

## *Evaluation of Physical and Chemical Properties of Saliva on Retention of Complete Denture (In Vitro Study)*

### ABSTRACT

**Aims** of this research to study the effect of surface area on retention of complete denture. **Materials and methods:** Oral examination clinically, some of physical and biochemical analysis of human unstimulated whole saliva have been studied. They constituted in three groups (25 in each the first and the third group) and (50 in the second group). The first group was healthy and aged (20-30) years, the second group was healthy and aged (50-70) years, also the third group aged (50-70) years but with systemic diseases. The following parameters were studied: flow rate, pH, total protein, density, surface tension, viscosity and film thickness. Total protein concentration was responsible for physical and chemical changes specially viscosity. There was a direct positive correlation between total protein and surface tension, viscosity, density and film thickness, while there was a negative correlation between total protein and with flow rate and pH. The measurement of force due to surface tension of these samples have been done by preparing two pairs of circular discs of (PMMA) of (5.2,6.5) cm in diameter and compare of force between these groups, also compare these physical analysis with mathematic analysis. **Results:** indicated that in both the first and the third groups, the physical and mathematic analysis for retention showed non significant changes while in the second group showed significant changes. **Conclusions :** there was a positive correlation between force and wetted area and this force was due to wettability and not to water sorption.

### الخلاصة:

تتناول الدراسة الفحص السريري لبعض الخواص الفيزيائية والكيميائية لللعاب فم الإنسان. تم جمع اللعاب بعد ساعتين من الفطور الصباحي من قبل (١٠٠) مائة متطوع حيث قسموا إلى ثلاثة مجاميع المجموعة الأولى ضمت (٢٥) متطوع تتراوح أعمارهم بين (٢٠-٣٠) سنة والمجموعة الثانية ضمت (٥٠) متطوعاً لا يعانون من أي مرض تتراوح أعمارهم بين (٥٠-٧٠) سنة وضمت المجموعة الثالثة (٢٥) متطوعاً تتراوح أعمارهم أيضاً بين (٥٠-٧٠) سنة ولكن تم اختيارهم من المتطوعين الذين يعانون من أمراض مختلفة. تم دراسة الخواص التالية لللعاب: معدل الجريان، الدالة الحامضية، البروتين الكلي، الكثافة، الشد السطحي، اللزوجة والسمك الفلمي.

ان البروتين الكلي هو المسؤول عن التغيرات الفيزيائية والكيميائية لللعاب وخاصة اللزوجة. حيث لوحظ ان هناك علاقة طردية مباشرة بين البروتين الكلي وكذلك الشد السطحي، اللزوجة، الكثافة، والسمك الفلمي. بينما لوحظ علاقة عكسية بين البروتين الكلي وكل من معدل الجريان والدالة الحامضية.

ان قياس القوة للشد السطحي لهذه النماذج تم بواسطة تحضير زوج من أقراص دائرية مصنوعة من (مادة بولي ميثيل ميثايل اكريليت) وبقطر (٢,٥-٦,٥ سم) وتمت المقارنة لهذه القوة بين المجاميع الثلاثة، وكذلك تم تحليل باقي القياسات حسابياً و أظهرت التحليلات الإحصائية ان كلاً من المجموعة الأولى والمجموعة الثالثة ليس هناك أي فرق معنوي بين النتائج الحسابية والنتائج المختبرية للقوة اللاصقة. بينما المجموعة الثانية كان هنالك فرق معنوي. ان نتائج المجاميع الثلاثة أعطت نتائج قوية بين القوة ومساحة المنطقة الرطبة وكذلك أثبتت ان القوة كانت نتيجة الرطوبة وليس نتيجة امتصاص الماء.

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Several studies reported age-related atrophic changes in the morphologic appearance of human salivary glands <sup>(1)</sup>.

One quarter of the morphologically active parenchymal cell volume found in the childhood is lost by old age due to the change in secretory activity by the replacement of parenchymal element by fat, connective tissue and onocytes <sup>(2)</sup>. The loss of 50 percent may occur in more severely affected submandibular glands. This loss reduces the functional salivary reserve normally present associated with functional reduction of salivary flow rate.

