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Color stability of a composite resin modified by ZnO and TiO_2 antibacterial nanoparticles

Estabilidade de cor de uma resina composta modificada por nanopartículas antibacterianas de ZnO e TiO_2

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ABSTRACT

Objective: To evaluate the color stability of a composite resin modified with ZnO and TiO_2 nanoparticles (both pure and silver decorated), synthesized via polymeric precursor and hydrothermal methods. **Material and Methods:** Filtek™ Z350 XT resin was modified with 2 wt% of nanoparticles, specimens were prepared (n=180), and photoactivated. Specimens were immersed in coffee and artificial saliva; color changes were measured spectrophotometrically, converted to NBS units, and analyzed using repeated measures ANOVA ($p<0.001$). **Results:** Coffee immersion caused the greatest discoloration, with the unmodified resin reaching $\Delta E^* = 15.48$ after 90 days. Resins containing ZnO/Ag and TiO_2/Ag exhibited even higher color instability ($\Delta E^* = 23.14$ and $\Delta E^* = 18.71$, respectively). **Conclusion:** While ZnO and TiO_2 tested nanoparticles may offer antimicrobial advantages, their incorporation, particularly in silver-decorated forms, can negatively impact the color stability of composite resins exposed to chromogenic agents like coffee.

KEYWORDS

Coloring agents; Composite resins; Nanoparticles; Titanium; Zinc oxide.

RESUMO

Objetivo: Avaliar a estabilidade da cor de uma resina composta modificada com nanopartículas de ZnO e TiO_2 (puras e decoradas com prata), sintetizadas pelos métodos de precursor polimérico e hidrotermal. **Materiais e Métodos:** A resina Filtek™ Z350 XT foi modificada com 2% em peso de nanopartículas, preparados os corpos de prova (n=180) e fotoativados. As amostras foram armazenadas em café e saliva artificial, e as alterações de cor foram mensuradas por espectrofotometria, convertidas em unidades NBS e analisadas estatisticamente por ANOVA de medidas repetidas ($p<0,001$). **Resultados:** A imersão em café induziu as maiores alterações cromáticas, com a resina não modificada atingindo $\Delta E^* = 15,48$ após 90 dias. As resinas contendo ZnO/Ag e TiO_2/Ag também demonstraram instabilidade de cor significativa ($\Delta E^* = 23,14$ e $\Delta E^* = 18,71$, respectivamente). **Conclusão:** As nanopartículas de ZnO e TiO_2 testadas podem conferir propriedades antimicrobianas, porém sua incorporação, especialmente nas formas decoradas com prata, pode comprometer a estabilidade da cor de resinas compostas expostas a agentes cromogênicos, como o café.

PALAVRAS-CHAVE

Agentes corantes; Resinas compostas; Nanopartículas; Titânio; Óxido de zinco.

INTRODUCTION

Conventional composite resins exhibit low to no antibacterial activity. To enhance this property, metal and metal oxide nanoparticles (NPs), such as silver (Ag), zinc oxide (ZnO) and titanium dioxide (TiO_2) have been incorporated into composite resin formulations [1-10]. Although composite resins are the primary restorative material used in clinical practice to replicate the natural aesthetics of teeth, their major drawback is the susceptibility to discoloration [11-14].

Furthermore, the inclusion of NPs as filler content in composite resins can affect their physical properties, including color stability [15-18]. While previous studies have demonstrated the antibacterial efficacy of NP-modified composite resins, color stability appears to be adversely affected [16-19].

This study aimed to evaluate the color stability of a commercially available composite resin when modified with Ag, ZnO and TiO_2 NPs synthesized using different chemical methods. The null hypothesis was that the inclusion of these NPs would not significantly alter the color stability of the composite resin.

MATERIALS AND METHODS

Experimental design

This study is an in vitro experimental investigation. The dependent variable was color stability, while the independent variables were the immersion medium and the types of nanoparticles used.

Characteristics of nanoparticles

The NPs used in this study were synthesized at the Research Group of Nanomaterials and Advanced Ceramics of the São Carlos Physics Institute (IFSC), University of Sao Paulo (USP), São Carlos, Brazil. Data regarding the synthesis (polymeric precursor and hydrothermal methods), size and surface area of ZnO (zinc oxide), TiO_2 (titanium dioxide), ZnO/Ag (Ag-doped ZnO) and TiO_2/Ag (Ag-TiO_2) are summarized in Table I.

