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Original Article

In Vitro Evaluation of Leaching from Various Clear Aligner Systems

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ABSTRACT

This study aimed to evaluate and compare the leaching behavior of four clear aligner systems: Invisalign®, Eon®, SureSmile®, and Clarity®. For each system, three sets of aligners were obtained, sectioned, and immersed in glass vials containing various ethanol–water mixtures: 100% ethanol, 75% ethanol/25% water, 50% ethanol/50% water, 25% ethanol/75% water, and 100 percent water. The samples were incubated at 37 °C for two weeks. Leached compounds were analyzed using gas chromatography–mass spectrometry (GC-MS), revealing a total of eleven chemical substances. Benzene-1,3-bis(1,1-dimethylethyl) was consistently detected across all four aligner systems in the 100 percent and 75 percent ethanol solutions. No statistically significant differences were observed among the systems regarding the confirmed leaching, except for Eon®, which showed significant variation in the number of leached substances across different immersion solutions. Overall, the systems demonstrated variable leaching levels, with Invisalign® exhibiting the lowest and Eon® the highest number of leached chemicals. Importantly, none of the aligners released detectable amounts of bisphenol-A (BPA).

Keywords: Gas chromatography–mass spectrometry, Clear aligners, Leaching, Benzene, Chemical compound

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Introduction

Leaching is the process by which compounds migrate from a solid material into surrounding fluids, serving as a crucial indicator of the material's biocompatibility [1]. The oral cavity represents a particularly complex environment due to the continuous presence of microorganisms, their metabolic byproducts, and salivary enzymes, all of which can contribute to the degradation of materials in contact with oral tissues. Water, for instance, acts as a plasticizer in polymeric materials by disrupting intermolecular forces and promoting chemical breakdown, while higher temperatures, mechanical stress, and enzymatic activity can further accelerate this degradation [2].

In recent years, clear aligner therapy has gained widespread use, supported by the development of multiple systems from different manufacturers [3]. Align Technology leads the market with Invisalign®, made from SmartTrack® material (Invisalign; Align Technology, Santa Clara, CA, USA) [4], whereas Eon® aligners, introduced by Eon® Holdings in 2011, are fabricated from medical-grade polyurethane [5]. 3M™ launched the Clarity® system in 2018, characterized by a strong and nearly invisible polymer [6], and SureSmile® aligners, developed by Dentsply-Sirona in 2019, are produced from Essix® plastic, a thermoformed polyurethane [7]. Except for Invisalign®, the potential for leaching from these aligners has not been thoroughly explored [8]. Considering that the acrylic resins used in dentures and removable orthodontic appliances share similar chemical features with clear aligner polymers, previous findings regarding the biological risks of these materials remain relevant. Residual monomers in denture acrylics, for example, have been reported to adversely affect oral tissues [9]. The dynamic oral environment, with its temperature fluctuations,

variable pH, mechanical forces, and enzymatic and microbial activity, further facilitates leaching into saliva [10–12].

A variety of polymers are employed in orthodontics, including polycarbonate, polyurethane, polyethylene, polyamides, and polymethyl methacrylate [13]. Polyurethane serves as the primary component of transparent trays and clear aligners, yet it is not chemically inert [8]. Polymers consist of chains of organic units connected by urethane linkages [14], and one drawback of synthetic polymers is the release of residual monomers into saliva, which may trigger adverse biological reactions [15]. Thavarajah and Thennukonda [16] suggested that these leached substances could accumulate over time, potentially eliciting allergic, anaphylactic, or nonspecific responses. The extent of leaching depends on monomer content, polymerization method, and storage conditions, and high concentrations of leached compounds may induce inflammation, irritation, or allergic reactions in oral tissues [9, 17].

In addition to degradation, toxicity in polymeric appliances can stem from additives, contaminants, or incomplete polymerization, which can leave behind monomers, oligomers, low-molecular-weight polymer fragments, catalysts, solvents, or incorporated chemicals such as bisphenol-A (BPA) [18]. BPA has raised concerns due to its potential biological effects, prompting the World Health Organization to release a detailed toxicity report in 2010. Despite these risks, BPA is often incorporated into polymers to enhance transparency and mechanical strength, properties essential for appliance fabrication [19, 20].