Elfenbaum <sup>(3)</sup> indicated that in old age, the flow of saliva is reduced, but mostly of the serous component. The thick mucous flow is not reduced as much as serous secretion and the resultant secretion is thick and ropy.

This study was assessed to find out the effect of saliva on retention of complete denture in vitro study and this can be assessed by the following clinical and physical parameters of saliva by the measurement of physical and chemical parameters of saliva samples in order to better understand the effectiveness on the retention of complete denture according to these parameters, the effect of surface area on retention of complete denture, the measurement of physical and chemical parameters of saliva samples in different age young and old, healthy and with systemic disease and compare between them, to evaluate the correlation between water sorption by polymethylmethacrylate (PMMA) and its wettability on standard discs of denture base PMMA and which principle sources of denture retention.

The aims of this study .The measurement of physical and chemical parameters of saliva samples in order to better understand the effectiveness on the retention of complete denture according to these parameters and study the effect of surface area on retention of complete denture

## **MATERIALS AND METHODS**

Total of (100) people were included throughout the period of this study and they have been divided into three groups. First group twenty – five healthy persons were taken, ten of them were males and fifteen were females. Their ages range between (20-30) years. Second group fifty healthy persons were taken, twenty – five of them were males and the other twenty – five were females. Their ages range between (50-70) years. Third group twenty – five persons were taken, ten of them were males and fifteen of them were females. Their ages range between (50-70) years. In this group the subjects complain of systemic disease and they had drugs such as antihypertensive drugs, antihistamine drugs and antidiabetic drugs.

### **Collection of Saliva**

The samples of saliva were collected from (100) persons by using spitting method since it is an easy chair side procedure that can be performed by the dentist or trained dental auxiliary. The time of collection was (10) minutes, and the collection was performed at least (2) hours after breakfast because it is a suitable time to determine its unaffected physical properties, and to avoid diurnal variations. Then, the samples were immediately centrifuged for about (3) minutes to remove any foreign materials in saliva and stored at (-20 °C) in deep freeze until the time of analysis <sup>(4)</sup>.

The measurement of flow Rate was done according to method described earlier <sup>(1,5)</sup>. The determination of pH–value in saliva was done immediately after collection of saliva using electric pH meter <sup>(6)</sup>. Measure of saliva density was done by measuring its mass per unit volume <sup>(7)</sup>. The determination of its mass was done by using electronic precision balance (Sartorius). Surface tension of saliva samples was measured by the most accurate method which consists of measuring the height to which the saliva rises in a capillary tube <sup>(8,9)</sup>.

Determined of viscosity was done by Ostwald viscometer which is a simple device and accurate for measuring the viscosity of liquid<sup>(9)</sup>. The determination of film thickness of saliva samples was measured by using the micrometer<sup>(10)</sup>. Total protein in saliva samples was done according to the most commonly used method for saliva total protein employs the biuret method<sup>(9)</sup>.

### **Preparation of Disc Plates and Wax**

Four discs of PMMA were done according to the following method: The wax plates were applied on a glass surface and cut into a uniform discs of dimensions (5.2, 6.5) cm using KDS-Hi with thickness of 2.5 mm representing the average thickness of denture base. The two surfaces of wax disc were smoothed with a piece of nylon – clothes<sup>(11)</sup>. After that the wax discs were ready for the flasking procedure. The lower half of flask was filled with freshly mixed dental stone according to the manufacture's instructions (100 gm of powder to 23 ml of distilled water). To facilitate the flow of stone into the whole surface of wax, using gentle vibration for 1 minute to allow the air to come out. Stone mixture was poured into the wax, vibrated, and then poured on the stone in the lower half of the flask.

The stone in the lower half of the flask was left to harden, then the glass was removed, so that the level of the wax would be with the level of stone, then the stone was coated with separating medium (cold mold seal). The upper half of the flask was positioned on the lower half and dental stone was poured into the flask and the flasking procedure was continued as a usual manner.

