In vitro analysis of aligner leachates

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ABSTRACT

Objective: To expose clear aligners from different companies to accelerated aging environments (ethanol and dichloromethane) and water to detect release of potentially hazardous byproducts that may be causing adverse events in patients.

Materials and Methods: Aligners from four different companies (Invisalign, Clear Correct, Smart Moves, and Durasoft) were: cut into fragments, weighed on an analytic balance (M1), and placed in separate solvents (dichloromethane, 99.5% ethanol, and water) in quadruplicate. M2 (mass directly after removal from solvent), and M3 (equalized mass after desiccation), were measured to calculate water sorption (WS) and solubility (SL), and leachates were analyzed via Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy for: Invisalign at days 3, 7, and 14; Smart Moves, Clear Correct, and Durasoft at day 14.

Results: Aligner materials took up water (sorption tests) but were essentially insoluble in ethanol and water (solubility tests). The lack of solubility was possibly due to the inability to desiccate samples completely, because ^{1}H-NMR spectra showed potential monomer and oligomers were released from these materials, especially in accelerated aging environments.

Conclusions: Leachates were produced from the aligners, however, at this time, it is unknown if these are causal to patients' adverse reactions. Aligners will most likely remain prevalent as a modality to treat our patients' malocclusions, so it is pertinent to understand potential hazardous effects.

INTRODUCTION

Kesling introduced the first clear aligner therapy in the 1940s.[1] Though, at the time it did not gain much popularity, this has changed with the development of dental materials and three-dimensional digital technologies. Now, orthodontists and general dentists elect to treat a variety of moderate to severe malocclusions with clear aligners.[2]

Aligner Usage

Few clinical studies have been published that adequately assess the effectiveness of treatment with clear aligners.[3] However, a recent study showed promise in that both clear aligners (Invisalign by Align Technology Santa Clara, Calif.) and fixed orthodontic appliances were able to improve malocclusions. Initial severity of malocclusion was matched for 48 aligner patients and 48 fixed appliance patients. The average pretreatment peer assessment rating (PAR) scores were 20.81 for the aligners and 22.79 for fixed appliances and the post treatment PAR scores were not statistically different between the two groups with both scoring less than 5 (P =0.7420).The PAR is an occlusal index that not only measures how much a patient deviates from ideal occlusion but also quantitatively evaluates orthodontic treatment outcomes by comparing pretreatment and posttreatment casts.[4] It is thought that at least a 30% reduction in PAR score is required for a case to be considered improved[5] and the cutoff point for treatment need is 17.[6]

Many advantages have been claimed of orthodontic treatment with removable aligners over fixed orthodontic appliances. Fixed orthodontic appliances include the traditional bracket and wire system to idealize tooth positions. In contrast, with clear aligner therapy, set of clear aligner trays are constructed from a series of dental models that are customized for serial incremental tooth movements from initial to ideal tooth positions for a given patient. The construction is done by heating a thermoplastic material and then using a vacuum (“vacuum-formed”) to suck it over the dental model. Thermoplastic is a term denoting a substance (like synthetic resins) that becomes plastic on heating and rigid on cooling. The models could be made from stone or from a scan through computer-aided design/computer aided manufacturing. The patients are instructed to wear each aligner in a custom sequence for about 22 hours per day for 1-2 weeks (dictated by the provider) before changing to the next aligner in a sequence designed to achieve gradual tooth movement.[7,8] Furthermore, some clinicians use “accelerating devices” to attempt to decrease the length of treatment and therefore prescribe a faster aligner change rate (~ 5 days).[9] Some would say the greatest advantage of clear aligner over conventional fixed appliance therapy is improved esthetics[10], comfort[11,12], and possibly oral hygiene[13-16] for the patient. However, as the popularity of clear aligner therapy has increased a number of adverse events have been reported.

Adverse Events

Adverse reactions to orthodontic materials due to the release of constituent substances from appliances are not novel events, and furthermore, intraoral ageing has the ability to affect the biologic properties of materials.[17] The amount of substances released are usually too low to cause any overt systemic toxic effects but even small amounts may lead to clinical manifestations of allergic contact dermatitis and urticaria[18]

Awosika et al. (2017) published a case report of a female adult patient who developed urticaria on extremities and flanks after 2 days of wearing clear aligners with subsequent development of facial and periorbital swelling. This patient was treated with oral prednisone and removal of the aligners, which lead to the resolution of her symptoms.[19]

Allareddy et al. (2017) searched the Manufacturer and User Facility Device Experience (MAUDE) database, which houses all medical device reports (MDRs) that are reported to the United States Food and Drug Administration (FDA) from November 1, 2006 to November 30, 2016. From November 1, 2006 to December 31, 2010, only 6 MDRs were reported. During the remaining 6-year span, 167 MDRs were reported, which demonstrates the marked increase in the number of reports in more recent years.

The most frequently reported adverse event (number of MDRs) was difficulty breathing (56), followed by sore throat (35), swollen throat (31), hives and itchiness (31), anaphylaxis (30), swollen lips (27), feeling of throat closing or airway obstruction (24), chest pain (19), cough (19), nausea (18), difficulty swallowing (12), dry mouth (11), headaches (10), swelling of eyes (9), blisters or ulcerations (6) and swelling of gums (5). In all of these cases no further evaluation were completed because the product performed to specifications and was used according to the labeled indications. Additionally, there was no conclusive evidence that a true causal relationship existed between use of the clear aligners and the side effects exemplified by the MDRs.[20]

Another article also reviewed the FDA's MAUDE database but from October 1, 2010 to September 30, 2015.[21] The search of the same database overlaps the time frame in the aforementioned article and further confirms the findings of adverse events from aligners. Of the 175 cases reported, 129 (73.71%) instances were mandatory reports filed by the manufacturer. Of all adverse event reports, 32 (18.29%) cases had been diagnosed with an allergic reaction, 20 (11.43%) with anaphylaxis, and 4 (2.29%) with angioedema.

Lesions involving tongue, throat, and lip were commonly reported and attributed to soreness, inflammation, and hives. In addition, 12 cases (6.86%) of nausea were reported, 11 (6.29%) of gastrointestinal issues (upset stomach, diarrhea, and vomiting), 13 (7.43%) of neuromuscular issues (muscle cramps, spasm, and pain), 13 (7.43%) of cough, 10 (5.71%) of persistent headache, 3 (1.71%) of fever, and 12 (6.86%) of cardiac-related issues.[21]

A possible reason for an increase in reports after 2010 may be due to the FDA's involvement. On November 17, 2010, the FDA issued a warning letter to Align Technology based on an inspection conducted in August of 2010 for failing to report serious adverse events associated with their clear aligner system (Invisalign).[22] However, Align technology took action and a closeout letter issued by the FDA dated July 2015 indicated that the violation had been addressed.[23]

Aligner Materials

Limited investigation of these aligners and their effects on intraoral and general health has been conducted. Multiple aligner companies exist and each use proprietary materials to construct their thermoplastic aligners. For example, some aligners (i.e. Invisalign and Clear Correct) are composed of thermoplastic polyurethanes (TPU; Figure 1), which require isocyanate for synthesis. The health effects of isocyanate have been well documented in the literature, including increased risk of asthma and contact dermatitis.[24-27] There has been usage of thermoplastic polyurethanes in other forms in the medical field and the degradation of these polyurethanes remains of concern. In a 2014 study, the deterioration of thermoplastic polyurethanes induced by UV radiation or thermal load was analyzed, but the material was not exposed to biologic fluid.[28] Another study exposed a segmented of polyetherurethane that would be used in blood-contact devices such as catheters to proteolytic enzymes for 1-6 months at 37° C and then analyzed the biodegradation by the enzymes via gel permeation chromatography and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) analysis. This study found that proteolytic enzyme are able to degrade polyetherurethanes.[29] Other aligners can be made of polyester polyethylene glycol terephthalate (PET-G) whose precursor molecule chemical structure (Figure 2).[30]

Specifically, Invisalign is composed of a polyurethane with added methylene diphenyl diisocyanate and 1,6 hexanediol.[33] Clear Correct is made by Bay Materials from Zendura a proprietary formulation of medical-grade polyurethane.[34] Clear Correct providers have access to the Aligner material information through “ClearComm”. This indictates that the polymer is a 1,6-hexanediol polymer with 1,1’-methylenebis(isocyanatobenzene).[35] Clear Correct most likely uses the newest generation Zendura FLX that has three distinct layers: Inner shell for “grip”, the outer shell provides a barrier against grinding, and the center area provides “elastic rebound”.[36] Smart Move aligners are made from a polypropolene/polyethylene copolymer-based material (Invisacryl Ultra, Great Lakes Orthodontics, Towanda, NY). The Material Safety Data Sheet (MSDS) states that the polymer is proprietary.[37] Durasoft is composed of a 0.7 mm “hard” polyethyleneterephthalat-glycol copolyester (PET-G) portion and 0.5 mm “soft” thermoplastic polyurethane portion[38] (Table 1).

Ryokawa et al. (2013) studied the physical properties of dental thermoplastic materials in a simulated intraoral environment. These products included Bioplast, Copyplast, Duran, Hardcast, Imprelon “S”, Essix A+, Essix C+ and Invisalign. These authors reported that Invisalign plastic absorbed the highest amounts of water after 24 and 36 hours among the materials tested and did not achieve complete saturation during the measurement periods. The authors hypothesized that water absorption may induce a breakup of the intrachain and/or interchain hydrogen bonds and modify the free volume of the polymers, and that the absorbed water might also wash out soluble substances from the polymers.[39]

Release of Byproducts from Aligners into Solution

Several in vitro studies have been conducted to identify molecules or byproducts released from clear aligner materials. One method utilized to analyze leachates was to place aligners into 75% ethanol-25% water (volume/volume) immersion medium for 2 weeks at 23°C to simulate accelerated aging.[40] The substances leached in the solution were characterized with gas chromatography-mass spectrometry (GC-MS) equipped with an electron impact ionization detector. To do this, approximately 10 mL of the samples were extracted with 10 mL High Performance Liquid Chromatography (HPLC)-grade dichloromethane and the extract was dried over anhydrous sodium sulfate, immersed in a solvent and analyzed with GC-MS. These authors found no traceable amount of substances in the ethanol aging solution.[33]

A study by Gracco et al. (2009) investigated short term chemical and physical changes in Invisalign appliances after immersion in artificial saliva or after exposure to intraoral wear during use. One ‘as-received’ Invisalign aligner, one ‘as-received’ Invisalign aligner immersed in artificial saliva for 14 days and 10 ‘worn’ Invisalign aligners were investigated. Fourier transform infra-red microspectroscopy was used to characterize molecular changes, spectrophotometry was used to evaluate changes in color and transparency, scanning electron microscopy and energy dispersive X-ray microanalysis were used to examine the surface morphology and composition of surface deposits, and GC-MS was used to identify substances released from the aligners into the artificial saliva. These authors did not identify any by-products released from the aligner simply immersed in the artificial saliva for 14 days. However, the clinically worn aligners had microcracks, abraded and delaminated areas, calcified biofilm deposits and loss of transparency.[41]

