Afterwards, a 0.1 M HCl solution was added dropwise until a 1 unit variation in pH value was detected. Taking into account the added solution amount, HCl concentration on the final volume was calculated and buffering capacity was expressed in mmol H+ /L. All experiments were conducted in triplicate.

**Surface tension analysis**

This was done using a tensiometer (KSV - Sigma 703d). All results of pH, density and spinnbarkeit were obtained by the same instrument, according to the procedure described by Meurman et al., used a method named the Dentubuff-strip method, which was repeated three times. Nevertheless, the same approach used here was also used by Gittings et al.® in their research managed to use a dynamic rheometer that at the experimental conditions applied a shear rate of approximately 50 s⁻¹. With those conditions, they determined a viscosity value of 5.3 mPa s for Saliva Orthana® while we detected 3.178 mPa s; this is likely accountable to the different applied shear rate. Since UWS viscosity values, at shear rate present in oral cavity, don’t exceed 10 mPa s, but preferably ranging between 1 and 2 mPa s, as described in previous work (2,3). Saliva Natura®, Saliva Orthana® and Xerostom® are those closer to the human natural value, suggesting that the lower viscosity is achievable with mucin and/or lipid based product. Since it is not possible to control shear rate, it is only possible to quantify the evaluated saliva substitutes in two different groups: such as Saliveze® and Xeroton®, which have a high viscosity and a low shear rate, and the others, Saliva Natura®, Saliva Orthana®, Glandosane® and Xerostom®, which have a low viscosity and a high shear rate. Vissink et al.® reported a clinical study in which three groups of patients, suffering from severe xerostomia, were treated with CMC and mucin based artificial saliva. They found out that patients preferred mucin containing saliva substitutes, since those such as Saliveze® and Xeroton®, which have a low viscosity and a high shear rate, and the others, Saliva Natura®, Saliva Orthana®, Glandosane® and Xerostom®, which have a low viscosity and a high shear rate.

**RESULTS**

Results obtained from Biotene characterization are not reported since it is a semi-solid paste and its viscosity ranges were undetectable by our instrument, as well as all other characterizations. For this reason, it was considered having too different features from other artificial salivas and it was not considered in this comparison. Viscosity of saliva substitutes and corresponding shear rate value are displayed in Table 1. Viscosity values ranging from a min. value of 1.682 mPas to a max. value of 21.727 mPas (about 13 times greater), correspond to Saliva Natura® and Xeroton® products. Saliveze® and Xeroton®, which are CMC based products, have the highest viscosity values. All average viscosity values for each product have a relative standard deviation of less than 5%, except for Xeroton® which was more than 10%. Analysis temperature did not affect drastically samples viscosity, apart from Saliva Orthana® and Xerostom® resulting in a variation of 32% and 35% respectively (data not reported). Intra-batches results of pH, buffering capacity, density, surface tension and spinbarkeit were highly reproducible, having a RSD lower than 2.5% (Table 1), this due to the industrial nature of the products. It is possible to notice how four products, Saliva Natura®, Saliva Orthana® and Xerostom®, have a pH value higher than 6.00. Buffering capacity characterization reveal broad results between the samples, in some case one product (Saliva Natura®) have a buffering capacity about ten times than other (Saliva Orthana®). All density and spinbarkeit products values lying in narrow figures, corresponding to less than a 3% variation. Surface Tension values were different between the analysed products, giving 2 products having a surface tension higher than 60 mN/m and other 3 with a surface tension lower than 50 mN/m.

**DISCUSSION**

**Viscosity**

Human saliva pH bibliographic data are summarized in Table 1, it is known that values change depending on subjects age, collection methods, cohort selection. Except for pH indication on product leaflet or brochure, only Saliva Orthana® pH value is available, evaluated by Christersson et al.®, having a value of 5.7; this is lower than our determination, nevertheless both values can fall in the so called neutral pH as declared on product package. Since pH values range about 6.49–7.28®, Saliva Natura® and Glandosane® are the only ones which don’t exceed 10 mPas, and mucin based artificial saliva. They found out that patients preferred mucin containing saliva substitutes, since those such as Saliveze® and Xeroton®, which have a low viscosity and a high shear rate, and the others, Saliva Natura®, Saliva Orthana®, Glandosane® and Xerostom®, which have a low viscosity and a high shear rate. Vissink et al.® reported a clinical study in which three groups of patients, suffering from severe xerostomia, were treated with CMC and mucin based artificial saliva. They found out that patients preferred mucin containing saliva substitutes, since those such as Saliveze® and Xeroton®, which have a low viscosity and a high shear rate, and the others, Saliva Natura®, Saliva Orthana®, Glandosane® and Xerostom®, which have a low viscosity and a high shear rate.