The measurement of the Force Exerted to the Denture Base Due to Surface Tension was done according to the method described<sup>(10,12)</sup> by a fixed – volume drop of liquid (saliva) between a pair of parallel surfaces has been a common model for the denture – mucosa system. Two pairs of parallel circular flat disc of (PMMA) of diameter of (5.2, 6.5) cm respectively were prepared and had been adjusted to zero, when the discs were in contact (at zero load) the actual separation of their surfaces was displayed directly during the experiment. The test liquids were dispensed from an adjustable micropipette in nominal drop size and five replicates were performed. In the small disc the size of drop was 40µl, while in the large disc, the size of drop was 60 µl. In each instance, drop size was checked by weighing (for water). The surface of discs had been exposed for (5) minutes to saliva. In this case and between drops, the discs were cleaned from soluble materials by streaming deionized water over their surfaces and drying occurred in a clean air blast. All saliva samples were taken and kept under the same conditions.

For measuring the force required to separate the complete denture from underlying tissue, a device was manufactured for this purpose. It consists from a base plate fixed on it an aluminum equilibrium arm of (30) cm length raised from the base plate by aluminum support of (20) cm height connected together by a hinge. The equilibrium arm carry on one side a dish for weights connected by a high strength polyethylene threads, and on the other side carry one of the two parallel (PMMA) disc connected by the same type of threads, the second disc was fixed on the base plate by three adjustable screws held on a special case. Measurement of Water Sorption Water sorption (absorption and adsorption) exerts significant effect on the mechanical and dimensional properties of the polymer<sup>(13-15)</sup>.

Two pairs of acrylic circular discs were prepared. The diameter of the first pair was (5.2) cm. The surface area of first discs were (21.23) cm<sup>2</sup>. The diameter of the second pair was (6.5) cm, so the surface area was (33.17) cm<sup>2</sup>. The force (retention) between two parallel discs of the same diameter attached by a thin layer of unstimulated whole mixed saliva due to surface tension was affected by their physical and biochemical properties of saliva.

## RESULTS

The flow rate, pH, total protein, density, surface tension, viscosity and film thickness in unstimulated whole saliva, also Retention of (A<sub>1</sub>) and (A<sub>2</sub>) were tabulated in (Table1).

Table 1: Physical and Biochemical Parameters and Retention of (A<sub>1</sub>) and (A<sub>2</sub>) in All Study Groups Mean ± SD

Parameters	First Group n=25 Mean ± SD	Second Group n=50 Mean ± SD	Third Group n=25 Mean ± SD
Flow Rate ml/min	0.11 ± 0.11	0.10 ± 0.15	0.07 ± 0.05
pH	5.94 ± 0.48	5.90 ± 0.68	5.82 ± 0.50
Total Protein g/dL	2.98 ± 1.94	3.59 ± 3.00	3.96 ± 2.32
Density g/cm <sup>3</sup>	1.03 ± 0.02	1.04 ± 0.06	1.05 ± 0.37
Surface Tension D.cm <sup>-1</sup>	80.59 ± 4.05	80.77 ± 4.58	82.05 ± 3.95
Viscosity D/cm <sup>2</sup>	0.01 ± 0.009	0.017 ± 0.0229	0.019 ± 0.0229
Film Thickness mm	0.04 ± 0.011	0.044 ± 0.017	0.045 ± 0.013
Retention (A <sub>1</sub> ) mN	120.59 ± 4.73	117.18 ± 3.66	122.10 ± 2.79
Retention (A <sub>2</sub> ) mN	188.18 ± 5.67	184.57 ± 5.16	188.84 ± 3.89

It has been showed that total protein concentration in the first group were lowered, when compared with second group and third group as shown in (Table 1). It has been found when there was a decrease in the mean value of flow rate and pH of the saliva, there was an increase in the mean value of total protein concentration. When the mean value of total protein concentration was decreased in the study groups, there were decreased in the physical parameters (Surface tension, viscosity and film thickness) of unstimulated whole mixed saliva samples, so there was a positive relationship between the concentration of total protein and surface tension, viscosity and film thickness of the saliva. In all study groups, there was a direct positive correlation between flow rate and pH, but there was a negative correlation between total protein concentration and both flow rate and pH of the saliva.

