Investigation of the effect of the water to powder ratio on hydraulic cement properties

Koutroulis, Andreas; Batchelor, Hannah; Kuehne, Sarah; Cooper, Paul; Camilleri, Josette

DOI:
10.1016/j.dental.2019.05.011

Document Version
Peer reviewed version

Citation for published version (Harvard):
Koutroulis, A, Batchelor, H, Kuehne, S, Cooper, P & Camilleri, J 2019, 'Investigation of the effect of the water to powder ratio on hydraulic cement properties', *Dental Materials*, vol. 35, no. 8, pp. 1146-1154. https://doi.org/10.1016/j.dental.2019.05.011

Manuscript Details

Manuscript number	DEMA_2018_810_R1
Title	Investigation of the effect of the water to powder ratio on hydraulic cement properties
Article type	Full Length Article

Abstract

Objectives. The use of rheological properties to determine the optimal water: powder ratio of tricalcium silicate-based prototype materials incorporating alternative radiopacifiers and fillers. Determination of how the proportion of water incorporated affected the physicochemical behaviour of the materials. Methods. Endodontic cements replaced with 30% radio-opacifier, and additions of calcium phosphate and micro-silica were tested. The unmodified cements were mixed with a 0.35 water: powder ratio which served as control. At this water: powder ratio, the paste had an adequate clinical consistency and furthermore these pastes have been well characterized. Assessment of material rheological properties enabled adjustment of the water: powder ratio in each material to provide comparable viscosity values to those of the controls. The flowability, phase analysis and calcium release were measured for both viscosity-matched and the standard 0.35 water: powder ratio blends. The prototype materials with the adjusted water ratio were also characterized by scanning electron microscopy, energy-dispersive spectroscopy and evaluated for radio-opacity. Results. The use of the 0.35 water: powder ratio is not appropriate when changing the radiopacifier and incorporating additives. Zirconium oxide did not vary the water: powder ratio but tantalum oxide and calcium tungstate resulted in an increase and decrease in water demand respectively. Using the standard 0.35 ratio when the mixture had a low water demand resulted in higher flowability values and calcium release in solution. Micro-silica and calcium phosphate altered the hydration of the materials. All materials were adequately radiopaque. Significance. Rheological assessment is an easy reproducible way to determine the water: powder ratios of materials with varying amounts of additives and radiopacifiers during development. Modifications to the water: powder ratio affects material properties.

Keywords	Tricalcium silicate, rheology, characterisation, water:powder ratio

Corresponding Author	Josette Camilleri
Corresponding Author's Institution	University of Birmingham
Order of Authors	Andreas Koutroulis, Hannah Batchelor, Sarah Kuehne, Paul Cooper, Josette Camilleri

Submission Files Included in this PDF

File Name [File Type]

submission letter .pdf [Cover Letter]

rebuttal_final.docx [Response to Reviewers (without Author Details)]

title page.docx [Title Page (with Author Details)]

revised manuscript.docx [Manuscript (without Author Details)]

figure 1a.pdf [Figure]

Figure 1b.pdf [Figure]

Figure 1c.pdf [Figure]

Figure 1d.pdf [Figure]

figure 2_ICP.pdf [Figure]

figure 3.pdf [Figure]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
No data was used for the research described in the article

UNIVERSITY OF BIRMINGHAM

SCHOOL OF DENTISTRY

Dr. J. Camilleri
B.Ch.D., M.Phil., Ph.D., FICD, FADM, FIMMM, FHEA
Senior Lecturer
Restorative Dentistry
(Endodontics)

8th October 2018

Dear Prof Watts

I would like to submit a manuscript entitled "**Investigation of the effect of the water:powder ratio on hydraulic cement properties**" to Dental Materials for peer review. The work carried out is original and has been performed by the authors mentioned. This manuscript has not been submitted elsewhere for consideration for publication. All authors have no conflict of interest.

Kind regards

Josephine (Josette) Camilleri

Dental School, College of Medical & Dental Sciences
5 Mill Pool Way, Edgbaston, Birmingham B5 7EG,
United Kingdom

Dear Prof Watts,

We would like to thank you and the 3 reviewers for taking the time to evaluate our manuscript entitled “Investigation of the effect of the water: powder ratio on hydraulic cement properties” Ref: “DEMA_2018_810”. Comments from the referees were particularly constructive and raised interesting issues in terms both of the methodological process followed and the rationale for the study.

In the section below, a point-by point response to the comments of the reviewers is provided. Accordingly significant changes to the manuscript have been made and these are highlighted with underlined text.

Prior to the addressing of the comments, we would like to highlight the relevance of our study and emphasize why we consider it suitable for publication in “Dental Materials”. The investigation was performed on prototype dental materials with a realistic potential of clinical use as their chemical compositions are highly relevant to commercially available formulations, with the main difference being the absence of setting accelerators or water reducing polymers. Apart from the importance of the current study in the field of material development, it is imperative to identify the importance in understanding the respective water:powder ratios in clinical practice. Indeed a number of papers are published on prototype cements where the changes and benefits reported are dependent on the material changes caused by an improper water:cement ratio rather than determining the real benefits of the additives. From the clinical perspective dentists might often mix materials with different water amounts to give them subjectively acceptable handling characteristics, without recognizing that this can potentially alter the material’s properties when clinically placed. We hope to have addressed all the comments and issues raised.

Response to reviewer comments

Reviewer 1

General comment:

The premise here seems to be that using alternative radio-opacifiers has undesirable effects on the behaviour of the material, with the addition of other "filler" as well for reasons that remain obscure. The treatment should be in terms of volume fractions, and the consequences for the rheological behaviour (determined properly), radio-opacity and so on dealt with theoretically - we know how to do this. If then there are deviations we need to know. But the assumption that the water can be changed without taking into account the reactable fraction is unwise. There are other consequences.
It is simply wrong to say that anything "altered the hydration of the materials". There is no expectation of this, nor any evidence.

General reply

We kindly disagree with the comment of the esteemed reviewer. There is a large amount of publications investigating the incorporation of alternative radiopacifiers to cements thus creating a new MTA or a similar material. Furthermore, there are so many of such materials even available clinically which are not even researched properly that we wonder why the reviewer finds this investigation obscure. The same goes for the comment that we know how to do this. Notably, all such materials are dosed by wt fractions and the radiopacifiers are changed by weight fractions and no attention is given to the water:cement ratios. This has led to our investigations and we therefore feel this issue needs to be examined more thoroughly. Indeed there are only two papers coming from the same group from the many investigating the alternative radiopacifiers who use volume fractions.

Arias-Moliz MT, Farrugia C, Lung CYK, Wismayer PS, Camilleri J. Antimicrobial and biological activity of leachate from light curable pulp capping materials. J Dent. 2017 Sep;64:45-51. doi: 10.1016/j.jdent.2017.06.006. Epub 2017 Jun 20.

Farrugia C, Lung CYK, Wismayer PS, Arias-Moliz MT, Camilleri J. The relationship of surface characteristics and antimicrobial performance of pulp capping materials. J Endod. 2018 Jul;44:1115-20. doi: 10.1016/j.joen.2018.04.002. Epub 2018 Jun 1.

It is appreciated that working with volume fractions is more relevant, however since nobody undertakes this for endodontic materials and in particular for MTA, we felt it was not relevant in this case to work with volume fractions.

The additives have an effect on the hydration and the leaching pattern. This has been tested previously and published (see reference below). Thus there is a need for our investigation which is underpinned by a sound hypothesis. Indeed microsilica is very well known to react with calcium hydroxide and in fact is used in the construction industry to remove the calcium hydroxide as this is an unwanted part of the reaction by product. This approach is not currently used for dental materials. The use of calcium phosphate has also been investigated and it did affect the hydration. We therefore feel our investigations are necessary, are based on previous published literature and necessary for the development of the many materials based on MTA.

Schembri-Wismayer P, Camilleri J. Why Biphasic? Assessment of the Effect on Cell Proliferation and Expression. J Endod. 2017 May;43(5):751-759. doi: 10.1016/j.joen.2016.12.022. Epub 2017 Mar 11.

It is evident that the additives do alter the material hydration. Indeed this is a well-recognized fact which strengthens our case for the need to investigate and publish our findings. The readers, researchers and clinicians need to be made aware of how important it is to be careful with what is added to materials and how much water is needed for each mixture.

C1: *No page numbers*

A1: Page numbers have now been added in the center of the footer of each page.

Comments in abstract:

C2: *mixed with a 0.35 … : specify basis of ratio*

A2: The rationale for using the 0.35 ratio is specified in lines 7-8 of the Abstract (Page 1). It is also addressed in Section 2.1, 4^{th}-6^{th} line, page 4. This is the ratio that is used

by all MTAs and related materials. These pastes at a water: powder ratio of 0.35 are well characterized.

C3: *viscosity matched >> viscosity-matched, radiopacity >> radio-opacity.*

A3: Thank you for the correction. Changes are highlighted in the 11th and the 13th line of the Abstract (1st page) and throughout the manuscript for the "radio-opacity".

C4: *Modifications to the water:powder ratio affects material properties : ! This is a result? Sorry to be sarcastic, but the credibility of the paper is now rather low.*

A4: The statement in the significance section of the abstract summarizes the results of the physicochemical properties that were tested by the current study, namely hydration, viscosity properties and calcium release water: powder ratio modifications are well known to affect material properties. Please refer to the book Lea's Chemistry of Cement and concrete where this phenomenon is very well explained.

Comments in introduction:

C5: *Tricalcium silicate (TCS) -based : oversimplification*

A5: The TCS term was introduced as it corresponds to materials which present a significant concentration of tricalcium silicate phase in their composition. This term is used internationally. May be oversimplified but we are using it here to enable the readers to follow and understand what we are discussing. Alternatively we can use the term coined by Darvell, hydraulic calcium silicate. Please let us know what you prefer.