Specimen preparation

The unmodified Z350 XT resin composite resin was used to prepare the control group. For the experimental groups, the NPs were incorporated into the composite resin (at a weight percentage defined by compositions that demonstrated good antibacterial activity without sacrificing the mechanical properties in preliminary studies) via manual mixing for one minute using a metal spatula and a glass slab. A total of 180 specimens were prepared (20 per group: 10 specimens for solution 1 and 10 specimens for solution 2) using Filtek™ Z350 XT (3M do Brasil), a nanofilled composite resin in color A2 Body. Table II summarizes the composition of the composite resin matrix and its inorganic fillers.

Table I - Features of the nanoparticles used in this study

Material	Synthesis method	Surface area BET (m^2/g)	Diameter by BET (nm)
ZnO	**Polymeric Precursor method**	2.18	491
ZnO/Ag	**Polymeric Precursor method**	8.60	125
TiO_2	**Polymeric Precursor method**	58.02	24
TiO_2/Ag	**Polymeric Precursor method**	56.26	25
ZnO	**Hydrothermal**	40.57	26
ZnO/Ag	**Hydrothermal**	40.72	26
TiO_2	**Hydrothermal**	322.58	4
TiO_2/Ag	**Hydrothermal**	447.60	3

Table II - Composite resin used

Nanofilled RBC	Batch number	Manufacturer	Resin Matrix	Filler	Filler, weight/ volume
Filtek™ Z350 XT	726707	3M do Brasil Ltda. Sumaré, SP.	TEGDMA, UDMA, BIS-EMA	Combination of nonaggregated 20 nm silica, nonaggregated 4-11 nm zirconia, and aggregated zirconia/silica cluster filler	78.5/59.5

Table III - Control and experimental groups: composition and characteristics

Group	Material and composition in % weight (wt.)	Code
Control	**Z350 XT**	**Z350**
Experimental	**Z350 XT + ZnO (PP) 1% (wt.)**	ZP
	Z350 + ZnO/Ag (PP) 2% (wt.)	AZP
	Z350 + TiO_2 (PP) 2% (wt.)	TP
	Z350 + TiO_2/Ag (PP) 2% (wt.)	ATP
	Z350 + ZnO (HY) 2% (wt.)	ZH
	Z350 + ZnO/Ag (HY) 2% (wt.)	AZH
	Z350 + TiO_2 (HY) 2% (wt.)	TH
	Z350 + TiO_2/Ag (HYD) 2% (wt.)	ATH

Note: PP: Polymeric Precursor Synthesis; HY: Hydrotermal Synthesis.

The specimens were prepared by placing a single increment of composite resin into a metallic matrix (10 mm in diameter and 2 mm in thickness), which was covered with a transparent polyester strip and a glass plate. Another polyester strip and glass plate were placed on the opposite side of the matrix. The composite resin was then light-cured by using a LED LCU (Radii Plus, SDI, 440–480 nm wavelength, 1500 mW/cm^2 light intensity, SDI Limited Australia, Anvisa: 10282499002) for 40 seconds.

Table III presents the control and experimental groups. These groups were defined based on compositions that demonstrated adequate antibacterial activity in prior investigations.

Color measurements

Color measurements were recorded using a spectrophotometer (Minolta, CM 2600d) at the times: 1, 7, 14, 28 and 60 days after immersion in tested solutions. The modified and unmodified composite resin specimens by the NPs were immersed in two solutions: coffee (Pilão Tradicional, Jacob Douwe Egberts, Jundiaí, SP, Brazil) and artificial saliva (Arte & Ciência, Araraquara, SP, Brazil, pH 7.0)

Before each color measurement, the specimens were washed and cleaned with distilled water and gauze. The spectrophotometer was then positioned to measure color according to the CIEL*a*b* (Commission Internationale de l'Eclairage).