There was a positive correlation between the viscosity of unstimulated whole mixed saliva and film thickness of saliva in all study groups, also there was a direct positive correlation between total protein concentration and surface tension, viscosity and film thickness.

Table 2. Compares between Mathematic and Physical Analysis in All Study Groups

Groups	Retention (Mathematic Analysis)	Retention (Physical Analysis)	Retention (Mathematic Analysis)	Retention (Physical Analysis)
Group One n=25	121.25 ± 4.06	120.59 ± 4.73	189.44 ± 6.34	188.18 ± 5.67
Group Two n=50	121.88 ± 6.24	117.18 ± 3.66	190.37 ± 9.71	184.57 ± 5.16
Group Three n=25	123.37 ± 5.03	122.10 ± 2.79	193 ± 59.19	188.84 ± 3.89

The force (retention) between two parallel discs(A<sub>1</sub>) of the same diameter attached by a thin layer of un stimulated whole mixed saliva due to surface tension was affected by their physical and biochemical properties of saliva. The force (retention) between two parallel discs of diameter (5.2) cm and surface area of (21.23) cm<sup>2</sup> by a thin layer of saliva in between them(Table2). The force (retention) between two parallel discs(A<sub>2</sub>) of diameter (6.5) cm

and surface area of (33.17) cm<sup>2</sup> attached by a thin layer of saliva was affected by their physical and biochemical properties of the saliva.

## **DISCUSSION**

The present study showed that the flow rate in all study groups had a mean and  $\pm$  SD with a lowest value at the third group and at the second group was slightly lower than the first group. This result was in agreement with the results by Hall <sup>(16)</sup> that found a decrease in the flow rate of saliva due to age-related morphologic changes of the salivary gland. As a result of regressive changes in the salivary glands, particularly atrophy of the cell lining the inter mediate ducts; there is a decrease in salivary flow in the aged. <sup>(17)</sup>. Also the results were in agreement with the results observed by Riva et al., <sup>(18)</sup> who found that there is a diminish function of the glands as a result of physiochemical changes in saliva.

In the third group the mean and  $\pm$  SD had the lowest value of flow rate due to the effect of various drugs and most often associated with symptomatic xerostomia. The main groups of agents reducing salivary secretion are the antihypertensive, anticholinergic, psychotropic and sedative drugs. The reduction in salivary output secondary to intake of these medications is known as drug induced xerostomia. Mostly elderly people are the chief users of drugs, many of these drugs interfere with digestion, absorption, utilization or exertion of essential nutrients <sup>(19)</sup>.

The lack of an adequate supply of saliva has multiple negative outcomes for complete denture retention that results when the saliva becomes thick and ropy. These results were in agreement with Moghadam, and Scandrette <sup>(20)</sup>, as mentioned by the previous authors, there are many factors influencing salivary flow rates such as mechanical stimulation, age, sex and diet which may lead to alteration in the flow rate. The differences in the results founded by previously mentioned authors may be due to improper selection of groups, differences in the method and time of sample collection. The samplers of saliva in this study were collected at least two hours after first meal and this causes disappearance of diurnal and individual variations as reported <sup>(21,22)</sup>.

The present study showed that the pH value of all study groups was slightly different from one group to another. In the first group the pH value was higher than that in the second and the third group. In the third group of this study, the mean of pH value showed the lowest value (0.07) ml/min. These results were in agreement with the results observed by Tatsutomi et al., <sup>(23)</sup>. So there is a positive correlation between salivary flow rate and pH value and when the flow rate decrease the pH becomes more acidic. These results were in agreement with Dittmer <sup>(24)</sup> in that the pH value of saliva decreased by aging and become more acidic due to a decrease in salivary flow rate. This in turn, favors the growth of acidogenic microflora and enhances the cariogenic property of dental caries and dental plaque to increase oral diseases <sup>(6)</sup>.

