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ORIGINAL RESEARCH

# Crystalline phases involved in the hydration of calcium silicate-based cements: Semi-quantitative Rietveld X-ray diffraction analysis

Renata Grazziotin-Soares,MSc, PhD[1]; Mohammad H. Nekoofar,MSc, DoIBoE[2,3]*; Thomas Davies,PhD[4]; Roberto Hübler,PhD[5]; Naghmeh Meraji,DDS, MSc, DoIBoE[2,]*; and Paul M.H. Dummer,MScD, PhD, DDSc, FDSRCS (Ed), FHEA[3]

1 College of Dentistry, University of Saskatchewan, Saskatoon,SK, Canada
2 Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
3 School of Dentistry, College of Biomedical & Life Sciences, Cardiff University, UK
4 School of Chemistry, Cardiff University, Cardiff, UK
5 Materials and Nanosciences Laboratory, School of Physics, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil

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**Correspondence**
Dr Naghmeh Meraji, Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, North Karegar St, 14399-55991 Tehran, Iran.
Emails: meraji_n@yahoo.com & n-meraji@sina.tums.ac.ir and
Dr Mohammad H. Nekoofar, Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran & School of Dentistry, College of Biomedical & Life Sciences, Cardiff University, UK. Emails: nekoofar@tums.ac.ir & nekoofarmh@cardiff.ac.uk

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## Abstract

Chemical comparisons of powder and hydrated forms of calcium silicate cements (CSCs) and calculation of alterations in tricalcium silicate ($Ca_3SiO_5$) calcium hydroxide ($Ca(OH)_2$) are essential for understanding their hydration processes. This study aimed to evaluate and compare these changes in ProRoot MTA, Biodentine and CEM cement. Powder and hydrated forms of tooth coloured ProRoot MTA, Biodentine and CEM cement were subjected to X-ray diffraction (XRD) analysis with Rietveld refinement to semi-quantitatively identify and quantify the main phases involved in their hydration process. Data were reported descriptively. Reduction in $Ca_3SiO_5$ and formation of $Ca(OH)_2$ were seen after the hydration of ProRoot MTA and Biodentine; however, in the case of CEM cement, no reduction of $Ca_3SiO_5$ and no formation of $Ca(OH)_2$ were detected. The highest percentages of amorphous phases were seen in Biodentine samples. Ettringite was detected in the hydrated forms of ProRoot MTA and CEM cement but not in Biodentine.

## Introduction

Mineral trioxide aggregate (MTA) is a calcium silicate–based cement (CSCs) used in dentistry with various clinical applications, such as vital pulp treatment modalities, endodontic surgery, one step orthograde root end closure, sealing of perforations, orifice plugs and regenerative endodontic (1). Many disadvantages have been reported for this CSC such as difficult handling properties (1,2) and tooth discoloration (3,4). Several other CSCs have been developed with the aim of overcoming these disadvantages.

Biodentine (Septodont, Saint Maur des Fosses, France) is a fast setting CSC with high compressive strength (5) that is marketed as a dentin replacement material (6). The powder is composed of tricalcium silicate, zirconium oxide and calcium carbonate, whereas the liquid supplied for mixing includes calcium chloride as an accelerator and a hydrosoluble polymer as a water-reducing agent (7).

Calcium-enriched mixture cement (CEM cement) (Bionique, Tehran, Iran) is a white CSC, with no discoloration potential (3). It is composed of calcium oxide (CaO), sulphur trioxide ($SO_3$), phosphorus pentoxide ($P_2O_5$) and silicon dioxide ($SiO_2$).

X-ray diffraction (XRD) can be used to analyse and characterise the crystal phases in both powder and hydrated forms of CSCs (8,9). This technique recognises

specific diffraction peaks that are particular to the various crystalline phases present in each material (10) such as calcium hydroxide crystalline structures (8,9). Rietveld refinement, in turn, is used for the quantitative analysis of these detected crystalline phases (11).

The assessment, quantification and comparison of the crystalline phases in CSCs before and after hydration is important for improving our understanding of cement composition and changes occurring during the setting process (9). Therefore, the aim of this study was to quantitatively evaluate the crystalline phases present in the powder and hydrated forms of tooth coloured ProRoot MTA, Biodentine and CEM cement using Rietveld-XRD analysis.