Cytotoxicity

Previous in vitro cytotoxicity studies have also been conducted. It has been shown that polyurethane is not an inert material and is affected by heat, moisture, and prolonged contact with enzymes.[29,42] Several in vitro studies have analyzed leaching of components from Invisalign aligners into aqueous media. Schuster et al. exposed "as-received" aligners to in vitro aging in ethanol solution and analyzed "after retrieval" aligners. They found that no residual monomers or oxidative byproducts were released to the solution, but the worn appliances showed abrasion at the cusp tips and buccal segments showed increased hardness. This increased hardness was only seen in the retrieved clinical samples indicating that it might be due to intraoral forces compacting the material in that area and increasing its density.[33] A 2009 study tested Invisalign aligners for cytotoxicity to gingival fibroblasts and estrogenicity and found no evidence of either.[43] However, another in-vitro cytotoxicity study exposed oral epithelial cells to Invisalign leachates. The results showed increased cell death, compromised cell membrane integrity, and reduced cell-to-cell contact and mobility, which may be the mechanism for isocyanate allergy.[44]

Another study immersed “as-received” aligners in normal saline for 2 months at 37°C. The solutions were diluted to 5%, 10% and 20% volume/volume and tested for cytotoxicity on human gingival fibroblasts. This study found there was no effect indicated from the aligner leachates on human gingival fibroblasts at any concentrations nor could it stimulate cell proliferation which was attributed to the short time frame, the stability of the aligners and the in vitro method of extraction.[43] Another study also used “as-received” aligners but ground them to a powder using a file, which produced particles that were about 86 x 56 um to 186 x 161 um in size. The Invisalign eluate was obtained by soaking 0.1 g of the aligner particulates in either 1.0 mL of normal saline solution or 1.0 mL of artificial saliva for 2, 4, and 8 weeks. [44] The artificial saliva composition conformed to a formula published for the modified Meyer solution.[45] This study concluded that exposure to Invisalign plastic caused changes in viability, membrane permeability and adhesion of epithelial cells. Furthermore, they suggested that hapten formation secondary to the compromised epithelial integrity might lead to isocyanate allergy. All of these mentioned studies tested the cytotoxicity of the leachates to the cells, but did not analyze the leachates for specific chemical composition.

Martina et al. (2019) examined the in vitro cytotoxicity of different thermoplastic material for clear aligners: Duran (Scheu-Dental GmbH, Iserlohn, Germany), Biolon (Dreve Dentamid GmbH, Unna, Germany), Zendura (Bay Materials LLC, Fremont, CA, USA), and SmartTrack (Align Technology, San Jose, CA, USA), on human primary gingival fibroblasts and found that each material exhibited a slight cytotoxic effect after 14 days. They also found that the cytotoxic effect increased when the specimens were tested after the thermoforming process.[46]

As more companies develop their own aligners, it is of concern that patients, especially those without a supervising health care provider, may be subjecting themselves to increased risks for adverse events, some of which could be life threatening.

The purpose of the present study was to expose four different aligner materials from different manufacturers: Invisalign (Invisalign), Clear Correct (Straumann), Smart Moves (Great Lakes) and Durasoft (Scheu-Dental), to water and to accelerated aging environments to detect release of potentially hazardous byproducts that could potentially lead to adverse health events in wearers. The aims for this current study were two fold, to verify: 1. leachates were released from each of the different aligners, and 2. aligners release dthe most in dichloromethane> alcohol > water.

MATERIALS AND METHODS

Four different aligners were obtained for this study: unused aligners from previous patients were gathered for Invisalign, Clear Correct; and Smart Moves, and Durasoft aligners were supplied by Great Lakes in their pre-vacuum formed material. For Invisalign and Clear Correct, one arch of each aligner was cut into 3 segments; 2 posterior segments from distal surfaces of the canines and 1 anterior segment from canine to canine. For Smart Moves and Durasoft, the pre-vacuum-formed material was cut into 1 cm x 3 cm segments. Initial "dry" segments were weighed on an analytical balance (Mettler Instrument Corp Hightstown, NJ) to the nearest 0.00001 gm to provide starting weights (M1) (Figure 2).

One segment of the aligner material was placed into a 29 x 65 mm sealable vial (Shell Vial with Titeseal Closure; Fisherbrand) containing 35 mL of solvent. This was done in quadruplicate (Figure 3). Solvents were deionized water (stored at 37° C in a Thelco GCA Precision Scientific Model 16 Mechanical Convection Oven), ethanol (ACROS, 99.5%, ACS Absolute, 200 Proof), and dichloromethane (ACROS, 99.9%, extra dry, stabilized). Deionized water at 37° C was chosen to represent a simple, aqueous control solution in which the aligners should be minimally soluble of insoluble. Water, in contrast to saliva in vivo, there were no enzymes, no intraoral pressures, and no a turnover. Ethanol was selected since it is expected to be a better solvent for the aligner and it is a more severe solvent than applied by a normal intraoral environment. Dichloromethane does not have clinical relevance as it is an extreme organic solvent. However, it was expected to be capable of rapid and complete extraction of organics not bound within the polymer and demonstrate the extreme of available leachates.

The Invisalign aligner segments were incubated for 3, 7, and 14 days. After each of these time points, the aligner segments were removed from the water solvent and immediately weighed to obtain the “wet” weight (M2; Figure 4). The final “dry” weight, M3, was measured after 2 months of drying in a vacuum hood followed by 3 weeks in a glass desiccator under vacuum, weighing each week, to ensure the weight had stabilized.

Clear Correct, Smart Moves, and Durasoft aligner segments were incubated in the three solvents for the 14-day time period only. After 14 days, the aligner segments were removed from the water solvent was immediately weighed to obtain M2. The final “dry” weight, M3, was determined after 2 months of drying in a vacuum hood followed by 3 weeks in a glass desiccator under vacuum to ensure, by weekly weighing’s, that the weight had stabilized. This was recorded as M3 for all aligner segments removed from the water, ethanol and dichloromethane solvents.

M1, M2, and M3 values were used in the formulas below to calculate water sorption (WS) and solubility (SL) in order to determine how much leaching took place:

$$WS = (M2 - M3)/M3$$

$$SL = (M1 - M3)/M1$$

To analyze the composition of the leachates, the contents of the immersion solvent were lyophilized, and the product was analyzed via ^{1}H-NMR (proton nuclear magnetic resonance) spectroscopy (Bruker, 400MHz) (Figure 4).

Lyophilization was carried out for all the samples using the manifold drying method. Water leachate samples were placed in a -80° C freezer until frozen. The frozen samples were then lyophilized at room temperature for 24 hours to carry out sublimation. This resulted in solid leachates devoid of water.

The recovered leachates were resuspended in 0.5 mL deuterated chloroform solvent (Oakwood Chemicals). This removed a neutron from the H atoms, and subsequently the protons oriented themselves in an applied magnetic field and resonated at a specific frequency which is represented by a detectable chemical shift from a reference in parts per million (ppm). Dependent on how these protons were attached to other atoms, their signals appeared at different frequencies on the spectrum. NMR spectroscopy is a qualitative analysis to depict what kind of molecules are present. In the current study, measuring the leachate molecules from aligners was the aim.

Furthermore, ^{1}H-NMR spectroscopy uses the standards deuterated chloroform solvent ($CDCL_3$) and tetramethylsilane (TMS) as a scale to record the frequency of the molecule in question's signal in ppm on the-x axis with the y-axis being intensity. Thus, the peak located in all spectra at 7.26 ppm corresponds to the deuterated chloroform solvent ($CDCL_3$) while the peak 0 ppm is tetramethylsilane (TMS). Upfield is denoted as peaks more the right of the figure while downfield is to the left with midfield in between the two (Figure 5).

Statistical Analysis

Statistical analyses were conducted using commercial software (SigmaSTAT, San Jose, CA).

Sorption:

For the water sorption tests, mean WS for the four materials at the 14-day time period were analyzed with a Kruskal-Wallis test with a Newman-Keuls post-hoc comparison ($\alpha = 0.05$). This was done because the data was not normally distributed and did not allow the use of Analysis of Variance (ANOVA).

Solubility:

For the solubility tests, the Kruskal-Wallis test with the Holm-Sidak post-hoc comparison ($\alpha = 0.05$) test was performed for comparisons of the mean SL between days 3, 7, and 14 for Invisalign in water, ethanol and dichloromethane as well as mean SL for all four materials (Invisalign, Clear Correct, Smart Moves and Durasoft) in water, ethanol and dichloromethane at 14 days.

RESULTS

^{1}H-NMR

Invisalign

The NMR spectra for Invisalign in water for 3, 7, and 14 days had the calibration peaks in addition to the peaks at ~1.56 ppm which were characteristic of undeuterated water (Figure 6).[48] Only two very short peaks appeared in the spectra for Invisalign in water, which indicated that the compounds were present in a minute concentration. The peak at 1.25 ppm was the location in the spectra commonly associated with grease contamination from the rotary-evaporator joints (part of the spectrometer).[49]

The NMR spectra for Invisalign in ethanol for 3, 7, and 14 days showed that the same peaks for the deuterated solvent and TMS were present (Figure 7). However, double peaks were present due to ethanol being polar and increasing the dipoles during the ^{1}H-NMR spectroscopy. The peaks around 1.25 and 1.32 ppm could be the ethanol solvent or grease contamination, as mentioned.[48] Peaks presented between 5.5 and 3 ppm for 3, 7 and 14 days. The peak around 3.4 ppm increased in concentration with increasing days in ethanol denoted by its peak intensity compared to the peak of the TMS. The Invisalign in the ethanol at 3 days also shifted upstream by 0.1 ppm.

The NMR spectra for Invisalign in dichloromethane for 3, 7, and 14 days showed a peak above 8 ppm in the dichloromethane, characteristic of aromatics, alcohols or amines (Figure 8). The peaks found between 4-5.5 ppm are consistent across the 3, 7, and 14-day dichloromethane samples as well as broad peaks upfield (~2.24-0.75 ppm).

Clear Correct and Smart Moves

The NMR spectra for Clear Correct and Smart Moves in water, ethanol, and dichloromethane for 14 days showed peaks for the deuterated solvent, water, and TMS (Figure 9 and 10). In water, no other peaks were present. The ethanol spectrum had peaks between 0.5-4 ppm. The dichloromethane spectrum for Clear Correct showed broader peaks around ~1.5-0.6 ppm. For Smart Moves in dichloromethane, peaks are observed above 8 ppm, between 4-5.5 ppm and broader peaks around ~1-2.25 ppm.

Durasoft

^{1}H-NMR spectra were not obtained for the Durasoft sample in dichloromethane due to the leachates creating a film. Thus, the spectra are shown for the ethanol and water for the Durasoft 14-day samples only (Figure 11). The water spectrum showed very small intensity peaks at ~3.4 ppm and 1.25 ppm. The ethanol spectrum had peaks showing up mid-field.

Sorption

Sorption (WS) results had no difference in water sorption for Invisalign, Clear Correct and Durasoft, but all three took up significantly more water than Smart Moves (Figure 12).

Solubility

Invisalign at 3, 7, & 14 days

When comparing Invisalign at the three time periods (3, 7 and 14 days) in the three solvents (water, ethanol and dichloromethane), the solubility in dichloromethane was the greatest at 7 days followed by 14 days and then 3 days (Figure 13). Furthermore, there was no difference in the solubility for the three time periods for ethanol or water. When comparing the solvents at 3 days, all were equal. At 7 and 14 days, dichloromethane had significantly higher solubility than water and ethanol, with the latter two being equal (Figure 13).

