

pH Evaluation over a Period of 6 months of Two-bottle Water-based Self-etching Primers: An *In Vitro* Study

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ABSTRACT

Aim and objective: The objective of this study was to monitor the pH stability over a period of 6 months of two-bottle self-etching (SE) primers.

Materials and methods: Four commercially available two-bottle SE primer solutions, Adhese SE (Ivoclar Vivadent; Schaan, Liechtenstein), Clearfil SE (Kuraray; Tokyo, Japan), Contax Primer (DMG; Hamburg, Germany), and Enabond SE (Micerium; Avegno (Ge), Italy), containing water as the main solvent were selected. The pH values during a 6-month storage period at intermittent temperature were daily measured by means of a microprocessor-based pH/temperature meter and hence recorded, tabulated, and analyzed.

Results: The mean values of overall pH measurements and standard deviation for Adhese SE, Clearfil SE, Contax Primer, and Enabond SE are, respectively, 1.70 (± 0.01), 1.72 (± 0.01), 1.20 (± 0.01), 1.80 (± 0.01). One-way ANOVA did not detect any significant change of the mean pH values of all measurements over time.

Conclusion: The conventional 6-months storage did not affect the pH values of the water-containing primers of two-bottle SE adhesives.

Clinical significance: This study provides evidence that conventional storage under adequate conditions did not affect the pH values of the water-containing primers of two-bottle SE adhesives with different monomer formulations. Clinicians may rely on the known pH value of the selected SE adhesive during shelf-life.

Keywords: Adhesive, pH, Primers, Self-etching.

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INTRODUCTION

The strategy of self-etching (SE) adhesives is to simultaneously etch and prime enamel and dentin, integrating the dissolved smear layer and the partially demineralized tissues into the adhesive interface.^{1,2} Currently SE adhesives are applied in single-step or two-steps, depending on the treatment modalities employed by the manufacturers.³ The degree of enamel and dentin demineralization is dependent on the pH of the priming solution, which is related to the composition and concentration of the acids and/or polymerizable acidic resin monomers.² In addition, the type of solvents and co-monomers plays a major role, as they affect the film forming properties and adhesive strength.⁴

A well-established classification for SE adhesives is according to their acidity.^{1,5} The pH value of the most SE primers ranges between 0.4 and 2.6, higher than that of the conventional 37% phosphoric acid (around 0.5).^{1,3,6,7} The aggressiveness of the SE adhesives has been classified based on the extent of hybridization at the dentin-adhesive interface. The SE adhesives have been graded as strong (pH ≤ 1), exhibiting a hybrid layer of some micrometers in depth; intermediately strong or moderate (pH: 1–2), possessing a hybrid layer depth of 1–2 μm ; mild (pH: 2–2.5), demonstrating an ~ 1 μm hybrid layer of ultra-mild (pH > 2.5), with a nanosized hybrid layer.^{1,8} Since recent studies have critically pointed out the vulnerable stability of SE adhesives as an intrinsic disadvantage, a shelf-life evaluation has been advocated to be fundamental to verify the materials' behavior over time.^{9,10} To the authors' knowledge, there is no information about any possible pH variation of these adhesives during shelf-life. Thus, the aim of the present investigation was to monitor the stability of the pH values of water-containing primers of two-step SE adhesive systems during a 6-month storage period at 4°C. The first null hypothesis tested was that the pH changes during the storage period. The second null hypothesis tested

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was that pH variation during storage period differs between the materials tested.

MATERIALS AND METHODS

Four commercially available two-bottle SE adhesives, Adhese SE (Ivoclar Vivadent; Schaan, Liechtenstein), Clearfil SE (Kuraray; Tokyo, Japan), Contax Primer (DMG; Hamburg, Germany), and Enabond SE (Micerium; Avegno (Ge), Italy), were selected according to the presence of water as the main solvent in the SE primer components. Four bottles of each adhesive system were purchased from the suppliers and only the SE primer components were used as test materials. The dental adhesives monitored and their compositions

Table 1: Selected materials and their composition according to the information supplied in the safety data sheets and manufacturers' instructions

SE primer	Manufacturer	Composition
Adhese SE	Ivoclar Vivadent; Schaan, Liechtenstein	Phosphonic acid acrylate, bis-acrylamide derivative, camphorquinone, water
Clearfil SE Bond 2	Kuraray; Tokyo, Japan	2-HEMA, 10-MDP, hydrophilic aliphatic dimethacrylate, camphorquinone, water
Contax	DMG; Hamburg, Germany	Carboxylic acid, sodium fluoride, water
Enabond SE	Micerium; Avegno (Ge), Italy	2-HEMA, 10-MDP, water

2-HEMA, 2-hydroxyethyl methacrylate; 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate

are listed in Table 1. Storage, experimental part, and data evaluation were performed in the Dental Clinic of the Department of Surgical and Diagnostic Sciences at the University of Genova, Italy.