## Materials and methods

This study has been approved by the Endodontology Research Group of Cardiff University, UK.

### Sample preparation

Both powder and hydrated forms of three CSCs were investigated: tooth coloured ProRoot MTA (Dentsply Maillefer, Ballaigues, Switzerland); Biodentine (Septodont, Saint-Maur-des-Fosses, France); and CEM cement (BioniqueDent, Tehran, Iran).

To investigate the hydrated form of cements, mixing was performed as follows: i) for tooth coloured ProRoot MTA and CEM cement, the mixing of the material was standardised by placing 1 g of the powder in a sterilised amalgam capsule (2). Then, 0.33 g of their respective liquids, distilled water for MTA and phosphate-buffered solution (PBS) for CEM cement were added to the capsules. ii) For Biodentine, five drops of the liquid provided were added to the powder, which is packaged in a plastic capsule.

The encapsulated materials were then mixed mechanically for 30 s at 4500 rpm (2) using an amalgamator (Dentsply Caulk, York, PA, USA). Subsequently, mixed slurries were placed in three prefabricated cylindrical silicone moulds (Elite Double 22; Zhermack SpA, Rome, Italy) with dimensions of $6 \pm 0.1$ mm length and $4 \pm 0.1$ mm internal diameter and subjected to a constant vertical compaction force of 3.22 MPa applied for 1 min (12). The moulds were then incubated at 37°C in fully saturated conditions for 7 days.

### Rietveld-XRD analysis

Both the dry powders and the hydrated forms of the materials (2 g each group) were subjected to Rietveld-XRD analysis for evaluation of their crystalline structures.

The hydrated materials were crushed to fine powder before analysis. Phase compositions of specimens from each group were determined using an X-ray diffractometer (PANalytical X'Pert PRO, Almelo, Netherlands) and CuKα radiation (40 Kv and 40 mA). Scans were undertaken from 10° to 80° in the 2θ range with 0.02° step. In order to identify crystalline compounds, all patterns were matched using the database of the International Centre for Diffraction Data (ICDD, Pennsylvania, PA, USA). The Rietveld refinement tool was used for the quantitative analysis of phases resulted in six diffractograms: three for the original powder and three for the hydrated form.

### Data analysis

To semi-quantitatively analyse the main phases related to the CSC hydration process, Rietveld refinement with the PANalytical X'Pert PRO software was used to adjust XRD spectra (diffractogram) in each sample. For this numerical simulation, the standard crystalline phases reported in the literature were used, according to the Inorganic Crystal Structure Database (ICSD), as follows: tricalcium silicate ($Ca3SiO_5$) [64759], dicalcium silicate ($Ca2SiO_4$) [166637], calcium hydroxide ($CaOH_2$) [91882], tricalcium aluminate ($Ca_3Al_2O_6$) [163579], the radiopacifiers bismuth oxide ($Bi2O_3$) [15072], zirconium oxide ($ZrO_2$) [82543], and barium sulphate ($BaSO_4$) [167519]. Additional phases detected in the samples were added to the spectra during the adjustments and are reported descriptively (%) with their respective ICSD codes.

## Results

XRD patterns in the 10°–80° 2θ range and Rietveld analysis results are shown in Figures 1–3. All samples consisted of tricalcium silicate and dicalcium silicate.

Figure 1 reveals the following:

**(a)** a diffractogram for tooth coloured ProRoot MTA, powder form (G1 powder): showing mainly tricalcium silicate ($Ca_3SiO_5$) and bismuth oxide ($Bi_2O_3$) peaks, as well as dicalcium silicate ($Ca_2SiO_4$). Rietveld analysis identified and quantified the following phases: 14.4% $Bi_2O_3$ [15072], 79.6% $Ca_3SiO_5$ [64759], 0.3% $Ca_2SiO_4$ [166637], 3.9% $CaAl_2O_6$ [163579] and 1.8% $CaSO_4$ [15876]; and