Invisalign, Clear Correct, Smart Moves & Durasoft at 14 days

The solubility of Invisalign, Smart Moves and Durasoft in dichloromethane was greater than in water and ethanol, with the latter two being equal (Figure 14). For Clear Correct, statistically, the solubility in water was greater than in dichloromethane, while the solubility in water was equivalent to ethanol and ethanol was equivalent to the solubility in dichloromethane (which was a negative percent solubility).

When comparing the materials in DCM, all aligners had different solubilities, except Invisalign, which was equal to Smart Moves. However, there were no differences in their solubility between water or ethanol (Figure 14).

Subjective – physical appearance of aligners after aging

As the aligners were made from different materials, they had different appearances and seemed to react differently to the solvents (especially in regards to dichloromethane). Invisalign samples that were immersed in water, ethanol and dichloromethane (Figure 15) showed no apparent difference between the water and ethanol samples. However, two distinct layers peeled away from one another in the dichloromethane sample. Additionally, large pieces of exposed aligner flaked off the main section.

For Smart Moves, the water and ethanol samples appeared comparable while the dichloromethane sample turned a frosty white with frayed edges that left a film on the inside of the vial (Figure 16)

The samples of Durasoft in water and ethanol appeared the same, whereas the sample stored in dichloromethane was frosty white, although the edge appeared more intact and there was less of a film on the vial than the Smart Move samples. Additionally, distinct layers were present for the water and ethanol samples whereas the dichloromethane sample appeared to have one sole layer remaining (Figure 17).

Clear Correct samples stored in water appeared unaltered, and those in ethanol appeared slightly frosty. In dichloromethane, however, Clear Correct samples behaved differently from one another. Two of the four samples maintained their arch form but appeared frosty, while the other two appeared liquefied at the bottom leaving a thin film on the vial (Figure 18).

DISCUSSION

^{1}H-NMR

The degradation of Invisalign in water over time up to 14 days seems to be minimal, as no leachates were identified as determined by the absence of any significant peaks in NMR. This also confirms the very low solubility percentages reported in the solubility tests. However, Invisalign in ethanol over time showed very small peaks midfield, with the intensity increased at 14 days. This suggests that soaking for 14 days led to more leachates being released, which was not evident in the solubility tests. However, it needs to be pointed out that very small changes in weight could drastically change the SL results. The Invisalign in the ethanol at 3 days was also shifted upstream by 0.1 ppm, suggesting that this material was more susceptible to degradation in stronger inorganic solvents. At times a shift in the spectra could indicate a further reaction of the ethanol with the urethane bonds present in the aligner, though this is unlikely, since those bonds are known to be reversible only under different conditions that were not present in this study.[50] Invisalign spectra in dichloromethane appeared very similar at 3, 7 and 14 days, showing that all leachates were extracted by day 3 with possibly monomers shown midfield and oligomers shown upfield with broad peaks. This result slightly contradicted the solubility data where it appeared that Invisalign was most soluble at 7 and 14 days. The peaks found between 4-5.5 ppm were consistent across the 3, 7, and 14-day dichloromethane samples and could be attributed to an unreacted monomer presence, a by-product of a degradation reaction that took place, or a transformative product that formed in solution after leaching. The broad peaks upfield (~2.24-0.75 ppm) were likely related to the presence of oligomeric species, suggesting the degradation of the aligners, rather than leaching of unreacted components or impurities. Furthermore, there was an aromatic released in the dichloromethane which could be attributed to the ink numbers present on the Invisalign tray, and more possible leachates being released in dichloromethane than ethanol and none in water (Figure 19).

In general, it appears Smart Moves, Invisalign, Durasoft, and Clear Correct were fairly insoluble in water at 14 days (Figure 20) since only peaks attributed to the standards used to run the ^{1}H-NMR and contaminants were identified.

In ethanol at 14 days, Smart Moves and Clear Correct appeared to have similar spectra suggesting similar leachates, while Invisalign and Durasoft were more similar (Figure 21). This could be attributed to the similarities between the aligner materials. Smart Moves is a polypropolene/polyethylene copolymer-based material and Clear Correct is a 1,6-hexanediol polymer with 1,1'-methylenebis(isocyanatobenzene). Invisalign is a polyurethane with added methylene diphenyl diisocyanate and 1,6 hexanediol and Durasoft is polyurethane and polyethyleneterephthalate-glycol. The ethanol spectra for Smart Moves and Clear Correct present peaks between 0.5-4 ppm which could be unreacted monomers or oligomers (shown more upfield). The ethanol spectra for Durasoft and Invisalign had peaks mid-field which could be unreacted monomers or oligomers (more upfield).

^{1}H-NMR spectra were not obtained for the Durasoft sample in dichloromethane due to the fact that the leachates created a film on the vial. This was most likely due to the oligomers being slip cast on the vial, and when the solvent evaporated, they were deposited there as a film. NMR spectra for Smart Moves, Invisalign and Clear Correct in dichloromethane at 14 days showed, as expected, the degradation with dichloromethane was more marked than with the other solvents (Figure 22). It is possible that this increase came both from increased extraction of unreacted species/impurities, or from degradation of the material itself. In these spectra, all materials appeared to release oligomers, as denoted by the broad peaks upfield. Midfield, peaks were only present for Smart Moves and Invisalign, which could possibly represent other leachates. Downfield, there was a peak that could be attributed to an aromatic for Smart Moves and Invisalign. Again, but it cannot be discounted that this may have been the result of the numbers marked in ink on the tray for Invisalign. However, Smart Moves did not have any ink on the aligners, so that peak could be from a different aromatic or an alcohol or amine leachate. In dichloromethane, the Smart Moves spectrum appeared more similar to the Invisalign spectrum, suggesting similar leachates. Again, this could be attributed to the similarities in the original aligner material interacting with a more organic solvent.

There was no intention to reverse-engineer the products tested, but rather to try to identify some of the species being leached that could pose a potential toxicity concern. Species like PET and isocyanates (which are in the make-up of the aligners), are known to cause allergic reactions and even may be endocrine disruptors.[25-27,51] It is apparent that the water itself was not able to significantly degrade the aligners, nor extract any potential harmful compounds, and it was only under the extreme conditions of storage (dichloromethane) that appreciable amounts of leachates could be identified. This means that, although not easily extractable, those small molecules and oligomers could potentially cause allergic reactions – this is a concern because neither the nature of the leachates, nor their minimum harmful concentration, is known.

Sorption

It was not possible to measure the sorption of the aligners in the ethanol and dichloromethane solvents. These solvents rapidly desorbed from the aligners during the weighing process, making it impossible to weigh the specimen (for M2) with any certainty.

For the water sorption, there was no difference in sorption for Invisalign, Clear Correct and Durasoft, but all three took up more water than Smart Moves. This could be because Smart Moves was a single layer of polypropylene/polyethylene copolymer-based material and water might have absorbed into the aligner better when there were multiple layers present. Additionally, Smart Moves and Durasoft absorbed less than Invisalign and Clear Correct. This might be because Smart Moves and Durasoft were tested prior to thermoforming the arches or could be due to the differences in the composition of the polymer systems. Ryu et al. found that the water absorption ability increased for four materials (Duran, Essix A+, Essix ACE and eCligner) when tested after thermoforming as compared to prior to this procedure. This suggests that Smart Moves and Durasoft might absorb more solvent after thermoforming.[52]

Despite the fact that there was no significant difference between the water sorption of Clear Correct and Invisalign, the variance for Clear Correct was extremely high. It is noteworthy that there was one value for Clear Correct that was vastly different than the other three, accounting for this variability. If the data were replotted without this specimen, the chart clearly shows that Clear Correct and Invisalign were more comparable in terms of percent water sorption.

Solubility

When comparing Invisalign at the three time periods (3, 7 and 14 days) in the three solvents (water, ethanol and dichloromethane), the solubility in dichloromethane was the greatest at 7 days followed by 14 days and then 3 days. The difference between the percent solubility was comparable between 7 and 14 days. Thus, it appears that Invisalign aligners reach maximum solubility in dichloromethane at 7 days and there is no further loss of components. Additionally, there was no difference in solubility for any of the time periods for ethanol or water. However, there is a possibility that the solvent saturated with leachates so no more leachates are extracted even if they are present in the sample. However, this is unlikely considering the percent solubility was so low (and even negative in some cases). The amount leaching at 3 days for Invisalign was negligible. A study by Schuster et al. reported that the diphenyl structure of Invisalign material provided stability and sufficient reactivity to form a polymer free of byproducts, and reported no residual monomers or oxidative byproducts in a 75% ethanol 25% water solvent.[33] However, due to the appearance of peaks in the ^{1}H-NMR spectroscopy conducted in this study, some leachates were definitely released, likely in the form of oligomers.

When comparing the results from the aligners stored in solvents at 3 days, all were equal in percent solubility. This might be because insufficient time was given for the solvent to completely saturate the material, complete desiccation was not achieved and/or the aligners had very little extractable components. Since some aligners showed a negative solubility, it suggests that all the solvent could not be removed and the final product had remaining solvent that caused it to weigh more than the original weight. This could be because of the presence of initially bound water which could serve as an attractor of further water during exposure all of which would remain tightly bound. This would account for the inability to completely desiccate and measuring a final weight (M3) measurement that was higher than expected. At 7 and 14 days, samples stored in dichloromethane had higher solubility than in water or ethanol, which was expected since dichloromethane has the ability to dissolve a wide variety of organic compounds.

Subjective- physical appearance of aligners after aging

After the aligner materials were exposed to water and ethanol, they appeared comparable to untreated aligner material, being clear with no films present. Additionally, all materials appeared frosty after being exposed to dichloromethane. Invisalign material appeared to have two layers that separated after exposure and became flaky with pieces coming off the main section. Smart Moves maintained its original shape. Durasoft appeared to have had its outer layers disappear leaving only one layer. Clear Correct samples behaved differently, where dichloromethane liquified the polymer structure for half the samples while the other half maintained the original arch form. It is possible that there was manufacturer error in the original material and the aligner samples that liquified were sectioned from the same aligner arch. The chemical make-up of the proprietary material affects how the aligner degrades in dichloromethane.

Limitations

This study has many limitations. Firstly, obtaining aligners from companies was problematic as many refused to provide samples. Therefore, unused trays from previous patients were gathered for Invisalign and Clear Correct. Additionally, two of the aligner materials (Smart Moves and Durasoft) were sent pre-vacuuform processing so the mechanical and physical properties of the materials and their final chemical composition could be different than what is actually being introduced intraorally. Ryu et al. determined that transparency, water solubility and surface hardness is affected by the thermoforming of materials into transparent orthodontic aligners and therefore physical and mechanical properties of thermoplastic materials used for the fabrication of these aligners should be evaluated after thermoforming in order to accurately characterize their properties for clinical application.[52] However, due to the limitations of this study in terms of being able to access material, pre-vacuum-formed materials for Smart Moves and Durasoft were used for the exposure to the solvents so as to not introduce more variables by vacuum-forming in-house.