This study was designed to investigate and compare the leaching profiles of four different clear aligner systems—Invisalign®, Eon®, SureSmile®, and Clarity®—using gas chromatography–mass spectrometry (GC-MS). The null hypothesis posits that no significant differences in leaching exist among these aligner materials.

Materials and Methods

Three sets of maxillary and mandibular aligners were obtained from four manufacturers—Invisalign®, Eon®, SureSmile®, and Clarity®—and cut into uniform 5 × 5 mm squares. Each sample was placed in separate glass vials containing alcoholic solutions to accelerate material degradation. Five immersion media were used: 100% ethanol, 75% ethanol with 25% water, 50% ethanol with 50% water, 25% ethanol with 75% water, and 100% water as the control. Samples were incubated at 37 °C for two weeks, with daily shaking at 150 rpm for five minutes to simulate accelerated aging.

Leached substances were analyzed using gas chromatography–mass spectrometry (GC-MS). The system comprised an Agilent 7890A GC coupled with a 5975 C single quadrupole mass spectrometer. Separation was achieved with an Agilent HP 5MS column (30 m × 0.25 mm × 0.25 µm) using high-purity helium at 1 mL/min. The injector temperature was set to 280 °C with a 20:1 splitless mode. MS source and quadrupole temperatures were 230 °C and 150 °C, respectively. The oven program began at 50 °C for 1 min, ramped to 150 °C at 25 °C/min (1 min hold), and then increased to 300 °C at 25 °C/min (1 min hold). The mass range was 40–600 m/z with 70 eV electron energy and a solvent delay of 3 min. Compounds were identified by matching spectra against the NIST 2008 library [21]. Analysis of each sample required approximately 13 minutes. This study was approved by the Institutional Review Board, College of Medicine, King Saud University (#E-20-4759) and CDRC (#PR0112).

Statistical analysis

Quantitative GC-MS data for all aligner systems across different solution concentrations were processed using SPSS version 26.0 (IBM, Chicago, IL, USA). Descriptive statistics, including means, standard deviations, and frequencies, were calculated. Differences among aligner systems and solution concentrations were evaluated using one-way and two-way analysis of variance (ANOVA). All analyses were conducted by a single examiner and repeated twice to ensure reliability and reproducibility. A p-value ≤ 0.05 was considered statistically significant.

Results and Discussion

GC-MS analysis detected eleven distinct chemical compounds across the aligner samples **(Table 1)**. Most compounds were found in samples immersed in 100% ethanol, whereas only a single compound was detected in the 75 percent and 50 percent ethanol solutions. No leaching occurred in 25% ethanol or in 100% water (control). Among the systems, Eon® exhibited the highest number of leached compounds (seven compounds across two immersion media), followed by Clarity® (6 compounds across three solutions) and SureSmile® (five compounds across three solutions). The Invisalign® system showed the lowest leaching, with the same compound appearing in two immersion solutions **(Table 1 and Figure 1)**.

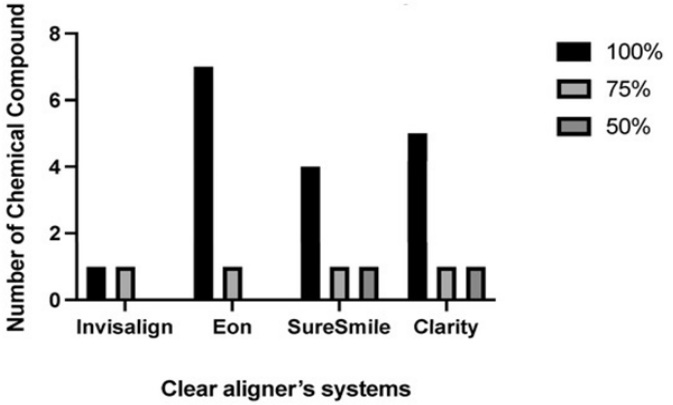

Figure 1. Number of leached chemicals among different systems at different concentrations.

Table 1. Detected and confirmed chemical compounds and their concentrations in relation to clear aligners' systems and immersion solution.