The color difference (ΔE*) was calculated using the equation:

$$\Delta E = \left[\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2 \right]^{1/2} \quad (1)$$

The data were then converted to the National Bureau of Standards (NBS) units to the ΔE* values to clinical relevance using the equation:

$$NBS\ units = \Delta E^* \times 0.92 \quad (2)$$

where critical remarks of color differences were expressed in terms of NBS units. NBS units classify color changes as follows: 0 to 0.5 = trace (extremely slight changes); 0.5 to 1.5 = slight (slight changes); 1.5 to 3.0 = noticeable (perceivable changes); 3.0 to 6.0 = appreciable (marked changes); 6.0 to 12.0 = much (extremely marked changes) and 12.0 or more = very much (change to another color).

Statistical analysis

The data were analyzed using IBM SPSS Statistics 20.0 (SPSS Inc. Chicago, USA) software. A mixed-model repeated measures ANOVA was employed to estimate the effects within-subject's factors (repeated measurements over time) and between- subject's factors (solutions) on ΔE* parameters, with a significance level of 5%. The Mauchly test was used to assess the assumption of sphericity (equality of variances in repeated measures ANOVA). Pairwise comparisons and contrasts were examined using a post hoc test for repeated measures, with Bonferroni adjustment applied.

RESULTS

To evaluate the influence of the type of nanoparticle type and immersion medium on color stability, a mixed-model repeated measures ANOVA was performed. The analysis revealed that the assumption sphericity was violated for both artificial saliva (Mauchly's W = 0.059;

$p < 0.001$) and coffee (W = 0.018; $p < 0.001$), leading to the application of Greenhouse–Geisser corrections for degrees of freedom.

Regarding all experimental groups, including those modified with ZnO, ZnO/Ag, TiO_2, and TiO_2/Ag nanoparticles, the overall analysis demonstrated a statistically significant effect of time ($p < 0.001$), solution ($p < 0.001$), and their interaction ($p < 0.001$) on ΔE* values. Trend analysis confirmed that color change progressed linearly over time for almost all groups, as illustrated in Figures 1 and 2. Specimens stored in coffee showed a rapid and progressive increase in ΔE*, regardless of nanoparticle type or synthesis method. In contrast, specimens stored in artificial saliva exhibited only mild increases over time, with color changes remaining below clinical thresholds for most groups. Notably, materials modified by hydrothermal synthesis displayed more aggressive color changes over time, even in saliva.

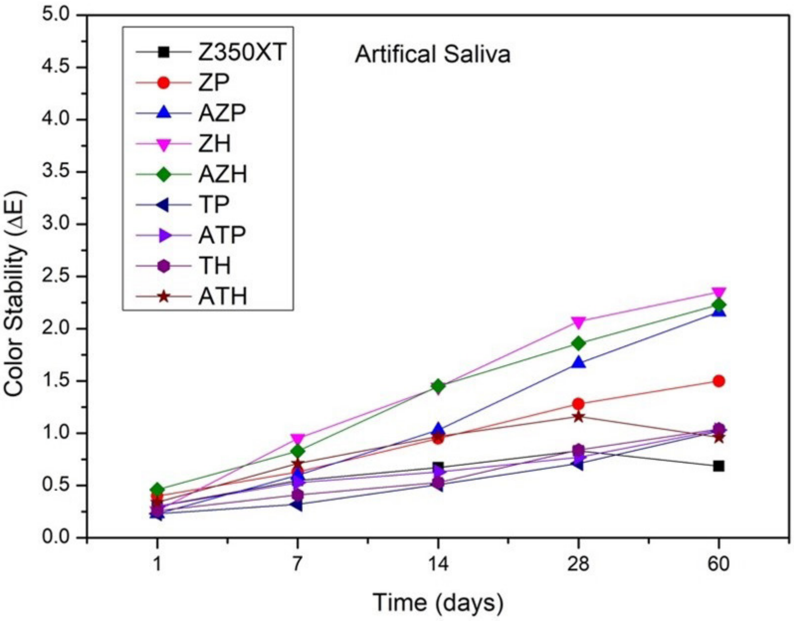

Figure 1 - Linear trends in color changes of modified and unmodified resin (for all tested NPs and synthesis) stored in artificial saliva. (ZP: ZnO via polymeric precursor; AZP: ZnO/Ag via polymeric precursor; ZH: ZnO via hydrothermal; AZH: ZnO/Ag via hydrothermal; TP: TiO_2 via polymeric precursor; ATP: TiO_2/Ag via polymeric precursor; TH: TiO_2 via hydrothermal; ATH: TiO_2/Ag via hydrothermal).