Additionally, this study represented an in vitro assessment, and therefore did not accurately simulate the complex intraoral environment which contains a mix of organic molecules, periods of acidity and alkalinity, temperature variations, mechanical function, and its individual-specific microbiomes. Under normal circumstances, enzymes are not thought to be able to significantly degrade synthetic polymers, though it has been pointed out that enzymes have the ability to reduce the activation energy of chemical reactions (a degradation process that usually takes place only at high temperature or in the presence of UV light) which may conceivably take place under physiological conditions in the presence of the proper enzymes.[29] Furthermore, degradation mechanisms of thermoplastic polyurethanes are not fully understood. This is due to the high chemical diversity of their composition and the complex interrelationships with the environment (temperature, ambient media) resulting in numerous degradation mechanisms and products[28]

Another limitation was the fact that the exact starting polymer structures for the aligners were unknown due to their proprietary nature, so classifying degradation products was very difficult.

Future studies could include in vitro analysis of a wider variety of aligners in artificial saliva simulating in vivo enzymes, pH, temperature, and possibly even bruxism/wear to provide more pertinent results of what happens in vivo. The physical properties, such as hardness and stiffness, can also be assessed for changes before and after exposure to the solvent. Furthermore, it could be done in conjunction with aligner leachate exposure to human epithelial cells or an allergic response cell.

CONCLUSIONS

The original aims were partially supported: 1. Leachates are were released from Invisalign, and other aligners, though likely of different chemical formulations due to their different original composition, and 2. aligners released the most leachates in dichloromethane> alcohol=water.

Aligners will likely remain prevalent as a modality to treat patients' malocclusions, so it is pertinent to understand there could be potentially hazardous effects, especially allergic reactions. Only a small amount of a hapten is needed to elicit an allergic response, so sensitive individuals may be more at risk.

TABLE

Aligner	**Company**	**Material Name**	**Chemical Makeup**
Invisalign	Invisalign	SmartTrack	polyurethane with added methylene diphenyl diisocyanate and 1,6 hexanediol
Clear Correct	Straumann	Zendura FLX by Bay Materials	1,6-hexanediol polymer with 1,1'-methylenebis(isocyanatobenzene).
Smart Move	Great Lakes	Invisacryl Ultra	Smart Move aligners are made from a polypropolene
Durasoft	Scheu-Dental	Durasoft pd	polyethyleneterephthalat-glycol copolyester and thermoplastic polyurethane

Table 1. Table of aligners utilized in this study, their respective company

FIGURES

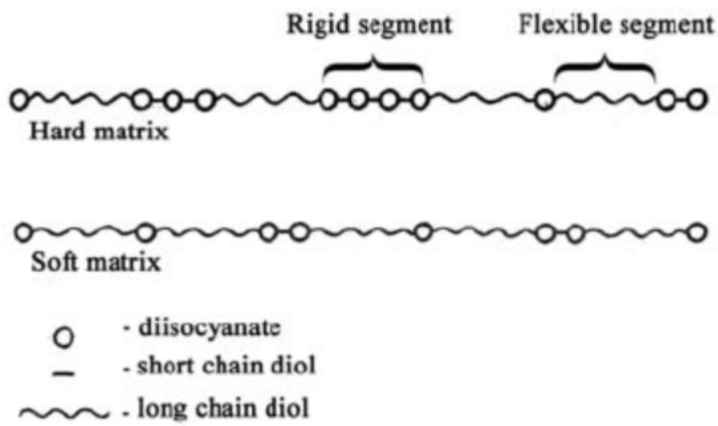

Figure 1. Depiction of the chemical structure of thermoplastic polyurethane (TPU)[31]

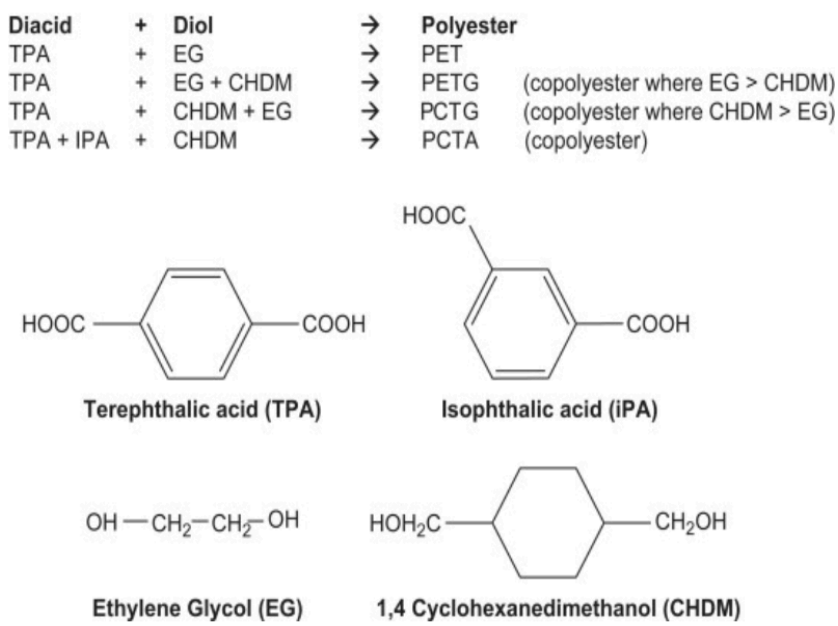

Figure 2. Chemical structure of precursor molecules for synthesis of polyester polyethylene glycol terephthalate (PET-G)[32]

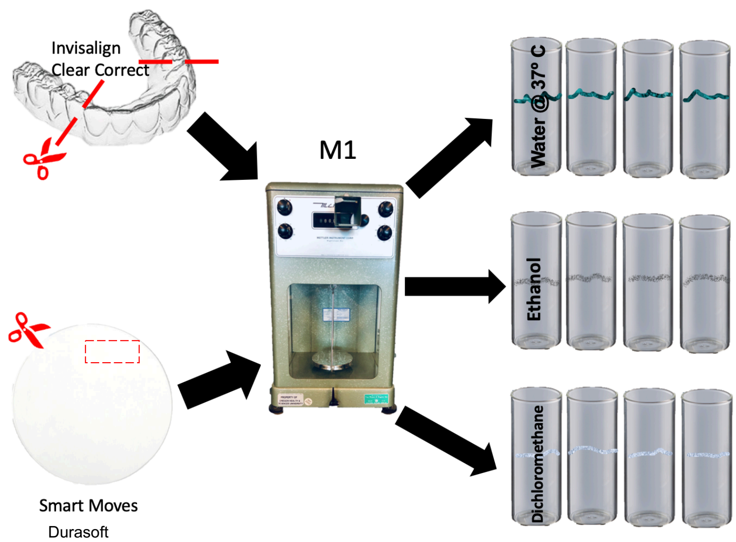

Figure 3. Method to obtain M1 and placement into solvents in quadruplicate

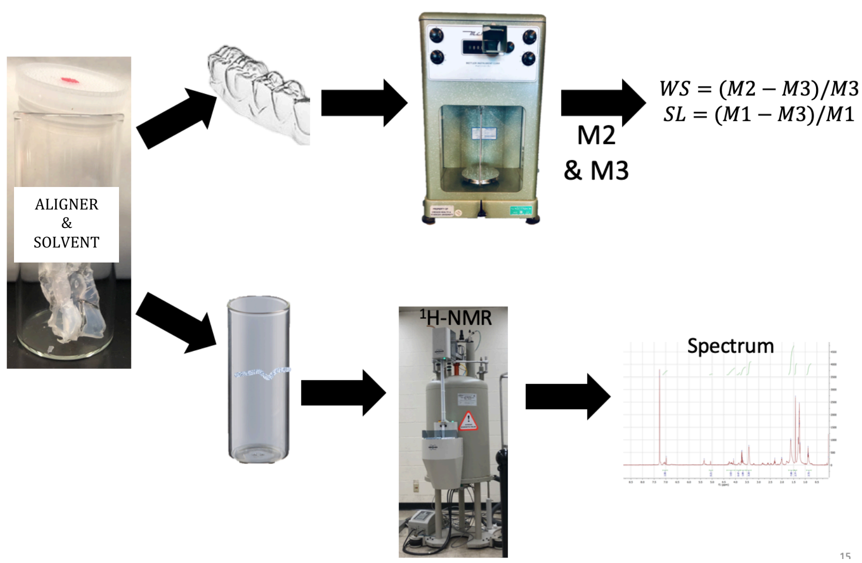

Figure 4. Method used to obtain M2, M3, and spectrum from ^{1}H-NMR spectroscopy

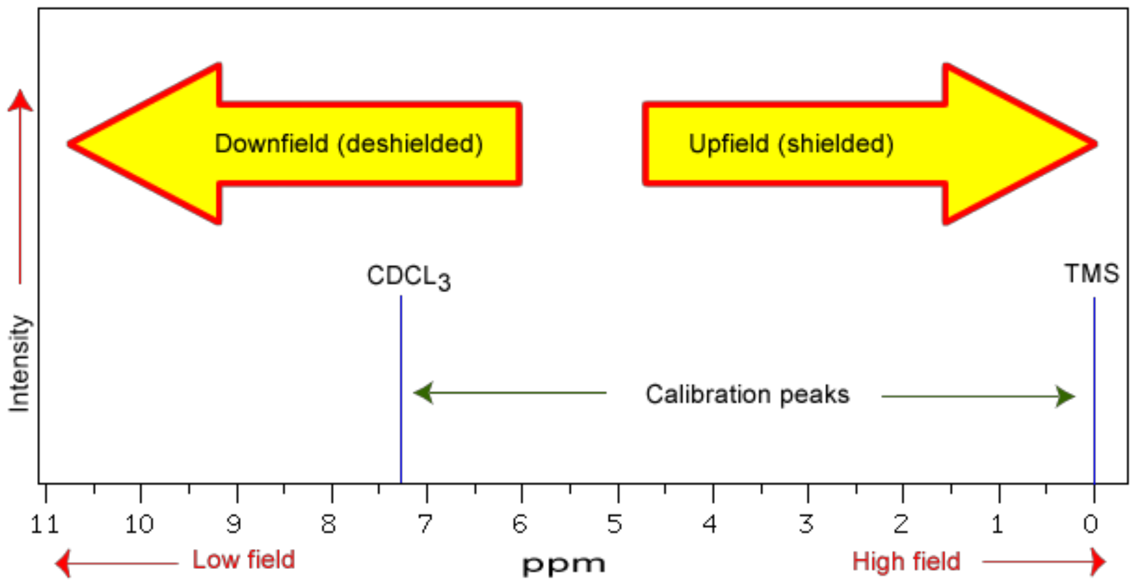

Figure 5. A ^{1}H-NMR field denoting a chemical shift and important terms[47]