Consequently, it was found that there were changing patterns in the pH of saliva in each subject, when there was some stimulation such as an inflammation or a physical irritation. In case of acute periodontal inflammation, the pH value showed tendency to change to the acidic side, conversely during the healing process or in case of chronic inflammation, the pH value showed a tendency to change to the alkaline side.

No significant correlation has been observed between retention of discs (A<sub>1</sub>) and the pH value. In the second group when the pH value is slightly lower than the first group, the retention was also lower than the first group that means, there is positive correlation between the pH value and retention. While in the third group the pH value had a lowest value (5.82) and retention had the highest one.

In discs ( $A_2$ ) retention of first and third group was the same and in the second group was the lowest value (184.57) mN. The total protein content of unstimulated whole saliva was the lowest value in the first group and was the highest value in the third group.

A total protein concentration of saliva layer in complete denture retention was studied by Murray<sup>(25,26)</sup>, who found that proteins and muco polysaccharides from the saliva adsorb to the acrylic rapidly and strongly and in so doing present a surface which is more wettable, so interfacial failure by a simple separation of denture and saliva does not occur. This strength is, therefore, quite adequate. If there were no wetting, no force would be needed to be applied to separate the denture from saliva and there would be no retention.<sup>(10)</sup> These results were in agreement with our present study where the highest value of total protein was found in the third group and had also high retention in this group with both discs ( $A_1$ ) and ( $A_2$ ), but disagreement with both the first group and the second group where total protein of saliva was higher in the second group than the first group but the retention value in the second group was lower than the first group with both discs ( $A_1$ ) and ( $A_2$ ). The results showed no significant increase in the density of unstimulated whole saliva at first, second and third groups. The results obtained were in agreement with Edgar<sup>(4)</sup> in that the salivary flow rate decreased during aging and when salivary flow rate decreased, there will be increase in the electrolytes contents of saliva and this will lead to increase their density, there was a negative correlation between salivary flow rate and density of saliva due to the presence of inorganic components of saliva such as sodium and potassium and the major osmotically active anions such as chloride and bicarbonate, also other electrolytes that are present such as chloride and magnesium<sup>(5)</sup>.

As saliva consist of 99% water and the remaining 1% consist of large and small inorganic and organic materials, the density of saliva was very slightly increase between the study groups and had very little effect on the retention of discs ( $A_1$ ) and ( $A_2$ ) and the difference in the retention value between groups was related to other physical and biochemical parameters of saliva sample rather than density. No significant changes in surface tension of unstimulated whole saliva of all study groups were observed. The mean and  $\pm$  SD of surface tension values showed that lowest value in the first group and the highest value in the third group, and these were related to total protein concentration of saliva which was mostly responsible for physical properties of saliva specially surface tension and viscosity. The retention which was found between two parallel discs ( $A_1$ ) and ( $A_2$ ) showed the highest value in the third group. These results were in agreement with O'Brien and Ryge<sup>(27)</sup>. The obtained results were in agreement with Swartz<sup>(28)</sup>, who stated that complete dentures were retained by the physical forces of adhesion, cohesion, surface tension and atmospheric pressure, acting either individually or in combination. These results obtained were also in agreement with Winkler<sup>(29)</sup>, who showed that the retention will increase as the surface tension increase, and the dentures become more wettable. He stated that a low contact angle indicates good wettability. Hence the wetting properties of saliva and distilled water can be evaluated by contact – angle.