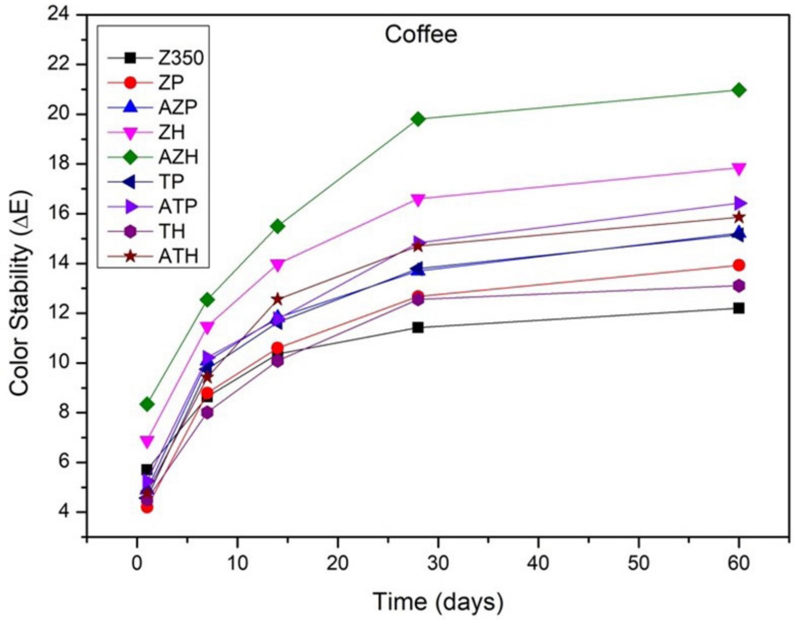

Figure 2 - Linear trends in color changes of modified and unmodified resin (for all tested NPs and synthesis) stored in coffee. (ZP: ZnO via polymeric precursor; AZP: ZnO/Ag via polymeric precursor; ZH: ZnO via hydrothermal; AZH: ZnO/Ag via hydrothermal; TP: TiO_2 via polymeric precursor; ATP: TiO_2/Ag via polymeric precursor; TH: TiO_2 via hydrothermal; ATH: TiO_2/Ag via hydrothermal).

A detailed comparison of the final main data (after 90 days of immersion) is presented in Table IV (for specimens containing NPs synthesized via hydrothermal method) and Table V (for specimens containing NPs synthesized via polymeric precursor method). Detailed data for all experimental groups, including measurements at all time points and specimens containing nanoparticles synthesized via both hydrothermal and polymer precursor methods, are provided in the Supplementary Material.

In coffee solution, the unmodified composite resin reached a ΔE* of 15.48 (95% CI: 14.61–16.35), classified as a “very much” color change according to NBS parameters, as demonstrated in Table IV. The modified resins containing hydrothermally synthesized ZnO/Ag and TiO_2/Ag exhibited even higher discoloration, with ΔE* values of 23.14 and 18.71, respectively, both exceeding the NBS threshold for complete color change. In artificial saliva, color stability was maintained across most groups. The control group showed a final ΔE* of 0.64 (CI: 0.37–0.89), considered a “slight” change. Among the experimental groups, ZnO/Ag (hydrothermal) exhibited the highest variation (ΔE = 2.77; CI: 2.51–3.03), still classified as “noticeable”. Luminosity (ΔL) measurements followed similar trends, with significant brightness loss observed in groups exposed to coffee, particularly in those containing Ag-doped nanoparticles (ΔL* = −6.94 for ZnO/Ag hydrothermal group, CI: −8.12 to −5.76).