C6: *through the use of Portland cement : not actually true.*

A6: To the best of our knowledge, Portland cement which encompasses approximately 74.7% TCS (1) was the first tricalcium silicate-based material used in dentistry , in a case report of root canal obturation with Portland cement by Dr Witte in 1878 (2). More than a century later, MTA, which consisted of a simple blend of Portland cement and bismuth oxide was patented as a material for tooth filling (3) and consequently commercialized. It would be greatly appreciated if you could explain further this argument.

C7: *mineral trioxide aggregate : trade name, use chemically-meaningful term*

A7: The trade name has been intentionally used on this occasion, as a reference is provided for the commercial introduction of the tricalcium silicate-based materials in dentistry. Furthermore, the chemical composition is reported in the following sentence.

C8: *homogenous >> homogeneous*

A8: Thank you for the correction, which has been now incorporated into the 1st sentence of the 3rd paragraph of the Introduction (Page 2).

C9: *not sufficient to hydrate the mixture : the meaning of this is not clear. Do you mean for wetting out or for full reaction? Or what?*

A9: Thank you for the comment. It has been now stated in the same sentence that the wetting out of the mixture is addressed, as we are discussing the pilot efforts of the operators to obtain a mixture with acceptable consistency (Introduction section, 3rd paragraph, 8th line, page 3).

C10: *visual inspection : on what basis? Not reaction, then.*

A10: Indeed, as is discussed in the above comment, the water proportion in the mixture of cements containing additives is usually determined by pilot studies resulting in a subjectively acceptable consistency.

C11: *there is no data in the literature … water demand : definitions again are essential*

A11: Thank you for indicating this. The term water demand corresponds to the amount of water added to obtain a mixture with acceptable consistency to be handled efficiently and to be adequately hydrated. We hope that the meaning is clearer now and it is provided in the 4th paragraph of the Introduction-3rd line, page 3. So far, there is no scientific basis to determine this amount, nor the effect of the alterations which are taken into consideration.

C12: *protocol … lacking : undoubtedly, but you do not indicate why or what should be done about it. Your experiment does not address this.*

A12: The gap in knowledge as is addressed in the current sentence derives as an observation from review of studies which test properties of materials under

development. As a solution, we suggest a rheological assessment which can match the rheological performance of formulations to a reference material. Furthermore, the effect of the alterations in the water:powder ratio can be independently identified following characterization of the materials under development.

C13: *The null hypothesis … change the water demand … modifies the material properties : fake hypotheses, for what we already know what the answer must be, are pointless. Rewrite to say what you want to know, properly.*

A13: The current hypothesis was submitted in the specified form not to draw conclusions in what appears as profound, but based on the current status of knowledge as evidenced from the literature. However, we do recognize that the null hypothesis should be negatively stated and therefore we have modified it, according also to the suggestion of the 3rd reviewer. This is now rewritten in the last 4 lines of the 5th paragraph of the Introduction (pages 3-4). The outcome has been also been changed accordingly (accept-reject null hypothesis) in the Discussion section (last line of the 2nd paragraph-page 14, 3rd line of the 6th paragraph-page 16).

Comments in Materials and Methods:

C14: *Methodology >> Materials and Methods -Meysieu >> Meyzieu*

A14: Changes have been incorporated (2nd line of Materials and Methods, page 4).

C15: *proportion : by what? I assume by mass. All following is therefore uninterpretable as by volume is the only sensible scientific approach.*

A15: The incorporations were indeed carried out in a weight percentage and this has now been reported throughout the manuscript. Proportions by volume have never been used for these material types. We are simply following on from what many other researchers have done (4) and what is used by the industry (5). We agree with the reviewer that volume is the only sensible way.

C16: *All products must be properly and fully identified (cat. id, essential, and batch no. where relevant), and in standard form.*

A16: Added to the manuscript. Thank you for your comment.

C17: *Portland cement : why? It is irrelevant, but there is no provenance anyway.*

A17: Unhydrated Portland cement contains 74.7 % TCS (1). Therefore it is also a TCS-based material. The rationale for the use of the Portland cement was mainly the availability in large quantities as is also stated in the manuscript.

C18: *described thoroughly : [11] is not relevant, on the face of it.*

A18: See above.

C19: *parallel plate geometry : now this is quite wrong. If the material shear-thins, then the results are totally confounded and uninterpretable. Cone and plate is essential, as a minimum.*

A19: The use of parallel plates is preferred for slurries and pastes as solid materials can accumulate under the "point" of the cone plate which can affect interpretation of the results. Parallel plates provide flexibility in selecting a gap to account for the particulate matter in the sample. Within this configuration the shear rate varies due to the gap size selected and according to the radius of the plate (6, 7). Additionally, a cross-hatched plate design was used to minimize slip and a solvent trap was included to minimize evaporation of the solvent.

C20: *2.5 g : how do we know that the space was filled? Not overfilled?*

A20: Thank you for the comment. Following the loading procedure of the sample in the rheometer, any potential excess of the material was trimmed using a spatula in a gap 100 µm above the loading gap. Consequently, the upper plate was loaded in the final gap (700 µm) and the experiment was started. This has now been reported in Section 2.1 Rheological assessment, 2nd paragraph, lines 9-10 at page 5. This type of sample preparation is consistent with previous publications (8).

C21: *an addition of 87.5µl … : therefore the mixing ratio is changed.*

A21: Indeed, the mixing ratio was altered due to the potential limitations described during the loading procedure. However, the addition of water that was used for all materials was standard and thus it was consequently subtracted from all proportions.

C22: *µl > µL*

A22: Formatted accordingly and has been highlighted throughout the manuscript.

C23: *established at 20oC >> established at 20 °C : but, why this temp? It is irrelevant.*

A23: Formatted at the 9th line of the 2nd paragraph of the Section 2.1 Rheological assessment at page 5.

The temperature was established at 20°C, as higher temperatures could potentially result in rapid dehydration of the test samples, which would lead to variability in the data. The primary purpose of the rheology experiments was to compare test formulations to a reference material. Therefore, matching rheological performance was our primary aim and the temperature was set to ensure reproducible data. Such temperatures have been reported in previous published work (9). Notably, experiments for analysis of flow or viscosity of materials are usually performed under laboratory conditions.

C24: *different water: powder ratios in order to obtain flow values comparable : why were these not calculated first to give the same reaction with the silicate? It is a straightforward thing to do.*

A24: We conducted this research because it is not possible to calculate the water: powder ratio. Thus the use of the rheological analysis and working backwards to provide the necessary information. We therefore do not agree that the suggested investigation is a straightforward thing to do.

C25: *shear rate : this is only true at the edge, hence the problem.*

A25: As per answers to previous comment (C18): the primary purpose of the study was to compare materials and this methodology allows the comparison. There is scope to undertake extensive work on the optimization of the rheological methodology however that is out of scope for this publication and will form the basis for future research.

C26: *viscosity values were proximated to null : not English, but 'null'is not zero anyway - unless, as is likely the material parted company with the plates. 100/ s is in any case a ludicrously high rate for a paste.*

A26: Thank you for the correction. This has been amended. New version: "Results of the values of viscosity per shear rate from 1 to 20 s^{-1} were plotted, as at higher shear rates the measurements were not always related to the samples as there was potential for separation of the upper plate from the sample." (Section 2.1, 2nd

paragraph, lines 14-16, page 5). Changes have been also introduced in the 2nd paragraph of the Discussion section, 2nd-3rd line, page 13.

C27: *values were translated to the TCS-based mixtures : what does this mean?*

A27: A rheological assessment was also carried out for tricalcium silicate mixed in a 0.35 water: powder ratio to evaluate the viscosity behavior of the material. Consequently, as experiments were performed for Portland cement-containing materials, values obtained from the rheological adjustment were used in the materials that contained TCS instead of Portland cement.

C28: *ISO 6876:2012 is a quick and dirty QC process, not the last word in scientific method.*

A28: The authors agree with the comment and highlight the limitations of the ISO 68767:2012 flow tests in the 3rd paragraph of the Discussion section, page 14. The particular test was used in order to compare results from the rheological technique that was introduced to a standard method which has been extensively applied in the literature(10-12). Comparison in results between these two methods indicated the lack of sensitivity of the ISO flow method.

C29: *ml >> mL*

A29: Modified accordingly throughout the manuscript.

C30: *Hank's balanced salt solution : meaningless, not relevant.*

A30: Hank's balanced salt solution was used as the extraction vehicle since its inorganic composition of ions is similar to that of human blood plasma. Additionally, the use of water as an extraction vehicle is not clinically relevant. This is also reported in the literature (13). The rate of extraction is different in different liquids.

C31: *vacuum desiccated : so there will be dehydration of key phases?*

A31: Thank you for your comment. The standard technique will be immersion in alcohol to remove water or acetone to block the reaction. We opted for desiccation only as we could then test immediately without allowing further hydration.

C32: *agate : a silicate …*

A32: The equipment used was an agate mortar and pestle.

C33: *inductively couple >> inductively-coupled*

A33: Changed as indicated (1st sentence of 2.4 Section-Ion release assessment, page 6).

C34: *HBSS : which one is now important …*

A34: the same HBSS was used throughout the experiment. The specific one is listed previously in the text so this is not repeated here.

C35: *6 calibration solutions : where is the calibration graph?*

A35: We understand that the inclusion of this is not routinely performed and therefore not necessary.

C36: *[SEM] vacuum desiccation … : how does this make sense?*

A36: The procedure that was followed was stated in brief to avoid repetition with descriptions from the XRD analysis. Specifically, samples were retrieved from the HBSS solution and were placed in a vacuum desiccator for 24 hours. Following this period, the pellets were embedded in plastic cylindrical moulds with resin and were left overnight. On the third day, the polishing procedures took place and specimens were then observed using SEM.

C37: *… grind … under water : what? What is the point of the dessication?*

A37: During the polishing procedure, materials were embedded in resin. Therefore, the bulk of samples was not exposed to environmental conditions. Immediately after the polishing procedure, specimens were vacuum desiccated and gold spattered for SEM observation.

C38: *Radiopacity evaluation >> Radio-opacity … , but why use ISO when there is a proper scientific method for attenuation coefficient? That can be done simply and meaningfully.*

A38: The term has been changed. The ISO 6876:2012 specifications for radio-opacity were used as it is a simple reproducible procedure that is routinely applied in the literature.