**(b)** a diffractogram for tooth coloured ProRoot MTA, hydrated form (G1 hydrated): showing the same phases of the powder sample, that is, tricalcium silicate ($Ca_3SiO_5$), dicalcium silicate ($Ca_2SiO_4$), and bismuth oxide ($Bi_2O_3$), plus calcium hydroxide (Ca[OH]$_2$) peaks and a small peak for ettringite (E). Rietveld analysis found 15.8% $Bi_2O_3$ [15072], 44.9% $Ca_3SiO_5$ [64759], 6.6% $Ca_2SiO_4$ [166637], 17.9% Ca

 

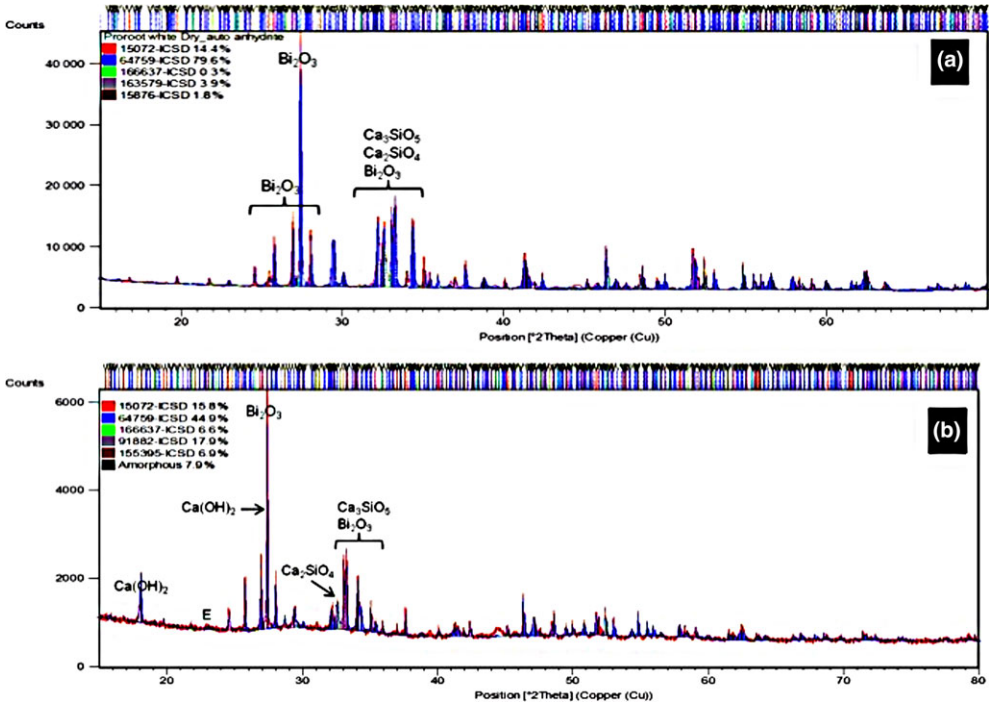


**Figure 1** Diffractogram and Rietveld analysis for tooth coloured ProRoot MTA, (a) powder and (b) hydrated form.

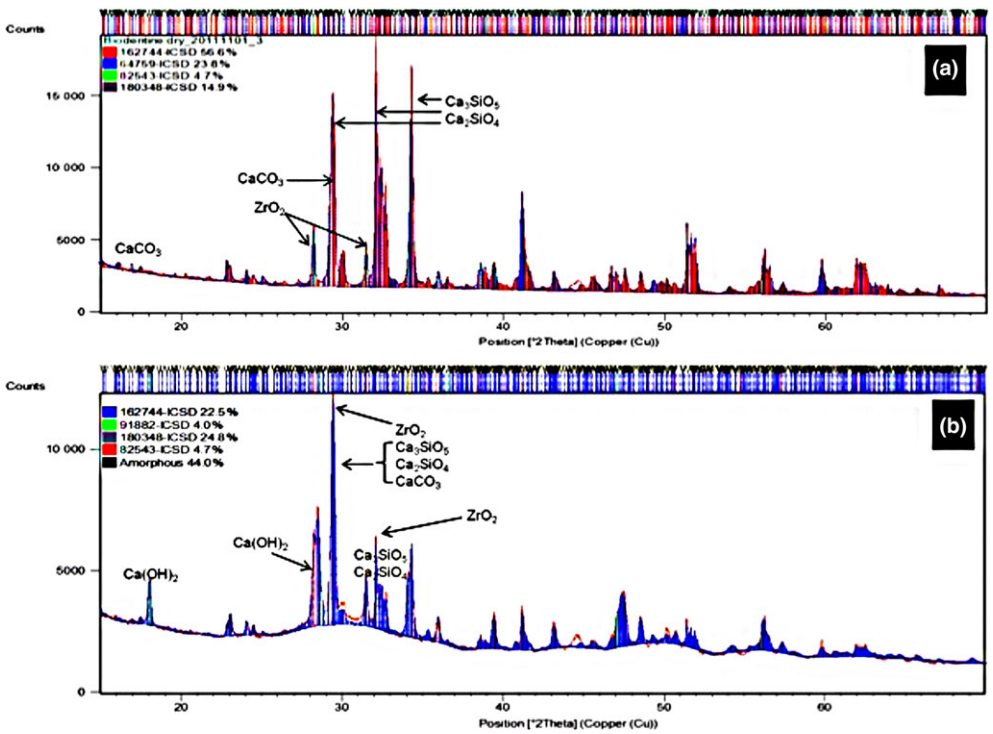


