# Impact of Ozonated Water on Physiomechanical Properties of PMMA Denture Base Material

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## Abstract

**Aim:** This contemplate aimed to compare the impact of normal tap water with ozonated one on both physical and mechanical criteria of acrylic resin denture base material after 7 and 30 days.

**Materials and Methods:** Acrylic resin specimens were fabricated following the ADA specifications No.12 for assessing the surface roughness, hardness, water sorption and solubility which took place after both one week and one month of soaking the acrylic resin specimens in the ozonated water (Group I) and tap water (Group II). Data was statistically analyzed utilizing both independent and paired t-tests.

**Results**: Group I revealed an initial significant increase in surface roughness after one week. While next to one month there was insignificant difference between the two groups. Considering surface hardness, water sorption and solubility, group I displayed a significant decline when compared to group II following both one week and one month.

**Conclusion:** Ozonated water as a soaking media had no impact on surface roughness and slight deteriorating effect on surface hardness. While water sorption and solubility revealed enhancement in Group I. Ozonated water (Group I) samples were significantly lower than tap water (Group II) ones regarding surface hardness, water sorption and solubility.

**Key Words:** Ozonated water, Surface Hardness, Surface Roughness, Water Sorption, Solubility.

## Introduction

Ozone is a naturalist triatomic oxygen molecule which is a prime participant in the earth's self-cleaning process via declining downwards from altitudes for its inflated density and merging with pollutants once approaching them. Ozone therapy is a multilateral bio-oxidative remedy where ozone is supplied either through gas, water or oil for curative services [1-4].

Owing to its unique features in handling diverse diseases via its immunostimulant, analgesic, detoxicating, biosynthetic, antimicrobial and bioenergetic activity, the ozone therapy is surprisingly beneficial on a broad scale in medicine [5, 6].

Furthermore, ozone has established its role in dentistry by being exceedingly beneficial once employed in diverse dental treatments due to the prevalence of the oral cavity transferrable diseases. Acting as disinfectant for controlling hemorrhage and wound cleaning in both bones and soft issues, as a mouth rinse for both gingivitis and periodontitis, oral cavity biofilms as well as, a disinfectant in dental surgery are few applications of ozone therapy in dentistry. Ozone water jet has influenced the endodontic remedy once employed for sanitizing cavities and root canals [7-9].

Additionally, Ozone therapy as a denture cleaner has been advantageous to the oral Prosthodontics as well via its influential and consistent antimicrobial impact antagonistic towards bacteria, fungi, protozoa, and viruses. Ozone, in the gaseous and aqueous phase, has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa, and viruses [9, 10].

Among the versatile denture base materials utilized in such field of dentistry yet heat cure acrylic resin has been the utmost extensively employed one. Denture hygenisity is merely defined as denture sanitary habits that should be followed to ensure its cleanliness especially while sleeping. This is a critical issue in both providing long standing successful prosthesis and maintaining hygienic oral tissues, otherwise further dramatic consequences can take place as inferior aesthesis, unpleasant breath, angular cheilitis and denture stomatitis [11-13].
Regardless of the denture cleansers' huge diversities and their major part in maintaining denture hygienic measures yet their demits are annoyable. Their harmful impact on the denture's both plastic and metal components, oral tissues and remaining dentation is a major issue which obligates dentists to search for the best and safest denture cleanser for the denture and oral tissues together [14, 15].

Denture stomatitis is one of the prime dilemmas which denture wearers are frequently facing due to the extensive plaque gathering on its surfaces. Hence claiming an efficient plaque control should take place to avoid such outcomes. Although denture cleansers are commonly employed for such defect especially by soaking such dentures in normal tap water overnight but ozone therapy proved in being an effective technique in cleaning dentures with fruitful outcomes. As the minimal exposure times of ozone therapy diminishes both Gram-positive and negative bacterial cell counts, viruses and fungi (Candida albicans) ones as well [16-18].

Merits of ozone therapy have extended to surface purification of implants and their sockets while placement by commencing renovation and stimulating tissue regeneration around implant's surface. Combining ozone with mechanical methods verified in advancing its impact as a denture cleanser together with being both extra favorable and affordable than the usual chemical ones [18-20].

Although the abundant qualities of ozone therapy yet inducing weakness, dizziness and allergic skin reactions are some of its demerits. Besides that, being contraindicated in pregnant patients and cases with wild hyperthyroidism, thrombocytopenia, ozone sensitivity, acute alcohol intoxication, epileptic and cardiac strokes, bleeding organs, blood coagulations impairment are some of ozone therapy drawbacks [16-21, 22].