C39: *Clarimat 300 : does this have autogain or autocontrast controls? If so were they disabled? If not, then the results are useless.*

A39: The aluminum step wedge was used in all radiographs and a unique equation expressing thickness of aluminum in the step wedge per pixel volume was created for every radiograph. Therefore, any potential effect of autogain or autocontrast performed during processing, would not affect the results as they were individually plotted in respect to the individual values of the step wedge.

C40: *PC_0.35 : not defined.*

A40: Thank you for indicating this. The term has now been defined in the 4th line of section 2.1 Rheological assessment, page 4.

C41: *mean values of viscosity : over the range of rotation rates? To what sense?*

A41: This was carried out as an additional method to compare the rheological performance of each material by obtaining the mean viscosity values for each material during rheological assessment.

Comments in results:

C42: *water:powder ratios : uninterpretable without reference to volume fraction. But particle size distribution is not even mentioned …*

A42: The weight vs volume fraction has been addressed at length as is described at the beginning of our response. We have applied an approach which is frequently used by all in this field. We appreciate the use of volume fraction but it is not appropriate in this context.

C43: *water demand : still undefined.*

A43: The comment has been addressed previously (C10).

C44: *Fig. 1 : legends need careful proof-reading.*

A44: Thank you for your indication. Figure 1 has now been removed according to the suggestion of the 2nd Reviewer.

C45: *standard change in the water : I do not understand what this is meant to be saying - esp. given "to hydrate".*

A45: The phrase "given to hydrate" corresponds to the water amount provided to mix each prototype material.

C46: *Table 1: PC?*

A46: This has been already defined in the table legend.

C47: *Table 2: percentage of a ratio is wrong*

A47: Thank you for the comment. In fact, the ratios have been calculated correctly, however, it has not been indicated that the water:powder ratios are provided with two decimals precision. This has been now elaborated on the legend of Table 1.

C48: *Tables 3 and 4: why do we not have proper graphical presentations? (And not like Fig. 3)*

A48: Table 4 is presented graphically in Figure 3 and has been now deleted. Layout of Table 3 has now been changed according to the indications of the 2nd Reviewer.

C49: *XRD: as far as I can see this tells us precisely nothing. There are no controls.*

A49: The chemical composition of materials with different water: powder ratios tested is the same, therefore a comparison of the effect of the change in the proportion of water can be drawn. The scope of the current study was therefore fulfilled as the effect of the water adjustment in the mineral phases of hydrated cements could be monitored.

C50: *Fig 4: pretty much pointless - the spectra especially are not helpful.*

A50: The SEM images provided in combination with XRD and ICP, giving a thorough characterization of the laboratory developed materials. Additionally, the hydration process is monitored. The EDX analysis is indicating the constituents of the respective formulations.

Reviewer 2

C1: *If I understand it correctly, % indicated wt%, right? If so, please use wt% to be more precise.*

A1: Thank you for the suggestion. It has now been corrected and highlighted throughout the manuscript.

C2: *Figure 1 is not needed as you already presented the information in Table 2.*

A2: Thank you for the comment. We included this figure in order to present the rheological performance of materials following the adjustment of the water: powder ratio. The figure has now been deleted.

C3: *In the Results, for 3.1, it was mentioned that "the replacement of each cement by inclusion of 30% radiopacifier resulted in a standard change in the water amount needed to hydrate the material". This statement is not correct as ZrO had no influence on water amount needed. Check all the statements again, as this is not the only sentence which is not precisely stated.*

A3: Thank you for the comment. We have now amended the statement in the 1st paragraph of the 3.1 Rheological adjustment (9th and 10th line), page 9.

C4: *Apart from rheological assessment, the other properties of TCS-CaP, TCS-mS10 and TCS-mS20 (without radiopacifier) were not evaluated. It will make the story more complete if the data can be added.*

A4: Thank you for the suggestion. It would be indeed interesting to identify properties of the non-radiopacified cements. However, the rationale of our study was to develop materials with a potential for use in clinical practice hence our evaluation of radio-opacity. The TCS used here served as a control in our study for all the prototype formulations rather than a test material. These materials always include radiopacifiers.

C5: *For 3.2, the result can be summarized in a more structured way. For instance, the first paragraph focused on the addition (CaP, mS), and the third paragraph mentioned again. The last sentence of the first paragraph "the different radio pacifiers did not have this effect on the flow values of cements" is very confusing. Which effect do you mean? Again in the next paragraph, "the incorporation of ZrO and CaWO did not change the flow characteristics". Please explain clearly which data support this statement. Please also check clearly for the rest of the results. Describe the data and then make the statement.*

A5: Thank you for the comment. The initial format of the flowability tests was to present comparisons of flow values in materials with the same chemical composition and different water: powder ratio (0.35 and adjusted). Consequently, the

radiopacifier effect was assessed, and finally the results of the comparisons with the control material were presented (pure tricalcium silicate mixed at a 0.35 water: powder ratio).
We have now changed the format of the paragraph presenting results on flowability in a more cohesive and explicit pattern (3.2 Section pages 9-10).

C6: *For Figure 2, summary the result of the same radiopacifier together to make the figure less complicated to read and mark the information that you wanted to show in arrow, and etc.*
A6: Thank you for your suggestion. We are now presenting the XRD plots as 4 per page. In each page, the diffractograms of materials with the same chemical composition appear regardless of the radiopacifier (1st page: only TCS, 2nd: TCS-CaP, 3rd: TCS-mS10, 4th: TCS-mS20). Additionally, we have included more links to the specific plots throughout the 3.3 Section- X-Ray diffraction analysis, pages 10-11. We consider that with these alterations result in the figure being more connected with the text and inclusion of arrow in the plots will complicate the diffractograms.

C7: *For Table 3, it is better to present it in a similar way as Table 1 to make it reader friendly. Add the data for TCS-CaP, and etc.*
A7: Thank you for your suggestion. Table 3 is now presented in a similar format to Table 1 on page 21.

C8: *Table 4 is not needed as Fig 3 presented the same information.*
A8: Thank you for indicating this. This table has now been deleted.

C9: *For the last figure, put all SEM images together and present the EDX result in a table to make it easy to read.*
A9: We have reduced the amount of SEM images presenting only a select few in Figure 3. Subsequently we left the EDS charts in the manuscript. If a table is preferable we can include this.

C10: *Discuss the clinical relevance of the result, as in clinical situation, the oral environment is wet and will this play a role.*
A10: These materials are used for pulp capping. They are never in contact with the saliva. There is some wetness form the dentine. This is not the scope of this

investigation. This investigation aims to indicate to clinicians not to add excess water as well as providing researchers with a robust way of determining water:powder ratios.

Reviewer 3

General comment:

Firstly, I would like to thank the authors for sharing their research and for giving us the opportunity to review their manuscript. Secondly, the researchers have carried out an extensive amount of work for which I would like to congratulate them.

However, the article entitled: *"Investigation of the effect of the water: powder ratio on hydraulic cement properties"* is, in my opinion, not of interest for a dental audience for which I recommend the paper to be rejected from publication in *Dental Materials*.

Nevertheless, in order to appreciate the effort of the authors I would like to give them my advice in case the researchers want to submit their article to another journal:

General reply:

We appreciate the reviewer's comments and opinion shared. We do think however that our research, on materials used for pulp capping in dentistry, should be of great interest to the dental community. As we previously described, the materials that we investigated have a realistic potential for clinical use. Alterations occurring to hydraulic cements' properties following inclusion of different fillers and radiopacifiers is not only important for material development. Clinicians should be made well aware on effects of different additives on physicochemical properties of TCS-based cements when they are studied on a standard basis (i.e adjustment of the water:powder ratio). Additionally, it should be made clear that properties can potentially be altered when manufacturers' water:powder ratios are not followed.

Comments in title:

C1: *OK (I suggest using "water to powder" instead of the colon punctuation mark but this is a minor issue).*

A1: Thank you for the suggestion. We have now changed the title accordingly.

Comments in abstract:

C2: *In the first 4 lines of 'objectives' the word "properties" has been used 3 times. Please, use synonyms to improve readability (characteristics, qualities,…).*

A2: Thank you, this has been now amended (Abstract section, page 1).

C3: *"Methods": use flowability instead of "flow".*

A3: This has now been amended throughout the manuscript.

Comments in introduction:

C4: *Line 13: "Indeed it is important…". This sentence is not clear. Please, rephrase.*

A4: Thank you for the suggestion. This phrase has been rewritten (page 2).

C5: *Last sentence of "Introduction": In my opinion, the null hypothesis is not correctly stated. Per definition, a null-hypothesis should be negatively stated. This is not the case in the article. Please, check it again.*

A5: Thank you for the correction. The null hypothesis has now been rewritten accordingly, in the last sentences of the Introduction, pages 3-4.

C6: *The authors should make it more clear why they performed the study. I see many contradictions. For example: Last paragraph: "Rheological properties of TCS-based materials…". The authors stated: "So far the flowability of hydraulic cements has not been studied; however, flow properties of TCS-based endodontic sealers are routinely reported…". TCS-based sealers are also hydraulic cements per definition. Their use is different because they have different properties. On the other hand, I find the flowability of endodontic sealers more important than the flowability of hydraulic cements. I think the authors should make more clear what they wanted to say here.*

A6: Thank you for the comment. We have now clarified that the current statement was addressing TCS-based cements with a potential of use as endodontic repair

materials, such as MTA (Introduction, 5th paragraph, lines 1-2, page 3). Additionally, in the same sentence, we explain why flowability of these repair materials is important, since it affects the material handling, delivery efficiency and stability of the material at the application field.

Comments in materials and methods:

C7: *At the beginning of this section, the authors mentioned the materials used but Portland cement (used as a control) is not mentioned here. Please, clearly state all the materials used (including those used as controls). Which brand/type of Portland cement was used?*

A7: Thank you for the correction. Portland cement has now been added to the materials used, along with the specific brand (Materials and Methods section, lines 11-12, page 4).