Storage

During the storage period of 6 months, the SE adhesives were kept at 4°C in a refrigerator. To simulate clinical usage, we removed the primer bottles from the refrigerator on a daily basis and exposed to an ambient temperature (20–25°C) for 8 hours, before storing them back to the refrigerator.

pH Measurements

For pH measurements a microprocessor-based pH/temperature meter with a nominal accuracy of ± 0.01 pH and $\pm 0.4^\circ\text{C}$ (HI-8424, Hanna Instruments, Woonsocket, Rhode Island, United States) was used, equipped with a semi-micro electrode (HI-1330B) and a temperature probe (HI-7662) for automatic temperature compensation. Before use, the instrument was calibrated using two buffered solutions provided with the pH meter (pH 7.01 and pH 4.01). For each pH measurement, the electrode and probe were submerged into the specimen vials to be tested, cleaned after each measurement and stored according to manufacturer instructions.

The pH measurements were performed daily, with the exception of the festive days for 6 months from June 2020 to November 2020. Four readings per day ($n = 4$), with an interval of approximately 2 hours, were made for each SE primer solution and they were averaged and considered as daily value for statistical analysis. During the monitoring period (22 weeks, namely, 110 days), 440 measurements have been done for each group (meaning 110 averaged daily value for each SE primer). The first measurement of each group was performed immediately after being unpacked and was considered as the respective initial pH value, hence, baseline for the analysis of the present study.

Statistical Analysis

Statistical methods used to assess the change over time of pH values of each product are analysis of variance (one-way ANOVA) and Pearson's product moment correlation coefficient (PMCC). A significance level of 5% was adopted in all tests. Homoscedastic distribution of data was assessed using the Kolmogorov-Smirnov test. The software used for the analysis was the IBM SPSS Statistics for iOS, Version 25.0 (IBM Corp., Armonk, New York, United States).

RESULTS

The baseline pH values that were recorded prior to the mean value of everyday recording were Adhese SE 1.70, Clearfil SE 1.73,

Contax Primer 1.21, and Enabond SE 1.80. The average pH value of four repetitions ($n = 4$) at baseline (day one) was for each material the mean of overall pH measurements and standard deviation has been calculated: Contax Primer had the lowest mean pH value 1.20 (± 0.01) and Enabond SE had the highest one 1.80 (± 0.01). The mean pH values of Adhese SE and Clearfil SE were approximately the same, 1.70 (± 0.01) and 1.72 (± 0.01), respectively.

Mean pH values, 95% confidence interval (CI), linear trends as well as PMCC and one-way ANOVA are represented in Figure 1. One-way ANOVA did not detect any significant change of the mean pH values of all measurements over time when considering the pH values of each product: Adhese SE ($p = 0.261$), Clearfil SE Bond 2 ($p = 0.380$), Contax ($p = 0.281$), Enabond SE ($p = 0.597$). Moreover, the correlation study (time vs pH) detected negligible correlations for each product: Adhese SE ($r = 0.108$), Clearfil SE Bond 2 ($r = 0.084$), Contax ($r = 0.104$), Enabond SE ($r = 0.051$). Homoscedastic distribution of data using the Kolmogorov-Smirnov test reported that each product was normally distributed: Adhese SE ($p = 0.179$), Clearfil SE Bond 2 ($p = 0.149$), Contax ($p = 0.174$), Enabond SE ($p = 0.188$).