Concerning its impact on the acrylic resin dentures' criteria is still not profoundly investigated yet. Hence the current study aimed to compare the impact of normal tap water which is most common and simply available for all patients with the ozonated one on both the physical and mechanical criteria of heat cure acrylic resin denture base material during cleaning. The research hypothesis was that the ozonated water as soaking media has no effect on physio-mechanical properties of acrylic resin denture materials.

## Materials and Methods

### Study Design:

This consideration was carried out employing polymethyl methacrylate (PMMA) acrylic resin samples soaked in two dissimilar soaking media; ozonated water (Group I) and tap water (Group II) for preserving and cleaning them at night. Surface hardness, roughness, water sorption and solubility were assessed after one week and one month after soaking samples overnight in the two groups.

**Sample Size Calculation:**

Sample size was considered based on a preceding study as a reference. Hence, the apparently passable sample size was 9/group, once each group's response was ordinarily spread thru standard deviation 0.05, difference 0.07, 80 % power and 0.05 type I error probability [23].

**Ethical Approval:**

The present study has been conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki in 1975. This research has been approved by the Medical Research Ethical Committee (MREC) of the National Research Centre, Cairo, Egypt with approval number 24311122022.

**Methods:**

**1- Specimen Fabrication:**

A total of 54 specimens were fabricated. Each group contained 9 specimens (n=9). A commercially accessible conventional heat cured acrylic resin (Acrostone; Acrostone dental factory - industrial zone -Salam city A.R.E-WHW Plastic, England) was utilized to fabricate the samples following the ADA specifications No. 12. A metal rectangular specimen measuring (65mm length x 10mm width x 2.5mm thickness) was utilized to make 36 samples for both surface hardness and roughness tests. While water sorption and solubility required fabrication of 18 circular specimens by employing a metal patterns model measuring (50 mm. diameter x 0.05 mm. thickness [24].

The traditional metal flask was utilized to attain molds of conventional heat cured acrylic resin samples. Dental flask's inferior surface was packed with dental plaster (Elite Rock Stone, Zhermack Clinical, Italy.) which was assorted fulfilling the industrialist`s guidelines. The metal pattern was coated with the separating medium then a layer of plaster mix was coated on metal pattern. Sequential to plaster setting (30 min.), both the plaster and metal patterns were coated with a separating medium and another layer of plaster was poured into the superior half of the flask with vibration by aid of mold vibrator. Plaster strengthening took place just by leaving for (60 min.), then flask unfastening, metal pattern detaching and finally the mold was gained.

Conventional heat cured acrylic resin for all tests carried out in the current contemplate was mixed and packed succeeding the manufacturer`s recommendations utilizing stainless steel spatula. Once approaching the dough stage, it was packed into the plaster mold. Successively, flask squeezing was applied employing the hydraulic press then positioned in water bath curing unit (Water bath curing unit: Type 5518, KaVo EWL, Biberach, Germany) for 30 min. at 70 °C then prolonged to extra 30 min. at 100°C for heat curing. Subsequently, the flask was segregated from the water bath and left to cool at room temperature prior to deflasking, then finishing and polishing of the specimens. Deflasking was performed by gentle mallet blows over the flask's hole and all the specimens were then finished and polished.

**2-Inclusion and Exclusion Criteria:**

There were some inclusion criteria limiting acrylic resin samples' selection for this consideration. Such criteria include; proper fabrication ensuing the manufacturer's guidelines in every individual step, free from any voids, cracks, bubbles or impurities. Moreover, they should be profoundly smoothed, finished and polished. Samples of each test should be perfectly fabricated following the required dimensions of its metal pattern specimen stated by ADA specification no.12. If there were any criteria other than these in the PMMA samples then they were directly excluded from this research.

**3-Soaking of the Specimens:**

Group I specimens were soaked in ozonated water which was prepared with a concentration of (2-4 mg/l) ozone for 1 min. in a 25 mg of double distilled water $37^{o}$ C employing an Ozone generator (Ozone generator type N 1888A, China) [25]. Whereas Group II ones were immersed in a normal running tap water $37^{o}C$.

**4- Assessment of Surface Roughness:**

Measuring of surface roughness took place through imaging the samples utilizing a Canon Power shot A520 digital camera (Canon Inc, USA) connected to a Technival-2 stereomicroscope (Carl Zeiss, Jena, Germany) and Ra factor was assessed employing an image analysis software (Image J 1.52, Image ware, USA).