Immersion Solution Concentration	Chemical Compound	Substance Concentration %			
		Invisalign®	Eon®	Sure Smile®	Clarity®
100% Ethanol	Benzene, 1,3-bis(1,1-dimethylethyl)	42%	16.1%	37%	32%
	Phenol, 2,4-bis(1,1-dimethylethyl)	ND	11%	25%	16%
	Undecane, 4,6-dimethyl	ND	5.4%	ND	8%
	Heptadecane, 2,6,10,14-tetramethyl	ND	2.1%	6%	ND
	Octane, 3,5-dimethyl	ND	4.3%	ND	ND
	Nonadecane	ND	5.3%	ND	ND
	Dodecanoic acid, ethyl ester	ND	16%	ND	ND
	1-Octadecanesulphonyl chloride	ND	ND	8.1%	ND
	Methoxyacetic acid, 2-tridecyl ester	ND	ND	ND	8%
	Ether, hexyl pentyl	ND	ND	ND	7.6%
75% Ethanol	Benzene, 1,3-bis(1,1-dimethylethyl)	20.3%	74.2%	80%	58%
50% Ethanol	Phenol, 3,5-bis(1,1-dimethylethyl)	ND	ND	95%	94%

ND: Non-detectable chemical compound.

Benzene, 1,3-bis(1,1-dimethylethyl), was the only compound consistently identified across all four aligner systems in both 100 percent and 75 percent ethanol solutions. In 50% ethanol, Phenol, 3,5-bis(1,1-dimethylethyl), was detected in the SureSmile® and Clarity® systems, with relative abundances of 95 percent and 94 percent, respectively **(Table 1)**. The concentration of leached benzene was higher in 100% ethanol than in 75% ethanol, with all systems showing comparable levels. At 100 percent ethanol, SureSmile® exhibited the greatest benzene abundance, whereas Eon® had the highest levels at 75% ethanol **(Figure 2)**.

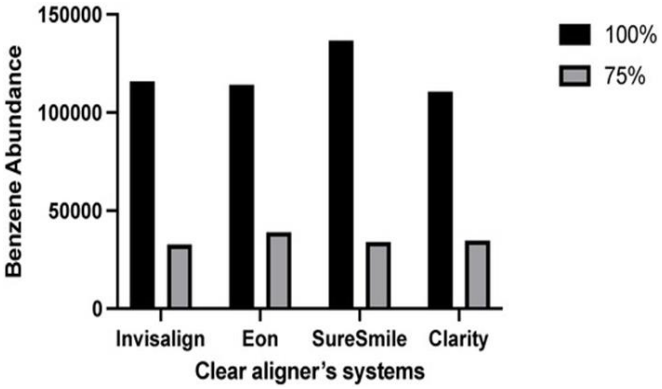

Figure 2. Abundance of leached benzene among different systems at different concentrations.

Leaching occurred in the tested aligner systems, with the extent varying according to the composition of the immersion solution **(Table 2)**. Statistically significant differences in the number of leached compounds across solutions were observed exclusively in the Eon® system **(Table 3)**. Specifically, Eon® released seven different chemical compounds when immersed in 100% ethanol, whereas only benzene was detected in 75% ethanol, and no leaching was evident in 50% ethanol. For Invisalign®, SureSmile®, and Clarity®, the quantity of leached substances remained consistent and did not vary significantly with solution concentration. Across all aligner systems, bisphenol-A (BPA) was absent.

Table 2. Comparison of different systems at variable immersion solution concentrations.

Immersion Solution Concentration	Aligner's System	N	Mean	Std. Deviation	Std. Error	F	p-Value	95% Confidence Interval	
								Lower Bound	Upper Bound
100% Ethanol	Invisalign®	11	10,535.82	34,943.36	10,535.82	0.961	0.42	−12,939.45	34,011.08
	Eon®	11	39,547.09	43,964.78	13,255.88			10,011.15	69,083.03
	SureSmile®	11	24,809.27	46,290.84	13,957.21			−6289.34	55,907.88
	Clarity®	11	22,472.73	34,562.63	10,421.02			−746.76	45,692.22
75% Ethanol	Invisalign®	11	2981.27	9887.76	2981.27	0.006	0.999	−3661.42	9623.96
	Eon®	11	3548.27	11,768.29	3548.27			−4357.77	11,454.32
	SureSmile®	11	3093.64	10,260.43	3093.64			−3799.42	9986.69
	Clarity®	11	3164.82	10,496.51	3164.82			−3886.84	10,216.47
50% Ethanol	Invisalign®	11	0.00	0.00	0.00	0.667	0.577	0.00	0.00
	Eon®	11	0.00	0.00	0.00			0.00	0.00
	SureSmile®	11	3137.64	10,406.36	3137.64			−3853.45	10,128.73
	Clarity®	11	2966.91	9840.12	2966.91			−3643.78	9577.59