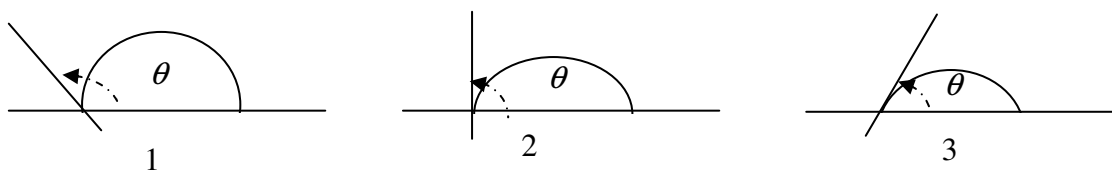


Fig 1: Contact angles associated with poor (1), either mediated (2) and good wetting (3).<sup>(24)</sup>

The results obtained were in disagreement with Ostlund<sup>(30)</sup>, who stated that increased retention was due to adhesion and not to surface tension and that therefore, the effective action of saliva was an adhesive and increased seating force of the denture base which increase its retentiveness. The present study showed that the viscosity of the study groups had a mean and  $\pm$  SD with the lowest value in the first group, and increase in the second group while in the third group showed the highest value. This result was in agreement with the results reported<sup>(5,17,31-33)</sup>.

The surface tension forces which act on two surfaces of acrylic discs of surface area ( $A_1$ ), ( $A_2$ ) showed that the mean and  $\pm$  SD with the highest value in the third group while the lowest value in the second group. The results obtained were in agreement with studies previously<sup>(12,34,35)</sup>. The results obtained were in disagreement with some studies<sup>(36,37)</sup>.

The study showed that the film thickness of saliva of the study groups had a mean and  $\pm$  SD, which was the highest value in the third group and with the lowest value in the first group. These results were due to a decrease in flow rate of unstimulated saliva in the third group and an increase in flow rate in the first group. The results were in agreement with the results reported<sup>(17)</sup>. And disagreement with the result reported by Zarb and Blonder<sup>(35)</sup>. They found that an increase in fluid viscosity cannot be accompanied by an equal increase in film thickness if displacement force is to be kept the same. The surface tension forces which act on two surfaces of acrylic discs of surface area ( $A_1$ ), ( $A_2$ ) showed that the mean and  $\pm$  SD with the highest value in the third group while the lowest value in the second group. This result was in agreement with the results obtained by previously<sup>(38)</sup>. The thickness of saliva layer in complete dentures was studied by Kawazoe and Hamada<sup>(39)</sup>. This result was agreement with the results obtained by Murray and Darvell<sup>(40)</sup>. Furthermore, it is the mucosa that is being considered, and it is actively secreting fluid. Since no physical inhibition of secretion by the presence of the denture base is expected, there will be a continuous flow of liquid into the space between and a

Consequent change in the pressure difference across liquid surface from the otherwise expected negative value to positive. Indeed, the separation must then tend to increase and the retention decrease. When immersing the acrylic discs ( $A_1$ ), ( $A_2$ ) in distilled water they showed a small increase in weight. As expected (PMMA) increase in weight when immersed in water, due to water imbibition. The increase in weight was found to be small after (24) h, (7) and (14) days.

However the nearly instant increase in wettability on contact with human saliva is consistent with clinical observations, and must be ascribed therefore to some chemical properties of saliva. This result was in agreement with the result reported.<sup>(25)</sup>, this improvement is almost immediate, with little later alteration. Improvement in complete denture retention usually observed at the first appointment is probably due to improved wettability.

### **Comparism between Mathematic and Physical Analysis in All Study Groups**

When comparing between the mathematic and physical analysis in this study(table.2) no clear dif ference in the mean values of retention in the first and the third group, and these changes were non significant. While in the second group, there were a clear difference in the mean values of retention and these changes were highly significant ( $P = 000$ ).

The difference of the results in the second group between mathematic and physical analysis may be due to the fact of increase in the viscosity of saliva samples. Adhesion was the greatest physical principle in denture retention and it represent an inverse ratio to the viscosity of the saliva – the thicker and more viscous the saliva, the less the adhesion, and the thinner the saliva the greater the adhesion. The greatest increase in adhesive force with liquids of lower viscosity and those patients with low viscosity saliva would, therefore, be

dependent to a greater extent on the surface tension factor and would benefit more from improved wettability.

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