**5-Assessment of Surface Hardness:**

Surface hardness was analyzed utilizing the Digital Vickers hardness tester (NEXUS 4000 TM, INNOVATEST, model No. 4503, Netherlands) using Vickers diamond indenter together with an objective lens of 20x. Such test was performed under load 25 g till 10 seconds. Three hollows were similarly sited on top of a circle and with a distance of 0.5 mm among the approaching depressions on each specimen's surface. The hollows' diagonal length was calibrated utilizing scaled microscope. As well as, Vickers values were transformed into hardness ones which was expressed in KHN and attained employing the succeeding equivalence: $HV = 1.854\ P/d^2$ HV is the Vickers hardness in $kgf/mm^2$, P is the load in kgf and d is the length of the diagonals in mm.

**6-Assessment of Water Sorption and Solubility:**

The samples were immersed in ozonated water and normal tap water for one week and for one month. After each period the samples were removed and wiped with a tissue and the specimens mass was measured 60 s after removal from the media (m1) with the help of high- accuracy digital weighing scale 0.0001 g (Electronic balance, Mettler PM 460, Germany) Then the sample were dried in a desiccator containing silica gel (Silica gel, static dehumidification, SUD-SHEMIE) at 37°C for 24 hours. Then an identical desiccator was employed for placing disks at normal room temperature till 1 hour and subsequently, weighed (m2)

The weights were regularly calibrated till a constant mass was achieved, indicating water saturation which was considered as the gained weight of the specimen (m3).

Water sorption of each specimen was assessed by means of specific formula; (Sorption % = (m2 − m3) /m1× 100). While water solubility was assessed by Solubility % = (m1 − m3)/m1×100).

**Statistical Analysis:**

Statistical analysis was accomplished via SPSS 16 ® (Statistical Package for Social Science, IBM, USA) besides Graph pad Technologies, USA and Microsoft cooperations, USA.) All data were offered as mean and standard deviation values. The resulting data was investigated via employing both Shapiro-Wilk and Kolmogorov-Smirnov tests for normality which disclosed that level of significance (P-value) was insignificant; as P-value > 0.05 demonstrating that all usual distribution outcoming datum (parametric data) approaching standard Bell curve. Sequentially, evaluation amongst dissimilar clusters was achieved employing the independent t-test, while contrast between different intervals was performed by aid of Paired t-test.

**Results**

**1-Surface Roughness:**

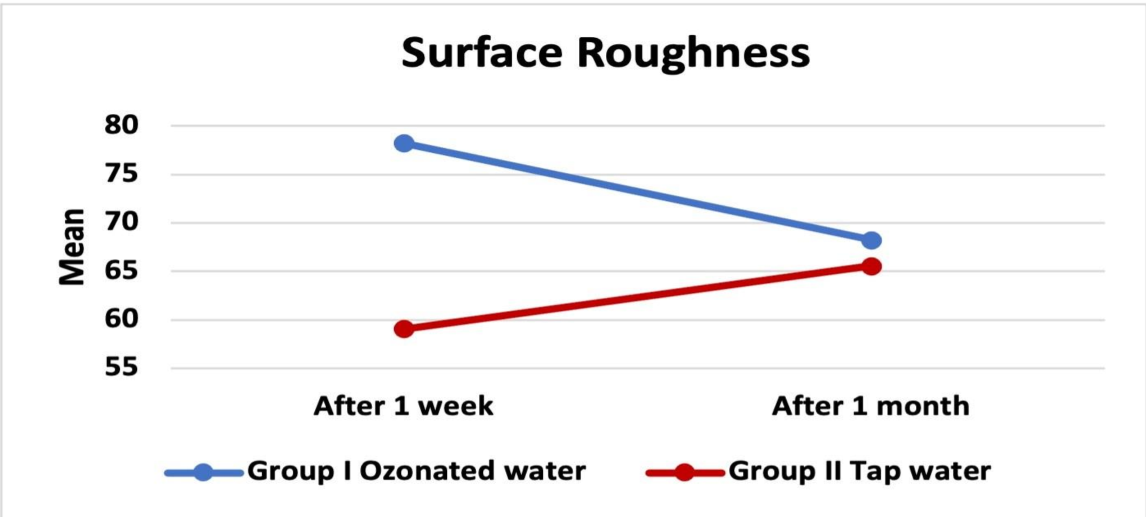
Table 1 revealed that in Group I surface roughness increased after one week while after one month it declined, and this difference was significant as P<0.05. Whereas, as an intra Group II comparison, the surface roughness increased after one month when compared to that post one week, and this was not statistically significant as P>0.05.