C8: *The correct reference to the brand/city/country of the materials/products used is missing many times. For example: Portland cement; graduated syringe; digital caliper; incubated at 37 degrees …where (incubator)?; ICP-OES; PSP plate; Progeny dental (city, country?); PASW…do you mean…SPSS???*

A8: Thank you for the comment. The information has been added at the indicated sections below:

Portland cement: Section 2-Materials and Methods, lines 11-12, page 4.

Graduated syringe: Section 2.2-Flowability measurements, 6th line, page 6.

Digital caliper: Section 2.2-Flowability measurements, 10th line, page 6.

Incubator: Section 2.3- Phase analyses, lines 4-5, page 6.

ICP-OES: Section 2.4-Ion release assessment, 2nd line, page 6.

PSP plate: Section 2.6-Radio-opacity evaluation, 5th line, page 7.

Progeny dental: Section 2.6-Radio-opacity evaluation, 7th line, page 8.

SPSS: Section 2.6-Statisitcal analyses, 2nd line, page 8.

C9: *The amount of samples used for each test is not clearly indicated. The authors performed parametric analysis for statistics which require a minimum amount of data in order to avoid mistake. I recommend to clearly specify the amount of data/samples used.*

A9: Amount of specimens tested is now included in the manuscript on page 4, for all experiments performed. Thank you for highlighting this.

C10: *The material and methods section is in my opinion the biggest problem of the article. At this moment, I don't understand the importance of determining the correct water to powder ratio for experimental hydraulic calcium-silicate cements. I understand this could be useful for companies in order to develop new materials but not for clinicians or researchers.*

A10: Thank you for raising this issue. Our study highlighted how the adjustment of the water: powder ratio can independently affect the material's chemistry. Therefore, several studies that have not taken into consideration this factor might have generated results that are somewhat uninterpretable in terms of the effect of additives in the formulation of hydraulic cements. In terms of the clinical significance, our study highlights the importance of respecting the specific instructions in the water amount used to mix the materials. Notably, issues with the grainy consistency of MTA and difficulties in placing this material have been addressed by several clinicians by mixing the powder with more water. It is important therefore to highlight, that alterations in the water: powder ratio will result in modified properties in materials following placement. The issue of water: powder ratio is important for both clinicians and researchers. Unfortunately this fact is often overlooked resulting in researchers attributing the changes to the additives when the water: powder ratio was not adjusted accordingly. This is the scope of the paper.

Comments in results:

C11: *Table 2 is not giving any extra information. The info is already provided in table 1.*

A11: Thank you for the comment. We consider that Table 2 highlights the trend in change identified following the adjustment of the water: powder ratio in exact values. If Table is withdrawn, the reader will not easily understand the standard change in the water demand which was calculated.

C12: *14 images in figure 4 is too much. I recommend selecting the most relevant ones. Moreover, it is very difficult for the reader to understand the information in the way it is presented at this moment.*

A12: Thank you for the suggestion. We considered it important to provide all images as part of the holistic characterization process that was carried out. Consequently, they could potentially serve as a supplementary material. We have now reduced the number of SEM images in Figure 3 down to 4 representative ones.

C13: *Section 3.5 SEM-EDX – third paragraph: "Zirconium particles appeared round in morphology…(figure 4)". Sorry, all of them are figure 4. In which one can Zr particles be seen??*

A13: Thank you for highlighting this. According to your previous comment, we are now providing representative figures. We consider it now easier to identify each particle in the figure.

Comments in discussion:

C14: *In general, the discussion is very nicely written and well-structured. It is very clear and it contains very good references. However, the general concept is still weak. Why is this important?*

A14: Thank you for your comment. We have highlighted the significance of our work in two fields: The first one, is the field of material development, as researchers should take into consideration the effect of the water adjustment when they evaluate the properties of additions of chemical in the conventional hydraulic cement formulation. Secondly, the clinical significance as is also discussed above, indicates it is important to respect the indicated water: powder ratios, as these can result in alterations in the properties of the material when placed by the clinician.

Comments in conclusions:

C15: *The conclusions are in my opinion a bit too strong. The authors mention that no other protocol for the adjustment of the water to powder ratio exist. My question is: How are current materials developed? Trial-error? If this is the case, then: why this method would be useful? And again, maybe this may be useful for companies or engineers but I don't see it very useful for dentists or dental researchers. Moreover,*

when researchers develop a new material, normally the water to powder ratio is not modified. Most researchers respect the instructions of the manufacturer, as recommended at the end of the discussion section.

A15: Thank you for the interesting points that are raised. Indeed, to the best of our knowledge, no protocol is evident on reproducible adjustment of the water: powder ratio. As we indicated in the introduction section, in several studies the water adjustment is performed in order to obtain mixtures with an acceptable consistency without a scientific base. This is carried out because the amount of water in the standard ratio is usually not adequate to perform the mixing procedure.
In a similar manner, clinicians might consider that a modifications in the water: powder ratio can be undertaken to improve handling characteristics of the cement. This study showed that such modifications can have significant effects on the materials' physicochemical properties.

Comments in references:

C16: *References 17, 32, 44, contains mistakes. Please check. Reference 48 is not in the text.*

A16: Thank you for the comment. The mistakes have been corrected and changes appear underlined within the reference section at pages 18-19.

REFERENCES

1. Marciano MA, Camilleri J, Costa RM, Matsumoto MA, Guimarães BM, & Duarte M AH. Zinc Oxide Inhibits Dental Discoloration Caused by White Mineral Trioxide Aggregate Angelus. J Endod 2017;43:1001–7.
2. Brasseler USA [Internet]. BC Sealer safety data sheet [cited 2018 27 September]. Available from: http://brasselerusa.com/wp-content/files/SDS-0003%20TotalFill%20BC%20Sealer%20SDS%20%20REV%20B.pdf.
3. Camilleri J. Characterization of hydration products of mineral trioxide aggregate. Int Endod J. 2008;41:408-17.
4. Witte. The filling of a root canal with Portland cement. German Quarterly for Dentistry; J Cent Assoc German Dent. 1878;18:153–4.
5. Torabinejad M, White DJ. Tooth filling material and method of use. United States patent US 5415547; May 16, 1995.
6. Feys D, Cepuritis R, Jacobsen S, Lesage K, Secrieru E, Yahia A. Measuring Rheological Properties of Cement Pastes: Most common Techniques, Procedures and Challenges [Internet]. RILEM Tech Lett; 2:129-35, 31Dec.2017 [cited 21Mar.2019]. Available from: https://letters.rilem.net/index.php/rilem

7. De Souza Mendes P, Alicke A, Thompson R. Parallel-plate geometry correction for transient rheometric experiments. Appl Rheol. 2014;24:52721.
8. Kim YJ, Cho B-Y, Lee S-J, Hu J, Wilde JW. Investigation of Rheological Properties of Blended Cement Pastes Using Rotational Viscometer and Dynamic Shear Rheometer. Advanc Mater Sci Engin. 2018;1-6.
9. Feys D, Asghari A. Influence of maximum applied shear rate on the measured rheological properties of flowable cement pastes. Cem Concr Res. 2019;117:69-81.
10. Asgary S, Shahabi S, Jafarzadeh T, et al. The properties of a new endodontic material. J Endod 2008;34:990–3.
11. Camilleri J. Evaluation of selected properties of mineral trioxide aggregate sealer cement. J Endod 2009;35:1412–7.
12. Tanomaru-Filho M, Garcia AC, Bosso-Martelo R, et al. Influence of addition of calcium oxide on physicochemical properties of Portland cement with zirconium or niobium oxide. J Conserv Dent 2015;18:105–8.
13. Kebudi Benezra M, Schembri Wismayer P, Camilleri J. Influence of environment on testing of hydraulic sealers. Sci Rep. 2017;7:17927.

Investigation of the effect of the water to powder ratio on hydraulic cement properties

[1]Koutroulis A, [2]Batchelor H, [1]Kuehne SA, [1]Cooper PR, [1]Camilleri J

[1]*School of Dentistry, Institute of Clinical Sciences, College of Medical and Dental Sciences, University of Birmingham,* [2]*School of Pharmacy, Institute of Clinical Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom*

Key words:

Tricalcium silicate, rheology, characterisation, water to powder ratio

Correspondence
Josette Camilleri
School of Dentistry,
Institute of Clinical Sciences,
College of Medical and Dental Sciences,
University of Birmingham
5 Mill Pool Way
Edgbaston B5 7EG
Birmingham
United Kingdom
J.Camilleri@bham.ac.uk

Investigation of the effect of the water to powder ratio on hydraulic cement properties

Abstract

Objectives. The use of rheological properties to determine the optimal water: powder ratio of tricalcium silicate-based prototype materials incorporating alternative radiopacifiers and fillers. Determination of how the proportion of water incorporated affected the physicochemical behaviour of the materials.

Methods. Endodontic cements replaced with 30% radio-opacifier, and additions of calcium phosphate and micro-silica were tested. The unmodified cements were mixed with a 0.35 water: powder ratio which served as control. At this water: powder ratio, the paste had an adequate clinical consistency and furthermore these pastes have been well characterized. Assessment of material rheological properties enabled adjustment of the water: powder ratio in each material to provide comparable viscosity values to those of the controls. The flowability, phase analysis and calcium release were measured for both viscosity-matched and the standard 0.35 water: powder ratio blends. The prototype materials with the adjusted water ratio were also characterized by scanning electron microscopy, energy-dispersive spectroscopy and evaluated for radio-opacity.

Results. The use of the 0.35 water: powder ratio is not appropriate when changing the radiopacifier and incorporating additives. Zirconium oxide did not vary the water: powder ratio but tantalum oxide and calcium tungstate resulted in an increase and decrease in water demand respectively. Using the standard 0.35 ratio when the mixture had a low water demand resulted in higher flowability values and calcium release in solution. Micro-silica and calcium phosphate altered the hydration of the materials. All materials were adequately radiopaque.

Significance. Rheological assessment is an easy reproducible way to determine the

water: powder ratios of materials with varying amounts of additives and radiopacifiers during development. Modifications to the water: powder ratio affects material properties.