**Figure 2** Diffractogram and Rietveld analysis for Biodentine, (a) powder and (b) hydrated form.

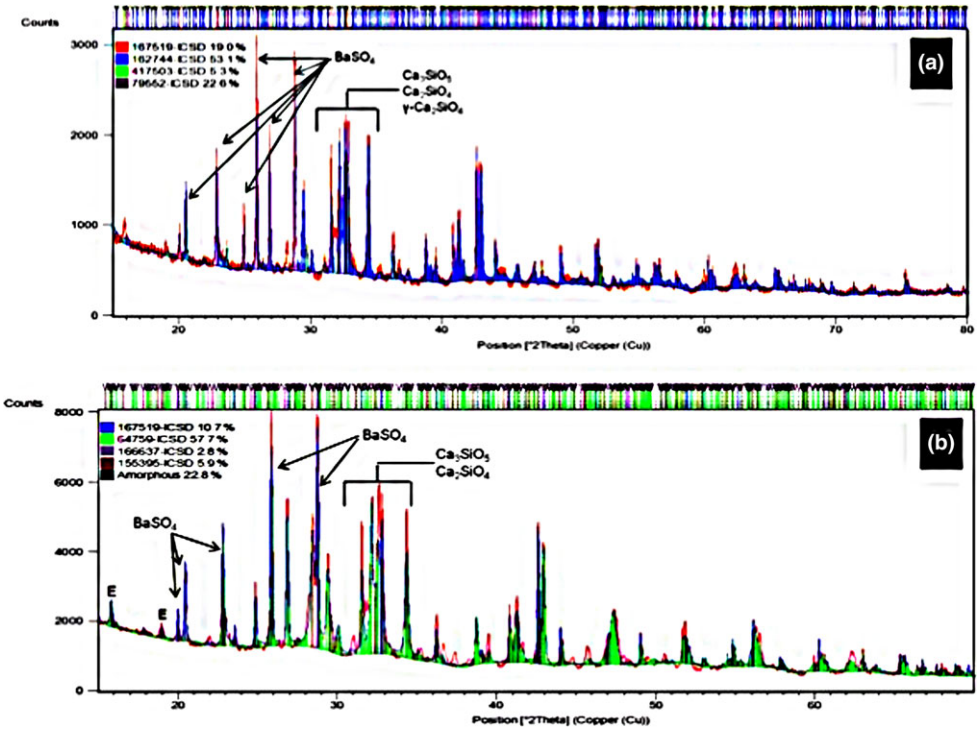


**Figure 3** Diffractogram and Rietveld analysis for CEM cement, (a) powder and (b) hydrated form.

$(OH)_2$ [91882] and 6.9% $Ca_6Al_2(SO_4)(OH)_{2\cdot26}H_2O$ [155395]. Material hydration revealed new crystalline phases, and the rearrangement caused some type of disorder, represented by 7.9% of an amorphous phase. Monoclinic and triclinic forms of tricalcium silicate were not distinguishable in MTA samples.