**Table 1: Comparison between mean and Standard deviation values of surface roughness after 1 week and 1 month (Intra group comparison) in both group I and II and comparison between both groups (Intergroup comparison).**

| Surface Roughness | N | Group I (Ozonated Water) | | Group II (Tap Water) | | Difference Inter group comparison (Independent t-test) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | MD± SEM | P value | 95% CI | |
| | | M | SD | M | SD | | | L | U |
| **After 1 week** | 9 | 78.25 | 6.34 | 59.09 | 17.76 | -19.16 ± 6.286 | 0.007[a] | -32.4 | -5.8 |
| **After 1 month** | 9 | 68.24 | 4.09 | 65.58 | 0.56 | -2.660 ± 1.376 | 0.07 | -5.5 | 0.25 |
| **% Of change** | | -12.79% | | 10.98% | | | | | |
| **P value (Paired t-test) Intra group comparison** | | 0.005[a] | | 0.31 | | | | | |

*M: mean SD: standard deviation MD: mean difference CI: confidence intervals [a-]Significant difference as P<0.05*

The comparison between Groups; I and II, Group I showed statistically remarkable rise in surface roughness after one week, as P < 0.05. Although, after one month there was no statistically significant difference between both groups, as P > 0.05; as displayed in Graph. (1).




**Graph (1): Line chart representing surface roughness changes in both groups.**

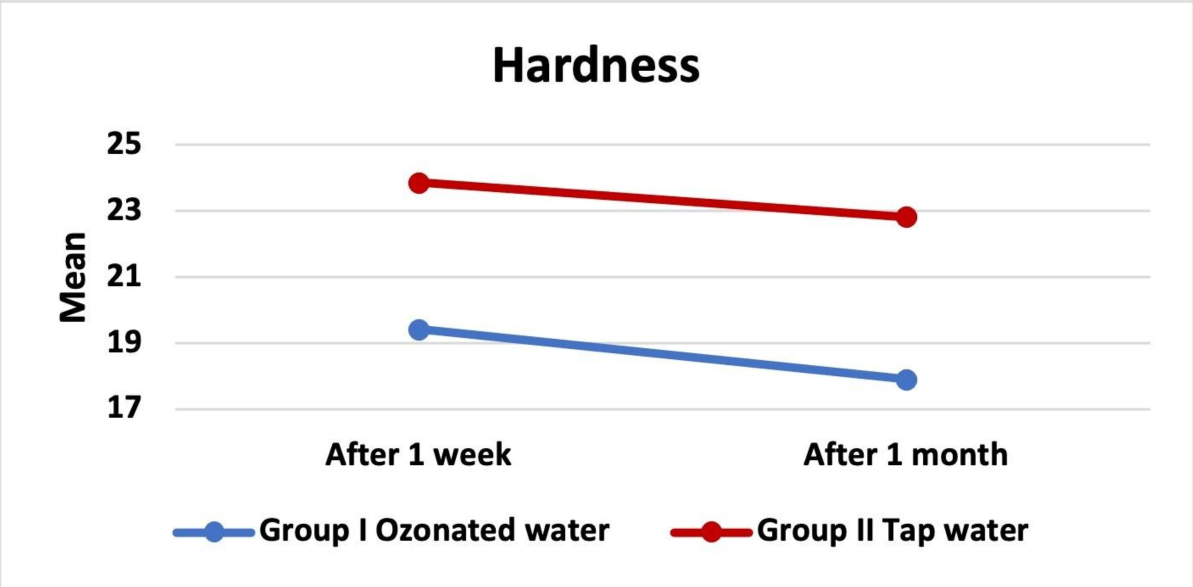
**2- Surface Hardness:**

As an intra Groups I and II comparison, table 2 displayed significant reduction in surface hardness after one week and after one month, as the P value was <0.05. Whereas, Groups I and II had a remarkable decline in the surface microhardness after one month when compared to that after one week, as the P value was <0.05; as revealed in Graph. (2).