1. Introduction

Tricalcium silicate (TCS) -based materials were introduced in dentistry through the use of Portland cement (PC) in mineral trioxide aggregate (MTA) [1]. The initial formulation of MTA, consisting of 80% PC and 20% bismuth oxide as radiopacifier [1], has been widely used for endodontic applications such as root-end filling [2], root-repair [3] and vital pulp therapy [4, 5]. However, several limitations exist in the original formulation, such as the inclusion of trace elements namely lead, chromium and arsenic in the cementitious phase [6-8] as well as the leaching of bismuth [9] which results in tooth discoloration [10].

In an effort to eliminate the leaching of trace elements from Portland cement, pure TCS is now used in several commercially available hydraulic cements [11, 12] and various compounds are also included to enhance the cement's physicochemical properties [13-15]. Similarly, inert materials with high molecular weight and which do not leach out have been incorporated as radiopacifiers [16]. Indeed it is important that compounds which serve as radiopacifiers in TCS-based formulations remain stable following cement hydration as a reduction in radio-opacity of the material can occur in the application field over long-term clinical use. Notably zirconium oxide, tantalum pentoxide and calcium tungstate have been used in commercial materials for this purpose [17-19].

TCS-based materials are mixed with water to obtain a homogeneous mixture. The cement particles hydrate in contact with the water and produce mainly calcium silicate hydrate and calcium hydroxide [9]. ProRoot MTA is mixed with water in a 0.33 powder to liquid ratio [4] as indicated by the manufacturer. A similar ratio is frequently used in studies

to assess properties of experimental TCS-based materials [15, 20]. Notably when the water: powder ratio is not sufficient to hydrate the mixture, alterations in the amount of water used are typically undertaken using visual inspection of the mixture by the operator to obtain acceptable consistency and handling efficiency. It has also been shown in experimental materials that addition of calcium phosphate or silica-based compounds, alters the hydration process of the cement [20-22].

Although a lot of work is undertaken to create novel materials based on TCS for clinical use, to date, there is no data in the literature regarding the exact alterations in the water demand in the powder to liquid proportion of hydraulic cements following replacement by different compounds and radiopacifiers in their formulation in order to be adequately hydrated. Notably, it has been reported that modifications in the water amount used in the mixture can have a significant effect in the physicochemical properties of cements [23, 24]. To the best of our knowledge, an accurate protocol for the determination of the water: powder proportion in hydraulic cements is still lacking.

Rheological properties of TCS-based materials with a potential of use as endodontic repair materials are important clinically since they affect material manipulation, placement, and stability. So far the flowability of hydraulic cements has not been extensively studied [25-27]; however, flow properties of TCS-based endodontic sealers are more routinely reported in the literature [18, 28, 29]. This could be attributed to the lack of a standardised model for flowability tests on hydraulic cements. The aim of this research therefore was to develop a standardized model for the calculation of water: powder ratio for hydraulic calcium silicate materials based on rheological properties and to investigate if the adjustment of the water: powder ratio affects the physicochemical properties of the materials. The null hypothesis was that the various additions will not affect the water

demand of the hydraulic calcium silicate cements. The other hypothesis studied was that materials mixed at different water: powder ratios will not present alterations in their physicochemical properties.

2. Materials and Methods

The materials studied were:

- Tricalcium silicate cement (TCS; Mineral Research Processing, Meyzieu, France)
- TCS and various radiopacifiers replacing the cement by 30% proportion including zirconium oxide (ZrO; CAS No: 1314-23-4) , tantalum oxide (TaO; CAS No: 1314-61-0), calcium tungstate (CaWO; CAS No: 7790-75-2) and a mixture of zirconium oxide and tantalum oxide in 15:15 wt% proportion (Sigma Aldrich, Gillingham, UK)
- Radiopacified TCS with 15 wt% replacement of calcium phosphate monobasic (CaP; Sigma Aldrich, Gillingham, UK, CAS No: 7758-23-8)
- Radiopacified TCS with 10 and 20 wt% addition of micro-silica (mS10, mS20 respectively; Meyco 610, MBT-FEB, Manchester, UK)
- Portland cement (PC; Blue Circle Portland Cement, Tarmac, Wolverhampton, UK, CAS No: 65997-15-1)

All the testing was performed in triplicate.

2.1. Rheological assessment

Portland cement was used in this experimental model system as this has been studied in the construction industry [30] and large quantities were available manufactured from a single batch. Portland cement manufactured to BS EN 197-1: 2000, type CEMI 52.5 N was mixed with water at a water: powder ratio of 0.35 (PC_0.35). This formulation was regarded as providing the ideal mixture with a clinically acceptable consistency and its hydration has been described thoroughly [11]. This water: powder ratio was used as the

baseline for all the experiments. The performance of Portland cement mixed with water in the above ratio served as a control for the establishment of the optimum proportion of water in all the other materials with additives or radiopacifiers.

A shear rheometer with adjustable temperature (Discovery HR-1, TA instruments, New Castle, USA) with stainless steel parallel plate geometry and a 40 mm diameter upper plate with crosshatched surfaces was used. After the mixing procedure using 2.5 g of each powder with the respective amount of water undertaken upon a glass plate with a metal spatula, an addition of 87.5 μL of water per gram was transferred to the initial mixture to ensure reproducible sample loading and homogenous contact surface with the upper plate.Samples were then loaded immediately upon the Peltier plate. The gap between the two plates was set at 700 μm and the temperature of the Peltier plate was established at 20^{o}C. The upper plate was initially loaded at 800 μm, where any potential excess of the material that was squeezed out was removed. Experiments were performed with a 2-minute flow-sweep test, during which the shear rate applied escalated from 1 to 100 s^{-1}. The prototype materials were mixed with different water: powder ratios in order to obtain flowability values comparable with those of the control. Results of the values of viscosity per shear rate from 1 to 20 s^{-1} were plotted, as at higher shear rates the measurements were not always related to the samples as there was potential for separation of the upper plate from the sample. An evaluation of the tricalcium silicate was also performed and the values were translated to the TCS-based mixtures.

2.2. Flowability measurement

Flowability tests of the materials mixed either in a 0.35 or an adjusted water: powder ratio established by the rheometer test were performed. The International Standards for dental root canal sealing materials (ISO 6876:2012) instructions for

assessment of flowability were followed [31]. More specifically, 0.05 mL (± 0.005 mL) of each material was transferred to a 40 mm x 40 mm glass plate (5 mm thick) with the use of a graduated syringe (BD Plastipak, Wokingham, UK). Two minutes after the start of mixing, a similar glass plate, weighing 20 g was centrally located upon the base plate, along with a 100 g weight. After a total of 10 minutes, the weight was removed. The minimum and maximum diameter of the material were assessed using a digital calliper with a 0.01 mm precision (Duratool, CPC Farnell, Preston, UK). If the variation was within 1 mm, then the mean diameter was calculated; otherwise the experiment was repeated. The TCS mixed at a water: powder ratio of 0.35 served as a control.

2.3. Phase analyses

For phase determination, X-ray diffraction analysis was performed. Materials mixed either with the 0.35 water: powder ratio or the adjusted ratio as determined by the rheometer were immersed in Hank's balanced salt solution (HBSS; Sigma Aldrich, Gillingham, UK) and incubated for 7 days at 37°C (Hybaid, Thermo Scientific, Thermo Scientific, Waltham, MA, USA). Subsequently, specimens were vacuum desiccated and crushed using an agate mortar and pestle. The diffractometer (Bruker D8 Advance, Bruker Corp., Billerica, MA, USA) with a CuKa radiation at 40 mA and 45 kV was set to rotate between 15-50° with a 0.02° 2θ step and a step time of 0.6 s. Phase identification was undertaken using a search-match software using the ICDD database (International Centre for Diffraction Data, Newtown Square, PA, USA).

2.4. Ion release assessment

Analysis of calcium leaching was performed using inductively coupled plasma-optical emission spectrometry (ICP-OES; Optima 8000 ICP-OES, Perkin Elmer, Waltham, MA, USA). Materials were prepared as described above, weighed (0.0001 g precision) and immersed in

vials containing 5 mL HBSS at 37ºC. After 7 days, pellets were retrieved from the vials and the solutions were analysed for calcium using pure HBSS as the blank sample and 6 calibration solutions. Results were assessed in respect to the weight of the material and the solution volume and expressed in μg/g using the following equation:

$$\text{Amount of calcium released per weight of pellet } (\mu g/g) = \frac{\text{amount of leachate per litre } \left(\frac{mg}{L}\right) \times \text{ volume of solution(L)} \times 1{,}000}{\text{weight of cement pellet (g)}}$$

2.5. Scanning electron microscopy (SEM) and X-ray energy dispersive analysis (EDX)

Materials mixed with the adjusted water: powder ratios were prepared in 15-mm rubber rings (3-mm thick) and allowed to set for 24h at 37ºC in relative humidity. Following vacuum desiccation for 24 hours, specimens were embedded in resin (EpoFix, Struers Ltd, Catcliffe Rotherham, UK) in cylindrical plastic moulds. A grinder/polisher (Phoenix Beta, Buehler, Coventry, UK) was used to grind the materials with progressively finer discs under water or appropriate lubricant irrigation (Diapro and OP-S, Struers Ltd, Catcliffe Rotherham, UK). Consequently, moulds were mounted on aluminium stubs, gold sputter coated (K550X Sputter Coater, Quorum Technologies Ltd, Kent, UK) and viewed under the SEM (EVO MA10, Carl Zeiss Ltd, Cambridge, UK) in backscatter mode at a range of magnifications. EDX analysis was performed at determined points (particles) or bigger fields.