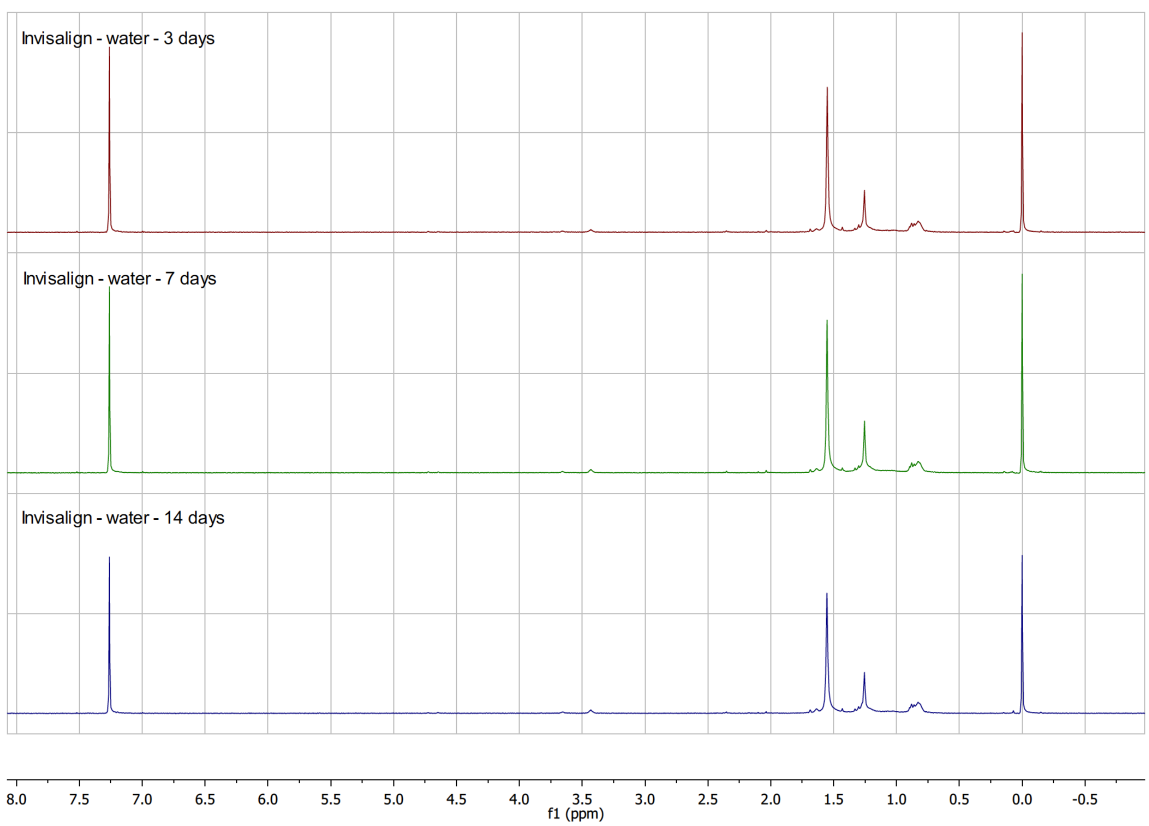

Figure 6. ^{1}H-NMR spectrum of potential leachates for Invisalign in water over 3, 7, and 14 days

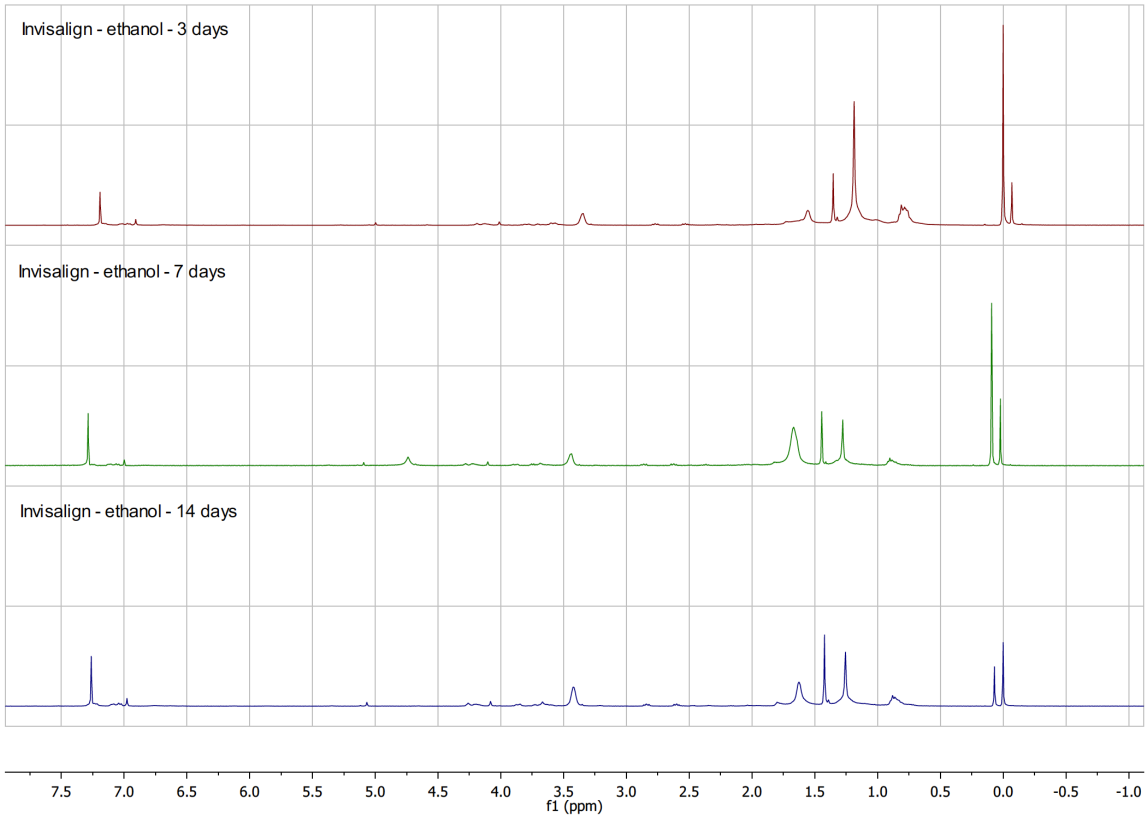

Figure 7. ^{1}H-NMR spectrum of potential leachates for Invisalign in ethanol over 3, 7, and 14 days

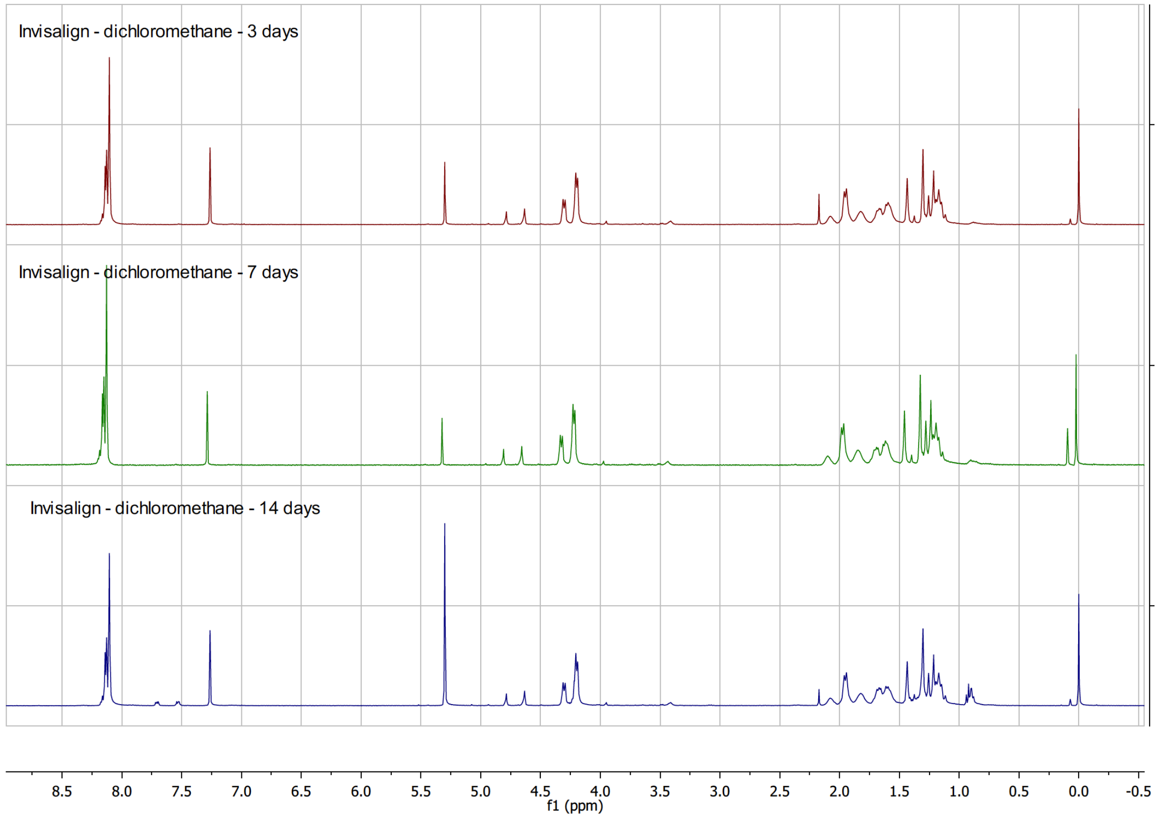

Figure 8. ^{1}H-NMR spectrum of potential leachates for Invisalign in dichloromethane over 3, 7, and 14 days

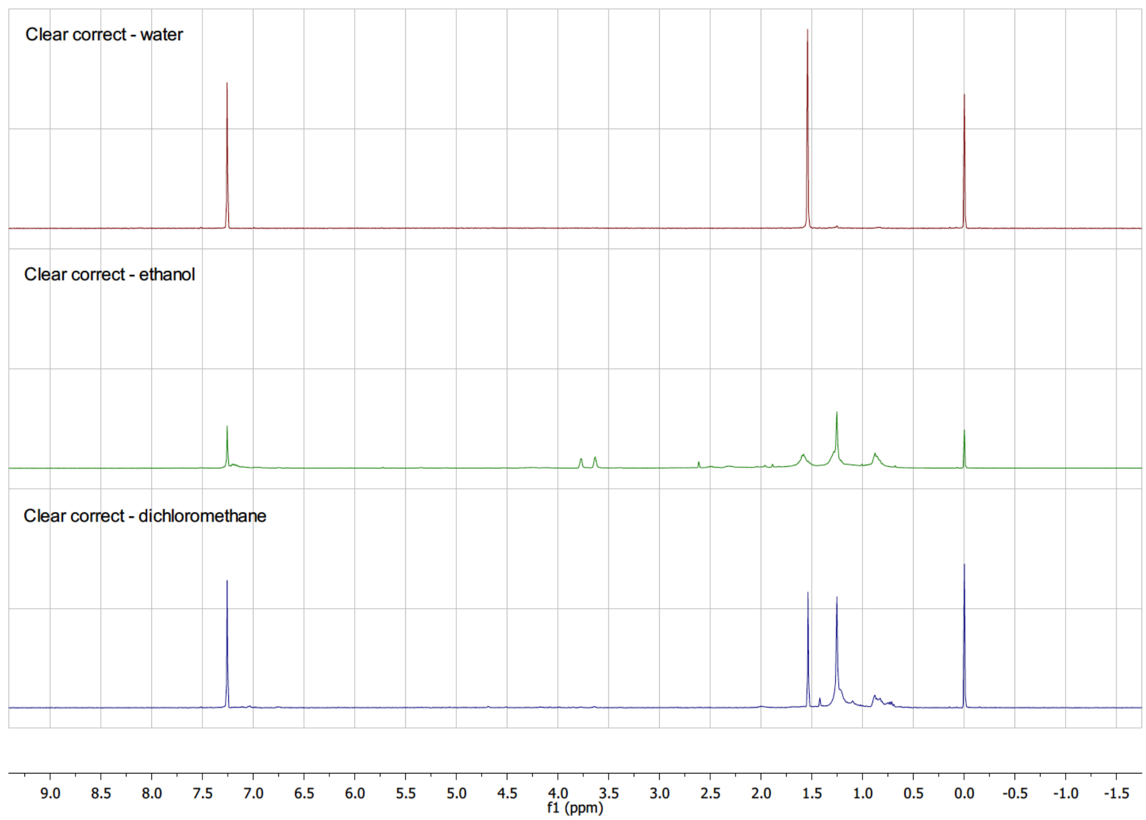

Figure 9. ^{1}H-NMR spectrum of potential leachates for Clear Correct in water, ethanol, and dichloromethane at 14 days