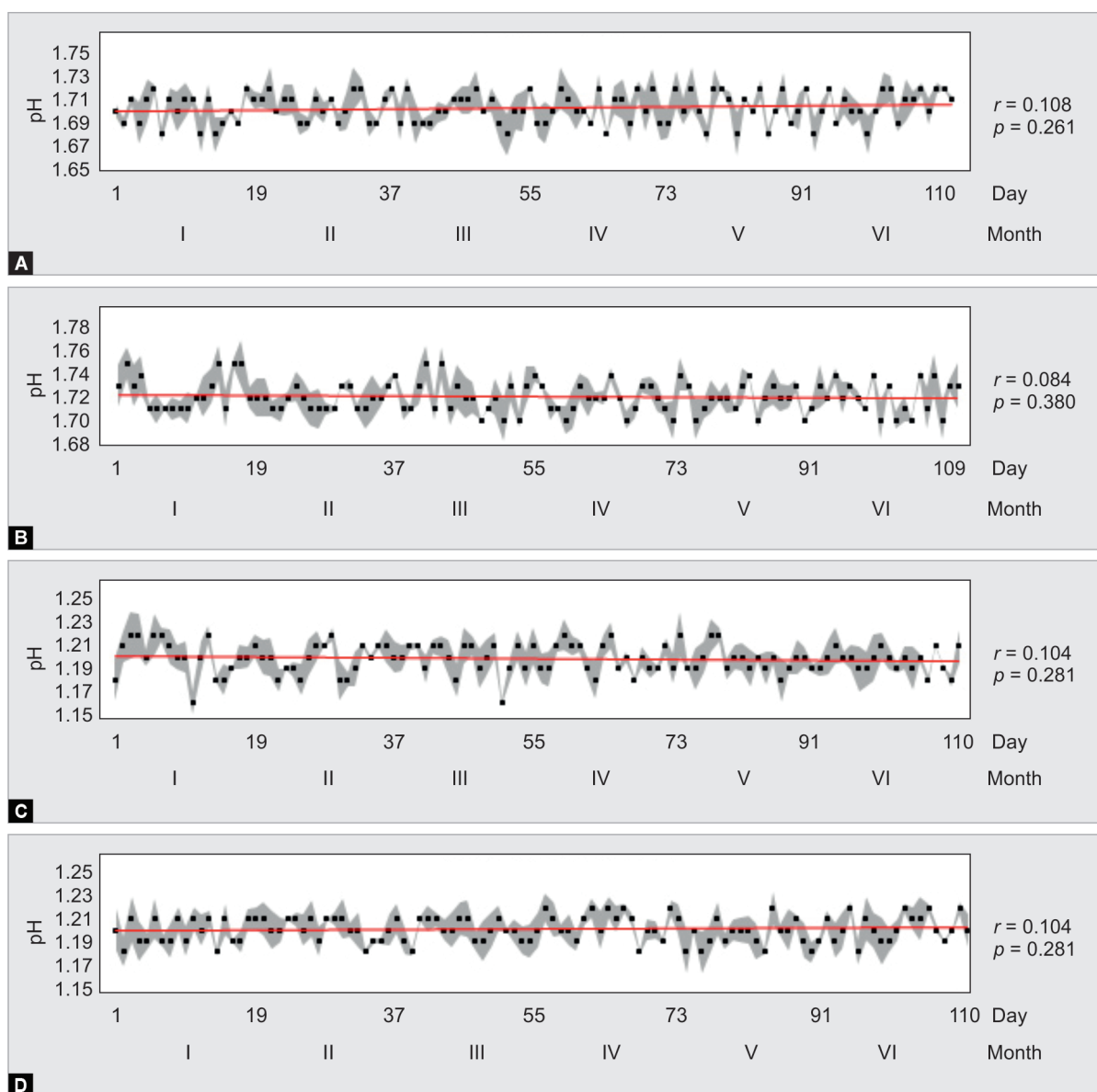
Based on the aim and objectives of the study, no statistical inference was observed, as the study is merely descriptive.

DISCUSSION

In daily practice the clinicians apply SE adhesive strategy being aware of the known pH value of the selected SE adhesive and of the corresponding interaction with enamel and dentin. Morphological studies demonstrated that enamel etching pattern and the degree of demineralization and interaction with dentin are correlated with the acidity of the SE primers.^{1,11–13} This implies that it is reasonable to speculate that a possible variation of the pH during shelf-life may affect the conditioning pattern and bonding performance on dental substrates.¹⁴

Based on the experimental design and the findings of the present study, storage duration and conditions did not influence the pH value of the tested SE primers. Although a 2-year shelf-life is commonly recommended for SE adhesives, in the current study, the testing period was limited to 6 months reflecting the actual clinical conditions.¹⁵ As these adhesives are consumed at a fast rate by most clinicians, a longer storage time has been considered clinically not corresponding to real life conditions.¹⁵