2.6. Radio-opacity evaluation

Materials mixed with the adjusted water: powder ratio were evaluated for radio-opacity values according to ISO 6876:2012 instructions [31]. Specimens were prepared in rubber discs measuring 10 ± 1 mm in diameter and 1 ± 0.1 mm thick and allowed to set for 24 hours at 37ºC in humidity. Specimens were arranged on a photo-stimulable phosphor plate (DenOptix, Gendex Dental Systems, Hatfield, PA, USA) adjacent to a calibrated

aluminium step wedge (Everything X-ray, High Wycombe, UK) with 3 mm increments. A standard X-ray machine (Progeny Dental, Midmark Corp, Kettering, OH, USA) was used to irradiate the specimens using an exposure time of 0.80 s at 10 mA, tube voltage at 65 ± 5 kV and a cathode–target film distance of 300 ± 10 mm. The radiographs were then processed (Clarimat 300, Gendex Dental Systems, Hatfield, PA, USA) and a digital image of the radiographs was obtained. For interpretation of results, a method previously described by Formosa et al. was used [32]. Briefly, an imaging programme, ImageJ (Rasband WS, ImageJ; US National Institute of Health, Bethesda, MD, USA) was utilised to calculate the gray pixel value on the radiograph of each step in the step-wedge. Consequently, data for the thickness of the aluminium against the gray pixel value on the radiograph was plotted; the best-fit logarithmic trend line was then identified.

2.6. Statistical analyses

Data were evaluated using the Statistical Package for the Social Sciences software version 24 (IBM SPSS Statistics, IBM Corp, Armonk, NY, USA). For the rheological assessment, Dunnett's test using the PC_0.35 as a control was conducted for viscosity values with similar shear rate applied (1 decimal precision) between 1 to 20 s^{-1}. The mean values of viscosity of each material were also compared. Independent sample t-tests between materials of the same components and with different ratios were conducted; for the flowability tests and calcium leaching, Dunnett's test using the TCS_0.35 as a control were conducted additionally. For the radio-opacity assessment, one-way analysis of variance and Tukey's multiple comparison tests were performed. The significance level of $p = 0.05$ was used for analyses.

3. Results

3.1. Rheological adjustment

The water: powder ratios of the different formulations in comparison to the one established for Portland cement mixed at a water: powder ratio of 0.35 are shown in Table 1. Both calcium phosphate and the micro-silica increased the water demand of the mixture. The replacement of the different radiopacifiers did not have the same effect. The zirconium oxide did not affect the water: powder ratio required while the tantalum oxide and calcium tungstate increased and decreased the water: powder ratios, respectively. It was also shown that replacement of each cement by inclusion of 30% radiopacifier resulted in a standard change in the water amount needed to hydrate the material, regardless of the composition of the cement, except for the zirconium oxide which had no effect in the water: powder ratio. These data are shown in Table 2 where the percentage increase or decrease in the water demand is provided.

3.2. Flowability tests

Table 3 shows results and comparisons between flowability of materials with the same components but different water: powder ratios, as well as comparisons with the TCS. The inclusion of calcium phosphate increased significantly the flowability of the materials in all the material combinations tested following the water: powder ratio adjustment, regardless of the radiopacifier used ($p < 0.05$). Calcium phosphate incorporation increased the water demand substantially as indicated in the rheology testing. Consequently, flowability was reduced for CaP- containing materials mixed with the 0.35 water: powder ratio in comparison with the pure TCS ($p<0.05$), except for the TCS-CaP/CaWO. The incorporation of calcium tungstate resulted in a relatively low water demand as indicated in the rheology testing (Table 2). Thus it compensated for the high water demand of the calcium phosphate in the mixture.

The micro-silica altered the flowability characteristics of the materials when the adjusted water: powder ratio was applied ($p < 0.05$), except for the TCS-mS10/ZrO-TaO and TCS-mS20/CaWO, where no significant change was reported ($p>0.05$).

The different radiopacifiers incorporated in the pure tricalcium silicate did not affect the flowability values of cements in comparison with the non-radiopacified control (TCS_0.35) at both water: powder ratios tested ($p>0.05$). The incorporation of zirconium oxide and calcium tungstate did not change the flowability characteristics following the adjustment of the water amount used to mix them, however tantalum oxide incorporation increased the material flowability at the adjusted water: powder ratio indicated comparing with the standard 0.35 ratio. The tantalum oxide replacement increased the water demand substantially as shown in Table 2 for the rheology study.

3.3. X-Ray diffraction analysis

Results from the XRD analysis are presented in Figure 1a-d. All materials exhibited peaks for calcium hydroxide (ICDD: 01-078-0315). The tricalcium silicate had completely reacted after 7 days and the only peaks visible in the trace were those of the calcium hydroxide at 18 and 34° 2θ. The micro-silica was amorphous and no peaks were detected in the respective materials (Figure 1c and 1d). The radiopacified materials exhibited peaks for zirconium oxide (ICDD: 00-037-1484), tantalum oxide (ICDD: 01-081-8067) and calcium tungstate (ICDD: 04-007-2671) depending on the respective radiopacifiers used. The use of tantalum oxide as radiopacifier enhanced the formation of crystalline calcium hydroxide in the materials with adjusted water: powder ratios (Figure 1a). This increase was also evident when calcium phosphate (Figure 1b) was included but not in the 10% micro-silica addition. When 20% micro-silica was added the formation of crystalline calcium hydroxide was

enhanced with the mixture of zirconium oxide with or without tantalum oxide and the calcium tungstate radiopacifiers (Figure 1d).

3.4. Ion release assessment

The calcium ion leaching data is presented in Figure 2. All the materials tested leached relatively high levels of calcium ions in solution after 7 days of immersion in HBSS. The reduction in the water added to the calcium tungstate radiopacified material resulted in a reduction in calcium ion leaching. Increased calcium ion leaching was shown for the additions of calcium phosphate with both zirconium oxide and the combination of zirconium oxide and tantalum oxide. The micro-silica addition did not affect the calcium ion release significantly except for the 20% addition with the tantalum oxide radiopacifier where a relatively higher calcium ion leaching pattern was observed in the adjusted water: powder ratio. Compared with the cement containing no radiopacifier, the zirconium oxide radiopacified TCS and the materials with additions of micro-silica and tantalum oxide mixed with the 0.35 ratio, released less calcium ($p < 0.05$). Only the material with 20% micro-silicaaddition and tantalum oxide presented significantly higher calcium release, following the adjustment of the water: powder proportion.

3.5. SEM-EDX characterization

Representative scanning electron micrographs in backscatter mode and EDX analysis of specific microstructures are presented in Figure 3. The prototype cements were all based on tricalcium silicate with different radiopacifiers replacing them by 30%. Calcium phosphate monobasic and micro-silica were also used as replacement materials. The cement particles in the pure TCS prototype cement exhibited a halo of hydration product surrounding unhydrated cement particles of a similar size in a dense matrix (Figure 3a). Some of the cement particles appeared fully hydrated and the purity of tricalcium silicate

was verified by the EDX analysis. The addition of the calcium phosphate monobasic changed the hydration kinetics of the tricalcium silicate cement. The calcium phosphate cement particles of the TCS-CaP based materials consisting of calcium, phosphorus and silicon was evident in the micrographs by large halo rims surrounding the unhydrated particles (Figure 3b). The addition of micro-silica also modified the hydration kinetics. In the 10% replacement (Figure 3c), a dense matrix of hydrated cement particles was evident. In the 20% replacement specimen (Figure 3d), fewer unhydrated cement particles were apparent in the micrograph. The matrix was densely filled with micro-silica particles.

Different radiopacifier particles and a combination of them (ZrO and TaO) were included in each cement. All of them appeared well distributed throughout the bulk of each material. Zirconium particles appeared round in morphology and exhibited a range of sizes (Figure 3c). The radiopacifier particles composed of tantalum oxide presented as a relative smaller size while they were occasionally organized in high proximity to each other (Figure 3a). In the materials consisting both of both zirconium and tantalum oxide, similar findings were evident (Figure 3b). Finally, the radiopacifier particles in the calcium tungstate containing prototypes, were spherical in morphology and relatively large in size (Figure 3d). The elemental analysis undertaken on each radiopacifier particle, verified its constituents.

3.6. Evaluation of radio-opacity

All materials exhibited acceptable radio-opacity (≥ 3 mm aluminium) after the adjustment of the water: powder ratio as specified by ISO 6876:2012 [31] (Table 4). The micro-silica addition with calcium tungstate and the 10% micro-silica with zirconium oxide, generated higher values of radio-opacity, with significant differences compared with the majority of the other materials, especially those containing tantalum oxide ($p<0.05$). No statistically significant differences were identified between the other materials tested ($p >$

0.05).

4. Discussion

The use of a rheometer has been proposed by several previous studies for the assessment of rheological properties of endodontic sealers [33, 34], impression materials [35], composites [36-39], as well as for determining the material setting time [40]. For endodontic sealers [41, 42], the materials are loaded between two parallel plates and different shear strain tests can be applied. The change in viscosity values as a result of the deformation of the material can thus be monitored. In the present study, a rheometer was used to determine viscosity values for increasing values of shear rate for each prototype material with different additives tested. Portland cement mixed with a 0.35 water: powder ratio was used as a control, since this mixture has the most acceptable handling properties for clinical use and the process of its hydration reaction has been extensively assessed [11]. A standard amount of water was added to each test material to render them adequately flowable and ensure reproducibility of the experiments (Table 2). This was considered important since the maximum loading force applied by the upper plate was not adequate enough to achieve an even contact surface throughout the mixture due to the cement's stiffness.

Although a 2-minute flow-sweep test was initially designed, it was noted that viscosity values for a shear rate above 20 s^{-1} could result in separation of the material with the upper plate and they were thus not significant for evaluation. Therefore, results were assessed for the first 23 seconds of the experiment. Data obtained from the rheological assessments showed an increase in the water demand when calcium phosphate, micro-silica or tantalum oxide was incorporated into the material. Replacement with zirconium oxide did not affect the water amount and resulted in similar viscosity values compared with the

unmodified cement, while calcium tungstate decreased the water demand. Apart from zirconium oxide, a standard percentage change of the water: powder ratio was determined when a 30% replacement of the radiopacifiers was incorporated compared with the unradiopacified cement. Thus, the hypothesis that different compounds will not alter the water demand of the cement was rejected.