Figure 2 reveals the following:

**(a)** a diffractogram for Biodentine, powder form (G2 powder): two different structures of tricalcium silicate ($Ca_3SiO_5$): triclinic [162744] and monoclinic [64759] were found. Rietveld analysis showed 56.6% $Ca_3SiO_5$ (triclinic structure), 23.8% $Ca_3SiO_5$ (monoclinic structure), 4.7% $ZrO_2$ and 14.9% $CaCO_3$ [180348]; and

**(b)** a diffractogram for Biodentine hydrated form (G2 hydrated): the same phases of the powder sample, that is, tricalcium silicate ($Ca_3SiO_5$), dicalcium silicate ($Ca_2SiO_4$), calcium carbonate ($CaCO_3$), calcium hydroxide ($Ca[OH]_2$) and zirconium oxide ($ZrO_2$) peaks were found. Rietveld analysis showed 22.5% $Ca_3SiO_5$ [162744] (only the triclinic structure), 4.0% $Ca(OH)_2$ [91882], 24.8% $CaCO_3$ [180348], 4.7% $ZrO_2$ [82543] and 44.0% amorphous phase.

Figure 3 reveals the following:

**(a)** a diffractogram for CEM Cement powder form (G3 powder): tricalcium silicate ($Ca_3SiO_5$), dicalcium silicate ($Ca_2SiO_4$), dicalcium silicate γ–phase (γ–$Ca_2SiO_4$) and barium sulphate ($BaSO_4$) peaks. Quantification with Rietveld analysis found 19.0% $BaSO_4$ [167519], 53.1% $Ca_3SiO_5$ [162744] (triclinic structure), 5.3% γ–$Ca_2SiO_4$ [417503] and 22.6% $Ca_2SiO_4$ [79552]; and

**(b)** a diffractogram for CEM Cement hydrated form (G3 hydrated): tricalcium silicate ($Ca_3SiO_5$), dicalcium silicate ($Ca_2SiO_4$), barium sulphate ($BaSO_4$) and ettringite (E) peaks. Rietveld analysis showed 10.7% $BaSO_4$ [167519], 57.7% $Ca_3SiO_5$ [64759] (monoclinic structure), 2.8% $Ca_2SiO_4$ [166637], 5.9% $Ca_6Al_2(SO_4)_3OH_{12}.H_2O$ [155395] and 22.8% amorphous phase.

## Discussion

Investigating the crystalline phases of the hydrated forms of CSCs and comparing it with their unhydrated powder and calculation of reduction in tricalcium silicate and formation of calcium hydroxide powder form was an attempt to better understand the hydration and setting of these cements (8,9,13). Differences in the formation of calcium hydroxide may result in various biological properties (14,15). The wide range of applications of CSCs (16,17) and the current scarcity of evidence regarding the hydration process of Biodentine and CEM Cement highlight the clinical importance of crystalline phase analysis and comparison of these materials.

 

According to the results of this study, as expected $Ca_3SiO_5$ was the predominant phase detected in all evaluated non-hydrated CSCs. During the initial stages of cement hydration, this phase reacts with water producing calcium hydroxide (7,9). Triclinic and monoclinic polymorphic phases of $Ca_3SiO_5$ were only distinguishable in Biodentine and CEM cement and not in ProRoot MTA. The triclinic form was predominant in Biodentine and this was consistent with the study by Grech and coworkers (18). The powder form of CEM cement only contained the triclinic form. After hydration, no triclinic form could be detected in CEM cement and only the monoclinic form was detected. After hydration of Biodentine, both forms of $Ca_3SiO_5$ reduced and no monoclinic form was found. The temperature used during the sintering process of cement production and presence of impurities determine the existing polymorphic phases of $Ca_3SiO_5$ (19,20), therefore indicating differences in the manufacturing temperatures used in the cements evaluated (21).

In the hydrated form of all three CSCs, an increase in the amorphous phase was seen. Greater amounts of amorphous phase tend to increase the hardness of materials (13). The highest percentage of amorphous phase was seen in Biodentine (44%), with the percentage of this phase being 22.8% and 7.9% for CEM cement and tooth coloured ProRoot MTA, respectively. This greater percentage in Biodentine may indicate an advanced hydration in relation to the other two materials. In this regard, it is important to point out that the liquid of Biodentine contains calcium chloride as a setting accelerator (18,22). Furthermore, this cement contains calcium carbonate, which acts as a nucleation site for C-S-H, reducing the duration of the induction period and consequently causing the setting reaction to start a few minutes after mixing (10).