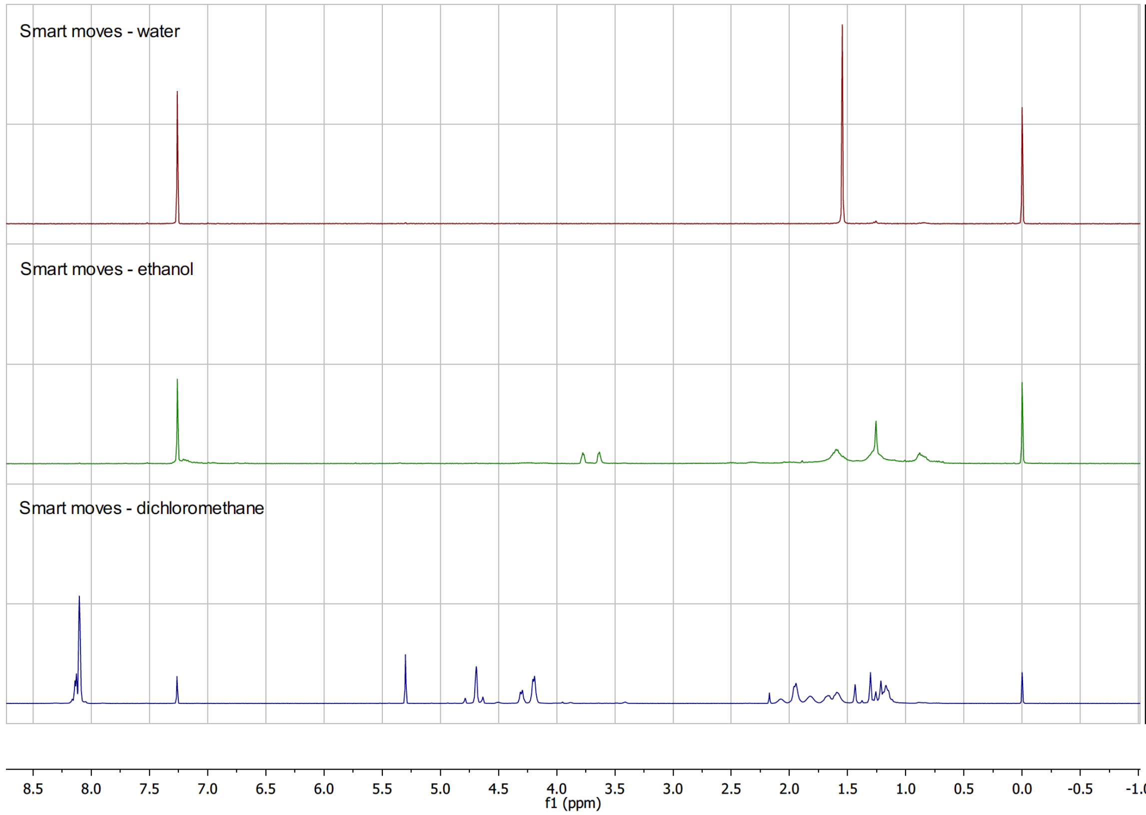

Figure 10. ^{1}H-NMR spectrum of potential leachates for Smart Moves in water, ethanol, and dichloromethane at 14 days

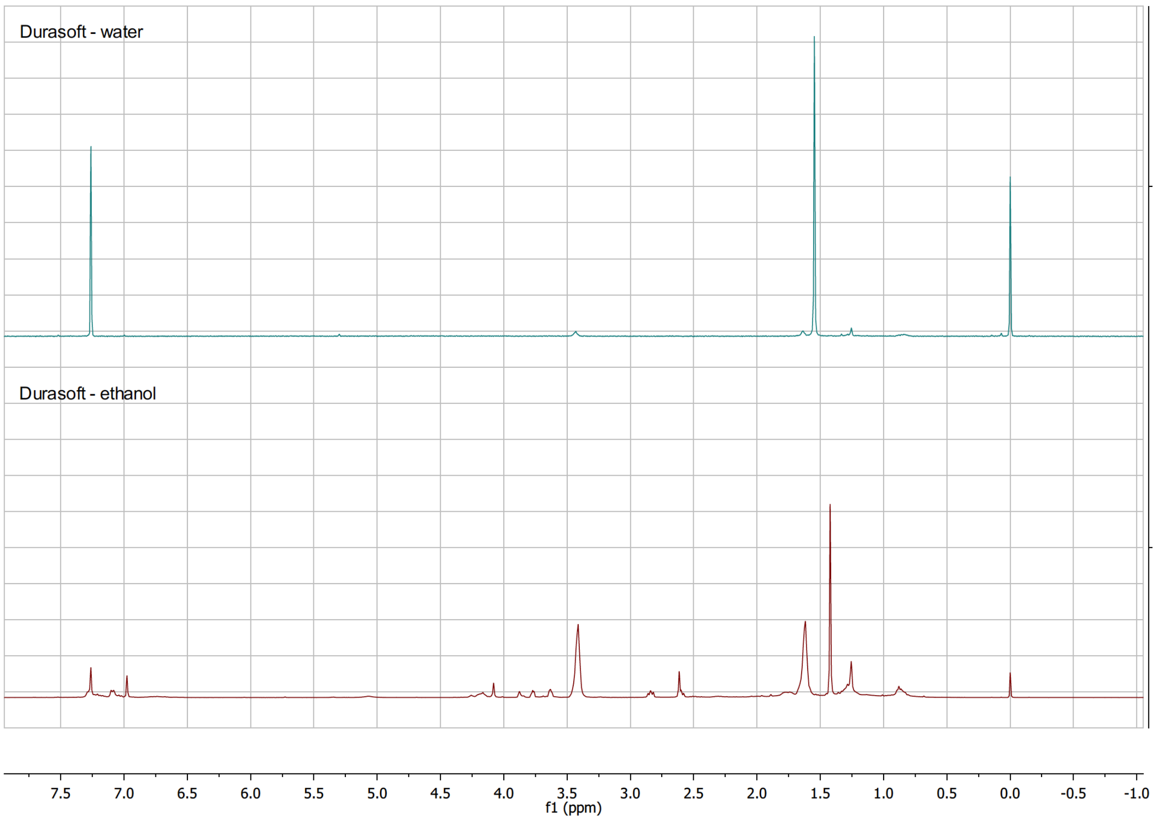

Figure 11. ^{1}H-NMR spectrum of potential leachates for Smart Moves in water and ethanol at 14 days

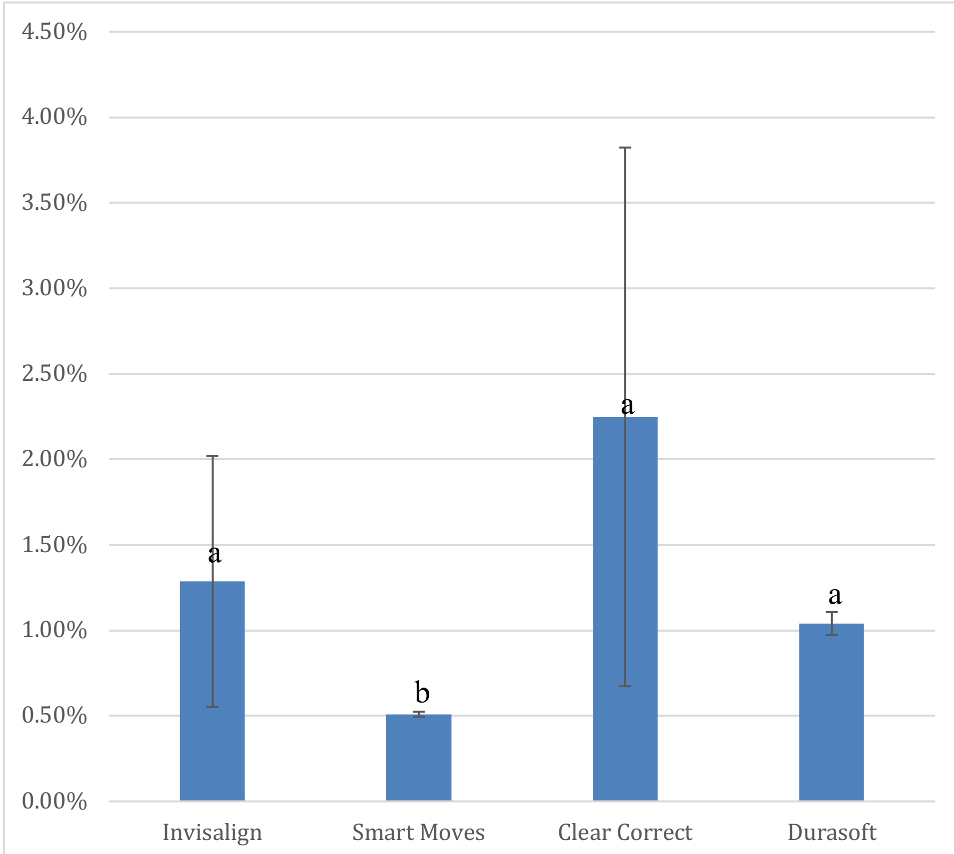

Figure 12. Percent sorption (WS) for four aligner materials in water at day 14 shows Smart Moves absorbed significantly less water than Invisalign, Clear Correct and Durasoft

Bars with the same letter are not significantly different (α>0.05)

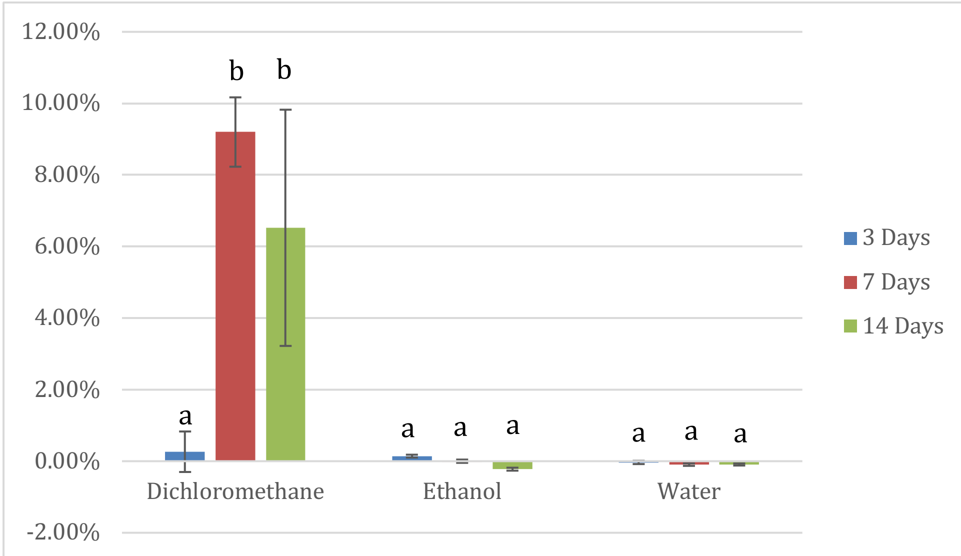

Figure 13. Percent solubility for Invisalign over time in three types of solvents