The results obtained from the polymer precursor synthesis method revealed that TiO_2/Ag nanoparticles exhibited the highest color change in coffee solution (ΔE* = 19.06), classified as “total change” according to the NBS scale. ZnO/Ag also showed significant alteration (ΔE* = 17.76), while the control group presented slightly lower values (ΔE* = 15.48). In artificial saliva, all groups demonstrated minimal color variation, with ΔE* values below 3.0, indicating “trace” to

Table IV - Mean values of color stability (ΔE*), luminosity (ΔL*), 95% confidence interval (IC) and NBS color change of modified by hydrothermal NPs and unmodified composite resin, immersed in coffee and artificial saliva

Group	NP	Synthesis Method	Solutions	ΔE* (90 days)	IC 95% ΔE*	ΔL* (90 days)	IC 95% ΔL*	NBS (90 days)	Color change (NBS)
Z350 XT	—	—	Coffee	15.48	14.61 – 16.35	−5.81	(−6.33) – (−5.29)	14.24	very much
AZH	ZnO + Ag	Hydrothermal	Coffee	23.14	20.08 – 26.19	−6.94	(−8.12) – (−5.76)	21.28	very much
ATH	TiO_2 + Ag	Hydrothermal	Coffee	18.71	17.25 – 19.17	−6.33	(−6.91) – (−5.75)	17.21	very much
Z350 XT	—	—	Artificial Saliva	0.64	0.37 – 0.89	+0.09	(−0.13) – (+0.31)	0.59	slight
AZH	ZnO + Ag	Hydrothermal	Artificial Saliva	2.77	2.51 – 3.03	−0.87	(−1.22) – (−0.52)	2.55	noticeable
TH	TiO_2	Hydrothermal	Artificial Saliva	1.50	1.28 – 1.73	−0.31	(−0.56) – (−0.06)	1.39	slight

Table V - Mean values of color stability (ΔE *), luminosity (ΔL *), 95%, confidence interval (IC) and NBS color change of modified by polymeric precursor NPs and unmodified composite resin, immersed in coffee and artificial saliva

Group	NP	Synthesis Method	Solutions	ΔE* (90 days)	IC 95% ΔE*	ΔL* (90 days)	IC 95% ΔL*	NBS (90 days)	Color change (NBS)
Z350 XT	—	—	Coffee	15.48	14.61 – 16.35	−5.81	(−6.33) – (−5.29)	14.24	very much
AZP	ZnO + Ag	Polymeric precursor	Coffee	17.76	16.46 – 19.06	−6.94	(−7.61) – (−6.27)	16.35	very much
ATP	TiO_2 + Ag	Polymeric precursor	Coffee	19.06	18.10 – 20.02	−6.87	(−7.45) – (−6.29)	17.54	very much
Z350 XT	—	—	Artificial Saliva	0.64	0.37 – 0.89	−0.12	(−0.31) – (+0.07)	0.59	slight
AZP	ZnO + Ag	Polymeric precursor	Artificial Saliva	2.87	2.60 – 3.13	−0.87	(−1.22) – (−0.52)	2.64	noticeable
TP	TiO_2	Polymeric precursor	Artificial Saliva	1.44	1.21 – 1.66	−0.19	(−0.44) – (+0.06)	1.32	slight

"notable" changes. TiO_2/Ag and the control group showed the lowest ΔE* values in this medium (0.90 and 0.637, respectively), suggesting greater stability under physiological conditions. Regarding luminosity (ΔL), all specimens stored in coffee exhibited a marked decrease in luminosity, with TiO_2/Ag (−6.87) and ZnO/Ag (−6.94) showing the most pronounced reductions, indicating strong pigment absorption and surface alteration. In artificial saliva, ΔL* changes were minimal across all groups, with the control (−0.12) and TiO_2 (−0.19) showing the least variation, suggesting that brightness remained relatively stable under non-chromogenic conditions.

DISCUSSION

The incorporation of nanoparticles significantly affected the color stability of composite resins, rejecting the null hypothesis. ZnO and ZnO/Ag nanoparticles synthesized via the hydrothermal method induced greater discoloration compared to those produced through the polymeric precursor technique, particularly at higher concentrations and after prolonged exposure to coffee. This effect simulates long-term clinical staining, equivalent to approximately 7.5 years of daily coffee consumption [20].