**Table 2: Comparison between Mean and standard deviation values of hardness after 1 week and after 1 month (Intra group comparison) in both group I and II and comparison between both groups (Intergroup comparison).**

| Surface Hardness | N | Group I (Ozonated Water) | | Group II (Tap Water) | | Difference Inter group comparison (Independent t-test) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | MD± SEM | P value | 95% CI | |
| | | M | SD | M | SD | | | L | U |
| **After 1 week** | 9 | 19.43 | 0.09 | 23.87 | 0.10 | 4.44 ± 0.04 | <0.0001[a] | 4.31 | 4.54 |
| **After 1 month** | 9 | 17.91 | 0.64 | 22.82 | 0.36 | 4.91 ± 0.24 | <0.0001[a] | 4.39 | 5.32 |
| **% Of change** | | -7.82% | | -4.39% | | | | | |
| **P value Intra group comparison (Paired t-test)** | | 0.001[a] | | 0.008[a] | | | | | |

*M: mean SD: standard deviation MD: mean difference CI: confidence intervals a-Significant difference as P<0.05*




**Graph (2): Line chart representing surface hardness changes in both groups.**

**3-Water Sorption and Water Solubility:**

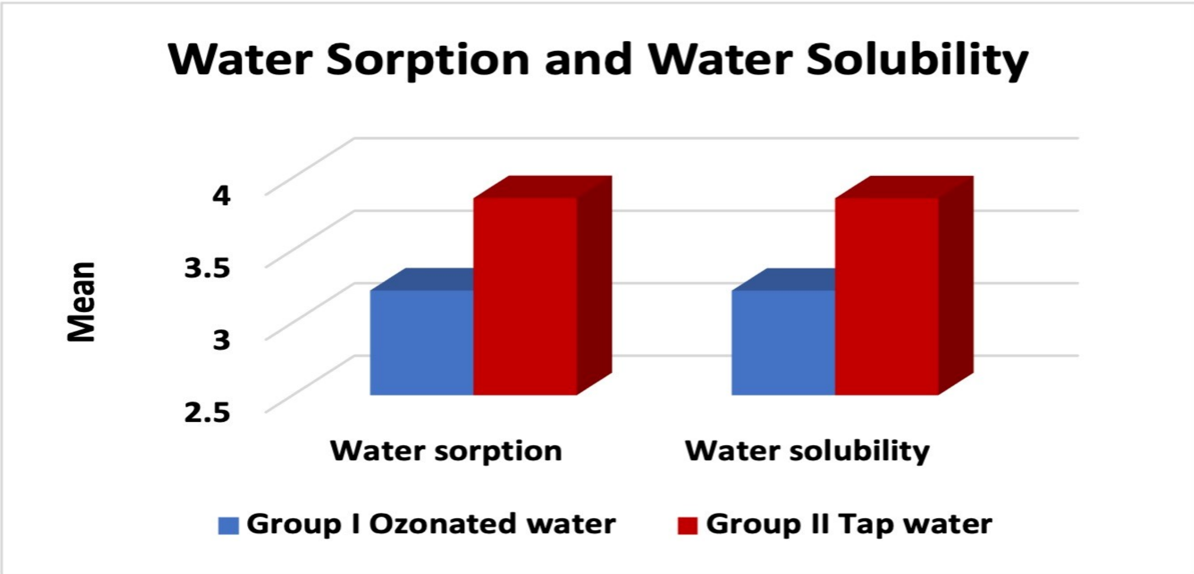
Table 3 displayed that Group I had a significant diminution in the water sorption and solubility after one week and one month when compared to Group II as P<0.05; as displayed in Graph. (3).

**Table 3: Comparison between Mean and standard deviation values of water sorption and water solubility in both group I and II comparison between both groups (Intergroup comparison).**

| | N | Group I (Ozonated Water) | | Group II (Tap Water) | | Difference Inter group comparison (Independent t-test) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | MD± SEM | P value | 95% CI | |
| | | M | SD | M | SD | | | L | U |
| **Water Sorption** | 9 | 3.222 | 0.0036 | 3.859 | 0.0015 | 0.64 ± 0.001 | <0.0001[a] | 0.634 | 0.637 |
| **Water Solubility** | 9 | 3.221 | 0.0037 | 3.858 | 0.0015 | 0.63± 0.001 | <0.0001[a] | 0.634 | 0.639 |

*M: mean SD: standard deviation MD: mean difference CI: confidence intervals a-Significant difference as P<0.05*

Hence, the ozonated water as a soaking media is better than the tap water one regarding the surface roughness, water sorption and solubility of acrylic resin samples. While concerning their surface hardness, the tap water proved to be better than the ozonated one.




**Graph (3): Bar chart representing water sorption and water solubility in both groups.**

## Discussion

The current consideration was conducted to evaluate the impact of ozonated water as a soaking media for PMMA denture base material compared to the tap one. Surface roughness and hardness, together with both water sorption and solubility of PMMA denture base material were evaluated after immersing acrylic resin samples in ozonated and tap water for one week and one month.

Conventional heat cured acrylic resin was utilized in this consideration to fabricate specimens for both groups since it is commercially accessible and most commonly used in diverse forms of oral removable prosthodontics [15].

Owing to the conserving and hygienic remarkable properties of ozonated water towards PMMA acrylic resin denture base material, it has been selected with that specific concentration, since it has previously been verified for being highly effective in killing pathogens, bacteria and fungi in acrylic resin dentures. Though, normal running tap water 37$^{o}$ C has been adopted as it is vastly common and simply handy for all patients aimed to both preserving and cleaning their dentures at night [24, 25].

Based on the current study's findings, the hypothesis was somewhat accepted. Due to the fact that immersion of heat-cured acrylic resin specimens in ozonated water does not affect their surface roughness, while surface hardness was diminished but water sorption and solubility were significantly improved. Outcomes of this research were in harmony with another study which determined that employing ozonated water as a disinfecting agent for heat-cured acrylic resin specimens significantly enlarged surface roughness and declined their hardness respectively [26].

Moreover, the present results were matching with another consideration which assessed ozonated water's influence on PMMA's surface versus that of other polymers. Consequently, it claimed that, though ozonated water had the skill of degrading organic compounds with its superior oxidizing ability of creating reactive oxygen species, yet it had no impact on PMMA. That might be clarified by the fact that PMMA lacks both C-C bond and benzene ring which are extremely sensitive to ozone itself [26, 27].

The Surface hardness outcomes of Group I acrylic resin samples were remarkably diminished. This might be attributed to the PMMA resin's polymerization procedure, which takes place by free addition and terminates by creating both free radicals and cross-linked polymer chains comprising peak levels of residual monomer. Accordingly, this has an adverse effect on PMMA's hardness owing to diffusion of monomer from the polymer and ozonated water into the resin respectively. Hence plasticizing effect is induced, declining the interchain forces, promoting effortless distortion together with noticeably drop in PMMA acrylic resin's hardness post immersion in ozonated water [27, 28].

Furthermore, this is also supported by other studies which claimed that resins' mechanical criteria were impaired consequent to their prolonged storage in water, since water manipulates acrylic resin's mechanical criteria and their prolonged exposure to water usually diminishes the denture's strength. This could be credited to resin's brittleness, poor tensile strength and loss of monomer by exudation. Thus, major decline in mechanical properties of the denture base resin is conditioned for long periods [28].

Immersion of acrylic resin samples in ozonated water and tap water displayed that there was a remarkable decrease of water sorption and solubility of acrylic resin samples immersed in ozonated water than that in tap one. This might be attributed to the simple and minor structured molecules of tap water compared to those of ozonated one. Thus, facilitating both entrance and exit of water molecules from the acrylic resin samples which eventually diminished PMMA denture base's porosity and hence recommend the hygiene maintenance as claimed by other studies [28, 29].

Additionally, on clinical bases once soaking PMMA denture base material for prolonged time in an aqueous intermediate for example; saliva, nasal secretion, water and cleansing operators as plasticizers and supplementary solvable constituents might leach out although saliva or water is captivated. Accordingly, absorbed water has an unfavorable impact on both physical and mechanical criteria of acrylic resin denture base material [29, 30].

**Limitation and recommendation:**

The fresh preparation of ozonated water was the only limitation faced in the current study. Further studies are recommended to take place regarding other forms of acrylic resin denture base materials with extended duration. Besides that, based on the outcomes of this study, clinicians are recommended to suggest ozonated water as a safe and healthier soaking media for patients wearing PMMA acrylic resin prosthesis.

**Conclusions**

Within the limitation of this research, it has been concluded that, soaking of acrylic denture base material in ozonated water had no effect on surface roughness and deceased the surface hardness, while it enhanced both water sorption and solubility of acrylic resin denture base material. Ozonated water can be used as a safe and healthier soaking media for patients wearing PMMA acrylic resin prosthesis. PMMA samples soaked in ozonated water were significantly lower than the tap water ones regarding surface hardness, water sorption and solubility.

**Conflicts of Interest: "**All authors declared that there was no conflict of interest."

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