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

Pier Luigi Foglio-Bonda* MD, DDSc Full Professor Department of Translational Medicine, University of Eastern Piedmont, Largo Solaroli 17, 28100 Novara, Italy. *Corresponding Author

Marco Farinone Junior Technician at APTSol srls. Largo Donegani 2/3, 28100 Novara Italy

Franco Pattarino Ph. Prof. Associated Professor Department of Pharmaceutical Science, University of Eastern Piedmont, Largo Donegani 2/3, 28100 Novara, Italy.

Andrea Foglio-Bonda M. Phar Department of Pharmaceutical Sciences, University of Eastern Piedmont, Largo Donegani 2/3, 28100 Novara, Italy.

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); Surface tension by using a tensiometer (KSV - Sigma 703d), while spinnbarkeit by own instrument built for the purpose. 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 3.333 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.

KEYWORDS : artificial saliva, saliva substitutes, rheological properties, spinnbarkeit

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, it 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[2]. At rest, without any stimulation, saliva is constantly produced and this phenomenon is denominated basal unstimulated secretion behaving 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 it is around 1 L, nevertheless regarding salivary flow rate (*FR*), there is a large biological variation[3]. A considerable work about *FR* has been conducted in 2013, data about unstimulated 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 not normally distributed ($p < 0.05$)[4]. Understanding daily production saliva is important as to know its biophysical properties such as viscosity: where values alteration has been associated with development of oral diseases[5]. A review of the literature indicated that there are several viscosity values obtained from population by different analytical techniques, giving different results but generally do not exceed 10 mPa·s[6]. Although saliva presence is often taken for granted, a decrease in his production or worst, its absence, can lead to a strong decrease in life quality; increasing, for example, cervical caries, mucosal infections, ulcerations. Xerostomia or hyposalivation (*FR* <0,16 mL/min) may occur in many different situations[3]. The most common is as a drug side effect; in this case an alternative medication may be suggested. Radiotherapy of the head and neck regions, which is used in upper aerodigestive cancer treatment, may indeed cause xerostomia. Immunological diseases, such as HIV, may affect saliva production as well. Those clinical pictures need to be treated; the most common approach is the use of palliative medicines (moisturizing products) together with oral complications preventive measures[7].

Artificial saliva substitutes are meant to have the same biophysical properties of natural saliva, such as lubricative and mucoadhesive function, still on the other hand they cannot act as substituents of the enzymatic-digestive actions. In order to obtain such properties, saliva replacers need to be as close as possible to human saliva composition[8]. There are many available approaches used to obtain rheological properties comparable to those of natural saliva, for example it is possible to add either mucins, either polymers, for example carboxymethyl cellulose (*CMC*) or polyethylenoxide (*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 effect[9]. 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 account[8]. Another interesting work, but not representative of all products that are currently commercially available, has been conducted by Vissink et al.[9], 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 polyethylenoxide. Hatton et al.[5] 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 surfaces[10]. This study was conducted over three different products based upon *CMC,* mucin and linseed 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-

physical properties: viscosity, pH, buffering capacity, superficial tension and density. Moreover, spinnbarkeit 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 Estearn Piedmont" was asked to spend, each one, 2 hours to web-search focused on artificial saliva products. Seven products were founded, to note that no student has been able to find all seven products (max four products), three different artificial salivas based on carboxymethylcellulose as rheological modifier component were obtained: Glandosane®, Saliveze® and Xerotin®. Biotene® is Carbomer® and hydroxyethylcellulose based. Saliva Orthana® and Saliva Natura® have been designated as mucin-based saliva substituents. 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.[11] 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 experiments were conducted over 20 mL samples at room temperature. From each product 2 samples were collected and every measurement was repeated three times.

Spinnbarkeit measurement

Spinnbarkeit analysis were performed by an own instrument built for the purpose. Each sample (50 µL) was transferred to the steel base and was brought into contact with the fixed punch, moving the base with a lifter. Subsequently, the lifter was lowered until the formed liquid wire broke; the distance was then measured using a graduated scale placed behind the instrument. Three samples from each product were collected and measurements were repeated ten times for every sample.

RESULTS

Results obtained from Biotene characterization are not reported since it is a semisolid 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 maximum value of 21.727 mPa·s (about 13 times greater), correspond to Saliva Natura® and Xerotin® products. Saliveze® and Xerotin®, which are *CMC* based products, have the highest viscosity values. All average viscosity values for each product have a relative standard deviation (RSD) lower than 5 %, except for Saliveze® product (5.6 %). 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 spinnbarkeit 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 (Saliveze®, Xerotin®, 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 spinnbarkeit 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