ISO 6876:2012 flowability tests were also performed [31]. Results from the compression glass plate analyses demonstrated a significant change in flowability values of most prototype materials after the adjustment of the water: powder ratio, especially for the calcium phosphate and micro-silica containing cements ($p < 0.05$). However, comparisons with the unradiopacified cement were only in partial agreement with results obtained from the rheometer. Therefore, the pattern in the adjustment of the water: powder ratio could not be supported in all cases. This can partially be attributed to the lower sensitivity of the compression glass plate test [33]. ISO flowability tests provide information on the compressed diameter of the material after a specific amount of load is applied. In contrast, the protocol design of the rheological adjustment is dynamic; apparent viscosity values are calculated as progressively higher shear strain is applied to the material. Moreover, the ISO flowability tests are designed for endodontic sealers; such materials are expected to present relatively higher flowability compared with hydraulic cements [31]. To overcome these limitations, a new method using micro-computed tomography has also been proposed recently for reproducible flowability assays of root-repair materials [25]. This model is applied in 3-dimensions and thus potentially more relevant to the clinical scenario.

X-ray diffraction was used for crystalline phase analysis. All materials formed calcium hydroxide while the peaks for tricalcium silicate were at reduced intensity indicated that a significant part of the hydration reaction had occurred by 7 days. The adjustment of the

water: powder ratio resulted in differences in the calcium hydroxide peaks mainly for the tantalum containing materials, where increase of the water amount included was correlated with higher intensity peaks. No differences in the diffraction patterns where detected in the 10% micro-silica replacement. Micro-silica affects material hydration and calcium ion release in the long term by formation of calcium silicate hydrate from the reaction of the calcium hydroxide and the silicon oxide [43]. This is very well documented in the construction industry and even exploited to enhance the strength of concrete mixtures in the long term [44]. The current testing was performed after 7 days so the effect of the micro-silica on the calcium ion release may have been masked. Calcium leaching in solution is considered an important parameter for endodontic materials as it has been correlated with the beneficial biological properties of the hydraulic cements, such as positively promoting pulp tissue responses and dentin bridge formation [45-47]. The increase of the water: powder ratio resulted in higher calcium release and this is in accordance with a previous study [24]. The material characterization was performed by SEM and EDX using the back-scatter detector after the adjustment of the water: powder proportion in order to monitor the hydration reaction. The hydration mechanism was altered after the addition of calcium phosphate and micro-silica as is evidenced by the micrographs. The different radiopacifiers were organized throughout the bulk of the material or spread as independent particles, especially following calcium tungstate inclusion.

Finally, we evaluated the radio-opacity of the materials after modifying the water amount. All the materials were adequately radiopaque after modification of the water amount according to ISO 6876:2012 specifications [31]. Notably it has been previously shown that an increase in the water: powder ratio is negatively correlated with the radio-opacity of hydraulic cements [24].

Results from the physicochemical analyses indicated that the null hypothesis regarding the modification of material's properties following the change in the hydraulic cement's water demand can be partially rejected. The effect of different amounts of water on the physical properties of Portland cement has been described previously [30]. Therefore, it should always be taken into consideration when evaluation of physicochemical properties of TCS-based cements is carried out after addition of different compounds and radiopacifiers. As a consequence, a potential alteration in characteristics could be incorrectly attributed to the additives. It is also evident that the suggested powder to liquid proportions suggested by the manufacturer, should be strictly followed.

Conclusions

The present study provides a reproducible method for the calculation of the water amount in hydraulic cements by establishing similar rheological properties using a reference material. To the best of our knowledge, no other protocol for this adjustment exists. The water demand for materials which include radiopacifiers and additives varies depending on the type of radiopacifier and additive. It is important to adjust the water: powder ratio based on the radiopacifier and additive type as this will affect the material properties.

Acknowledgments

Gabor Dravavolgyi and Jianguo Liu for their technical assistance in rheology and scanning electron microscopy respectively.

References

[1] Torabinejad M, White DJ. Tooth filling material and method of use. United States patent US 5415547; May 16, 1995.

[2] Chong BS, Pitt Ford TR, Hudson MB. A prospective clinical study of mineral trioxide aggregate and IRM when used as root-end filling materials in endodontic surgery. Int Endod J 2003;36:520-6.
[3] Torabinejad M, Parirokh M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview - part II: other clinical applications and complications. Int Endod J 2018;51:284-317.
[4] Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. J Endod 1999;25:197-205.
[5] Parirokh M, Torabinejad M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview - part I: vital pulp therapy. Int Endod J 2018;51:177-205.
[6] Schembri M, Peplow G, Camilleri J. Analyses of heavy metals in mineral trioxide aggregate and Portland cement. J Endod 2010;36:1210-15.
[7] Camilleri J, Kralj P, Veber M, Sinagra E. Characterization and analyses of acidextractable and leached trace elements in dental cements. Int Endod J 2012;45:737-43.
[8] Monteiro Bramante C, Demarchi AC, de Moraes IG, Bernadineli N, Garcia RB, Spångberg LS, Duarte MA. Presence of arsenic in different types of MTA and white and gray Portland cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106:909-13.
[9] Camilleri J. Characterization of hydration products of mineral trioxide aggregate. Int Endod J 2008;41:408-17.
[10] Marciano MA, Costa RM, Camilleri J, Mondelli RF, Guimaraes BM, Duarte MA. Assessment of color stability of white mineral trioxide aggregate angelus and bismuth oxide in contact with tooth structure. J Endod 2014;40:1235-40.
[11] Camilleri J. Characterization and hydration kinetics of tricalcium silicate cement for use as a dental biomaterial. Dent Mater 2011;27:836-44.[12] Camilleri J, Sorrentino F, Damidot D. Investigation of the hydration and bioactivity of radiopacified tricalcium silicate cement, Biodentine and MTA Angelus. Dent Mater 2013;29:580-93.
[13] Camilleri J. Characterization and chemical activity of Portland cement and two experimental cements with potential for use in dentistry. Int Endod J 2008;41:791-9.
[14] Grech L, Mallia B, Camilleri J. Characterization of set Intermediate Restorative Material, Biodentine, Bioaggregate and a prototype calcium silicate cement for use as rootend filling materials. Int Endod J 2013;46:632-41.
[15] Gandolfi MG, Spagnuolo G, Siboni F, Procino A, Rivieccio V, Pelliccioni GA, et al. Calcium silicate/calcium phosphate biphasic cements for vital pulp therapy: chemicalphysical properties and human pulp cells response. Clin Oral Invest 2015;19:2075-89.
[16] Cutajar A, Mallia B, Abela S, Camilleri J. Replacement of radiopacifier in mineral trioxide aggregate; characterization and determination of physical properties. Dent Mater 2011;27:879-91.
[17] Brasseler USA [Internet]. BC Sealer safety data sheet [cited 2018 27 September]. Available from: http://brasselerusa.com/wp-content/files/SDS-0003%20TotalFill%20BC%20Sealer%20SDS%20%20REV%20B.pdf.
[18] Khalil I, Naaman A, Camilleri J. Properties of tricalcium silicate sealers. J Endod 2016;42:1529-35.
[19] Kebudi Benezra M, Schembri Wismayer P, Camilleri J. Interfacial characteristics and

cytocompatibility of hydraulic sealer cements. J Endod 2018;44:1007-17.
[20] Akbari M, Zebarjad SM, Nategh B, Rouhani A. Effect of nano silica on setting time and physical properties of mineral trioxide aggregate. J Endod 2013;39:1448-51.
[21] Viapiana R, Guerreiro-Tanomaru JM, Hungaro-Duarte MA, Tanomaru-Filho M, Camilleri J. Chemical characterization and bioactivity of epoxy resin and Portland cementbased sealers with niobium and zirconium oxide radiopacifiers. Dent Mater 2014;30:1005-20.
[22] Schembri-Wismayer P, Camilleri J. Why Biphasic? Assessment of the effect on cell proliferation and expression. J Endod 2017;43:751-9.
[23] Fridland M, Rosado R. Mineral trioxide aggregate (MTA) solubility and porosity with different water-to-powder ratios. J End 2003;29:814-7.
[24] Cavenago BC, Pereira TC, Duarte MAH, Ordinola-Zapata R, Marciano MA, Bramante CM, et al. Influence of powder-to-water ratio on radiopacity, setting time, pH, calcium ion release and a micro-CT volumetric solubility of white mineral trioxide aggregate. Int Endod J 2014;47:120-6.
[25] Tanomaru-Filho M, Torres FFE, Bosso-Martelo R, Chavez-Andrade GM, Bonetti-Filho I, Guerreiro-Tanomaru JM. A Novel model for evaluating the flow of endodontic materials using micro-computed tomography. J Endod 2017;43:796-800.
[26] Camilleri J. Evaluation of selected properties of mineral trioxide aggregate sealer cement. J Endod 2009;35:1412-7.
[27] Tanomaru-Filho M, Garcia AC, Bosso-Martelo R, Berbert FL, Nunes Reis JM, Guerreiro-Tanomaru JM. Influence of addition of calcium oxide on physicochemical properties of Portland cement with zirconium or niobium oxide. J Conserv Dent 2015;18:105-8.
[28] Zhou HM, Shen Y, Zheng W, Li L, Zheng YF, Haapasalo M. Physical properties of 5 root canal sealers. J Endod 2013;39:1281-6.[29] Silva Almeida LH, Moraes RR, Morgental RD, Pappen FG. Are premixed calcium silicate-based endodontic sealers comparable to conventional materials? A systematic review of in vitro studies. J Endod 2017;43:527-35.
[30] Neville AM. Properties of concrete. 5th ed. Essex: Pearson; 2002.
[31] International Standards Organization. Dentistry- root canal sealing materials. ISO 6876;2012.
[32] Formosa LM, Mallia B, Camilleri J. The effect of curing conditions on the physical properties of tricalcium silicate cement for use as a dental biomaterial. Int Endod J 2012;45:326-36.
[33] Chang SW, Lee YK, Zhu Q, Shon WJ, Lee WC, Kum KY, et al. Comparison of the rheological properties of four root canal sealers. Int J Oral Sc 2015;7:56-61.
[34] Lacey S, Pitt Ford TR, Watson TF, Sherriff M. A study of the rheological properties of endodontic sealers. Int Endod J 2005;38:499-504.
[35] German MJ, Carrick TE, McCabe JF. Surface detail reproduction of elastomeric impression materials related to rheological properties. Dent Mater 2008;24:951-6.
[36] Ellakwa A, Cho N, Lee IB. The effect of resin matrix composition on the polymerization shrinkage and rheological properties of experimental dental composites. Dent Mater 2007;23:1229-35.
[37] Beun S, Bailly C, Dabin A, Vreven J, Devaux J, Leloup G. Rheological properties of experimental Bis-GMA/TEGDMA flowable resin composites with various macrofiller/microfiller ratio. Dent Mater 2009;25:198-205.