In this study, calcium hydroxide crystalline structures were only detected in the hydrated forms of ProRoot MTA and Biodentine. These changes occur consequent to the hydration of calcium silicate, which begins soon after the powder and liquid are combined and produces calcium silicate hydrate gel (C-S-H) and calcium hydroxide. Interestingly, in the case of CEM cement, no crystalline calcium hydroxide was detected after the hydration of CEM cement.

Soluble $Ca(OH)_2$ is released from the cement surface of CSCs during hydration (23), improving the alkalinity of the surrounding environment (24) and resulting in antibacterial properties (25). The reaction of soluble Ca $(OH)_2$ with phosphate present within body fluids and/or the phosphate present within the cement's liquid can result in the formation of hydroxyapatite (26,27). Therefore, the bioactivity and biocompatibility of CSCs are enhanced by releasing $Ca(OH)_2$ (27). Furthermore, when soluble $Ca(OH)_2$ reaches saturated concentrations, crystalline $Ca(OH)_2$ will be formed (14,28). Therefore, in the case of CEM cement, lack of crystalline $Ca(OH)_2$ may contribute to either early hydroxyapatite formation or sub-saturated concentrations of soluble $Ca(OH)_2$. Considering that no reduction was seen in the peaks of tricalcium silicate in CEM cement, the latter is more likely to be the cause but more research is required regarding this matter.

Ettringite was detected in the hydrated forms of ProRoot MTA and CEM cement as aluminate is present in their composition. This crystalline complex contains calcium, aluminium, silicon and sulphur and is responsible for resistance and hardening in the early hydration stage (9). The absence of ettringite peaks in the hydrated form of Biodentine may be a consequence of the reduction (or absence) of the aluminate phase in its powder form (10). The reduction/absence of the aluminate phase is an added benefit with respect to the workability of the fresh cement paste (10). It is associated with biological improvements, for example, avoiding the undesirable effects of aluminium (e.g. risks of Parkinson's and Alzheimer's disease) (29) and avoiding the leaching of trace elements into surrounding tissues (30).

The alternative radiopacifiers detected in Biodentine ($ZrO_2$) and in CEM cement ($BaSO_4$) are an attempt to improve their chemical and mechanical properties. $Bi_2O_3$ has the potential to increase the setting time, decrease mechanical strength and increase the porosity of CSCs (13,22). It has been suggested that $Bi_2O_3$ present in ProRoot MTA, unlike other radiopacifiers, is not inert during the cement hydration process (9,21). Rather, it has been reported to be present in the hydrated phase, forming a structure comprised of calcium silicate bismuth hydrate (C-S-H-Bi), which would affect $Ca(OH)_2$ precipitation and, consequently, the bioactivity of the hydrated material (9,21). In addition, a reduced amount of C-S-H will produce a poorer and slower hydration reaction, resulting in a longer setting time and deterioration of mechanical properties (13), factors that would impact negatively on the clinical use of ProRoot MTA. On the contrary, the findings of this study revealed no reduction in the amount of $Bi_2O_3$ detected. This inconsistency may be due to different methodologies. For instance, in the study by Camilleri *et al.* (9,21), analysis was conducted 1 month after cement preparation. Further research is suggested to evaluate the amount of $Bi_2O_3$ at different time intervals after cement preparation.

## Conclusion

The hydrated form of CEM cement had a different behaviour in relation to tricalcium silicate and $Ca(OH)_2$ peaks

 

when compared with the hydrated forms of Biodentine and ProRoot MTA. In CEM cement, no reduction in tricalcium silicate occurred and formation of calcium hydroxide was not detected. Biodentine did not have ettringite in its hydrated form, in contrast to the other cements. The highest percentage of amorphous phase was seen in Biodentine.

## Author Contribution

All authors have contributed significantly and are in agreement with the manuscript.

## Disclosure of interests

Authors deny any conflict of interest.

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