Viscosity is known to be non-Newtonian across the range of shear rates present in the oral cavity: a shear rate of 4 s^{-1} corresponds to the movement of particles across the tongue whilst 60 s^{-1} and 160 s^{-1} correspond to swallowing and speech respectively[11]. This feature is a peculiar characteristic of human saliva thus; it is important in its substituents as well. During the analysis of our saliva viscosity data it is necessary to consider all the problems related to our analytical methods and since in every other study, where saliva viscosity has been determined in a lot of different ways[7]. Viscosity determination was accomplished using a falling ball viscometer, which is not the most suitable instrument to study in detail the rheological properties of fluids, because is not possible to set the Shear Rate. Nevertheless, since it allows to perform rapid testing and the shear rates generated are similar those observed in the oral cavity, we'll be using it to fulfil further studies on *WS* and, in this way, data comparison with a broad donor population will be achievable. Comparison with literature data might not be straightforward since different methods are adopted; for example, Christersson et al.[10] 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[6,8,11], Saliva Natura®, Saliva Ortana® 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 classify the evaluated saliva substitutes in two different groups: those such as Saliveze® and Xerotin®, which have an 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.[12] 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 could ensure an improvement of the oral functioning. Thus, a correlation between products with low viscosity and patient relief from symptoms is noticeable.

pH

Human saliva pH bibliographic data are summarized in Table 1, it is known that values change depending on subjects age, collection methods, cohort selection[3]. Except for pH indication on product leaflet or brochure, only Saliva Orthana® pH value is available, evaluated by Christersson et al.[10], 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[11], Saliva Natura® and Glandosane® are the only ones which deviate from those, having a lower pH (5.40 and 4.97 respectively); this could be attributed to some ingredients usage, such as ascorbic acid in Glandosane®.

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[11] 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.[13] who found the *UWS* buffer capacity ranging from 3.1 to 6.0 mmol H^+/L. Other comparisons are very difficult due to the different methods adopted for buffering capacity evaluation: Meurmanet al.[14], used a method named the Dentubuff-strip method, while Moritsuka et al.[15] and Kitasako et al.[16] 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)[17] nevertheless Glandosane® have 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.[11] found a mean value of 58.98 ± 2.18 mN/m, a similar value was obtained in 2000 by Kirkness et al.[18] and Adamczyka et al.[19] in an older study (1997), while Kazakov et al.[20] 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 range[10], while Preetha et al. reported the values of 64.17, 66.15 and 64.89 respectively for Saliveze, Xialine 1® and Xialine 2®[8]. 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[11]. Noticeable that the lower surface tension was found in product 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 value[21] that ranges from 1.002 to 1.012 g/cm^3. In our study, we detected that Saliva Natura®, Saliva Orthana® and Xerostom® have a density value higher than human saliva and *CMC* based products; this could be accounted to the presence of more substances. All other *CMC* based products, on the other hand, have a value that falls within the range of human saliva density. It is evident that between all the products and human saliva there is not a sensible change in density values.

Spinnbarkeit

Saliva spinnbarkeit is related to its elasticity and viscosity properties[22], having 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 salivas spinnbarkeit. Human saliva spinnbarkeit has been reported to be in the range between 1.9 and 4.9 mm by use of a new automatic device for measuring the saliva spinnbarkeit (Neva Meter®)[22]. In contrast with Neva Meter®, it is necessary to bear in mind the operator error during the measure with our equipment. Spinnbarkeit detection, using our instrument, occurs in a naked-eye way with the occurrence of sample wire breakage while in the Neva Meter equipment the spinnbarkeit detection occurs automatically due to the break of the electric flow by the sample wire breakage. Moreover, Neva Meter equipment is electrically actuated having a more reliable and constant speed than our equipment. Spinnbarkeit values fall into the range reported by Ghoara K[22], nevertheless the obtained results are too similar among them, in contrast with the differences obtained in viscosity determination (using falling ball viscometer), suggesting that spinnbarkeit analysis could not be the suitable method for artificial saliva characterization.

CONCLUSION

In this study, different chemical-physical characterizations we conducted over different saliva substitutes easy available on market, giving useful information about the artificial saliva offer. It is our main concern to continue improving our analysis techniques (such as viscosity) to get more detailed results. Moreover, could be appreciated to compare clinical studies data with chemical-physical characterizations, understanding what are the aspects in which saliva substitutes need to be improved. Saliva substitutes are supposed to be as faithful as possible to human saliva's features, to fully replace its functions in oral cavity. Nevertheless, despite several R&D efforts it is difficult to reproduce, in one device, all the different features that belongs to natural saliva. There 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.

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Table 1: summary of samples characterization at 20 °C.

Sample	Viscosity (mPas); Shear rate (s^{-1})	pH	Buffering capacity (mmol H^+/L)	Surface Tension (mN/m)	Density (g/ cm^3)	Spinnbarkeit (cm)
Saliva Natura®	1.682; 353.1	5.40	3.333	49.97	1.0508	1.38
Saliveze	14.908; 37.5	6.38	0.909	58.89	1.0072	1.30
Glandosane®	3.784; 161.6	4.97	1.176	68.19	1.0102	1.32
Xerotin®	21.727; 26.1	6.29	1.519	60.94	1.0074	1.37
Saliva Orthana®	3.178; 187.9	6.29	0.324	42.58	1.0205	1.32
Xerostom®	2.879; 207.7	6.70	1.189	40.94	1.0544	1.28
Natural saliva	2.330; 450[24] 2.520; 90[25] 15.500- 2.8; 0.5-94.5[8] 6< × <7; 90 [26] 1.090; n.d. [27]	6.97[11] 6.95[4] 6.79[23] 7.40[10]	5.930[11]	58.98[11] 53.00[19] 68.70 - 44.90[20]	1.0020 - 1.0120[18]	1.90 - 4.90[22]

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