**Buffering capacity**

In our study saliva substitutes buffering capacity is principally around 1 mmol H+ /L, except for Saliva Natura® which has a higher value. A UWS buffering capacity value of 5.93 mmol H+ /L was obtained by Gittings et al.® using the same method, but unfortunately, before the evaluation the authors flash freezed and stored at −80 °C the saliva samples. Nevertheless, the same approach used here was also used by Bardow et al.® who found the UWS buffering capacity ranging from 3.1 to 4.0 mmol H+ /L. Other comparisons are very difficult due to the different methods adopted for buffering capacity evaluation: Meurman et al.®, used a method named the Dentubuff-strip method, while Moritsuka et al.® and Kitasako et al.® used another one, ranking the samples into three categories due to the pH values obtained after acid addition. Thus, from the review of literature we could consider artificial saliva buffering capacity lower than natural one.
Surface tension

All artificial surface tension values were lower than water (71.99 ± 0.05 mN/m) nevertheless Glandosan® has a similar value (68.19 mN/m) while other products generally have a surface tension value lower than 60.00 mN/m. Literature reports variable values about natural saliva surface tension. For example, recently Gittings et al. found a mean value of 58.98 ± 2.18 mN/m, a similar value was obtained in 2000 by Kirkness et al. and Adamczyk et al. in an older study (1997), while Kazakov et al. suggest a broad values range 68.7 to 44.9 mN/m. Some data about artificial saliva surface were found. Saliva Orthana® having an interval 41.9 - 36.0 and our data fall in this range², while Preetha et al. reported the values of 64.17, 66.15 and 64.89 respectively for Saliveze, Xialine® 1 and Xialine® 2⁷. Unfortunately, we were not able to find on the market Xialine products so there is no way to compare the results, while Saliveze® showed a lower surface tension in our study (58.89 vs 64.17), this difference could be ascribed to an intra-batches difference and a different measurement method. Proteinaceous and glycoproteinaceous material has been attributed to the surface activity of saliva, in particular proline rich proteins, moreover, lipidic materials such as phospholipids, fatty acids and triglycerides are known to influence surface tension.³ Noticeable that the lower surface tension was found in products being mucin and/or lipid based, while CMC based products having the highest values.

Density

Human saliva, since it consists of water for 99%, has a density value³ that ranges from 1.002 to 1.012 g/cm³. In our study, we detected that Saliva Natura®, Saliva Orthana® and Xerostem® have a density value higher than human saliva and Saliva Natura®, Saliva Orthana® and Xerostem® have a density value higher than human saliva and Saliva Natura®, Saliva Orthana® and Xerostem® have a density value higher than human saliva. All other artificial saliva substitutes are few data to indicate the superiority of any of the products, form our data and the literature review we can assert that the most important features that obtain patient compliance are viscosity and surface tension, indeed lower values thereof; though products selection will be based on market availability and personal preference.

Spinnbarkeit

Spinnbarkeit saliva spinnbarkeit is related to its elasticity and viscosity properties, giving a significant correlation with viscosity and, for this reason, spinnbarkeit evaluation was included in the study. Our data are not comparable because there no data about artificial saliva spinnbarkeit.

Table 1: summary of samples characterization at 20°C

<table>
<thead>
<tr>
<th>Sample</th>
<th>Viscosity (mPas)</th>
<th>Shear rate (s⁻¹)</th>
<th>Buffering capacity (mmol H⁺/L)</th>
<th>Surface Tension (mN/m)</th>
<th>Density (g/cm³)</th>
<th>Spinnbarkeit (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural saliva</td>
<td>2.330 - 450⁴</td>
<td>2.520 - 90⁴</td>
<td>6.97⁴</td>
<td>5.93⁴</td>
<td>58.98⁴</td>
<td>1.0020 - 1.0120⁶</td>
</tr>
<tr>
<td>Saliva Natura</td>
<td>1.682 - 353.1⁵</td>
<td>5.40</td>
<td>3.333</td>
<td>49.97</td>
<td>0.909</td>
<td>1.0508</td>
</tr>
<tr>
<td>Saliveze</td>
<td>14.908 - 37.5⁶</td>
<td>6.38</td>
<td>0.909</td>
<td>58.89</td>
<td>68.19</td>
<td>1.0072</td>
</tr>
<tr>
<td>Glandosan</td>
<td>3.784 - 161.6³</td>
<td>4.97</td>
<td>1.176</td>
<td>68.19</td>
<td>60.94</td>
<td>1.0102</td>
</tr>
<tr>
<td>Xerostem</td>
<td>2.879 - 207.7⁷</td>
<td>6.70</td>
<td>0.324</td>
<td>42.58</td>
<td>40.94</td>
<td>1.0205</td>
</tr>
<tr>
<td>Preetha</td>
<td>3.178 - 187.9⁷</td>
<td>6.29</td>
<td>1.189</td>
<td>40.94</td>
<td>60.94</td>
<td>1.0544</td>
</tr>
<tr>
<td>Natural saliva</td>
<td>15.000 - 0.5-94.5⁸</td>
<td>6×&lt;7; 90⁹</td>
<td>40.94</td>
<td>68.70 - 44.90</td>
<td>1.90 - 4.90⁸</td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES


