SORPTION AND SOLUBILITY OF A DENTURE BASE ACRYLIC

by

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DECLARATION

I, Abduraghman Latief, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

Statement of problem

It is well documented that water sorption and water solubility by auto-polymerizing resins have a negative impact on their physical properties and may lead to harmful tissue reactions. The presence of residual monomer is often identified as the main cause for adverse tissue reactions. To optimize the polymerization reaction, the use of the proper powder/liquid ratio is recommended in the fabrication of a dental appliance. It is also recommended that a dental appliance should be soaked in water for at least 24 hours before delivery to a patient, in order to reduce the possible adverse effect. For auto-polymerizing resins, associated with higher residual monomer levels than heat-cured resins, soaking the appliance at elevated temperatures (65ºC for 60 minutes), would reduce the residual monomer content more efficiently than at room temperature. This requires additional processing conditions from the technician or dentist. Changing the powder/liquid ratios, deliberately or not, may modify the residual monomer content of the final product. A relationship exists between the levels of residual monomer and water sorption. Also, residual monomer leaching into the oral fluids may lead to adverse effects such as, oral tissue irritation or a delayed hypersensitivity reaction.

Aim of the study

The aim of this study was to evaluate the effect of different powder/liquid ratios and different water temperatures on the levels of sorption and solubility of an auto-polymerizing resin material used for denture bases. The null-hypothesis tested was that there is no difference in sorption and solubility among groups of specimens made from an auto-polymerizing resin material soaked in water at different temperatures and/or fabricated with different powder/liquid ratios.

Material and methods

Specimens were made from cold-cure pour-type denture base resin (Type 2, Class 2) using different powder/liquid ratios and soaked at different soaking temperatures. One group of specimens fabricated with the manufacturer's recommended powder/liquid

ratio and soaked in water at 37ºC, served as the control group for both experiments. Custom-made stainless steel moulds were used to fabricate resin disks, with a diameter of 50mm and a thickness of 0.5mm.

For the temperature-controlled experiment, identical specimens were prepared and stored in distilled water at 37ºC; 45ºC; 55ºC and 67ºC. For the ratio-controlled experiment, the ratios were increased incrementally for each group, starting with a 10% increase, followed by a 15%; 20% and 25% increase in monomer. Water sorption and solubility were tested in accordance with ISO Standard 1567 (1999). Specimens were weighed before and after water immersion, and desiccation. Water sorption and solubility were calculated using the difference in wet and dry mass and the volume of the specimens. The water sorption and solubility results were analyzed by means of analysis of variance. For multiple comparisons, Bonferroni simultaneous confidence intervals (α=0.05) were applied.

Results

For the ratio-controlled experiment, *water sorption* mean values varied from 24.148 µg/mm^3 to 25.1333 µg/mm^3. Statistically significant differences in mean values were found between the following groups: 0%-10%; 0%-15%; 0%-25%; 10%-20%; 15%-20% and 20%-25% ratio groups (P<.0001). *Water solubility* mean values varied from 0.616µg/mm^3 to 0.932µg/mm^3. Statistically significant differences in mean values were found between the following groups: 0%-15%; 0%-20%; 0%-25% and 10%-25% and 20%-25% ratio groups (P<.0001).

For the temperature-controlled experiment, *water sorption* mean values varied from 24.185µg/mm^3 to 26.434µg/mm^3. Statistically significant differences in mean values were found between the following groups: 37ºC-45ºC; 37ºC-55ºC; 37ºC-67ºC; 45ºC-67ºC and 55ºC-67ºC temperature-controlled experiments (P<.0001). *Water solubility* mean values, for the same experimental groups, varied from 0.616µg/mm^3 to 2.752µg/mm^3. Statistically significant differences in mean values were found among all the 6 pairs of groups (P<.0001).

Despite statistical differences, the water sorption and water solubility values of the tested resin for both experiments and all groups were within the ISO Standard 1567 (1999) specification limits.

Conclusion

For the ratio-controlled experiment, there was an inverse relationship between the mean sorption and solubility values with an increase in liquid in the mixture: low water sorption levels are associated with high solubility levels. The lower water sorption and higher solubility results for more fluid mixtures could be related to initial and residual high monomer content characteristic of auto-polymerizing materials. These higher levels of free monomer are consequently released upon immersion in water; hence the higher water solubility levels.

For the temperature-controlled experiment, a higher soaking temperature resulted in an increase in water solubility levels. The higher solubility levels could be attributed to the higher soaking temperatures causing higher or faster monomer diffusion from the resin material. Except for the 67ºC group, sorption is also lower with higher temperatures. It may be assumed that an additional polymerization process takes place and a subsequent more inaccessible polymer matrix is produced. For the 67ºC group, thermal expansion may explain the higher sorption level.

Clinical Implications

In terms of the sorption and solubility results, this auto-polymerizing pour-type resin may be used as a denture base resin. Even though statistical differences were demonstrated, the material satisfies the ISO 1567 (1999) requirements not only for auto-polymerizing but also for heat-polymerizing resins. Therefore, within limits, the mixture may be prepared more fluidly in order to improve flow of the material, without negatively affecting its sorption and solubility properties. Because solubility is higher at higher soaking temperatures, this property can be used to minimize monomer content of the appliance. Therefore, it is recommended that the dental appliance be soaked in warm water, below 67ºC, prior to delivery to the patient.

ACKNOWLEDGEMENTS

I wish to thank:

DEDICATION

To my family for the time sacrificed so I may complete my thesis.

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GLOSSARY

KEYWORDS AND EXPLANATIONS

Sorption

The process or state of being sorbed - absorption or adsorption (Dorland's Medical Dictionary for Health Consumers, 2007). For denture base polymers, the increase in volumetric mass should not exceed $32\mu g/mm^3$ (ISO Standard 1567, 1999).

Solubility

Solubility is described as the maximum amount of solute that can dissolve in a specific solvent under a given temperature (Mosby's Medical Dictionary, 2009). For denture base polymers, the loss in volumetric mass (soluble matter) should not exceed $1.6\mu g/mm^3$ for types 1, 3 and 5 and should not exceed 8.0 $\mu g/mm^3$ for type 2 polymers (ISO Standard 1567, 1999).

Auto-polymerizing polymethyl methacrylate resins (auto-polymerizing PMMA)

Auto-polymerizing PMMAs refer to resins whose polymerization is initiated by a chemical activator (The Glossary of Prosthodontic Terms, 2005:17).

Fluid resin technique

The fluid resin technique is used with auto-polymerizing resins (also known as "pour-type" or "fluid resins") as opposed to conventional flask, pressure and heat-polymerizing techniques for fabricating dentures (Phillips & Eugene, 1991:177-203).

CHAPTER ONE

LITERATURE REVIEW

1.1   Introduction

New materials are introduced to the dental market, often claiming improvements on previous products. Independent research is needed to investigate these claims.

Traditionally, dentures are processed by means of flasking the wax denture in dental stone, packed with resin dough, placed under pressure and heat-polymerized which is considered a time-consuming procedure. More recently, a new denture base resin has been introduced using an auto-polymerizing acrylic resin, but without the disadvantages previously associated with auto-polymerizing acrylics (Vertex Dental BV, n.d.).

Manufacturers claim excellent flow of the unpolymerized resin, less shrinkage during polymerization, no deformation or fractures during deflasking, no problem with excess monomer after water immersion and easy cleaning. The investing flask is plastic and the investing material is a reversible hydrocolloid gel.

However, auto-polymerizing resins have a reputation of higher residual monomer in the polymerized product (McCabe et al., 2008:110-123). This is of concern both to the researcher and clinician, because the leaching of components from the denture base into the oral environment may have a negative effect on the health of the tissues of the patient. Cytotoxicity and allergic reactions have been reported (Jorge et al., 2003:190-193).

1.1.1   Denture base polymers

According to Manappallil et al. (2003:98-142) acrylic resins were so well received by the dental profession that by 1946, 98% of denture bases were constructed from polymethyl methacrylates (PMMA). Its many advantages include ease of use, versatility and variety of colours. However, there are disadvantages as well, such as low strength, sorption, solubility and flexibility (Cucci et al., 1998:434-438). Owing to water absorption and the relative ease

with which they are scratched, acrylic resins are not easily kept clean and free from deposits and stains as are metal dentures (Dhir et al., 2007:465-472).

Denture base polymers can be categorized into the following types and classes (ISO Standard 1567, 1999):

Type 1: Heat-polymerizable polymers
    Class 1:   Powder and liquid
    Class 2:   Plastic cake
Type 2: Auto-polymerizable polymers
    Class 1:   Powder and liquid
    Class 2:   Powder and liquid pour-type resins
Type 3: Thermoplastic blank or powder
Type 4: Light-activated materials
Type 5: Microwave cured materials

Bhola et al. (2010:129-136) investigated the more frequently used polymer prosthetic materials used in dentistry at present, and focused primarily on PMMAs. Despite the advantages of PMMA resin, there are still problems associated with manufacturing and it's functioning in the oral environment (Bhola et al., 2010:129-136). Dental appliances may be subjected to high levels of biting force and fluctuating oral environmental conditions. Toxic eluates or breakdown of the resin material may also contribute to injury of soft tissues and surrounding structures that are in contact with these denture polymers (Bhola et al., 2010:129-136).

Denture base polymers should be chemically stable, that is, the resin should not deteriorate inside the oral cavity, bringing about probable chemical reactions or resulting in undesirable conditions within the oral cavity (Bhola et al., 2010:129-136). The resin material should ideally polymerize to completion, without leaching any residual components (Bhola et al., 2010:129-136).

Side effects, reported by Huang et al. (2000:17-21), due to residual monomer release into saliva, include redness, swelling and pain of the soft tissues. This monomer may be converted into secondary harmful substances by oxidation (formation of formaldehyde) and hydrolysis (formation of methacrylate acid). Effective and practical methods should be considered in order to reduce the

amounts of chemicals released from the resin material during clinical use (Huang et al., 2000:17-21).

Bhola et al. (2010:129-136) reported the development and need of newer radio-opaque, high strength denture polymers, with enhanced physical properties. This may be achieved by altering the primary polymer matrix with a number of additions. Experiments are underway in order to develop the ideal denture polymer, which includes testing of different fillers such as glass, borosilicates and fused quartz (Bae et al., 2001:33-39; Bhola et al., 2010:129-136). Binders as well as processing techniques, which include the use of rods, fibres or matte, are also being researched. There is particularly a need for the development of non-leachable plasticizers (Bhola et al., 2010:129-136).

1.1.2   Autopolymerising PMMA

Chemical activation does not require the application of thermal energy and therefore may be completed at room temperature. As a result, chemically activated resins are also referred to as cold-curing, self-curing or auto-polymerising resins. Auto-polymerisation progresses in a manner similar to that described for heat-activated systems. However, the degree of polymerisation achieved using chemically activated resins is generally not as complete as that achieved using heat-activated systems (Anusavice et al., 1996:237-255). Ingredients of auto-polymerizing PMMA's are summarized in Table 1.1 (Bhola et al., 2010:129-136).

Table 1.1: Auto-polymerized poly methyl methacrylate resin (adapted from Bhola et al., 2010:129-136).

Powder System	Liquid System
Poly (methyl methacrylate)	Methyl methacrylate
Benzoyl Peroxide	Dibuthyl phthalate
Mercuric Sulphide, Cadmium Sulphide	Glycol dimethacrylate (-2%)
Zinc Oxide, Titanium Oxide	Hydroquinone (0.006%)
Dibutyl phthalate	Dimethyl-p-toluidine
Dyed particles-glasses, beads	

The polymerization process of cold-cure acrylic resins is initiated when the tertiary amine activates the benzoyl peroxide to produce free radicals. The hydroquinone initially inhibits the reaction by destroying the free radicals; this process increases working time of the material. When the hydroquinone is depleted the rubber stage is reached. This reaction is exothermic and goes quickly from warm to hot and at this stage the material is hard and stiff (Hatrick et al., 2003:249-256). The propagation and termination phases are the same as with heat-cured resins, and the polymerization process is exothermic in nature, but, unlike heat-cured resins, external heat is not applied, and the maximum temperature reached is therefore not efficient to sustain and complete the polymerization process.

The degree of polymerization caused by the tertiary amine is not as high as with heat-activation (Vallittu et al., 1995:338-342). This results in an end-product with a lower molecular weight and a higher level of residual free monomer, with a slight palatal shrinkage of 0.25mm. (Hatrick et al., 2003:249-256).

Doğan et al. (1995:313-318), citing McCabe and Basker (1976:347-350), reported that for heat polymerized PMMA's the curing time of the acrylic resin determines the level of residual monomer in the cured resin material. Previous studies found that the level of residual monomer was significantly higher when specimens were processed with shorter curing cycles compared to longer curing cycles (Doğan et al., 1995:313-318). However, there seems to be no reported literature on the effect of curing temperature for auto-polymerized acrylic resin. Huang et al. (2000:17-21) reported that the levels of released residual monomer depend on the processing method and composition of the resin material. Huang et al. (2000:17-21) compared self-, heat- and light-cured resins and found that, as a result of the repeated pressure being applied to the heat-cured resin during the packing stage, a reduction in voids would be observed, in contrast to self-cured resins. This was also said to be the reason for lower levels of free monomer found in heat-cured resins compared to self-cured resins. Denture base resins consist of PMMA and MMA, with the addition of cross-linking agents. Depending on the curing temperature, time and type of resin material, various levels of free monomer would therefore be present in the polymerized resin.

1.1.3 Fluid resin material and technique

Research on pour-type resins has been documented since the late 1960's. The studies have focused on the gradual change in physical and mechanical properties of the resin material (Dixon et al., 1991:510).

Massad et al. (2006:122-126) reported that the fabrication of dentures utilizing the fluid-resin technique, compression moulding and injection moulding techniques provided clinically acceptable outcomes depending on the skill of the operator. For the fluid-resin technique flexible mould materials such as hydrocolloid and silicone allows for easy divesting and recovery of the processed prosthesis. However, it requires multiple sprues and a highly fluid mixture (extra light viscosity), to create an even flow through the sprues. The author further emphasised the continual ongoing investigations of the most consistent processing techniques. Bahrani et al. (2012:171-175) mentioned that the cold cure fluid-resin technique also presented with disadvantages such as tooth movement, high creep rates, reduced stiffness, lower fatigue strength, colour instability, and solubility in the denture base resin during processing. The author found a significant difference in polymerization shrinkage between fluid-resin, or chemically-cured resin, and heat-cured resins. The greater polymerization shrinkage observed with the fluid-resin material was attributed to the higher monomer to polymer ratio utilized. Phillips and Eugene (1991:177-203) also mentioned disadvantages such as air inclusions, shifting of teeth, decreased vertical occlusion, incomplete flow of material and poor bonding.

Due to these limitations of the material, it has not been widely used as denture base material. However, techniques and materials are continuously being improved. Advantages claimed for current fluid-resins used for denture bases include better tissue fit, less open bites, less fracture of teeth during deflasking and reduced material cost (Zenith Dental, 2009).

Pour-type resins have smaller powder particles, and upon mixing with the liquid present with a fluid consistency (van Noort, 2002:213). The mixture is poured into an agar-hydrocolloid mould and polymerized under moderate pressure (van Noort, 2002:213). The water, which is present in the hydrocolloid, does not

interfere with the polymerization of the polymer and monomer mixture (Powers & Sakaguchi, 2006:534-536).

Pour-type denture resins present with a lower molecular weight than heat-cured resins, which results in significantly lower internal strain build-up during polymerization (van Noort, 2002:213). Yau et al. (2002:622-629) conducted a study measuring the pressure and temperature changes of heat-cured acrylic resins during the processing procedure. They reported that the pressure within the dough inside the clamped flask could rise to twice the initial pressure: it ranged from an initial 11.5 atmospheric pressure (atm), (±1.1145MPa) to 22.0atm (2.22915MPa) during the polymerization cycle. For pour-type resin, an internal pressure of only 0.1 to 0.2MPa has been calculated (Powers & Sakaguchi, 2006:534-536). It may be assumed that lower pressure inside the flasks, leads to lower strain build-up in the prosthesis during polymerization and less deformation when pressure is released.

The agar-hydrocolloid mould eases flasking and deflasking procedures, reduces finishing time and involves the utilization of less expensive equipment.

1.1.4 Sorption

PMMA may absorb water. The absorption of water is facilitated by the polarity of the resin molecules (Miettinen & Vallittu, 1997:531-534). Water sorption into polymer results due to the polarity of the monomer by unsaturated bonds of the molecules (Miettinen & Vallittu, 1997:531-534). Resin polarity will determine the degree and rate of water sorption into polymer networks, depending on a) the concentration of available polar sites, which can form hydrogen bonds with water, and b) the network structure (Tuna et al., 2008:191-197). High water uptake can have a negative effect on the physical properties of the denture base material, such as *flexural strength* and Young's modulus, because water acts as a plasticizer of PMMA (Anusavice et al., 1996:237-255; Miettinen & Vallittu, 1997:531-534).

Studies have indicated that high levels of residual monomer influence the mechanical strength of the acrylic resin. This reduced mechanical strength is primarily due to the loss of residual monomer which leaches out into saliva or water, and the absorption of water (Patel & Braden, 1991:653; Arima et al., 1996:476-480). Dhir et al. (2007:465-472) also reported that, immersing a

dental appliance in water would decrease the mechanical properties of the resin material and subsequent expansion of the polymers. The latter is due to the water molecules pushing the polymers apart.

Water sorption of the dental appliance should be within limits. According to ISO Standard 1567 (1999), water sorption should not exceed 32µg/mm^3 for all types of denture base materials (Table 1.2.)

Table 1.2: ISO-determined limits for sorption and solubility for each type of denture resin.

Requirement	Sorption (µg/mm^3) max	Solubility (µg/mm^3) max
Type 1, 3, 4, 5	32	1.6
Type 2	32	8.0

Water sorption and solubility of denture base resins affect *dimensional behaviour* (Cucci et al., 1998:434-438; Pfeiffer et al.*,* 2004:72-78). Due to volumetric changes, the resin is subjected to internal stresses which may result in crack formation and eventually fracturing of the denture (Tuna et al.*,* 2008:191-197). An advantage of water sorption is that polymerization shrinkage is to some extent compensated for. However, prolonged use could create an affinity for water, resulting in a long-term plasticizing effect on the resin material (Dhir et al.*,* 2007:465-472). This reduces its transverse strength; hardness and fatigue limit (Cucci et al., 1998:434-438; Tuna et al.*,* 2008:191-197).

Cucci et al. (1998:434-438) compared the water sorption of two denture base materials (auto- and heat-polymerized acrylic resins), which was performed according to ISO Standard 1567 (1999), for denture base materials. The results, which were within the specification limit, indicated that there was no significant difference in water sorption between the autopolymerizing acrylic resin and heat-polymerizing resin.

Doğan et al. (1995:313-318) investigated water sorption of acrylic resins and found the dispersion of water as voids in the polymer matrix. These voids may be due to gas bubbles or entrapped residual monomer, which forms voids in the polymer matrix. They reported that when a resin present with high levels of residual monomer, the amount of voids, after leaching off the residual

monomer, will also be high, with a subsequent higher level of water sorption. It has been established that there is a parallel relation between the level of residual monomer and percentage water sorption (Doğan et al., 1995:313-318; Miettinen & Vallittu, 1997:531-534). A reduced amount of monomer conversion may result in increased sorption and solubility (Pfeiffer & Rosenbauer, 2004:72-78).

1.1.5   Solubility

Solubility is described as the maximum amount of solute that can dissolve in a specific solvent under a given temperature (Mosby's Medical Dictionary, 2009). For denture base polymers, the loss in volumetric mass (soluble matter) should not exceed 1.6μg/mm^3 for types 1, 3 and 5 and should not exceed 8.0 μg/mm^3 for type 2 polymers (Table 1.2.) (ISO Standard 1567, 1999).

Besides the fact that solubility affects the dimensional behaviour of denture base resins, it may contribute to irritation and allergic reactions to the oral mucosa (Pfeiffer & Rosenbauer, 2004:72-78). Eluates of PMMA denture base acrylic resins caused *in vitro* cytotoxic effects (Cimpan et al., 2000:59-69). Residual monomer is often blamed as main cause for these harmful tissue reactions. Because these cytotoxic effects were more pronounced for autopolymerized resins than for the heat-polymerized ones (Cimpan et al., 2000:59-69; Craig et al., 2004:271-286) may indicate that there is a greater amount of unreacted monomer in denture bases fabricated via chemical activation and improper processing. This unreacted monomer creates two major difficulties: Firstly, the residual monomer serves as a potential tissue irritant, thereby compromising the biocompatibility of the denture base. Secondly, it acts as a plasticiser, which results in decreased transverse strength of the denture resin (Anusavice et al., 1996:237-255).

Although the residual monomer is considered as the main cause for tissue irritation, other water-soluble materials have been reported as being potentially harmful. Cimpan et al. (2000:59-69) listed the following potentially toxic substances commonly found in denture base resins: formaldehyde, methyl methacrylate, methacrylic acid, benzoic acid, dibutyl phthalate, phenyl benzoate, phenyl salicylate, and dicyclohexyl phthalate. Jorge et al. (2003:190-

193) also identified possible leachable toxic substances such as formaldehyde, methacrylic acid, plasticizers, organic additives, benzoic acid, biphenyl and phenyl benzoate. According to Lung and Darvell (2005:1119-1128), the amount of methyl-methacrylate diffusing out of the denture base is proportional to the amount of residual monomer present. Thus the highest observed levels of leaching were from cold-cured resins.

Solubility is measured based on the loss in weight by the denture base resin. The polymerized denture base material is entirely insoluble in water and fluids present in the oral cavity (Miettinen & Vallittu, 1997:531-534). Cucci et al. (1998:434-438) established that most of these soluble materials leached out of the specimens during the first 7 days of immersion in water.
Cucci et al. (1998:434-438) reported that acrylic resins present with low solubility, and the minimal amount leaking into the oral cavity is due to residual monomer or water-soluble additives. There is however a concern regarding the leaching of residual monomer into the oral cavity, as it may produce harmful soft tissue reactions.

1.1.6 Mixing ratio

Anusavice et al. (1996:237-255) recommend a polymer/monomer ratio of 3/1 by volume. This should provide sufficient monomer to wet the polymer particles and avoid excess monomer that would lead to increased polymerization shrinkage. Powder/liquid ratio could influence the cytotoxicity of a denture base acrylic (Jorge et al., 2003:190-193). The more liquid (monomer) is added to the mixture, the greater the residual monomer content after polymerization and higher potential for cytotoxicity (Kedjarune et al., 1999:25-30). Often the powder/liquid ratio of PMMA resins is changed to modify the handling properties of the material (Geerts and Du Rand, 2009:110-116).

For the pour-type resins, the manufacturer supports the powder/liquid consistency to be that of water to increase the flow of the mixture into smaller details of the mould (Zenith dental, 2009). This consistency is substantially lower than the type 1, class 1 and 2, and type 2 class 1 denture base acrylic resins.

McCabe and Walls (2008:110-123) advises that the polymer/monomer ratio should be kept as high as possible, to significantly reduce shrinkage. A polymer/monomer ratio of 2.5/1 by weight is therefore recommended. This would allow for volumetric polymerisation shrinkage of 5-6% (McCabe & Walls, 2008:110-123). According to van Noort (2002:213) a powder/liquid ratio of 2/1 by weight is recommended.

1.1.7   Soaking in water and soaking temperature

According to Vallittu et al. (1995:338-342), chemically-cured resins release more methyl methacrylate into the surrounding water than heat-cured resins. The more porous structure of chemically-cured resins may therefore affect the release of monomer by enhancing the diffusion/dispersion between PMMA and water. This diffusion/dispersion is dependent and enhanced by an increase in temperature. Several studies examined the influence of time and temperature when PMMA is immersed in water.

Martin et al. (2003:225-227) reported a decrease in hypersensitivity reaction after immersion of dentures (from autopolymerizing resin) in heated water for 1 hour. Jorge et al. (2003:190-193), citing Tsuchiya et al. (1994:618-624), reported that immersing the denture in water for at least 24 hours or in hot water at 50° reduces residual monomer as a result of further polymerisation in water by 75 per cent or more; free radicals of the monomer lead to continued polymerisation, resulting in less cytotoxicity.

Wala (2007:553-560) emphasized the importance of soaking a chemically-cured appliance in water one 1 week before placement, in order to reduce the percentage of residual monomer.

In 1994, Tsuchyia et al. recommended that for auto-polymerizing resins, the appliance is soaked at a temperature of 50°C for 60 minutes to reduce the residual monomer content. Sheridan et al. (1997:73-77) reported that the longer a prosthesis is soaked in water, the less its cytotoxic effect. Therefore it is recommended that dentists soak dentures in water for at least 24 hours before delivering them to the patient.

Kedjarune et al. (1999:25-30) based their study on different mixing ratios utilizing different processing methods to establish the amount of residual monomer released into the oral cavity after the first 24 hours of processing, and 24 hours thereafter. They established from their experiment that specimens (auto- and heat- polymerized) which presented with the highest level of residual monomer released higher levels of monomer within the first 24 hours of processing. The level of released monomer after the second 24 hours was significantly lower (for both auto- and heat- polymerized).

Therefore, to reduce substances leaching from newly processed dental appliances into the oral cavity, they need to be immersed in water, ideally for 1 day after processing, or immersion in water at a higher temperature of at least 50ºC (also for 1 day or shorter) (Kedjarune et al., 1999:25-30).

Vallittu et al. (1995:338-342) compared the content and amount of residual monomer during storage in water for both a heat-cured and chemically-cured acrylic resin. They established that a higher storage temperature reduced the monomer content of chemically-cured resins and that a longer storage period in water was needed before a constant level of monomer was reached. The latter is significant as it indicates that the polymerization reaction in chemically-cured resins continues longer compared to that of heat-cured resins.

Jorge et al. (2003:190-193) also established that storage time plays an important role in the cytotoxicity of resin materials. The effect of cytotoxicity was reported to be the greatest in the first 24 hours after processing, and decreased the longer the appliance was soaked in water. Previous studies have speculated that these toxic substances released into the water within the first 24 hours are either broken down as time progresses or complexes with other chemicals in the water, thereby altering their cytotoxicity. Immersion of the appliance in heated water allows the monomer molecules to disperse/diffuse at a more rapid rate, and in the presence of free radicals, an additional polymerization reaction occurs.

1.2   STATEMENT OF RESEARCH PROBLEM

Acrylic resin is the most commonly used material for dentures. Denture base resins are subject to sorption and solubility. The properties of sorption and solubility influence the strength, colour stability and biocompatibility of a

material (Dhir et al., 2007:465-472). Dental technicians may change the powder/liquid ratio to influence the handling properties of a material without realizing that this may influence the properties of the final product like its sorption, solubility, strength or cytotoxicity. This is of concern because the leaching of components from the denture base into the oral environment may have an effect on the health of the tissues. Cytotoxicity and allergic reactions have been described (Jorge et al., 2003:190-193).

The pour-type acrylic resins are classified as auto-polymerizing resins. Auto-polymerizing resins have a reputation of higher residual monomer in the polymerized product (McCabe & Walls, 2008:110-123). Also, the "fluid" consistency of the material raises concern that the monomer content in the mixture is excessively high which may leach during clinical use of the polymerized appliance.

1.3. AIM AND OBJECTIVES

The aim of this study was to assess the influence of different soaking temperatures and mixing ratios on the sorption and solubility properties of an auto-polymerizing pour-type denture base.

The objectives of this study were to determine:
1. To determine sorption and solubility of an auto-polymerizing denture based resin at different soaking temperatures.
2. To determine sorption and solubility of an auto-polymerizing denture based resin using different powder/liquid mixing ratios.

1.4. NULL–HYPOTHESES

The null-hypotheses of this study were:
1. There is no difference in sorption and solubility among denture base specimens soaked in water at different soaking temperatures.
2. There is no difference in sorption and solubility among denture base specimens made with different powder/liquid mixing ratios.

1.5   SIGNIFICANCE OF RESEARCH

The standard practice in many laboratories in South Africa for manufacturing denture bases is still the conventional compression moulding technique and heat-polymerization. Before a standard procedure is changed, it is desirable that results from independent research are available. This study will add to the scientific evidence necessary for making a choice between the established conventional systems or moving towards a newer more convenient system, with improved handling properties without compromising the quality of the product.

1.6   ETHICS STATEMENT

All specimens testing were of a mechanical nature and no animal or human being testing took place.

The material involved is part of standard dental laboratory products used in any laboratory and posed no specific or additional risk to the operator or the environment.

All tested products were purchased by the researcher. The researcher has no direct or indirect interest in these companies.

CHAPTER TWO

EXPERIMENTAL DESIGN

2.1   Introduction

Two experiments were completed. The first experiment concentrated on the influence of different temperatures of the soaking water on the sorption and solubility of the autopolymerizing acrylic resin; The second experiment was identical except that the powder/liquid ratios were altered.

All the procedures were performed by the researcher. The methodology for the sorption and solubility experimentation followed was done according to the ISO Standard 1567 (1999) for the ratio-controlled experiment. For the temperature-controlled experiment the recommended temperature of 37ºC was used as the control. The manufacturer's instructions of the material that was used for the experiments were followed, except when the powder/liquid ratios were changed. In this instance, the recommended ratio was used as the control.

All specimens were prepared using a custom-made stainless steel mould and cover, as specified by ISO Standard 1567 (1999). The specimens were allocated to one of the 2 experiments. The group of specimens prepared with the recommended powder/liquid ratio and soaked in water at 37ºC as specified by ISO Standard 1567 (1999), served as the control group for both experiments.

The study design is shown in table 2.1. A total of 30 specimens per group were made. The sorption and solubility values of all groups were vertically and horizontally compared.

Table 2.1: Study design
(Powder/liquid ratio is given by weight)

Control group 37ºC and recommended ratio 1.70/0.95		
Experiment 1 : Temperature-controlled experiment		*Experiment 2: Ratio-controlled experiment*
45ºC and recommended ratio 1.70/0.95		1.70/1.045 and 37ºC
55ºC and recommended ratio 1.70/0.95		1.70/1.0925 and 37ºC
67ºC and recommended ratio 1.70/0.95		1.70/1.140 and 37ºC
		1.70/1.1875 and 37ºC

For the temperature-controlled experiment the different water temperatures were as follows: 37ºC, 45ºC, 55ºC and 67ºC, whilst the powder/liquid ratio was according to the manufacturer's instructions. For the ratio-controlled experiment the water temperature for soaking the specimens was the same (37ºC), and the liquid volume was increased by 10%, 15%, 20% and 25%. The ratios by weight are indicated in Table 2.1.

2.2 Preparation of specimens

Specimens were processed in batches as per experimental group described above. Each group was tested on its own.

The material for testing the sorption and solubility was Vertex Castavaria Cold Curing Pour Type denture base material (Type 2, Class 2) as the resin powder,

together with Vertex Castavaria Liquid. The product information related to Vertex Castavaria is indicated in Table 2.2.

Table 2.2: Acrylic resin specimen information

POWDER		LIQUID
Vertex-Dental B.V. Netherlands	**Manufacturer**	Vertex-Dental B.V. Netherlands
Vertex Castavaria Cold Curing Pour Type	**Product**	Vertex Castavaria Liquid
Type 2, Class 2	**Classification**	Type 2, Class 2
XY 324 P04	**Lot**	XY 262 L01
2016-02	**Expiry Date**	2016-01
1.70g	**Ratio**	1ml = 0.950g
23.2 $\mu g/mm^3$	**Water Sorption**	23.2 $\mu g/mm^3$
1.8 $\mu g/mm^3$	**Water Solubility**	1.8 $\mu g/mm^3$

A custom-made stainless steel mould was used to fabricate the specimens. The dimensions of this circular mould and cover are indicated in Figures 2.1 and 2.2. This produced specimens in the shape of disks, 50mm in diameter and 0.5mm in thickness.

Figure 2.1: Dimensions of stainless steel mould and cover

Acrylic wafer

±80mm

± 5mm
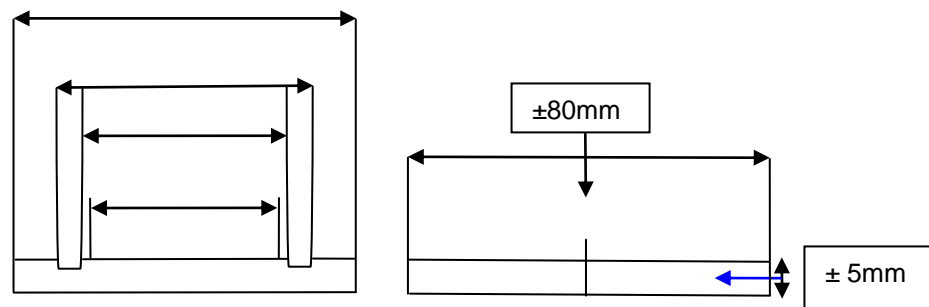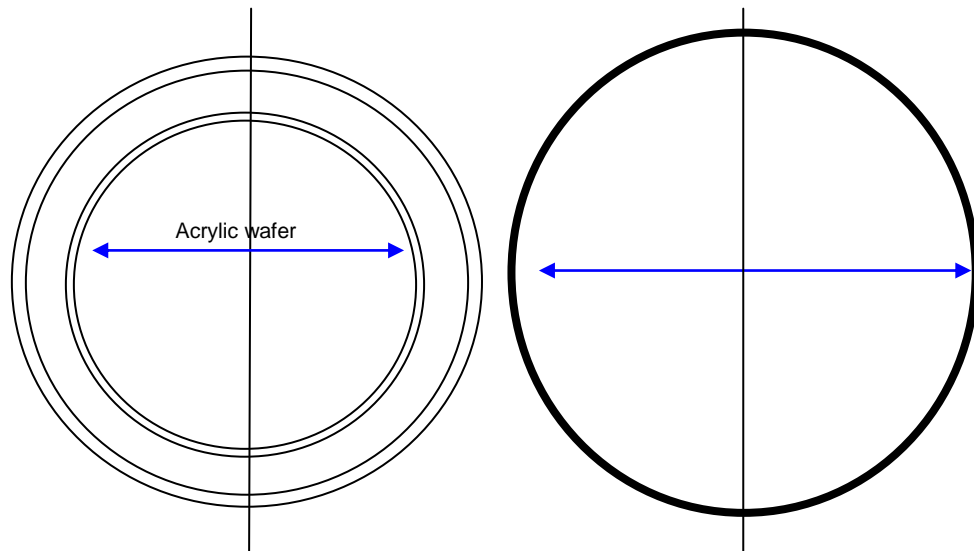
Figure 2.2: Stainless steel moulds and cover

Figure 2.3: Close-up view of stainless steel mould

The liquid and the powder were measured following the manufacturer's instructions using a powder/liquid ratio of 1.70g/0.95g. The liquid was drawn from its container using an analogue pipette (Biohit Proline Pipette 100-1000µl, Helsinki, Japan). The pipette was set to draw 100ml of liquid through D1000 Gilson diamond tips.

The templates were filled with the mixed resin material, and polythene sheets, with a thickness of 50±25 µm, were used as a separating medium between the circular mould and its cover. The poured templates were allowed to bench sit for 10 seconds, after which the templates were carefully submerged in the water-filled pressure pot to polymerize the resin material.

The pressure pot (Futuramat, Schütz Dental Group, Rosbach Germany) was set for 30 minutes at 2.5 bar, with a water temperature of 55ºC. After curing the specimens were carefully removed wearing latex powder-free gloves and using a stainless steel instrument, the edges removed with a scalpel and stored in polythene to prevent possible evaporation of monomer during transport from the dental technology laboratory at the Cape Peninsula University of Technology to the research laboratory at the Oral Health Centre of the University of the Western Cape.

After fabrication, the specimens were transferred to a desiccator based with silica gel for drying. Specimens were weighed every 24 hours until a constant mass was obtained (m_1), after which the specimens were submerged in distilled water for 7 days and weighed for a second time (m_2). The specimens were again reconditioned to a constant mass in the desiccator (m_3). The volume of each specimen was calculated (mm^3) after the first desiccation. Water sorption (wsp) and water solubility (wsl) was calculated using the following equations:

$$wsp = m_2-m_3/V \qquad and \qquad wsl = m_3- m_1/V.$$

A scale (Mettler PE3600, Delta Range, Mettler Instrument Corporation, New Jersey, U.S.A), with an accuracy of 0.01g, was used to weigh the powder and the liquid. Small porcelain bowls (100ml) were used to measure the powder and liquid respectively. The weight of these porcelain bowls were subtracted by using the tear function on the scale, which reset the display screen to zero, thereby the weight of only the content of the porcelain bowl was measured. This process was repeated for each individual specimen fabricated.

The room temperature was monitored, before commencing the experiment and monitored throughout, using two glass thermometers (N.T. Laboratory Supplies (Pty), 76mm Immersion type). A digital thermometer (TH03 digital hygro-thermometer) was used to indicate a third reading and to monitor humidity.

Figure 2.4: Specimen according to ISO before testing (specimen was scanned using an Epson 1680 Flatbed scanner at 1600dpi)

2.3   Testing of specimens

After processing, each individual specimen was numbered with a waterproof marker and placed in parallel position, in a custom made glass rack (Figure 2.5), using polymer-coated tweezers.

Figure 2.5: Glass rack containing specimens.
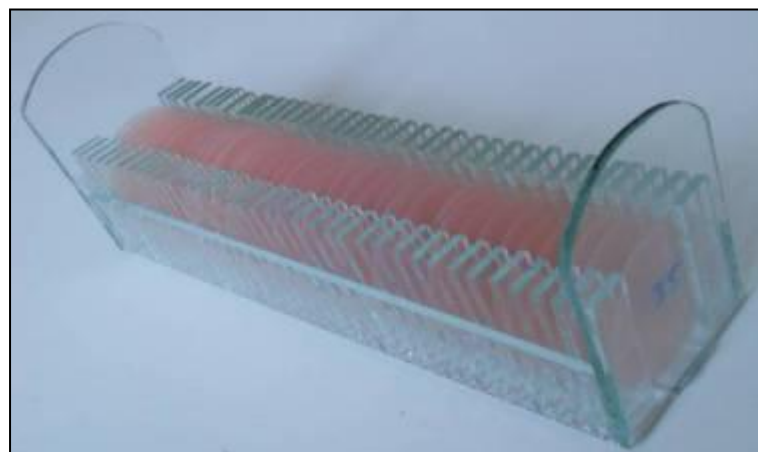
The specimens were inspected to ensure that they were not in contact with one another, after which the glass rack containing the 30 specimens were placed in a desiccator (300mm diameter knobbed desiccator with lid). For the drying process, the desiccator contained 500 grams of freshly pre-dried silica crystals (dried for 300 min ± 10 minutes at 130ºC ± 5ºC), with a particle size of 2.8 - 7.0 mm. The silica was dried for every group of specimens.

The securely closed and air-tight desiccator was placed in a thermostatically controlled incubator (Memmert Incubator, Schwabach, Germany) which was set at 37ºC ± 1ºC and left for (23±1) hours. After this cycle, the desiccator was removed from the incubator and left on the bench until the inside temperature of the desiccator was 23±2ºC (the same as the room temperature). Retrieval of the specimens commenced for weighing.

The specimens were weighed using a Mettler A240 analytical scale with an accuracy of 0.0001 g. The room temperature and humidity were monitored. The cycle of incubating at 37ºC and cooling off before weighing was repeated every 23±1hr until a constant weight/mass or state of equilibrium was reached, which is referred to as m1 or "conditioned mass 1". A constant weight was achieved if the loss in weight between two consecutive weightings was not more than 0.02mg.

After dry weight was determined, the thickness and width of the specimens were measured in order to calculate the volume (mm^3) of each sample. For the diameter, an electronic digital vernier calliper (Mitutoyo Digimatic caliper, Japan) to an accuracy of 0.01mm, was used and three diameter measurements were recorded. The mean of these three measurements was calculated. Using an electronic digital micrometer (Model no., EOSAMO-24- IP54) to an accuracy of 0.001mm, five thickness measurements were taken at four equally spaced locations at the circumference of the specimen, together with a centre measurement. The mean of these five thickness measurements was calculated and recorded for each specimen. Non-powdered latex gloves were worn when the specimens were handled for measurement.

Volume was recorded as follows: $V = pi.r^2.h$

where:

pi   =   3.14;

radius (r)   =   mean diameter of sample;

height (h)   =   mean thickness of the sample

The specimens were then replaced in the glass rack and immersed in distilled water at 37°C ± 1°C, which was retrieved after 7 days for weighing (m2 – "wet' weight).

The specimens were wiped with a clean dry towel to remove all visible moisture. They were waved in the air for 15±1 seconds and then weighed 60±10 seconds after removal from the distilled water. Each individual specimen was transferred from the glass rack to the measuring pan in the scale. After weighing, the specimen was placed in a second dry glass rack. Polymer coated tweezers were always used when handling specimens, thereby ensuring accurate weight values by not contaminating the samples with skin moisture. Before each individual specimen reading, the display screen was reset to zero, the sample placed on the measuring pan and the sliding glass door closed. After closing the sliding glass door, the stability light flickers, indicating that the weight is stable, and the sample's weight recorded to an accuracy of 0.0001 g.

When the m2 of all specimens was established, the glass rack with all the specimens was returned to a desiccator with freshly dried silica crystals. Specimens were again stored and weighed using the same sequence as described earlier for m1, until a constant weight (m3) or "reconditioned mass" was reached. This was achieved when the difference between 2 consecutive weightings was not more than 0.02mg (m2).

m1   =   "Dry" weight / pre-immersion weight

m2   =   "Wet" weight / at removal from water

m3   =   "Dry" weight / post immersion

Water sorption (Wsp) = $(m_2-m_3) / V$

Water solubility (Wsl) = $(m_1-m_3)/ V$

2.4   Statistical analysis

The mean sorption and solubility values were used for the analysis of the results. For statistical purposes an average (mean) is defined as the number measuring a central tendency of a set of data values. It refers to the value obtained when a set of data values are added together and then divided by the total number of data values used in the set.

Box and whiskers plots were used to graphically represent the symmetry of data. Box and whiskers plots indicate a *median* value, which is displayed as a solid horizontal bar surrounded by 50% of the data within the box. This 50% falls between the 25th and 75th percentile mark, the 25th being the bottom and the 75th the top. The whiskers indicate the *maximum* (highest) and *minimum* (lowest) values that are not *outliers*. *Outliers* are data that show 1.5 box lengths from the 25th and 75th percentile and are represented by a circle (O) (Kerr et al., 2004:9-28).

All the statistical analysis was performed by a professional statistician. The mean sorption and solubility values of all groups were vertically and horizontally compared and statistically analysed by means of pairwise comparison.

The data were analysed by means of one way analysis of variance. SPSS version 19 statistical software was used. Bonferroni simultaneous confident intervals (95%) were applied for multiple comparisons by means of the Post Hoc test.

CHAPTER THREE

TEST RESULTS

3.1 Introduction

All specimens, from both ratio-controlled and temperature-controlled experiments, were measured according to ISO 1567 (1999) specifications. Those specimens that reached stable weight at day 3 (m1) and again at day 2 after soaking (m3) were included in the study. Those that did not reach stable weight at these specific times were excluded from the study. The raw data for both experiments are presented in Appendix A.

3.2 Ratio-controlled experiment

A total of 139 specimens were used for testing the influence of powder/liquid ratio on sorption and solubility. For the 0.00% group all specimens (n=30) were used. For the 10% ratio group an additional number of specimens were fabricated after too many specimens of the initial group of 30 did not reach stable weights within 3 days. None of the specimens of the second batch were excluded. This resulted in a total of 32 specimens for the 10% ratio group. For the 15% ratio group (n=27) 3 specimens were not used, for the 20% (n=25) and 25% ratio groups (n=25) 5 specimens were not used.

Table 3.1 gives the summary of the number, mean, standard deviation, minimum and maximum for the volume, sorption and solubility for each group. The highest mean values are highlighted in yellow; the lowest mean values are highlighted in green.

Table 3.1: Case summaries for the different ratio-controlled experiments

Group		Volume in mm3	Sorption in µg/mm3	Solubility in µg/mm3
0.00%	N	30	30	30
	Mean	1024.372667	25.133333	.616667
	Std. Deviation	34.2918599	.8603661	.1723736
	Std. Error of Mean	6.2608084	.1570806	.0314710
	Minimum	959.7200	23.0000	.2000
	Maximum	1098.8800	26.0000	.9000
10.00%	N	32	32	32
	Mean	1023.913460	24.281250	.768750
	Std. Deviation	34.1910593	.7288690	.2375106
	Std. Error of Mean	6.0441825	.1288471	.0419863
	Minimum	952.3940	22.0000	.2000
	Maximum	1102.1578	25.0000	1.3000
15.00%	N	27	27	27
	Mean	1020.584253	24.148148	.837037
	Std. Deviation	29.3546861	.4560452	.1572557
	Std. Error of Mean	5.6493120	.0877659	.0302639
	Minimum	966.2483	23.0000	.5000
	Maximum	1073.6341	25.0000	1.1000
20.00%	N	25	25	25
	Mean	1011.953390	24.960000	.824000
	Std. Deviation	29.3691907	.4546061	.1451436
	Std. Error of Mean	5.8738381	.0909212	.0290287
	Minimum	967.5484	24.0000	.6000
	Maximum	1062.8548	26.0000	1.2000
25.00%	N	25	25	25
	Mean	1004.284935	24.280000	.932000
	Std. Deviation	29.3013822	.7371115	.1625833
	Std. Error of Mean	5.8602764	.1474223	.0325167
	Minimum	952.9423	23.0000	.7000
	Maximum	1057.0331	25.0000	1.3000
Total	N	139	139	139
	Mean	1017.684487	24.561151	.788489
	Std. Deviation	32.0973784	.7812579	.2067972
	Std. Error of Mean	2.7224641	.0662654	.0175403
	Minimum	952.3940	22.0000	.2000
	Maximum	1102.1578	26.0000	1.3000

The group with the highest mean *sorption* was the 0.00% group and the group with the lowest mean *sorption* was the 15% group.

The group with the highest mean *solubility* was the 25% group and the group with the lowest mean solubility was the 0.00% group.

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One way analysis of variance (ANOVA) with a 95% confidence interval of the means showed significant differences among sorption and solubility among the ratio groups. No significant differences were detected among the volumes between the groups (p>0.94) (Table 3.2).

Table 3.2: ANOVA for the ratio-controlled experiments:

		Sum of Squares	df	Mean Square	F	Sig.
Volume in mm3	Between Groups	8120.426	4	2030.107	2.029	.094
	Within Groups	134052.929	134	1000.395		
	Total	**142173.355**	**138**			
Sorption in µg/mm3	Between Groups	22.887	4	5.722	12.499	.000
	Within Groups	61.343	134	.458		
	Total	**84.230**	**138**			
Solubility in µg/mm3	Between Groups	1.508	4	.377	11.500	.000
	Within Groups	4.393	134	.033		
	Total	**5.902**	**138**			

For sorption, multiple comparisons by means of the Post Hoc test identified significant differences between the following pairs (Appendix B):

> 0% and 10%
>
> 0% and 15%
>
> 0% and 25%
>
> 10% and 20%
>
> 15% and 20%
>
> 20% and 25%

For sorption, no significant difference was identified between groups:

> 0% and 20%
>
> 10% and 15%
>
> 10% and 25%
>
> 15% and 25%

For solubility, multiple comparisons by means of the Post Hoc test identified significant differences between the following pairs (Appendix B):

> 0% and 15%,
>
> 0% and 20%
>
> 0% and 25%
>
> 10% and 25%
>
> 20% and 25%

There were no significant differences between the other pairs:

> 0% and 10%,
>
> 10% and 15%,
>
> 10% and 20%,
>
> 15% and 20%,
>
> 15% and 25%.

The box plots for the volume, sorption and solubility for the ratio-controlled experiment groups are shown in Figures 3.1; 3.2 and 3.3.

According to the box plots for the Volume specifications, there were no specimens with outlier or extreme values.
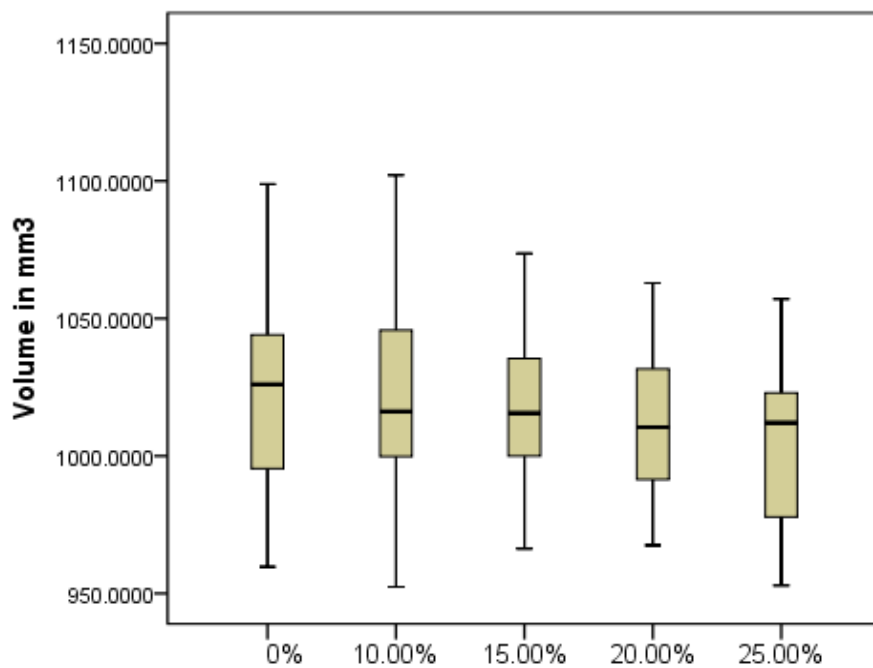
Figure 3.1: Box plot for the volume of the 5 ratio-controlled groups.

For sorption, there were 2 outliers for the 0% group (control), 1 outlier for the 10% group, 4 extremes for the 15% group and 4 extremes for the 20% group.
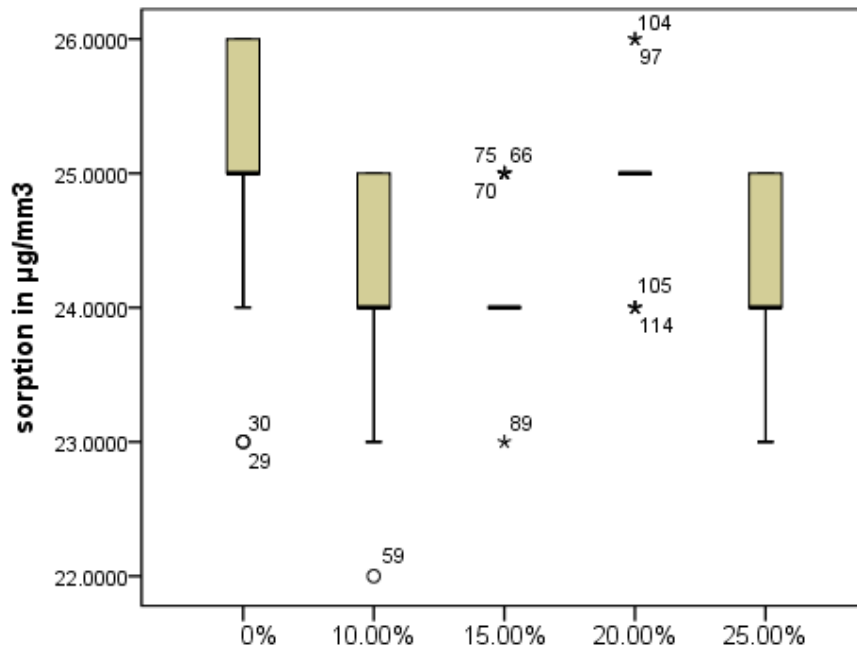
Figure 3.2: Box plot for the sorption of the 5 ratio-controlled groups.
o = outlier; * = extreme value.

For solubility, there were 3 outliers for the 10% group and 1 outlier for the 25% group.
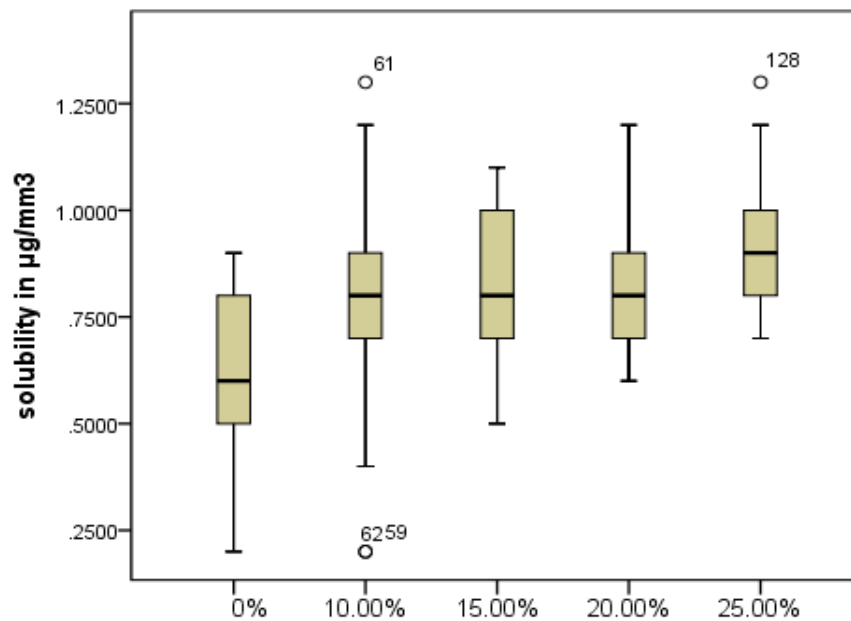
Figure 3.3: Box plot for the solubility of the 5 ratio-controlled groups. o = outlier.

3.3 Temperature-controlled experiment

A total of 108 specimens divided over 4 ratio groups of 37ºC, 45ºC, 55ºC and 67ºC were used. Table 3.3 gives the summary of the number, mean, standard deviation, standard error, minimum and maximum for the volume, sorption and solubility for each group. No specimens were excluded for the 37ºC group (n=30). For the 45ºC group (n=27) 3 specimens were excluded, for the 55ºC group (n=28) 2 specimens were excluded, and for the 67ºC group (n=23) 7 specimens were excluded.

Table 3.3: Case summaries for the temperature-controlled experiment

Group		Volume in mm3	sorption in µg/mm3	solubility in µg/mm3
37(0%)	N	30	30	30
	Mean	1024.372667	25.133333	.616667
	Std. Deviation	34.2918599	.8603661	.1723736
	Std. Error of Mean	6.2608084	.1570806	.0314710
	Minimum	959.7200	23.0000	.2000
	Maximum	1098.8800	26.0000	.9000
45	N	27	27	27
	Mean	1013.091481	24.185185	1.288889
	Std. Deviation	40.3529460	.9622504	.3129676
	Std. Error of Mean	7.7659281	.1851852	.0602306
	Minimum	884.9300	23.0000	.9000
	Maximum	1077.6300	28.0000	2.3000
55	N	28	28	28
	Mean	1042.543929	24.285714	1.821429
	Std. Deviation	34.7119112	.9759001	.1343217
	Std. Error of Mean	6.5599346	.1844278	.0253844
	Minimum	990.4300	21.0000	1.6000
	Maximum	1110.6700	25.0000	2.1000
67	N	23	23	23
	Mean	1027.086957	26.434783	2.752174
	Std. Deviation	34.4519475	.5068698	.2921232
	Std. Error of Mean	7.1837276	.1056897	.0609119
	Minimum	987.0000	26.0000	2.3000
	Maximum	1103.4700	27.0000	3.3000
Total	N	108	108	108
	Mean	1026.841481	24.953704	1.551852
	Std. Deviation	37.1002105	1.2104240	.8033204
	Std. Error of Mean	3.5699694	.1164731	.0772995
	Minimum	884.9300	21.0000	.2000
	Maximum	1110.6700	28.0000	3.3000

Again a one way analysis of variance (ANOVA) with a 95% confidence interval of the means showed significant differences among sorption and solubility among the ratio groups. For the volume, no differences were detected among the groups (p>0.29) (Table 3.4).

Table 3.4: ANOVA for the temperature-controlled experiment

		Sum of Squares	df	Mean Square	F	Sig.
Volume in mm3	Between Groups	12192.796	3	4064.265	3.129	.029
	Within Groups	135084.745	104	1298.892		
	Total	**147277.541**	**107**			
Sorption in µg/mm3	Between Groups	79.861	3	26.620	35.998	.000
	Within Groups	76.907	104	.739		
	Total	**156.769**	**107**			
Solubility in µg/mm3	Between Groups	63.277	3	21.092	379.984	.000
	Within Groups	5.773	104	.056		
	Total	**69.050**	**107**			

For the sorption, multiple comparisons by means of the Post Hoc test identified significant differences between the following pairs (Appendix B):

37ºC and 45ºC

37ºC and 55ºC

37ºC and 67ºC

45ºC and 67ºC

55ºC and 67ºC

There was no significant difference between the 45ºC and 55ºC temperature-controlled experiment groups.

For the solubility, multiple comparisons by means of the Post Hoc test identified significant differences among all the pairs (Appendix B).

The box plots for the volume, sorption and solubility for the temperature-controlled experiment groups are shown in Figures 3.4; 3.5 and 3.6.
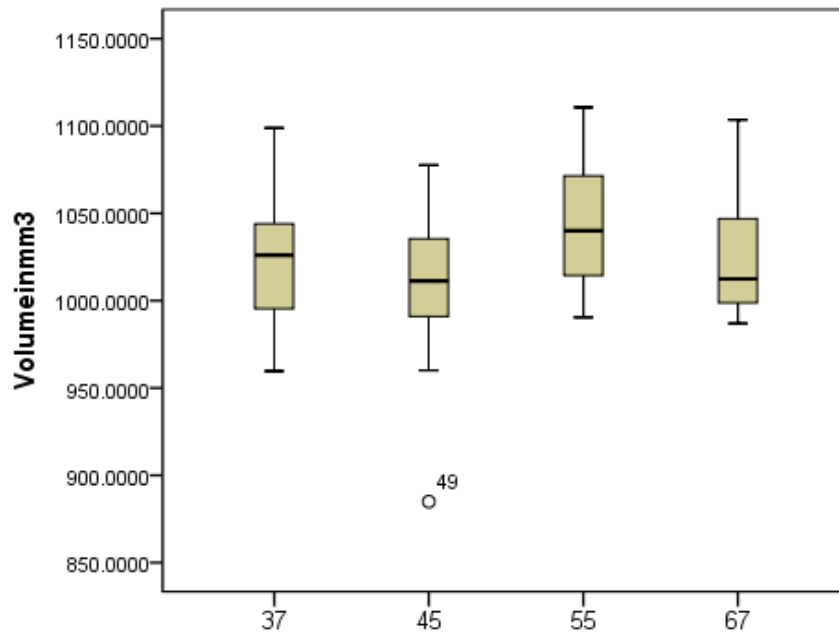
Figure 3.4: Box plot for the volume of the 4 temperature-controlled groups. o = outlier.

For the volume, there was 1 outlier present for the 45°C temperature-controlled experiment group.
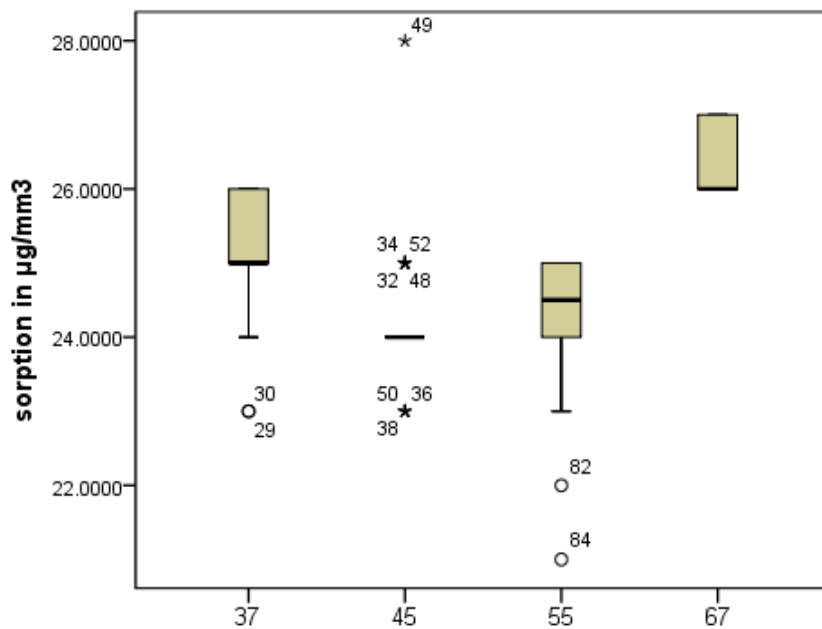
Figure 3.5: Box plot for the sorption of the 4 temperature-controlled groups.

o = outlier; * = extreme value.

For the sorption, there were 2 outliers present for the 37°C temperature-controlled experiment group, 8 extremes for the 45°C group, and 2 outliers for the 55°C group.
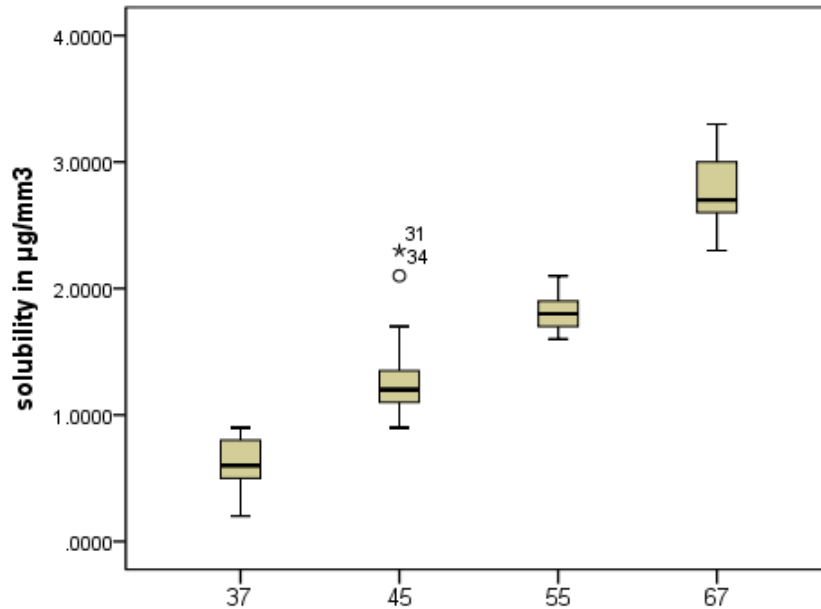
Figure 3.6: Box plot for the solubility of the 4 temperature-controlled groups. o = outlier; * = extreme value.

For the solubility, there were 1 outlier and 1 extreme value present for the 45°C temperature-controlled experiment group.

CHAPTER 4

DISCUSSION

4.1   Introduction

The purpose of these experiments was to investigate the influence of different P/L ratios and different water soaking temperatures on the sorption and solubility of a Type-2 Class-2 denture base acrylic. The volume was also analysed as a quality control mechanism to ensure consistent specimen fabrication among groups.

The methods followed for specimen fabrication and the analysis of sorption and solubility were strictly according to the specifications described in the ISO standard 1567 (1999) except for the temperature-controlled experiment, where the influence of soaking temperatures was investigated. In the latter case, the ISO standard specification of 37ºC of soaking was used as the control group. The PMMA material was handled according to the manufacturer's instructions, except when the testing condition required a change in the ratio. The recommended ratio-controlled experiment group served as a control for this experiment. Therefore, for both experiments, the same group served as the control, i.e. the group with all specimens made according to manufacturer's instructions and soaked in 37ºC water.

4.2.   Factors contributing to the inclusion and exclusion of specimens

According to the methodology, 30 specimens were initially fabricated for each group, allowing the loss of a few specimens during testing. Several pilot studies showed that the majority of specimens would reach m1 after 2 days, and m3 after 3 days. This meant that the drying out of most specimens would fit within the timeframe of one working week (Monday to Friday). Specimens that took longer than 5 working days to reach stable weight were not used for the statistical analysis. This was a convenience and security measure for the

researchers, avoiding having to access the laboratory over weekends for weighing a few specimens only.

The only groups requiring the fabrication of additional specimens were the 55ºC degree temperature group and the 10% ratio group. Too many specimens did not reach m1 and m3 within the timeframe of 5 working days. It is speculated that the reason for this was the hot summer weather and air conditioning not coping efficiently at the time. Additional specimens, again allowing for a small margin of specimen loss, were made and added to the first batch of specimens. Of these additional specimens, none were lost, resulting in a total number of specimens in excess of the planned 30 specimens per group.

The statistical analysis of the volumes among the ratio-controlled experiment and temperature-controlled experiment groups respectively, did not show significant differences, outliers or extreme values. Therefore, it is accepted that the method of fabrication produced consistent specimens. As a result, no specimens had to be excluded due to varying volumes. Similarly, extreme values, outliers or statistical differences among groups in terms of sorption and solubility cannot be attributed to differences in volume.

4.3   ISO requirements

For the industry, the material passes the ISO 1567 (1999) sorption and solubility tests, if 4 of the manufactured 5 specimens comply with the specifications for that specific category of material. For a Type-2 Class-2 denture base material, sorption and solubility limits are set at 32 and 8.0 $\mu g/mm^3$ respectively. If 3 of the 5 specimens exceed these values, the material fails. Should this be the case, a further 6 specimens have to be processed of which 5 have to pass. Because this is a research study and the data required statistical analysis, more specimens than the required 5 or 6 specimens were fabricated for each group. It is important to note that all specimens of all groups satisfied the requirements for Type-2 Class-2 denture base materials.

For Type 1, 3, 4 and 5 denture base materials, limits for sorption and solubility are set at 32 and 1.6 $\mu g/mm^3$ respectively. It is interesting to note that all the specimens of the control group of the Type 2 material tested in this study

satisfied these requirements as well. It is only when soaking temperatures are increased that solubility results exceed the limits. However, testing conditions determined soaking during a period of 7 days at a particular temperature, something that is not likely to happen in the clinical environment.

Technically, the ISO requirements can only be applied to the control group in this study, because it is the only group that is made to both ISO specifications *and* manufacturer's instructions.

The manufacturer of the denture base material used in this experiment conducted tests comparing the performance of heat-cured resin and their cold-cure denture base resin. The experiments were in accordance with the new ISO 20795 specifications (2008) for Base Polymers part 1 Denture base polymers. The results obtained from their tests fell well within the ISO specification limit for heat-cured resins, with a water sorption value of $25.5 \pm 0.3 \mu g/mm^3$ and a water solubility level of $1.3 \mu g/mm^3 \pm 0.23 \mu g/mm^3$. This manufacturer concluded that their cold-cure Type-2 product can therefore be referred to as superior as it reaches all specifications (flexural strength, flexural modulus and impact strength which were also tested) in accordance with ISO 20795 (2008), Type 1, Class 1 for heat-cured denture base polymers (Vertex Dental, 2010). Therefore testing of newer materials should be completed by independent research having no conflict of interest.

Comparing the results of this study with the product specification supplied by the manufacturer (Table 2.2.), the control group compares favourably for solubility. However, results recorded a slightly higher mean sorption value as specified by the manufacturer.

4.4   Sorption and solubility for the ratio-controlled experiment

Five different powder/liquid ratios were compared in order to determine the influence of powder/liquid ratio on sorption and solubility, of which one was the control group (0%). The control group (0%) was prepared according to manufacturer's instructions. Powder/liquid ratios were prepared with an increase of 10%, 15%, 20% and 25% in liquid.

Often the powder/liquid ratio of PMMA resins is changed to modify the handling properties of the material (Geerts & Du Rand, 2009:110-116). The theory behind increasing the liquid content is to improve the flow of the material to reproduce fine detail (Zenith Dental, 2009). During the pilot study it was observed that the 25% ratio group was already water-like in consistency, but the specimens remained visually acceptable. It was decided that further diluting the mixture with more liquid would not result in further benefits in terms of clinical handling and detail reproduction.

4.4.1. Powder/liquid ratios and sorption

For the ratio-controlled experiment, the highest mean *water sorption* value was obtained from the control group (25.13330μg/mm^3). The powder/liquid ratio used in this experimental group was per manufacturer's instructions, and these results should therefore be the norm in laboratories. According to ISO 1567 (1999), the water sorption level for Type-2, Class-2 should not exceed 32μg/mm^3, and the highest result obtained from the ratio experiment is still well below these ISO standards. Therefore, even though there are statistically significant differences among the ratio groups, water sorption for the control group and all the different powder/liquid ratios pass the ISO Standard.

The lowest mean value of sorption was for the 15% group (24.1481μg/mm^3).

By means of multiple comparisons, significant differences were identified in sorption (p<0.0001) between all the ratio-controlled experiment groups, except for the 0% - 20% groups; 10% - 15% groups; 10% - 25% groups and the 15% - 25% groups (p>0.0001). The only test group that did not differ from the control group (0%) was the 20°C group. The null hypothesis that there is no difference in *sorption* using different powder/liquid ratios is therefore partially rejected.

A few outliers and extremes are observed in Figure 3.2. The 0% group has 2 outliers (specimens 29 and 30). Looking at the raw data and Table 3.1., it can be observed that the two outliers for the 0% group actually fall on the boundary of the minimum value, which is 23μg/mm^3. The 10% mixing ratio group presents with one outlier (specimen 59). This outlier presents with a slightly smaller value than the minimum sorption value for this group.

When compared to the (0%) control group (0%), the 10%, 15% and 25% ratio-controlled experiment groups show mean sorption values significantly lower than that of the 0% group. This was expected, as the increase in powder/liquid ratio levels will have an influence on the level of water-soluble additives that can be absorbed by the specimens that are already more saturated with monomer than the 0% control group (0%). These findings are in agreement with those of Cucci et al. (1998:434-438). Auto-polymerizing resins present with higher residual monomer levels than other denture base resins. Therefore, with a high monomer to polymer ratio, residual monomer levels would be high in the polymerized resin.

Sorption and solubility are 2 different processes, involving different molecules. Monomer has a larger molecular structure than water. Observing the values of sorption and solubility, considerably more matter is "sorbed" than "solved" and it is clear that residual monomer is not simply replaced by water upon immersion.

The 15% group presented with four extremes. The reason for these extremes is that they fall on the minimum and maximum values of this ratio-controlled experiment group, and the values of the other specimens are congested around the mean sorption value of $24.14823 \mu g/mm^3$. These extremes are therefore not regarded as substandard specimens.

The 20% ratio-controlled experiment group presented with interesting results. The mean sorption value is not considered significantly different compared to the control group (0%). It can only be hypothesised that the 20% ratio group was less saturated with residual monomer than the other ratio groups, with a subsequent higher amount of water sorption taking place. The 20% group also presents with four extremes. The same reasons for these extremes apply as for the 15% ratio group, that is, they fall on the boundaries of the maximum and minimum values of this ratio group.

Properties that could be affected by residual monomer in a dental appliance include hardness, biocompatibility, tensile strength, flexural strength and water sorption (Bayraktar et al., 2006:340-345). Since sorption is not higher for specimens made with higher liquid ratios, the negative influence of the effect of water sorption on the physical properties on the material should also not be

higher compared to the control group (0%). When a dental appliance absorbs water, a decrease in the mechanical properties of the material could be observed, making this material more flexible and resilient, due to the plasticizing effect water has on the resin material (Cucci et al., 1998:434-438).

Miettinen & Vallittu (1997:531-534) suggested that high solubility of a resin material is not favourable. Bayraktar et al., (2006:340-345) reported low water sorption levels, as confirmed in this study with the 10%, 15% and 25% ratio-controlled experiment groups compared to the control group (0%). These specimens should not be considered favourable because of their higher solubility levels. It may be assumed that these specimens fabricated with higher liquid to powder ratios only presented with low mean water sorption levels due to the saturated bonds of the polymer chains. These specimens still contain unfavourable amounts of residual monomer, which, upon leaching when they are exposed to e.g. high temperatures may result in a weak resin material. These may be prone to fracture due to the number of voids filled up by water left by the previously occupied monomer. When considering biocompatibility of the material while in-service, a higher risk to toxicological reactions could therefore be expected (Bhola et al., 2010:129-136).

4.4.2. Powder/liquid ratios and solubility

The highest mean *water solubility* value obtained in the ratio-controlled experiment was from the 25% ratio-controlled experiment group (0.9320µg/mm^3). As the 25% ratio group presents with the highest initial liquid levels in relation to the other ratio groups, more unpolymerized residual monomer may be present, and higher levels of monomer may consequently be released upon immersion in water. The specification limit set by ISO 1567 (1999) is 8.0µg/mm^3 which again is substantially higher that the value obtained for the 25% ratio group.

Even though there are statistically significant differences among the ratio groups, the results obtained from this experiment indicate that water solubility for the control group and the different mixing ratios, were well within the ISO Standard 1567 (1999) specification limit of 8.0µg/mm^3 for a Class 2 Type 2 denture base material. In fact, the values are also well within the requirements

specified for heat-polymerized denture base materials. According to the ISO 1567 (1999) specification limit regarding Type 1, Class 1 heat-polymerizable denture base polymers, and water solubility levels should not exceed 1.6µg/mm^3. The highest mean water solubility level obtained from the ratio-controlled experiment is 0.9320µg/mm^3 (25% ratio group), whereas the 0% control group (0%) presented with an even lower mean solubility level of 0.61668.0µg/mm^3.

For solubility, all pairs were significantly different (p<0.0001), except for the 0% - 10% groups; 10% - 15% groups; 10% - 20% groups 15% - 20% groups and 15% - 25% groups (p>0.0001). The null hypothesis that there is no difference in *solubility* using different powder/liquid ratios is therefore partially rejected.

A few outliers are observed in Figure 3.3: the 10% ratio-controlled experiment group presents with 3 outliers. Looking at the raw data and Table 3.1, it can be observed that these outliers fall on the boundaries of the minimum and maximum values, which is 0.2µg/mm^3 and 1.32µg/mm^3 respectively. The only other ratio-controlled group presenting with an outlier is the 25% ratio group, which also indicates the maximum boundary. Compared to the control group (0%), the 10% to 25% ratio groups show mean solubility values significantly higher than that of the 0% group. The increase is incremental, from the control group (0%) up to the 25% group. This increase in solubility levels was the expected outcome for this experiment. According to Cucci et al. (1998:434-438) the high residual monomer content is related to higher solubility levels, especially for auto-polymerizing resins. This experiment also shows an inverse relationship to the mean sorption values within the ratio-controlled experiment, that is, low water sorption levels correspond to an increase in solubility levels.

Theoretically, both a low sorption and low solubility value would result in a clinically superior product. However, the inverse relationship between sorption and solubility means that this cannot be achieved clinically. Therefore, a decision needs to be made based on the consequences and impact of both properties: low sorption leading to a more durable product, or low solubility leading to a biologically more acceptable product.

Martin (2003:225-227) reported that several studies have shown that unpolymerized methyl methacrylate is a primary irritant to denture wearers,

which is localized to the immediate soft tissue area contacting the dental appliance. Martin (2003:225-227) emphasises that delayed hypersensitivity is rare and can occur in and around some patients' mouths. Allergic hypersensitivity reactions can either be immediate or delayed, and with MMA, the reaction is usually delayed (Hochman, 1997:93-96). This delayed reaction is referred to as stomatitis venenata (Hochman, 1997:93-96, citing Weaver & Goebel, 1980:138-142). From a clinical standpoint, proper powder/liquid ratios are therefore recommended in order to minimize reactions to the dental appliance, thereby minimizing possible trauma and denture discomfort, and improving patient satisfaction.

Lamb et al. (1982:155-159) reported that residual monomer present in a dental appliance will eventually leach out in the oral cavity of the denture wearer. Based on cell culture studies (Cimpan et al., 2000:59-69), it may be extrapolated that monomer has a direct cytotoxic effect on cells *in vivo*. Since toxic effects are usually time and dose dependent, lower solubility values are desired to minimize the release of harmful substances into the oral cavity, reducing cytotoxicity and allergic reactions. The results obtained from this experiment indicate that an increase in the liquid ratio produced increased solubility. A higher risk of cytotoxicity and allergic reactions could therefore be anticipated, and an accurate powder/liquid ratio as indicated by the manufacturer is therefore recommended.

4.5 The influence of water immersion temperature on sorption and solubility

The rate of dispersion between PMMA and water may be enhanced, and is also dependent on, an increase in temperature (Vallittu et al., 1995:338-342). The influence of water immersion temperature on sorption and solubility was therefore examined within this experiment. Four groups, were submerged into distilled water at different temperatures. All specimens were made according to manufacturer's instructions and the recommended powder/liquid ratio. The 37ºC group also complied with ISO specifications and thus was regarded as the control group.

The four temperature-controlled experiment groups, 37ºC; 45ºC; 55ºC and 67ºC were chosen to represent everyday intra-oral temperature changes during

the consumption of hot and cold foods and beverages. By means of multiple comparisons, significant differences were identified in sorption (p<0.0001) between all the temperature groups, except for the 45ºC - 55ºC groups (p>0.0001). For solubility, all pairs were significantly different (p<0.0001). The null hypotheses that there is no difference in sorption and solubility among denture base specimens soaked in water at different temperatures is therefore rejected.

In this experiment, the soaking period was set at seven days, as recommended by ISO Standard 1567 (1999) for testing water sorption and solubility. Clinically, the control group using a water temperature of 37ºC may represent the oral environment. However, it is unlikely that higher temperatures would persist continuously in the mouth for a period of seven days. Further research may test the effect of thermocycling during soaking on PMMA's sorption and solubility properties.

4.5.1   The influence of water immersion temperature on sorption

Immersion of the dental appliance at an elevated water temperature ultimately reduces water sorption. Bayraktar et al. (2006:340-345) recommended soaking the appliance in hot water of at least 65ºC. There seems to be a correlation between temperature and the level of sorption reduction, as higher temperatures ranging between 65ºC-70ºC seems to be associated with the highest levels in sorption reduction. Within the limitations of the present study, the results do not conform with Bayraktar et al.'s observation: It was found that a soaking temperature of 67ºC *increased* both sorption and solubility. It is assumed that for the material under investigation, 67ºC may have caused enough expansion or structural changes in the polymer network to modify the sorption and solubility behaviour compared to the lower temperatures.

Vallittu et al. (1995:338-342) used high-performance liquid chromatography to measure free monomer after storage at 22ºC and 37ºC respectively. They found that there was less free monomer after storing at the higher temperature for both heat and cold-cure resins. It is well documented that auto-polymerized resins contain higher levels of free monomer than heat-cured resins, and

consequently more free monomer may be released into the surrounding water from auto-polymerized resins.

The results obtained from this experiment indicate that for all groups, water sorption was within the ISO Standard 1567 (1999) specification limit of $32\mu g/mm^3$ maximum. A few outliers and extremes are however observed in Figure 3.5. Figure 3.5 indicates 2 outliers for the 37ºC group ($\pm23\mu g/mm^3$). Looking at the raw data and Table 3.3., it can be observed that the two outliers for the 37ºC group actually fall on the boundary of the minimum value, which was calculated by statistical software. The reason for these outliers is therefore only statistically significant as they fall well below the specification limit. The 45ºC group presented with 8 extremes. Seven of these outliers present with water sorption values ranging between $23\text{-}25\mu g/mm^3$, which is closely grouped around the mean water sorption value ($24,1851\mu g/mm^3$) calculated for the 45ºC group. The eighth extreme ($28\mu g/mm^3$) actually indicates the maximum value specimen for the 45ºC group. If these extremes are however observed within the context of this experiment, they are not considered as extremes as they too fall well within the ISO specification limits.

The two outliers present in the 55ºC group fall either on or just below the minimum value ($21\mu g/mm^3$) of this experimental group. These two specimens are only considered as outliers due to the fact that the values of the other specimens lay so close around the mean value of the 55ºC group. Both the 45ºC and 55ºC temperature-controlled experiment groups presented with mean water sorption values lower than the 37ºC control group (0%).

The latter contradicts Dhanpal et al. (2009:122-132) finding that an increase in temperature increases the rate of diffusion, and thereby the degree of water sorption, as found with the specimens in these two temperature groups. During the higher soaking temperature an additional polymerisation process occurs resulting in a lower water sorption. This may be explained by a continued polymerization process when auto-polymerizing resin is exposed to moderate higher temperatures, such as 45ºC and 55ºC. The polymer structure becomes more dense with the result that upon immersion in water, lower levels of liquid penetrate the structure, hence the lower sorption values. However, once the temperature rises even higher, such as at 67ºC, more energy in the polymer structure may cause expansion forcing higher amounts of free monomer to disperse from the resin material and simultaneously more water absorption.

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4.5.2 The influence of water immersion temperature on solubility

All pairwise comparisons of the mean solubility values showed a significant difference. Therefore, the null hypothesis that water temperature does not influence solubility is totally rejected.

A closer look at the results displayed in the box and whisker plot of sorption and solubility (Figures 3.5 and 3.6), which was obtained from this experiment, indicates that a higher soaking temperature leads to a lower sorption and a higher solubility. From these results it may therefore be assumed that a higher soaking temperature allows the monomer molecules to disperse at a more rapid rate, and in the presence of free radicals an additional polymerization reaction occurs. As a result, a closer polymer matrix is produced, making the matrix less accessible to water molecules, with a subsequent decrease in water sorption. On the other hand, the higher water solubility levels may be contributed to the fact that the higher temperatures bring about a higher percentage of the monomer content to leach out.

The results obtained from this experiment demonstrate that water solubility, for all temperature-controlled experiment groups is within the ISO Standard 1567 (1999) specification limit (8.0µg/mm^3) for type 2 denture base materials. In addition, the 37ºC and 45ºC groups are also within ISO 20795 (2008) specifications for Type 1 (heat-cured) denture base resins. One outlier and one extreme are however observed in Figure 3.6 for the 45ºC group. The outlier and extreme can be contributed to the closely grouped values of the other specimens around the mean water solubility value (1.288µg/mm^3). The solubility value of the outlier and extreme are however still well within the specification limit, and is therefore not of a concern in affecting the results of this experiment.

When compared to the 37ºC control group (0%), an incremental increase in solubility can be observed with the increase in temperature (45ºC; 55ºC; 67ºC). The outcome of the results for solubility testing was within our expectations. Figure 3.6 confirms previous studies and literature that an increase in temperature results in an increase in solubility levels. Lamb et al. (1983:80-88) suggests that, due to additional polymerization at the sites of active radicals, monomer molecules would disperse at a more rapid rate if stored at increased

temperatures, thereby increasing the rate at which free monomer and water-soluble additives are reduced.

Because the conversion of MMA to PMMA is incomplete for auto-polymerizing resins, it is recommended to store the appliance in hot water at higher temperature ranges, thus promoting the release of residual monomer and increasing the solubility of the resin material. A decrease in water sorption will be observed as well, thereby preventing the possible weakening in the properties of the dental appliance.

Previous studies have established that storage time influences the level of residual MMA in processed specimens. However, it would be highly impractical and non-profitable to soak a newly processed appliance for periods even ranging up to one week before delivery to the dentist. Proper patient education related to handling their new appliance would be recommended instead. As mentioned before, one of the aspects investigated in this study was the influence of increase in temperature on the water sorption and solubility of the resin material. Possible future studies could examine the effect storage time and storage temperature will have on sorption and solubility values.

For the water temperature experiment, the 67ºC temperature-controlled experiment group presented with both the highest mean *water sorption* value (26.4347µg/mm^3) and highest mean *water solubility* (2.7521µg/mm^3) value. This high mean water sorption value may be attributed to the higher dispersion rate of monomer occurring at an elevated temperature such as 67ºC. This accelerated monomer release is accompanied by an increase in water sorption.

Except for the 67ºC group, a generally inverse relationship exists between sorption and solubility values. This may be explained by a continued polymerization process when auto-polymerizing resin is exposed to higher temperatures. The polymer structure becomes more dense with the result that upon immersion in water, lower levels of liquid penetrate the structure, hence the lower sorption values. The higher average solubility value for the 67ºC could be due to this elevated temperature creating more energy within the resin and expansion forcing higher amounts of free monomer to disperse from the resin material. But still, both the highest water sorption and water solubility levels in the temperature-controlled experiment group is again below ISO 1567

(1999) specification limits for type-2 denture base resins. In terms of their sorption values, all the temperature groups comply with ISO 1567 (1999) requirements set for the other types of denture base resins as well (Table 1.3). In terms of solubility, temperature-controlled experiment groups 37°C and 45°C also comply with ISO 1567 (1999) requirements set for the other types of denture base resins as well (Table 1.3). It is therefore recommended from these results that soaking an appliance at moderately elevated temperatures could be advantageous as higher levels of soluble material is allowed to disperse from the resin, thus reducing the amount of residual monomer in the appliance when it is delivered to the patient.

4.6.   Study limitations and future research possibilities

4.6.1. Solution

The solution used in this experiment was distilled water. A medium such as distilled water has been reported to be a highly effective method to reduce traces of unreacted MMA and water-soluble additives within the processed dental appliance, especially prior to insertion in a patient's mouth (Vallittu et al., 1995:338-342). Therefore, by immersing the resin material in water, pre-leaching will be encouraged, thus reducing the subsequent intra-oral leaching of residual MMA and formaldehyde (Tsuchiya et al., 1994:618-624). These studies were all conducted using a water temperature of 37°C. While distilled water is effective for leaching prior to delivery, it is not known what the solubility would be in intra-oral fluids and fluids consumed by the patient wearing the appliance. It should be kept in mind that the test conditions did not simulate intra-oral conditions. The study could be repeated by thermocycling the specimens instead of keeping them at the same temperature for 7 days.

During laboratory testing procedures, the quantity of distilled water used to cover the specimens in the glass rack before placing them in the incubator was not measured as this is not specified by ISO Standard 1567. Enough water was however used to cover all specimens and to compensate for evaporation that would take place during the 7 days of placement in the incubator.

For the duration of the 7 day soaking period, the water was not stirred. In the mouth however, new saliva is produced and swallowed continuously. Baker et al. (1988:1295-1299) reports that intra-orally, a dental appliance is covered in a flow of saliva that is constantly replaced, and it could therefore be speculated that an equilibrium between the saliva and monomer content from the dental appliance is never reached. It is not known if equilibrium between the water and monomer content from the specimen groups was reached after the 7 day soaking period. If it did, solubility values after 7 days of soaking in the same water would be lower compared to soaking in water that was replaced at certain time intervals.

The water bath was covered with cling wrap (polythene) to prevent evaporation of the water in the incubator during the 7-day soaking period. The cling wrap was separated from the water by an air pocket. Therefore, monomer being volatile, and heavier than air, could evaporate from the solution into the air pocket, and establish an equilibrium here as well. In this experiment, the water bath may be defined as a system, and the area outside of the system the environment. When a system is covered and/or isolated, it is referred to as a closed system. Changes will occur in such a closed system until an equilibrium state is reached (when no net changes occur after a period of time). Within the enclosed water bath, water and monomer molecules will escape into the air pocket by absorbing energy from the environment and the vaporised monomer and water molecules will therefore eventually reach a state of equilibrium vapour pressure.

Specimens fabricated for each experimental group were all placed in the same water bath. Therefore, monomer released from specimen x may have been ad/absorbed by specimen y. For more accurate results, each specimen could have been placed in individual baths. Specimens from different experimental groups were not placed in the same bath. Further research could be done standardizing the volume of water and placing each specimen in its own bath.

Not all PMMA appliances are placed in a wet environment. Maxillofacial prostheses and obturators are placed in regions where they are not necessarily covered by saliva, but by other mucous fluids. In the case of cancer patients, being subjected to radiation therapy, the saliva production may be severely compromised. Evaluation of water sorption by acrylic resins stored in an

atmosphere of 100% relative humidity at 37ºC, instead of soaking may be considered for future research. Tsuboi et al. (2005:382-388) suggests that for water sorption testing, the acrylic resins could be stored in an atmosphere of 100% relative humidity at 37ºC, which is considered typical intra-oral conditions for these types of prostheses. The results obtained between testing samples at 100% humidity and the test conditions used in this experiment could then be compared.

The substances being lost or absorbed during water immersion were not identified. Only weight gain and loss were measured during sorption and solubility testing, proving an exchange of substances. The solubility results could be supplemented by means of in-vitro cytotoxicity testing.

4.6.2 Temperature

PMMA is considered to be very stable and almost insoluble in the oral environment (no "depolymerisation"). With regards to chemical stability, these polymers are stable to heat, and at 125ºC the polymer begins to soften, whereupon it can be moulded, as observed with thermoplastic resins. However, at elevated temperatures between 125ºC and 200ºC, polymer unzipping or depolymerisation occurs, and at about 450ºC it has been estimated that almost 90% of the polymer would have been depolymerised to form monomer. Polymer unzipping or depolymerisation is therefore simply the reversal of the polymerization process whereby polymers are converted into monomers (Anusavice et al., 1996:237-255).

Exposing the specimens to a higher water temperature or by microwave irradiation could increase the rate of diffusion of the monomer from the specimen into the water (Patil et al., 2009:293-297). According to Jorge et al. (2004:340-344), a higher degree of polymerization is obtained with microwave irradiation. The authors described that these microwaves only have an effect on the monomer molecules, with a subsequent decrease in the amount of monomer relative to the increase in the degree of polymerization. The diffusion of monomer into water is therefore not considered to be due to depolymerisation of polymer into monomer, but by a post-polymerization

process in the presence of free radicals in the polymer chains, thereby facilitating the release of residual monomer (Jorge et al., 2006:203-207).

It should be kept in mind that, with denture wearers, a reduction in the concentration of MMA from the denture (and saliva) is a temperature-dependent process (Baker et al., 1988:1295-1299). Therefore, with the consumption of hot fluids, the release of residual monomer may be induced, leading to transient periods of more residual monomer release from the dental appliance.

4.6.3. Time

Because this study was based on ISO specifications, only 1 time period (7 days) was used. Based on previous literature, it is known that the highest level of free MMA and water-soluble additives leaches from a newly processed denture appliance within the first few days (Jorge et al., 2003:190-193). The 7 days used in this study covers the period of the "first few" days of highest rate of solubility. Soaking an appliance for longer than 7 days prior to delivery to patient would probably not be realistic in clinical practice. Establishing the diffusion coefficient which gives a relation of water sorption over immersion time did not form part of this experiment and could therefore be explored further.

4.6.4 Specimens

The specimens in this experimental study were not polished, as it did not form part of the factors under testing influencing water sorption and solubility levels. It is also not required by ISO standards. Polishing the resin material does not change the chemistry of the material, but it does change the surface characteristics of the material by removing irregularities from the surface and hypothetically reducing the contact surface area between specimen and solution.

Even though water sorption will always be observed in resin materials, the degree of water sorption is increased by an increase in surface roughness. The porous structure of the resin material subjected to abrasion would therefore

reduce the wettability of the resin surface, and subsequently water sorption levels (Rahal et al., 2004:225-230).

Namen et al. (2008:239-243) explains that the contact angle at the liquid-solid boundary influences the wettability of the solid surface. They report that a reduction in the contact angle on the solid surface is observed proportional to an increase in surface roughness. Therefore, the smoother the solid surface, the higher the contact angle, and the more hydrophobic the solid surface is to liquids. Because the contact area between the solid and liquid is reduced by means of polishing, this may reduce the rate of its solubility, as found by Rahal et al. (2004:225-230).

The specimens were fabricated with a thickness of 0.5mm, as prescribed by ISO 1567 (1999). Fabricating the prescribed specimens using a pour-type resin was possible due to the fluid consistency of the material. There were no serious difficulties encountered in fabricating the prescribed specimen thickness. No modification of the specimens, as observed from mentioned literature, was therefore required. Since dentures are not of an equal thickness, the influence of difference in thickness of the specimens on water sorption and solubility could be considered for further research (Dhir et al., 2007:465-472).

The specimens were not subjected to loading. The effect of loading conditions on sorption and solubility, as would happen when a PMMA appliance is subjected to masticatory forces, can be investigated.

4.6.5 Material

This experiment was conducted using only one product representing a Type-2, Class-2 denture base resin. Results from one material should be generalized to other materials within the same category, with caution.

The material under investigation is classified as a cold-cure denture base resin. The sorption and solubility values compare well with those of heat-cure values, with result below the minimum requirements as stipulated by the ISO 1567 (1999) standards. During processing and polymerization of the pour-type material, it is not subjected to high temperatures and high pressure as heat-

cured resins are (Anusavice et al., 2003:166). McCabe and Walls (2008:110-123) describes that resins cured at elevated temperatures present with a higher molecular weight than resins cured at lower temperatures. Resins with low molecular weight also result in poor mechanical properties as well as possible adverse tissue reactions

The polymerization reaction is exothermic, i.e., heat is generated during the polymerization process, accompanied by the release of energy. This additional heating may cause expansion and increased pressure within the flask of heat polymerized resins, which may introduce stresses during polymerization, cooling off and deflasking of heat cured materials that does not happen with the pour-type cold-cure materials (Yau et al., 2002:622-629). A continuous increase in internal flask pressure was observed by the authors from clamping to curing the resin material (11 atmospheric pressure (atm) to 22 atm). As the temperature during the curing process is increased, pressure is generated within the enclosed flask, due to acrylic resin's higher coefficient of thermal expansion. The pressure within the flask only decrease upon polymerization shrinkage, and released upon deflasking (Yau et al., 2002:622-629).

The powder of cold-cure resins, consisting of the polymer, consists of a lower molecular weight chain, releasing less energy and consequently less shrinkage during polymerization. The manufacturers of the pour-type material indeed claim less polymerization shrinkage compared to heat-cured materials. Comparing shrinkage between pour-type and heat-cured denture base resins may be investigated further.

This experiment could be repeated using other brands of pour-type or cold-cure denture base resins. Even though the material used for this study is classified as a "cold-cure" resin, manufacturers instruct that the polymerization process should be carried out at 55°C for 30 minutes. Laboratories should utilize curing units properly monitoring water temperature during polymerization. The effect of modifying curing temperature and time on water sorption and solubility levels could be explored.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

Within the limitations of this study, the following conclusions and recommendations may be made:

1. For auto-polymerizing resins, it is recommended to store the appliance in water at moderately higher temperature ranges (under 67ºC), thus promoting the release of residual monomer.

2. At a moderately increased temperature (under 67ºC), water sorption is not increased, preventing a possible weakening effect of the material by water.

3. Soaking auto-polymerizing resins at higher temperatures (e.g. 67ºC) increases solubility, but also sorption of water, hence this practice is not recommended.

4. As the liquid ratio was increased there was a decrease in sorption (except for group 20%) and an increase in solubility. Therefore, an increase in fluid in the mixture does not affect the sorption and its negative consequence on the mechanical properties of the finished product, but it does affect the solubility. However, in terms of bio-compatibility, the use of the manufacturer's guidelines for powder/liquid ratios is recommended, resulting in the lowest solubility.

5. When the fluid content in the mixture is increased, it is recommended to pre-soak the polymerized product longer to optimize pre-leaching of residual monomer and other possible harmful substances.

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APPENDIX A: RAW DATA

37ºC Temperature-control experiment group (n=30)

Specimen number	M1 in µg	M2 in µg	M3 in µg	Volume in mm^3	M2-M3	sorption in $µg/mm^3$	M1-M3	solubility in $µg/mm^3$
1	1203510	1229370	1202960	1035.06	26410	26	550	0.5
2	1193540	1219420	1192910	1013.44	26510	26	630	0.6
3	1168330	1193870	1167890	1001.81	25980	26	440	0.4
4	1211430	1238280	1211170	1047.55	27110	26	260	0.2
5	1272120	1300060	1271680	1098.88	28380	26	440	0.4
6	1149690	1174090	1149190	959.72	24900	26	500	0.5
7	1155710	1181170	1155050	1004.57	26120	26	660	0.7
8	1162520	1187350	1161600	1008.05	25750	26	920	0.9
9	1189720	1215340	1189140	1023.69	26200	26	580	0.6
10	1151570	1176640	1151080	986.86	25560	26	490	0.5
11	1246300	1272880	1245590	1098.26	27290	25	710	0.6
12	1223820	1250180	1223240	1062.92	26940	25	580	0.5
13	1197240	1223000	1196700	1040.13	26300	25	540	0.5
14	1150980	1174520	1150260	980.78	24260	25	720	0.7
15	1193490	1217480	1192680	1028.81	24800	24	810	0.8
16	1248120	1275190	1247610	1043.32	27580	26	510	0.5
17	1160510	1185180	1159930	992.43	25250	25	580	0.6
18	1211770	1237660	1211400	1034.59	26260	25	370	0.4
19	1214760	1240570	1214290	1036.74	26280	25	470	0.5
20	1154540	1178920	1153950	995.47	24970	25	590	0.6
21	1150590	1174270	1149820	992.58	24450	25	770	0.8
22	1157870	1181610	1157150	985.28	24460	25	720	0.7
23	1200180	1225150	1199530	1028.42	25620	25	650	0.6
24	1215860	1241290	1215030	1061.80	26260	25	830	0.8
25	1211530	1236210	1210730	1048.28	25480	24	800	0.8
26	1242690	1268480	1241950	1072.82	26530	25	740	0.7
27	1203230	1228060	1202720	1021.54	25340	25	510	0.5
28	1150660	1173750	1149910	995.41	23840	24	750	0.8
29	1128410	1150640	1127530	987.91	23110	23	880	0.9
30	1197700	1221240	1196810	1044.06	24430	23	890	0.9

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45ºC Temperature-controlled experiment (n=30)

Specimen number	M1 in µg	M2 in µg	M3 in µg	Volume in mm³	M2-M3	sorption in µg/mm³	M1-M3	solubility in µg/mm³
31	1.18423	1207520	1182230	1019.94	25290	25	2000	2.0
32	1.23888	1263260	1236920	1069.06	26340	25	1960	1.8
33	1.18334	1205860	1181050	1011.24	24810	25	2290	2.3
34	1.23230	1256910	1231170	1043.18	25740	25	1130	1.1
35	1.15063	1173270	1149410	992.12	23860	24	1220	1.2
36	1.15799	1180680	1155920	1005.50	24760	25	2070	2.1
37	1.19340	1217440	1192280	1035.04	25160	24	1120	1.1
38	1.13407	1155560	1132430	989.88	23130	23	1640	1.7
39	1.14200	1163950	1140640	976.76	23310	24	1360	1.4
40	1.16645	1188900	1164960	1025.17	23940	23	1490	1.5
41	1.21061	1234790	1209460	1042.92	25330	24	1150	1.1
42	1.23774	1261590	1236480	1064.45	25110	24	1260	1.2
43	1.15582	1178510	1154730	983.60	23780	24	1090	1.1
44	1.23837	1262820	1237160	1077.33	25660	24	1210	1.1
45	1.16458	1187840	1163430	1021.47	24410	24	1150	1.1
46	1.20360	1228090	1202450	1073.54	25640	24	1150	1.1
47	1.19104	1214630	1189910	1035.93	24720	24	1130	1.1
48	1.18457	1207680	1183170	1023.65	24510	24	1400	1.4
49	1.19571	1219300	1194390	1024.90	24910	24	1320	1.3
50	1.25281	1277780	1251480	1077.63	26300	24	1330	1.2
51	1.14416	1166870	1142960	974.12	23910	25	1200	1.2
52	1.18173	1205750	1180860	884.93	24890	28	870	1.0
53	1.23866	1262620	1237740	1074.22	24880	23	920	0.9
54	1.15041	1172690	1149100	987.95	23590	24	1310	1.3
55	1.12590	1148170	1124560	960.11	23610	25	1340	1.4
56	1.15450	1177100	1153280	1007.09	23820	24	1220	1.2
57	1.17019	1192820	1168850	1010.38	23970	24	1340	1.3
58	1.16094	1183700	1159910	1002.45	23790	24	1030	1.0
59	1.18884	1211740	1187660	1019.99	24080	24	1180	1.2
60	1.17130	1193290	1169960	1005.25	23330	23	1340	1.3

55⁰C Temperature-controlled experiment (n=35)

Specimen number	M1 in µg	M2 in µg	M3 in µg	Volume in mm^3	M2-M3	sorption in µg/mm^3	M1-M3	solubility in µg/mm^3
61	1176480	1200280	1174600	1014.97	25680	25	1880	1.9
62	1163700	1187260	1161800	1011.46	25460	25	1900	1.9
63	1164710	1188600	1162800	1014.22	25800	25	1910	1.9
64	1261630	1286880	1259500	1076.94	27380	25	2130	2.0
65	1195950	1219690	1193800	1037.44	25890	25	2150	2.1
66	1145600	1167800	1143100	1001.39	24700	25	2500	2.5
67	1160250	1183280	1158600	1014.68	24680	24	1650	1.6
68	1207870	1232100	1206000	1046.03	26100	25	1870	1.8
69	1243570	1268530	1241500	1079.90	27030	25	2070	1.9
70	1165960	1189570	1164200	1012.19	25370	25	1760	1.7
71	1154850	1177980	1152900	1000.82	25080	25	1950	1.9
72	1218430	1242800	1216600	1101.27	26200	24	1830	1.7
73	1188530	1212430	1186600	1091.22	25830	24	1930	1.8
74	1140800	1163820	1139100	1046.12	24720	24	1700	1.6
75	1219340	1243810	1217600	1110.67	26210	24	1740	1.6
76	1251470	1276660	1249700	1120.18	26960	24	1770	1.6
77	1237450	1262470	1235700	1121.97	26770	24	1750	1.6
78	1229420	1253980	1227600	1066.08	26380	25	1820	1.7
79	1192310	1215830	1190300	1042.70	25530	24	2010	1.9
80	1256350	1281270	1254500	1091.39	26770	25	1850	1.7
81	1256020	1281000	1253900	1098.87	27100	25	2120	1.9
82	1228580	1253050	1226700	1079.23	26350	24	1880	1.7
83	1206570	1230150	1204500	1048.57	25650	24	2070	2.0
84	1213490	1237500	1211500	1048.02	26000	25	1990	1.9
85	1232320	1256870	1230500	1068.96	26370	25	1820	1.7
250	1107740	1129900	1105970	990.43	23930	24	1770	1.8
251	1155090	1178140	1153220	1059.56	24920	24	1870	1.8
252	1179380	1202450	1177530	1031.13	24920	24	1850	1.8
253	1138510	1160840	1136720	1019.49	24120	24	1790	1.8
254	1108400	1130020	1106640	995.36	23380	23	1760	1.8
255	1185800	1185800	1209170	1183920	25250	25	1880	1.9
256	1150270	1150270	1172480	1148610	23870	22	1660	1.6
257	1229250	1229250	1252810	1227210	25600	25	2040	2.0
258	1114810	1114810	1135910	1112720	23190	21	2090	1.9
259	1167400	1167400	1189640	1165410	24230	24	1990	2.0

67ºC Temperature-controlled experiment (n=25)

Specimen number	M1 in µg	M2 in µg	M3 in µg	Volume in mm³	M2-M3	sorption in µg/mm³	M1-M3	solubility in µg/mm³
86	1193650	1217480	1190200	1034.84	27280	26	3450	3.3
87	1154870	1179050	1152300	991.32	26750	27	2570	2.6
88	1162070	1185730	1159000	1002.09	26730	27	3070	3.1
89	1263920	1290410	1261000	1103.47	29410	27	2920	2.6
90	1164220	1188200	1161200	1002.43	27000	27	3020	3.0
91	1220130	1245150	1216700	1067.08	28450	27	3430	3.2
92	1225330	1251320	1222900	1073.78	28420	26	2430	2.3
93	1151830	1175370	1149000	994.37	26370	27	2830	2.8
94	1149640	1173570	1147200	994.99	26370	27	2440	2.5
95	1143570	1166760	1140900	987.00	25860	26	2670	2.7
96	1234180	1259380	1231400	1066.81	27980	26	2780	2.6
97	1191010	1215360	1188300	1038.23	27060	26	2710	2.6
98	1164960	1188720	1162300	1005.15	26420	26	2660	2.6
99	1151460	1174830	1148700	997.75	26130	26	2760	2.8
100	1222230	1247840	1219800	1051.60	28040	27	2430	2.3
101	1205230	1230170	1202700	1042.32	27470	26	2530	2.4
102	1183510	1207670	1180900	1009.24	26770	27	2610	2.6
103	1156850	1180480	1154100	999.65	26380	26	2750	2.8
104	1156890	1179900	1153600	1012.47	26300	26	3290	3.2
105	1253610	1279080	1250700	1089.12	28380	26	2910	2.7
106	1201750	1226440	1199100	1029.11	27340	27	2650	2.6
107	1200730	1224530	1197600	1033.81	26930	26	3130	3.0
108	1231820	1256950	1229200	1032.13	27750	27	2620	2.5
109	1242560	1266970	1239100	1080.57	27870	26	3460	3.2
110	1159820	1182460	1156700	993.35	25760	26	3120	3.1

10% Ratio-controlled experiment (n=32)

Specimen number	M1 in µg	M2 in µg	M3 in µg	Volume in mm^3	M2-M3	sorption in µg/mm^3	M1-M3	solubility in µg/mm^3
111	1265900	1292770	1265160	1085.102022	27610	25	740	0.7
112	1160500	1184700	1159670	1004.407585	25030	25	830	0.8
113	1196400	1221100	1195630	1034.673537	25470	25	770	0.7
114	1214300	1239390	1213340	1052.174432	26050	25	960	0.9
115	1215900	1241280	1215090	1061.712099	26190	25	810	0.8
116	1205200	1230140	1204430	1044.44354	25710	25	770	0.7
117	1251400	1277230	1250360	1102.157793	26870	24	1040	0.9
118	1166900	1191180	1166120	1009.434988	25060	25	780	0.8
119	1156100	1180360	1155330	1006.817836	25030	25	770	0.8
120	1116400	1138820	1115250	976.6442918	23570	24	1150	1.2
121	1151900	1175750	1151470	1000.144834	24280	24	430	0.4
122	1141100	1164470	1140610	991.1612584	23860	24	490	0.5
123	1164900	1188790	1163980	1002.340477	24810	25	920	0.9
124	1155400	1179260	1154480	1009.207587	24780	25	920	0.9
125	1132500	1156260	1131730	987.4864817	24530	25	770	0.8
126	1181500	1206410	1180640	1032.725564	25770	25	860	0.8
127	1199400	1223950	1198570	1047.062822	25380	24	830	0.8
128	1071600	1093690	1070680	952.3940041	23010	24	920	1.0
129	1207000	1231620	1206350	1044.269212	25270	24	650	0.6
130	1195200	1219920	1194600	1028.114245	25320	25	600	0.6
131	1135600	1158010	1134800	983.6704373	23210	24	800	0.8
132	1168400	1191550	1167530	1010.520896	24020	24	870	0.9
133	1230200	1254540	1229080	1063.694345	25460	24	1120	1.1
134	1195900	1219670	1195100	1031.519271	24570	24	800	0.8
135	1198500	1222530	1197760	1036.038888	24770	24	740	0.7
136	1158100	1181140	1157460	999.4719015	23680	24	640	0.6
137	1157500	1180370	1156850	998.2189698	23520	24	650	0.7
138	1203300	1227470	1202570	1079.204675	24900	23	730	0.7
139	1141200	1163410	1141010	1021.789221	22400	22	190	0.2
140	1154900	1177840	1153940	993.9130527	23900	24	960	1.0
141	1232100	1256640	1230680	1064.911284	25960	24	1420	1.3
142	1171500	1194660	1171270	1009.803173	23390	23	230	0.2

15% Ratio-controlled experiment (n=30)

Specimen number	M1 in µg	M2 in µg	M3 in µg	Volume in mm^3	M2-M3	sorption in µg/mm^3	M1-M3	solubility in µg/mm^3
143	1095700	1118380	1094830	966.2482642	23550	24	870	0.9
144	1164700	1189190	1163940	1025.57383	25250	25	760	0.7
145	1125300	1148470	1124340	997.1248051	24130	24	960	1.0
146	1158000	1182100	1157030	1006.947775	25070	25	970	1.0
147	1134900	1157810	1133820	993.1585919	23990	24	1080	1.1
148	1221000	1245850	1219940	1067.837553	25910	24	1060	1.0
149	1144900	1168300	1144070	1000.41612	24230	24	830	0.8
150	1155500	1178910	1154450	996.271661	24460	25	1050	1.1
151	1213600	1238310	1212650	1060.469711	25660	24	950	0.9
152	1187000	1211090	1185980	1030.608014	25110	24	1020	1.0
153	1187200	1211450	1186530	1022.509866	24920	24	670	0.7
154	1141400	1164660	1140650	992.8750876	24010	24	750	0.8
155	1156100	1179920	1155230	1006.158708	24690	25	870	0.9
156	1204800	1229230	1203880	1063.498276	25350	24	920	0.9
157	1190900	1214870	1189930	1037.978389	24940	24	970	0.9
158	1229900	1254770	1228880	1073.634111	25890	24	1020	1.0
159	1157900	1181750	1157150	1021.91166	24600	24	750	0.7
160	1160500	1184170	1159720	1015.510412	24450	24	780	0.8
161	1226400	1251700	1225630	1066.733619	26070	24	770	0.7
162	1143400	1167060	1142790	999.598636	24270	24	610	0.6
163	1129800	1152850	1129100	1003.057298	23750	24	700	0.7
164	1174120	1198420	1173410	1018.077883	25010	25	710	0.7
165	1215400	1240360	1214610	1062.730349	25750	24	790	0.7
166	1145000	1168400	1144280	991.7899869	24120	24	720	0.7
167	1114300	1137120	1113590	977.7507538	23530	24	710	0.7
168	1153100	1176580	1152590	1001.385129	23990	24	510	0.5
169	1182680	1206430	1181640	1032.968059	24790	24	1040	1.0
170	1162620	1186120	1161850	1008.824477	24270	24	770	0.8
171	1233070	1257820	1232260	1069.110094	25560	24	810	0.8
172	1135330	1157740	1134520	1014.740267	23220	23	810	0.8

20% Ratio-controlled experiment (n=25)

Specimen number	M1 in µg	M2 in µg	M3 in µg	Volume in mm³	M2-M3	sorption in µg/mm³	M1-M3	solubility in µg/mm³
174	1106680	1129930	1105720	967.5483762	24210	25	960	1.0
175	1172300	1196730	1171500	1010.434997	25230	25	800	0.8
176	1181250	1206000	1180480	1012.375098	25520	25	770	0.8
177	1129930	1153630	1129090	971.1630358	24540	25	840	0.9
178	1150550	1174880	1149850	991.4870674	25030	25	700	0.7
179	1172520	1196990	1171780	1012.620877	25210	25	740	0.7
180	1208260	1233920	1207490	1051.400672	26430	25	770	0.7
181	1134470	1158790	1133840	974.9496198	24950	26	630	0.6
182	1139200	1163100	1138450	992.1915516	24650	25	750	0.8
183	1134200	1157720	1133420	983.8728724	24300	25	780	0.8
184	1169520	1194060	1168650	1004.686913	25410	25	870	0.9
185	1205230	1230480	1204490	1041.038334	25990	25	740	0.7
186	1121470	1144900	1120720	977.6558107	24180	25	750	0.8
187	1202060	1226590	1201060	1039.944749	25530	25	1000	1.0
188	1241500	1268310	1240880	1060.168079	27430	26	620	0.6
189	1147760	1171510	1147090	999.1651077	24420	24	670	0.7
190	1144630	1168500	1143720	977.8835688	24780	25	910	0.9
191	1164310	1188890	1163550	1004.099596	25340	25	760	0.8
192	1185260	1209930	1184500	1025.008428	25430	25	760	0.7
193	1132880	1156110	1132120	996.6087167	23990	24	760	0.8
194	1194170	1218450	1192970	1031.670089	25480	25	1200	1.2
195	1226590	1251610	1225440	1059.057348	26170	25	1150	1.1
196	1183570	1208060	1182730	1025.684324	25330	25	840	0.8
197	1220110	1245370	1219130	1062.8548	26240	25	980	0.9
198	1179610	1203670	1178650	1025.264708	25020	24	960	0.9

25% Ratio-controlled experiment (n=25)

Specimen number	M1 in µg	M2 in µg	M3 in µg	Volume in mm^3	M2-M3	sorption in µg/mm^3	M1-M3	solubility in µg/mm^3
199	1119620	1142690	1118800	963.7914903	23890	25	820	0.9
200	1127000	1150420	1126040	976.7807095	24380	25	960	1.0
201	1116510	1139300	1115380	969.1057324	23920	25	1130	1.2
202	1196740	1221370	1195780	1028.440872	25590	25	960	0.9
203	1188440	1212880	1187620	1016.616099	25260	25	820	0.8
204	1198460	1223920	1197650	1057.033098	26270	25	810	0.8
205	1091620	1113900	1090660	952.9422981	23240	24	960	1.0
206	1161740	1185500	1160540	1005.488192	24960	25	1200	1.2
207	1163980	1188350	1162990	1015.521845	25360	25	990	1.0
208	1180260	1204600	1179170	1021.579124	25430	25	1090	1.1
209	1185460	1208840	1184500	1037.192607	24340	23	960	0.9
210	1125680	1147890	1124660	961.1855656	23230	24	1020	1.1
211	1155470	1178120	1154520	996.2130796	23600	24	950	1.0
212	1161290	1182970	1160020	995.3602139	22950	23	1270	1.3
213	1119640	1141000	1118700	961.9846742	22300	23	940	1.0
214	1167390	1191530	1166480	1014.801681	25050	25	910	0.9
215	1188620	1212760	1187770	1022.929093	24990	24	850	0.8
216	1161400	1184940	1160540	1012.539478	24400	24	860	0.8
217	1176920	1200340	1176080	1005.525346	24260	24	840	0.8
218	1134610	1156970	1133930	977.8229239	23040	24	680	0.7
219	1112700	1135050	1111970	984.4449651	23080	23	730	0.7
220	1166410	1190140	1165540	1012.056319	24600	24	870	0.9
221	1208210	1233160	1207390	1037.517034	25770	25	820	0.8
222	1219500	1244010	1218750	1049.095762	25260	24	750	0.7
223	1204360	1227740	1203290	1031.155162	24450	24	1070	1.0

Ratio-controlled experiment - Multiple Comparisons							

Bonferroni

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95%Confidence Interval	
						Lower Bound	Upper Bound
Volume in mm3	0.00%	10.00%	.4592066	8.0379626	1.000	-22.483225	23.401638
		15.00%	3.7884138	8.3903615	1.000	-20.159856	27.736683
		20.00%	12.4192771	8.5651795	1.000	-12.027968	36.866523
		25.00%	20.0877321	8.5651795	.205	-4.359513	44.534978
	10.00%	0.00%	-.4592066	8.0379626	1.000	-23.401638	22.483225
		15.00%	3.3292072	8.2652290	1.000	-20.261902	26.920316
		20.00%	11.9600705	8.4426384	1.000	-12.137411	36.057552
		25.00%	19.6285255	8.4426384	.216	-4.468956	43.726007
	15.00%	0.00%	-3.7884138	8.3903615	1.000	-27.736683	20.159856
		10.00%	-3.3292072	8.2652290	1.000	-26.920316	20.261902
		20.00%	8.6308633	8.7788078	1.000	-16.426133	33.687860
		25.00%	16.2993183	8.7788078	.656	-8.757678	41.356315
	20.00%	0.00%	-12.4192771	8.5651795	1.000	-36.866523	12.027968
		10.00%	-11.9600705	8.4426384	1.000	-36.057552	12.137411
		15.00%	-8.6308633	8.7788078	1.000	-33.687860	16.426133
		25.00%	7.6684550	8.9460382	1.000	-17.865860	33.202770
	25.00%	0.00%	-20.0877321	8.5651795	.205	-44.534978	4.359513
		10.00%	-19.6285255	8.4426384	.216	-43.726007	4.468956
		15.00%	-16.2993183	8.7788078	.656	-41.356315	8.757678
		20.00%	-7.6684550	8.9460382	1.000	-33.202770	17.865860
sorption in µg/mm3	0.00%	10.00%	.8520833*	.1719451	.000	.361307	1.342859
		15.00%	.9851852*	.1794834	.000	.472893	1.497478
		20.00%	.1733333	.1832231	1.000	-.349633	.696300
		25.00%	.8533333*	.1832231	.000	.330367	1.376300
	10.00%	0.00%	-.8520833*	.1719451	.000	-1.342859	-.361307
		15.00%	.1331019	.1768067	1.000	-.371550	.637754
		20.00%	-.6787500*	.1806017	.003	-1.194234	-.163266
		25.00%	.0012500	.1806017	1.000	-.514234	.516734
	15.00%	0.00%	-.9851852*	.1794834	.000	-1.497478	-.472893
		10.00%	-.1331019	.1768067	1.000	-.637754	.371550
		20.00%	-.8118519*	.1877929	.000	-1.347862	-.275842
		25.00%	-.1318519	.1877929	1.000	-.667862	.404158
	20.00%	0.00%	-.1733333	.1832231	1.000	-.696300	.349633
		10.00%	.6787500*	.1806017	.003	.163266	1.194234
		15.00%	.8118519*	.1877929	.000	.275842	1.347862
		25.00%	.6800000*	.1913703	.005	.133780	1.226220
	25.00%	0.00%	-.8533333*	.1832231	.000	-1.376300	-.330367
		10.00%	-.0012500	.1806017	1.000	-.516734	.514234
		15.00%	.1318519	.1877929	1.000	-.404158	.667862
		20.00%	-.6800000*	.1913703	.005	-1.226220	-.133780
solubility in µg/mm3	0.00%	10.00%	-.1520833*	.0460158	.012	-.283424	-.020742

		15.00%	-.2203704*	.0480332	.000	-.357470	-.083271
		20.00%	-.2073333*	.0490340	.000	-.347289	-.067377
		25.00%	-.3153333*	.0490340	.000	-.455289	-.175377
	10.00%	0.00%	.1520833*	.0460158	.012	.020742	.283424
		15.00%	-.0682870	.0473169	1.000	-.203342	.066768
		20.00%	-.0552500	.0483325	1.000	-.193204	.082704
		25.00%	-.1632500*	.0483325	.010	-.301204	-.025296
	15.00%	0.00%	.2203704*	.0480332	.000	.083271	.357470
		10.00%	.0682870	.0473169	1.000	-.066768	.203342
		20.00%	.0130370	.0502570	1.000	-.130410	.156484
		25.00%	-.0949630	.0502570	.610	-.238410	.048484
	20.00%	0.00%	.2073333*	.0490340	.000	.067377	.347289
		10.00%	.0552500	.0483325	1.000	-.082704	.193204
		15.00%	-.0130370	.0502570	1.000	-.156484	.130410
		25.00%	-.1080000	.0512144	.368	-.254179	.038179
	25.00%	0.00%	.3153333*	.0490340	.000	.175377	.455289
		10.00%	.1632500*	.0483325	.010	.025296	.301204
		15.00%	.0949630	.0502570	.610	-.048484	.238410
		20.00%	.1080000	.0512144	.368	-.038179	.254179

*. The mean difference is significant at the 0.05 level.

Temperature-controlled experiment							
Multiple Comparisons							
Bonferroni							
Dependent Variable	**(I) Group**	**(J) Group**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95%Confidence**	**Interval**
						Lower Bound	**Upper Bound**
Volume in mm3	37(0%)	45	11.2811852	9.5605175	1.000	-14.433361	36.995731
		55	-18.1712619	9.4702368	.347	-43.642984	7.300460
		67	-2.7142899	9.9884908	1.000	-29.579939	24.151359
	45	37	-11.2811852	9.5605175	1.000	-36.995731	14.433361
		55	-29.4524471*	9.7209102	.019	-55.598395	-3.306499
		67	-13.9954750	10.2264685	1.000	-41.501203	13.510253
	55	37	18.1712619	9.4702368	.347	-7.300460	43.642984
		45	29.4524471*	9.7209102	.019	3.306499	55.598395
		67	15.4569720	10.1421175	.783	-11.821881	42.735825
	67	37	2.7142899	9.9884908	1.000	-24.151359	29.579939
		45	13.9954750	10.2264685	1.000	-13.510253	41.501203
		55	-15.4569720	10.1421175	.783	-42.735825	11.821881
sorption in µg/mm3	37(0%)	45	.9481481*	.2281192	.000	.334585	1.561711
		55	.8476190*	.2259650	.002	.239850	1.455388
		67	-1.3014493*	.2383308	.000	-1.942478	-.660420
	45	37	-.9481481*	.2281192	.000	-1.561711	-.334585
		55	-.1005291	.2319462	1.000	-.724386	.523327
		67	-2.2495974*	.2440091	.000	-2.905899	-1.593296
	55	37	-.8476190*	.2259650	.002	-1.455388	-.239850
		45	.1005291	.2319462	1.000	-.523327	.724386
		67	-2.1490683*	.2419964	.000	-2.799957	-1.498180
	67	37	1.3014493*	.2383308	.000	.660420	1.942478
		45	2.2495974*	.2440091	.000	1.593296	2.905899
		55	2.1490683*	.2419964	.000	1.498180	2.799957
solubility in µg/mm3	37(0%)	45	-.6722222*	.0624991	.000	-.840324	-.504121
		55	-1.2047619*	.0619090	.000	-1.371276	-1.038248
		67	-2.1355072*	.0652969	.000	-2.311134	-1.959881
	45	37	.6722222*	.0624991	.000	.504121	.840324
		55	-.5325397*	.0635477	.000	-.703461	-.361618
		67	-1.4632850*	.0668526	.000	-1.643096	-1.283474
	55	37	1.2047619*	.0619090	.000	1.038248	1.371276
		45	.5325397*	.0635477	.000	.361618	.703461
		67	-.9307453*	.0663012	.000	-1.109073	-.752418
	67	37	2.1355072*	.0652969	.000	1.959881	2.311134
		45	1.4632850*	.0668526	.000	1.283474	1.643096
		55	.9307453*	.0663012	.000	.752418	1.109073
*. The mean difference is significant at the 0.05 level.							