Bars with the same letter are not significantly different (α>0.05)

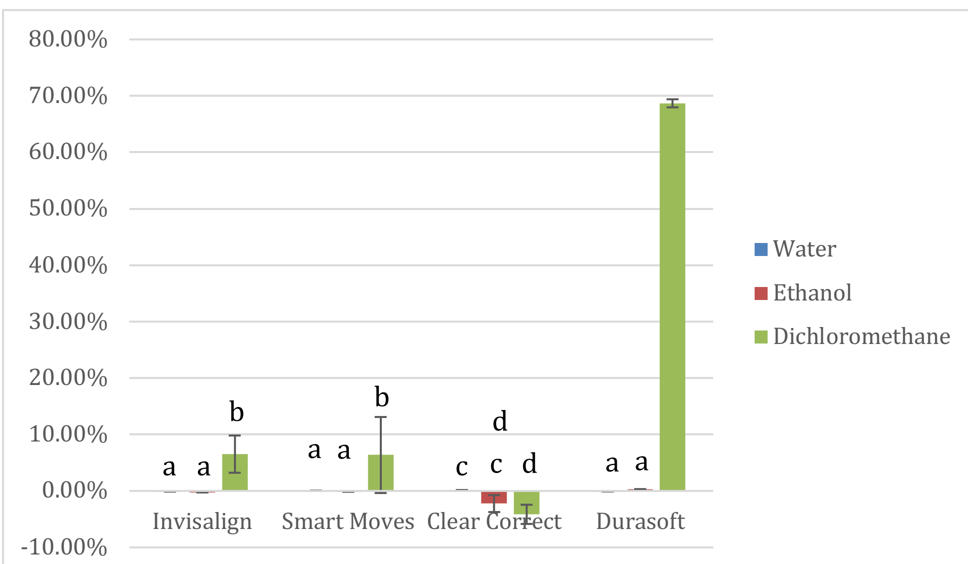

Figure 14. Percent solubility in three solvents for four types of aligner materials at day 14

Bars with the same letter are not significantly different (α>0.05)

Figure 15. Depicts 14-day Invisalign samples that were in water, ethanol and dichloromethane, from left to right, respectively

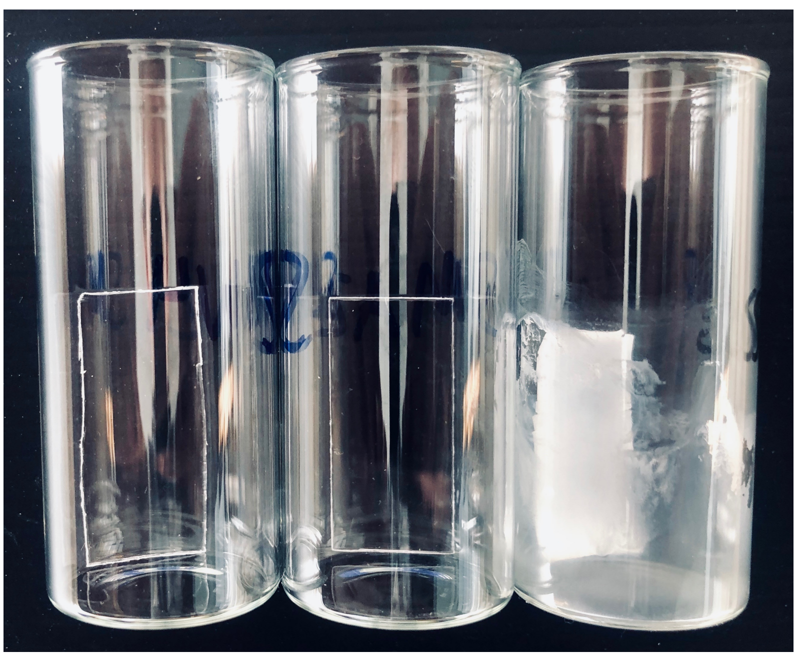

Figure 16. Depicts 14-day Smart Move samples that were in water, ethanol and dichloromethane, from left to right, respectively

Figure 17. Depicts 14-day Durasoft samples that were in water, ethanol and dichloromethane, from left to right, respectively

Figure 18. Depicts 14-day Clear Correct samples that were in water, ethanol and dichloromethane (the last 2), respectively

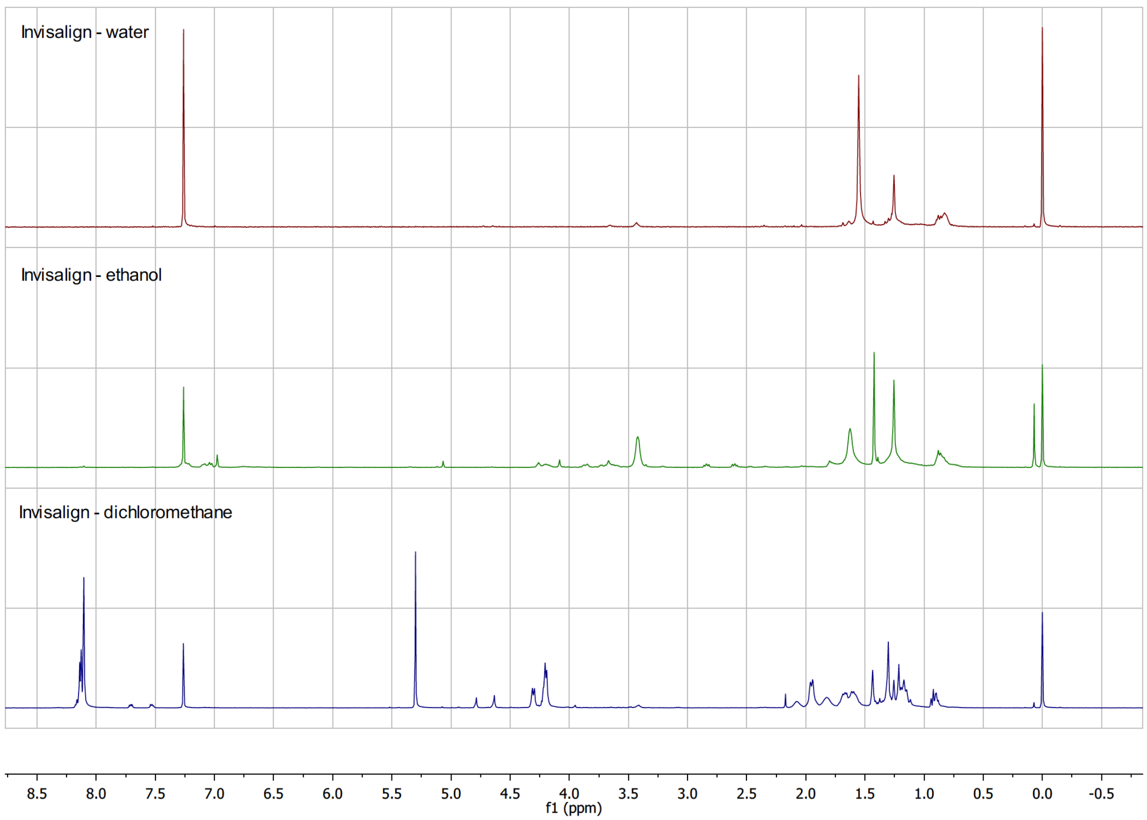

Figure 19. ^{1}H-NMR spectrum of potential leachates for Invisalign in water, ethanol, and dichloromethane

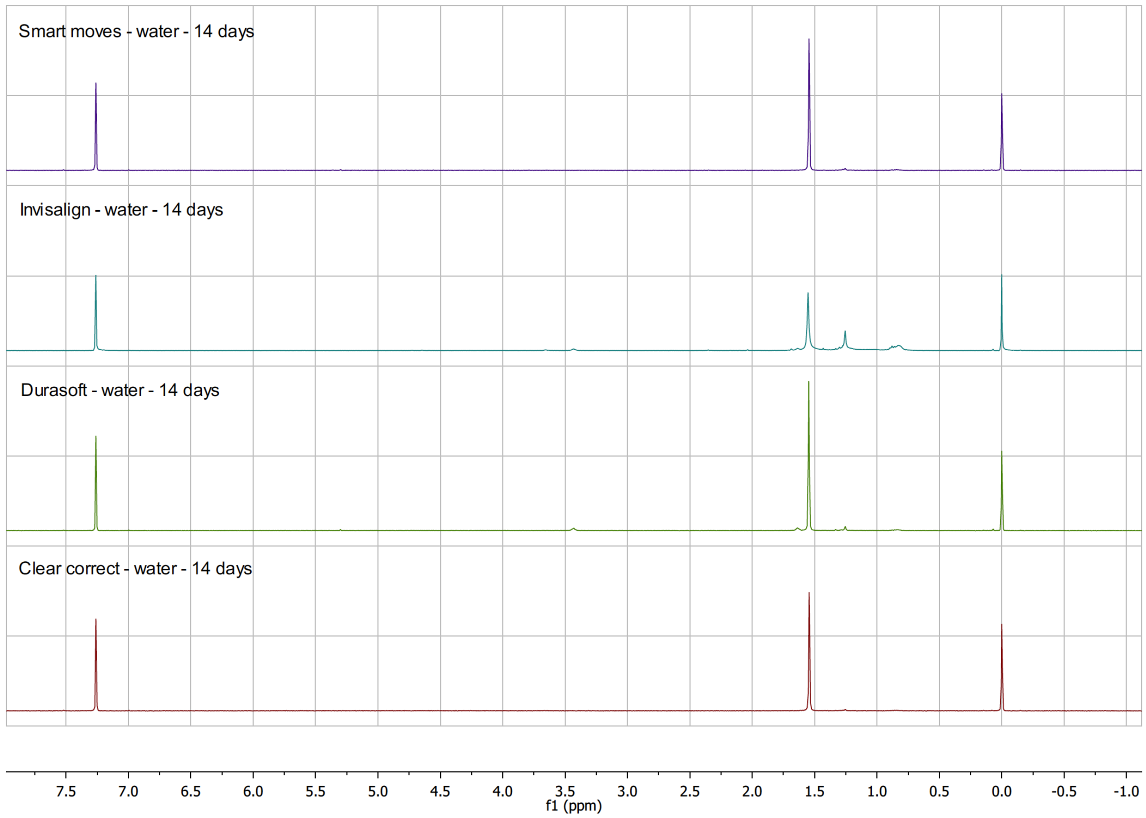

Figure 20. ^{1}H-NMR spectrum of potential leachates for Smart Moves, Invisalign, Durasoft and Clear Correct in water