No significant pH differences were identified in any SE primers as a function of time. This leads to rejection of the first null hypothesis that the pH of the tested materials changes during the storage period. The current study, however, found fluctuations in the pH for all evaluated SE adhesives during recording period. It is worth mentioning that in multiple pH measurements realistic



Figs 1A to D: Mean pH values \pm 95% CI and PMCC of (A) Adhese SE; (B) Clearfil SE Bond 2; (C) Contax; (D) Enabond SE. One-way ANOVA p -value and PMCC r are reported for each product

accuracies of between ± 0.03 and ± 0.05 pH units can be achieved even assuming adequate temperature compensation, with a well calibrated, properly cleaned, and stored electrode connected to an accurate pH measuring instrument.¹⁶ Variations in measurement values have been observed in accurate pH meter and are related to several factors, such as limitations inherent in the electrode membrane, in the two-point calibration and temperature probe.¹⁷ Despite that apparent limitation of the pH measurement technology, pH deviations to the second decimal remaining close to the theoretical values are considered acceptable.

Moreover, despite the different formulations, insignificant mean pH variations have been observed between the SE primers during storage time. Therefore the second null hypothesis, which claims that the pH variation during storage period differs between the tested materials, should be rejected as well.

It is worth to mention that the Safety Data Sheet (Section 9: Physical and chemical properties) of the investigated materials, with the exception of Enabond SE, does not provide precise data about the pH; therefore, any direct comparison with the information provided by the manufacturer would be misleading. Moreover, to date no scientific data about any possible pH variation of SE adhesive primers during shelf-life are available.

Water-free (ethanol- or acetone-based) SE priming solutions were not included in this study, due to different volatility and dielectric constant of these solvents, which influence the dissociation behavior of acids.⁶ The SE adhesives included in the study were water-based primers. This type of primers usually contain 30–40% water, which ionizes the acidic functional monomers that attack the apatitic crystalline structure during conditioning.^{11,18} A possible evaporation of the solvent during storage time and

intermittent storage temperature or a hydrolytic degradation of the acidic monomers and co-monomers may change the original formulations leading to pH changes.^{9,10} Nevertheless, based on the results of the present study, it can be assumed that the extent of these phenomena should be limited, since the pH was not changed. Consequently, we may assume that the reactivity of the primers, regarding the demineralization capacity, at least, is not changed.

Accumulated evidence is showing that when the adhesives' shelf-life was tested by accelerated aging procedures, methacrylate monomers undergo rapid hydrolysis under acidic aqueous conditions.¹⁰ When water is mixed with the acidic monomers, a considerable amount of methacrylates is already decomposed by progressive acid-catalyzed hydrolysis of the ester bonds in the methacrylate monomers by water during the guaranteed shelf-life, especially if the material is stored under inadequate conditions.¹¹ Storage duration and conditions, such as temperature, which normally accelerates the degradation processes, greatly influence the hydrolytic stability.¹⁰ However, in a previous study of Nishiyama et al., the authors demonstrated that hydrolysis of functional methacrylate monomers occurs despite conservation carried out according to the manufacturers' instructions.¹⁵ The hydrolysis of the adhesive monomers completely changes the chemical composition and their physical properties.¹⁰

However, in our study design, direct correlation between degradation phenomena and pH stability was not detectable.

The experimental set-up used in this study had several limitations that deserve some comments. As already mentioned in the present investigation, only water-based SE priming solutions were tested. The results may have been different if SE primers with more volatile solvents, such as ethanol or acetone-based systems, had been investigated. Therefore, ideally, also ethanol- or acetone-based SE primers should be included in a foreseeable future study, to fully assess the suggested results. In addition, measuring the pH only is not sufficient to monitor the hydrolytic degradation of functional methacrylate monomers under acidic aqueous conditions. A lack of data assessing a direct correlation between degradation phenomena of functional monomers and pH stability encourages future investigations on this matter. The study limitations include the fact that an absolute worst case scenario was tested, by leaving the samples to an ambient temperature (20–25°C) for 8 hours on a daily basis. These limitations should be considered when interpreting the results.

CONCLUSION

Within the experimental limitations of this study, it is possible to conclude that the storage time under appropriate conditions does not affect primers' pH values of two-bottle SE adhesives. Whereas the pH stability over time of SE priming solutions is not influenced by different formulations.

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