Table 3. Comparison of different systems at variable immersion solution concentrations.

Aligner's System	Immersion Solution Concentration		Mean Difference	Std. Error	F	p-Value	95% Confidence Interval	
							Lower Bound	Upper Bound
Invisalign®	100%	75%	7554.55	8940	0.738	0.487	−14,486	29,595
		50%	10,535.82	8940			−11,504	32,576
	75%	100%	−7554.55	8940			−29,595	14,486
		50%	2981.27	8940			−19,059	25,021
	50%	100%	−10,535.82	8940			−32,576	11,504
		75%	−2981.27	8940			−25,021	19,059
Eon®	100%	75%	35,998.81818 *	11204	7.627	0.002 *	8377	63,621
		50%	39,547.09091 *	11204			11,925	67,169
	75%	100%	−35,998.81818 *	11204			−63,621	−8377
		50%	3548.27	11204			−24,074	31,170
	50%	100%	−39,547.09091 *	11204			−67,169	−11,925
		75%	−3548.27	11,204			−3,1170	24,074
SureSmile®	100%	75%	21,715.64	11,950	2.197	0.129	−7745	51,177
		50%	21,671.64	11,950			−7789	51,133
	75%	100%	−21,715.64	11,950			−51,177	7745
		50%	−44.00	11,950			−29,505	29,417

	50%	100%	−21,671.64	11,950			−51,133	7789
		75%	44.00	11,950			−29,417	29,505
Clarity®	100%	75%	19,307.91	9217	2.956	0.067	−3413	42,029
		50%	19,505.82	9217			−3215	42,227
	75%	100%	−19,307.91	9217			−42,029	3413
		50%	197.91	9217			−22,523	22919
	50%	100%	−19,505.82	9217			−42,227	3215
		75%	0	9217			−22,919	22,523

*: Statistically significant < 0.05.

Evaluating material leaching is crucial because it directly reflects potential toxicity and inversely correlates with the safety and biocompatibility of a material. This study examined the leaching behavior of clear aligners produced by four manufacturers: Invisalign®, Eon®, SureSmile®, and Clarity®. Using GC-MS analysis, eleven distinct chemical compounds were identified. Among these, benzene, 1,3-bis(1,1-dimethylethyl), was consistently detected across all systems in both 100 percent and 75 percent ethanol solutions, while it was absent in 50% ethanol and lower concentrations. As shown in **Table 1 and Figure 1**, the majority of leached compounds appeared in 100% ethanol. In 75% ethanol, benzene was present in all systems, whereas 50 percent ethanol yielded phenol, 3,5-bis(1,1-dimethylethyl), in SureSmile® and Clarity® only. No leaching occurred in 25% ethanol or pure water, suggesting that alcohol concentrations below 50% are insufficient to induce significant degradation of the aligner materials.

These findings contrast with a 2004 study in which no leaching was observed from Invisalign® aligners immersed in 75% ethanol; no residual monomers or oxidative byproducts were detected in that experiment [8]. The other three aligner systems had not been previously evaluated for leaching. More recent research in 2016 reported leaching of residual monomers from certain clear aligners at 75% ethanol, noting that the amount of leached material varied among thermoplastic sheets and was inversely related to their biocompatibility [22].

In the current study, benzene was the only compound detected in all systems at both high ethanol concentrations, which aligns with its common use in plastic manufacturing. The Centers for Disease Control and Prevention (CDC) has documented that benzene exposure can cause a range of biological effects, from skin irritation to cardiac arrhythmias, depending on the level and type of exposure [23]. The toxicity of benzene as a degradation product can vary with the polymer: it is considered highly or moderately toxic in relation to polyamide and polyvinyl chloride, respectively [18].