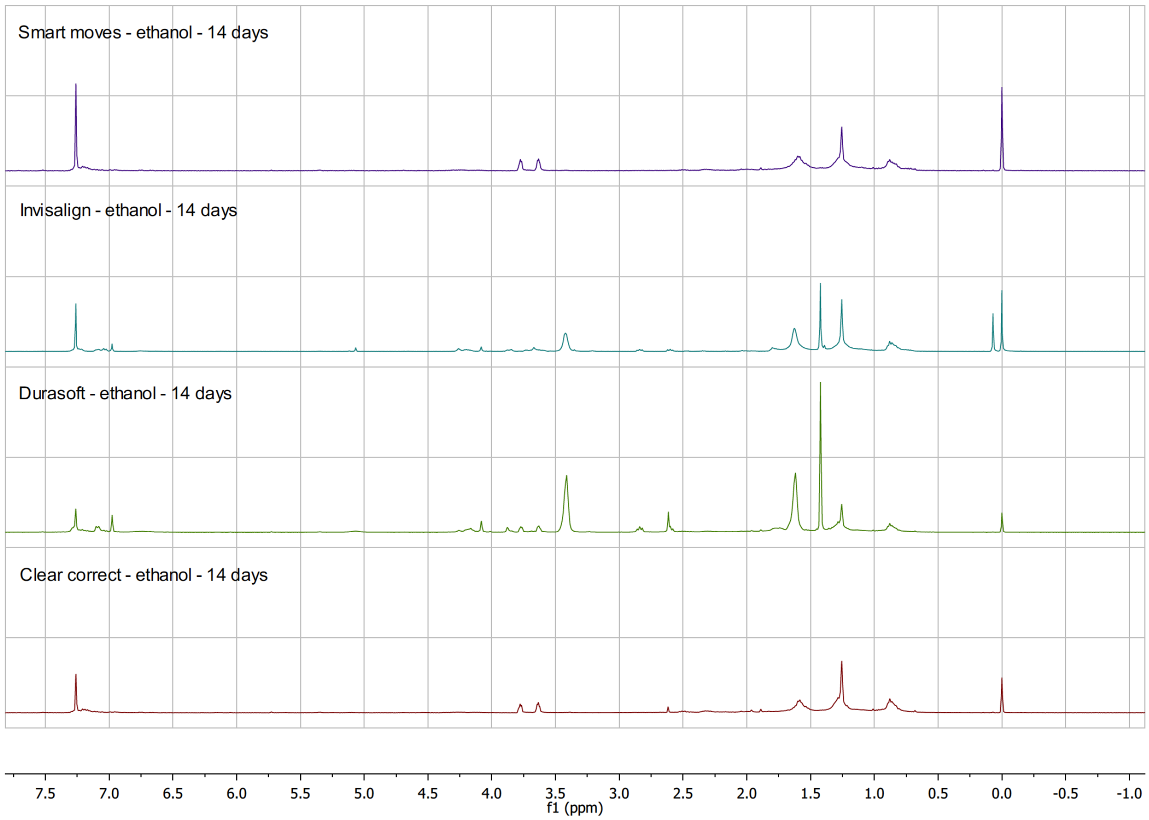

Figure 21. [1]H-NMR spectrum of potential leachates for Smart Moves, Invisalign, Durasoft and Clear Correct in ethanol

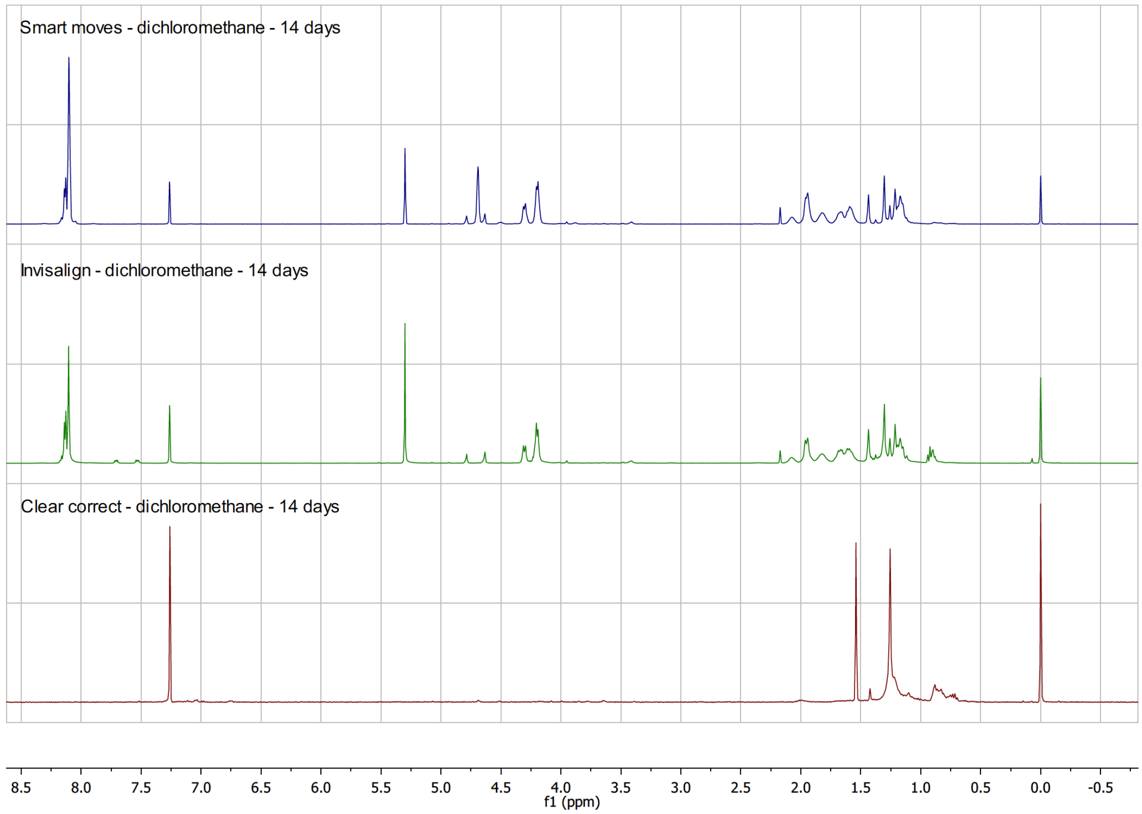

Figure 22. ^{1}H-NMR spectrum of potential leachates for Smart Moves, Invisalign, Durasoft and Clear Correct in water.

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APPENDICES

Solvent	Days in Solvent	Sample Number	M1 (g)	M2 (g)	M3 (g) after 1 month from removal from solvent	M3 (g) after 2 months from removal from solvent	M3 (g) after 3 weeks of desiccation
Dichlo-romet-hane	3	1	0.48569		0.50789	0.48954	0.48483
		2	0.50056		0.53312	0.50512	0.50214
		3	0.51126		0.53354	0.50512	0.50593
		4	0.40036		0.42022	0.40303	0.39974
	7	1	0.49915		0.45774	0.44835	0.44656
		2	0.51582		0.47722	0.47133	0.46954
		3	0.52464		0.487	0.47829	0.47704
		4	0.50871		0.48154	0.4688	0.46691
	14	1	0.50900		0.46953	0.46018	0.45918
		2	0.54225		0.52217	0.51439	0.51138
		3	0.60110		0.56205	0.55314	0.55099
		4	0.51348		0.65345	0.50865	0.5018
Ethanol	3	1	0.51449		0.51496	0.51531	0.51377
		2	0.52073		0.52125	0.52169	0.52017
		3	0.52008		0.52018	0.52077	0.51946
		4	0.58415		0.58422	0.58474	0.58299
	7	1	0.48289		0.48393	0.48454	0.48275
		2	0.59454		0.59833	0.59606	0.59425
		3	0.47859		0.47995	0.48025	0.47878
		4	0.51280		0.51442	0.51472	0.513
	14	1	0.47920		0.48331	0.48158	0.48005
		2	0.49520		0.49961	0.49884	0.49657
		3	0.42256		0.42599	0.42486	0.42345
		4	0.51770		0.52425	0.52259	0.51888
Water	3	1	0.51640	0.52589	0.51719	0.51791	0.5166
		2	0.51389	0.51696	0.51437	0.51545	0.51399
		3	0.58875	0.59141	0.58915	0.59023	0.58867
		4	0.48400	0.48671	0.48461	0.48552	0.48446
	7	1	0.51700	0.52091	0.51729	0.51886	0.51753
		2	0.51340	0.51901	0.51444	0.51527	0.51413
		3	0.52546	0.52669	0.52609	0.527	0.5258
		4	0.51400	0.51794	0.51469	0.51559	0.51437
	14	1	0.59258	0.60331	0.59369	0.59446	0.5931

		2	0.51738	0.52885	0.51916	0.5197	0.51803
		3	0.60047	0.60552	0.60224	0.60288	0.60106
		4	0.51891	0.52225	0.52019	0.52046	0.51919

Apendix 1: Raw data from weight measurements of Invisalign aligners in all three solvents at three time points

Aligner Material	Solvent	Number in Quadruplicate	M1 (g)	M2 (g)	M3 (g) after 1 month from removal from solvent	M3 (g) after 2 months from removal from solvent	M3 (g) after 3 weeks of desiccation
Smart Moves	Dichloromethane	1	0.64591		0.60581	0.58632	0.58318
		2	0.64468		0.60092	0.58155	0.57868
		3	0.65956		0.70095	0.68728	0.68409
		4	0.70908		0.67253	0.64685	0.6436
	Ethanol	1	0.67416		0.67689	0.67609	0.67481
		2	0.70143		0.70414	0.70346	0.70212
		3	0.70218		0.70529	0.70445	0.70316
		4	0.65092		0.65401	0.65307	0.65188
	Water	1	0.61385	0.61674	0.61424	0.61484	0.61362
		2	0.67558	0.67882	0.67621	0.67692	0.6754
		3	0.71919	0.72266	0.71995	0.72065	0.7191
		4	0.726	0.72954	0.72671	0.72736	0.7257
Clear Correct	Dichloromethane	1	0.28029		0.29749	0.28942	0.2882
		2	0.46806		0.51568	0.49944	0.49494
		3	0.27283		0.28968	0.28085	0.27974
		4	0.60798		0.66693	0.64627	0.6413
	Ethanol	1	0.42531		0.44699	0.44144	0.43812
		2	0.46209		0.48591	0.47964	0.47615
		3	0.2809		0.28129	0.28123	0.28087
		4	0.42906		0.45024	0.44475	0.44154
	Water	1	0.39848	0.40219	0.39845	0.39907	0.39794
		2	0.29947	0.30392	0.29932	0.30009	0.29912
		3	0.44551	0.46507	0.44766	0.44818	0.44475
		4	0.30312	0.30804	0.30301	0.30356	0.30274
Durasoft	Dichloromethane	1	0.82551		0.26164	0.26217	0.26161
		2	0.74765		0.22628	0.22712	0.22652
		3	0.79212		0.253	0.2535	0.25274
		4	0.76088		0.23868	0.23934	0.2389
	Ethanol	1	0.80481		0.8042	0.80469	0.80252
		2	0.71616		0.71529	0.71581	0.714

		3	0.8147		0.81389	0.81456	0.81228
		4	0.73838		0.73755	0.73817	0.73629
	Water	1	0.84259	0.852	0.84526	0.84634	0.84331
		2	0.77446	0.78277	0.77688	0.7786	0.77514
		3	0.87805	0.88866	0.88089	0.88189	0.87867
		4	0.739	0.74704	0.74125	0.74222	0.73959

Apendix 2: Raw data from weight measurements of Smart Moves, Clear Correct and Durasoft aligners in all three solvents at the 14 day time point