TiO_2 nanoparticles also showed different behaviors depending on the synthesis method; the hydrothermal route resulted in slightly more staining, possibly due to less homogeneous particle distribution within the resin matrix. Notably, samples immersed in artificial saliva exhibited minimal color changes, reinforcing that staining is primarily associated with external agents like chemicals, food substances and chromogenic bacteria resulting in staining of teeth and restorative materials [21,22].

Nanocomposites were more susceptible to discoloration due to filler morphology, resin–filler interface quality, and water sorption capacity [23]. Despite some agglomeration in ZnO and ZnO/Ag clusters, hydrothermally synthesized particles (mean size: 26 nm) caused greater color change, emphasizing the importance of the synthesis technique over nominal composition.

Trend analysis revealed progressive staining over time, aligning with previous studies and confirming that nanoparticle incorporation influences aesthetic outcomes, particularly in chromogen-rich environments [17,22-29]. While regular brushing may help mitigate staining, the substantial ΔE* values observed indicate that resin modifications should be carefully evaluated in clinical settings.

This study emphasizes that, although resin modifications can provide functional advantages, they also introduce significant color instability, especially when exposed to staining agents like coffee. The pronounced color changes highlight the importance of considering aesthetic outcomes in restorative dental applications.

CONCLUSION

The incorporation of ZnO, TiO_2, and Ag decorated nanoparticles, synthesized via hydrothermal and polymeric precursor routes, significantly altered the color stability of the composite resin, particularly after immersion in chromogenic solutions such as coffee. These findings demonstrate that both the type of nanoparticle and the synthesis method influence the aesthetic performance of the material over time.

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Data availability

Detailed data for all experimental groups are provided in the Supplementary Material. The data sets generated and analyzed during the current study are available in the UNESP Institutional Repository as part of the full doctoral thesis (URI: http://hdl.handle.net/11449/151883), or are available from the first author on reasonable request (herculesdias@ufpa.br).

Author's Contributions

HBD, ACH, ANSR: Conceptualization. HBD, ACH, ANSR: Methodology. HBD, ACH, ANSR: Software. HBD, MIBB, ACH, ANSR: Validation. HBD, MIBB, ACH, ANSR: Formal Analysis. HBD, MIBB, ACH, ANSR: Investigation. HBD, MIBB, ACH, ANSR: Resources. HBD, LS, MIBB, ACH, ANSR: Data Curation. HBD, LS, MIBB, ACH, ANSR: Writing – Original Draft Preparation. HBD, LS, MIBB, ACH, ANSR: Writing – Review & Editing. HBD, LS, MIBB, ACH, ANSR: Visualization. MIBB, ACH, ANSR:

Supervision. ACH, ANSR: Project Administration. ACH, ANSR: Funding Acquisition.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Regulatory Statement

Not applicable.

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Supplementary Material

Supplementary material accompanies this paper.

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Figure 1 - Linear trends for color changes of one composite resin modified by ZnO and ZnO/Ag 2% (wt) stored in artificial saliva

Figure 2 - Linear trends for color changes of one composite resin modified by ZnO and ZnO/Ag 2% (wt.) stored in coffee.

Figure 3 - Linear trends for color changes of one composite resin modified by TiO_2 and TiO_2/Ag 2% (wt.) stored in artificial saliva.

Figure 4 - Linear trends for color changes of one composite resin modified by TiO_2 and TiO_2/Ag 2% (wt.) stored in coffee.

Table 1. Mean values (x) of color stability (ΔE *) and 95% confidence interval (CI) of the FiltekTM Z350 XT composite resin modified with ZnO and ZnO / Ag NPs and immersed in coffee solution

Table 2. Mean values (x) of color stability color stability (ΔE *) and 95% confidence interval (CI) of the FiltekTM Z350 XT composite resin modified with ZnO and ZnO / Ag NPs and immersed in artificial saliva

Table 3. Mean values (x) of color stability color stability (ΔE *) and 95% confidence interval (CI) of the FiltekTM Z350 XT composite resin modified with TiO_2 and TiO_2/Ag NPs and immersed in artificial saliva

Table 4. Mean values (x) of color stability (ΔE*) and 95% confidence interval (CI) of the FiltekTM Z350 XT composite resin modified with TiO_2 and TiO_2/Ag NPs and immersed in coffee solution

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