Although limited information is available on specific compounds leached from clear aligners, some of the detected chemicals are known human metabolites. For instance, undecane, 4,6-dimethyl, occurs naturally in human metabolism; heptadecane, 2,6,10,14-tetramethyl, is associated with cancer metabolism; and octane, 3,5-dimethyl, functions in both human and cancer metabolism. Dodecanoic acid ethyl ester has also been classified as a metabolite [24, 25], primarily detected in the Eon® system. Other compounds exhibit recognized biological hazards, such as nonadecane, commonly found in essential oils from Artemisia armeniaca, which the Globally Harmonized System (GHS) classifies as a dangerous aspiration hazard if ingested or inhaled [24, 26].

Certain hazardous compounds were detected in specific aligner systems. For example, 1-octadecanesulphonyl chloride appeared in SureSmile® at 100% ethanol, while phenol, 3,5-bis(1,1-dimethylethyl), was observed at 50% ethanol in both SureSmile® and Clarity®. According to GHS classification, these substances pose risks of skin corrosion, irritation, and eye damage, with severity dependent on factors such as concentration, contact duration, and exposure route [24, 25]. Phenol, 2,4-bis(1,1-dimethylethyl), identified as a bacterial, antioxidant, and marine metabolite, belongs to the phenol family and is recognized as a health and environmental hazard, capable of causing organ toxicity, skin irritation, and eye damage [24, 27]. Methoxyacetic acid, 2-tridecyl ester, a phytochemical, has reported cytotoxicity [28], while the toxicological profile of ether, hexyl pentyl, remains largely unexplored [29].

The experimental conditions used in this study may not fully replicate the complex environment of the oral cavity, where additional factors influence aligner degradation. Intraoral conditions subject these appliances to mechanical wear, chemical attrition, temperature fluctuations, and enzymatic activity, none

of which are entirely reproduced in vitro [8]. While the composition of the aligner material and the type of immersion solution significantly affect leaching, polymer-specific characteristics—such as degree of polymerization, molecular weight, and polymer density—also play a crucial role [22, 30]. Aging of aligners in the mouth differs from laboratory conditions because it involves dynamic chemical and mechanical interactions, including the effects of salivary enzymes, bacterial metabolites, abrasion, and pH variations [12]. Additionally, the strength of the immersion medium is a determinant factor; earlier studies commonly used 75% ethanol, considered a potent solvent, whereas this study tested both higher and lower ethanol concentrations, revealing that lower concentrations produced no detectable leaching [8, 22]. Although bisphenol-A (BPA) was not detected under the experimental conditions, the overall safety of these appliances remains a matter of discussion. Biomaterial toxicity is generally proportional to both the number of leached chemicals and their concentrations [22]. Among the systems tested, Invisalign® demonstrated the lowest leaching, with only benzene detected. In contrast, Eon® released seven distinct chemicals, representing the highest leaching potential, followed by Clarity® and SureSmile®, which leached six and five compounds, respectively, across three immersion concentrations.

Fortunately, clear aligners are typically in contact with oral tissues for short durations (approximately 7–14 days) compared to long-term orthodontic or retention devices, as patients are advised to replace their aligners every one to two weeks. Nevertheless, potential cumulative effects should not be overlooked. Well-designed clinical studies are warranted to assess leaching of aligner materials into body fluids such as saliva and urine during actual treatment.

Conclusion

All four aligner systems exhibited some degree of leaching when immersed in alcoholic solutions. Maximum leaching occurred in 100% ethanol, with a total of eleven chemical compounds identified, whereas no leaching was detected in solutions with ethanol concentrations below 50%. Invisalign® showed the lowest leaching, with benzene, 1,3-bis(1,1-dimethylethyl), present at both 100 percent and 75 percent ethanol. Conversely, the Eon® system exhibited the highest leaching, releasing seven different chemicals at both 100 percent and 75 percent ethanol. Importantly, none of the tested aligner systems released detectable amounts of BPA under the conditions of this study.

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Ethics Statement: None

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