[38] Lee I-B, Min S-H, Kim S-Y, Ferracane J. Slumping tendency and rheological properties of flowable composites. Dent Mater 2010;26:443-8.
[39] Kim M-H, Min S-H, Ferracane J, Lee I-b. Initial dynamic viscoelasticity change of composites during light curing. Dent Mater 2010;26:463-70.
[40] Ha WN, Nicholson TM, Kahler B, Walsh LJ. Rheological characterization as an alternative method to indentation for determining the setting time of restorative and endodontic cements. Mater 2017;10(12)1451. https://doi.org/10.3390/ma10121451)
[41] Ørstavik D. Physical properties of root canal sealers: measurement of flow, working time, and compressive strength. Int Endod J 1983;16:99-107.
[42] Vermilyea SG, Huget EF, De Simon LB. Extrusion of rheometry of fluid materials. J Dent Res 1979;58:1691-5.
[43] Camilleri J, Montesin FE, Curtis RV, Ford TR. Characterization of Portland cement for use as a dental restorative material. Dent Mater 2006;22:569-75.
[44] Bache HH, Densified cement/ultra-fine particle-based materials. Proceedings of the 2nd International conference on superplasticizers in concrete, 1981 June 10-12; Ottawa, Canada. Aalborg Portland, 1981.
[45] Foreman PC, Barnes IE. Review of calcium hydroxide. Int End J 1990;23:283-97.
[46] Tamburic SD, Vuleta GM, Ognjanovic JM. In vitro release of calcium and hydroxyl ions from two types of calcium hydroxide preparation. Int Endod J 1993;26:125-30.
[47] Prati C, Gandolfi MG. Calcium silicate bioactive cements: Biological perspectives and clinical applications. Dental Mater 2015;31:351-70

Table 1. Adjusted water: powder ratio for Portland cement (PC) with different compounds or radiopacifiers as calculated by the rheological assessment. The numerical scale has been set in two decimal digits.

PC	No radiopacifier	*30% radiopacifier*			
		ZrO	**TaO**	**ZrO-TaO**	**CaWO**
No addition	0.35 *(control)*	0.35	0.42	0.37	0.26
15% CaP	0.50	0.50	0.60	0.53	0.37
10% mS	0.40	0.40	0.48	0.42	0.30
20% mS	0.50	0.50	0.60	0.53	0.37

Table 2. Standard percentage change in the water demand of hydraulic cements after 30% replacement of radiopacifier.

30% radiopacifier	*Modification in the water: powder ratio*
ZrO	Same
TaO	20%↑
ZrO-TaO	5.71% ↑
CaWO	25.71%↓

Table 3. Mean flow values and standard deviation of tested prototype materials mixed with different ratios according to ISO 6876(2012). A = Adjusted; The latin letter '*a*' indicates significant difference in flowability values of materials with the same components after mixing with different water amounts ($p<0.05$); the asterisk indicates significant difference in flowability values comparing to the TCS_0.35 control ($p<0.05$).

Material	**Ratio**	*30% radiopacifier*			
		ZrO	**TaO**	**ZrO-TaO**	**CaWO**
TCS	0.35	9.22 ± 0.1	8.63 ± 0.5	9.99 ± 0.9	10.8 ± 1.1
	A	-	10.91 ± 0.3^{a}	10.09 ± 0.6	9.38 ± 1
TCS-CaP	0.35	8.14 ± 0.04*	6.84 ± 0.3*	7.06 ± 0.5*	8.34 ± 0.4
	A	10.45 ± 0.3^{a}	10.3 ± 0.5^{a}	10.12 ± 0.2^{a}	9.17 ± 0.2^{a}
TCS-mS10	0.35	8.65 ± 0.3	8.34 ± 0.5	8.35 ± 0.4	10.75 ± 0.2^{a}
	A	11.36 ± 0.3*^{a}	10.72 ± 1.4^{a}	9.31 ± 0.5	9.37 ± 0.6
TCS-mS20	0.35	8.41 ± 0.2	8.42 ± 0.3	8.47 ± 0.4	10.57 ± 0.3
	A	10.57 ± 0.5^{a}	11.11 ± 0.4*^{a}	13.97 ± 0.3*^{a}	10.34 ± 0.4
TCS_0.35 (*control*)		9.65 ± 0.6			

Table 4. Mean and standard deviation of radio-opacity values of materials (mm aluminium) after the adjustment of the water: powder ratio. Latin letters *a*, *b* and *c* indicate statistical significant differences from TCS-mS10/CaWO, TCS-mS10/ZrO and TCS-mS20/CaWO respectively ($p<0.05$)

TCS	*30% radiopacifier*			
	ZrO	**TaO**	**ZrO-TaO**	**CaWO**
No addition	3.6 ± 0.2	3.1 ± 0.1^{abc}	3.5 ± 0.2^{b}	3.1 ± 0.1^{abc}
15% CaP	3.1 ± 0.1^{abc}	3.1 ± 0.02^{abc}	3.3 ± 0.2^{abc}	3.5 ± 0.1^{b}
10% mS	4 ± 0.1	3.3 ± 0.1^{abc}	3.6 ± 0.04	4.2 ± 0.4
20% mS	3.1 ± 0.2^{abc}	3.2 ± 0.3^{abc}	3.3 ± 0.05^{abc}	3.9 ± 0.3

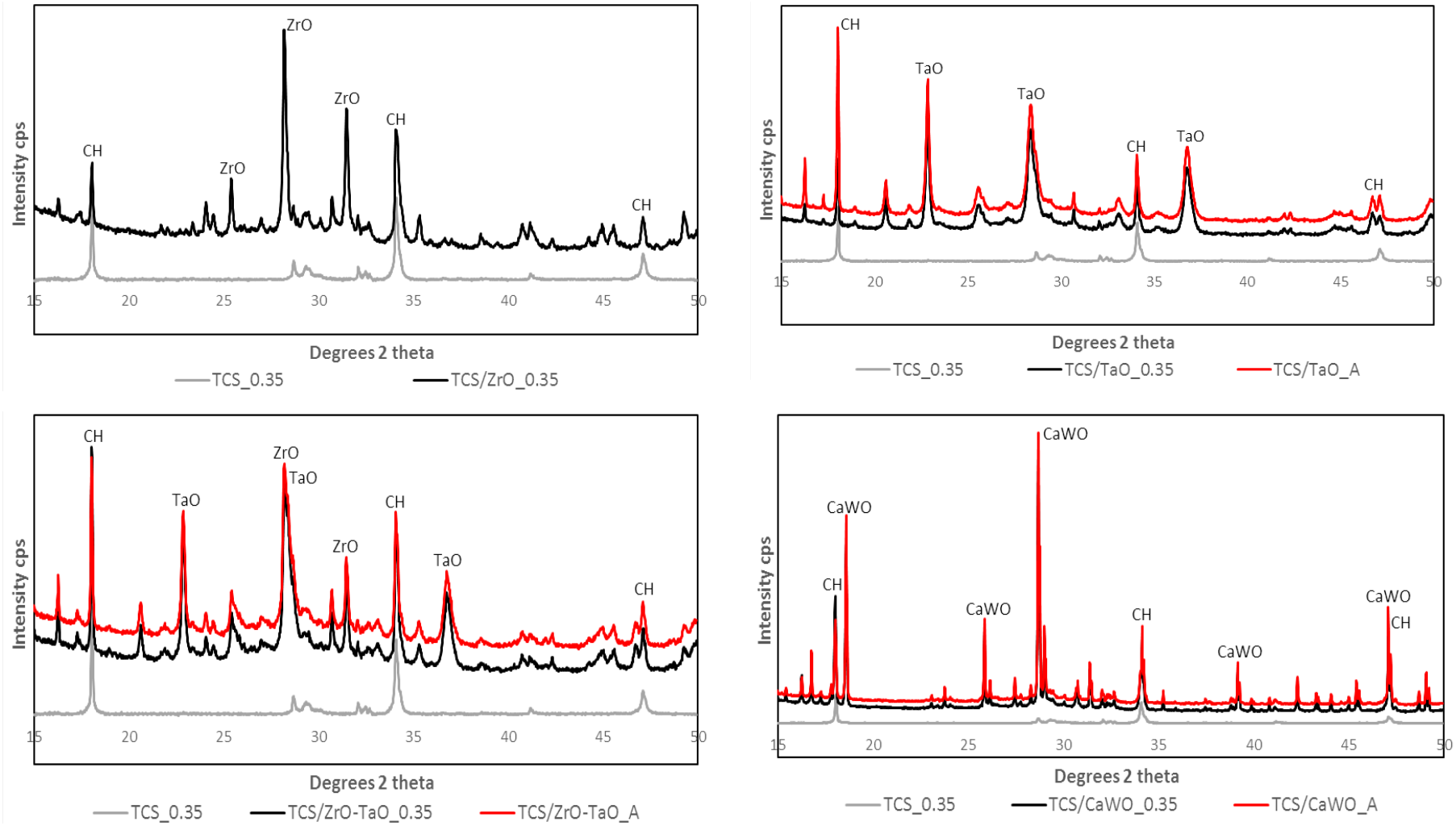


Figure 1a. X-ray diffraction plots of tricalcium silicate cement and test prototype materials replaced with zirconium oxide, tantalum oxide, a mixture of both zirconium oxide and tantalum oxide and calcium tungstate mixed in a 0.35 water: powder ratio or an adjusted ratio after immersion in Hank's balanced salt solution for 1 week. CH: calcium hydroxide.

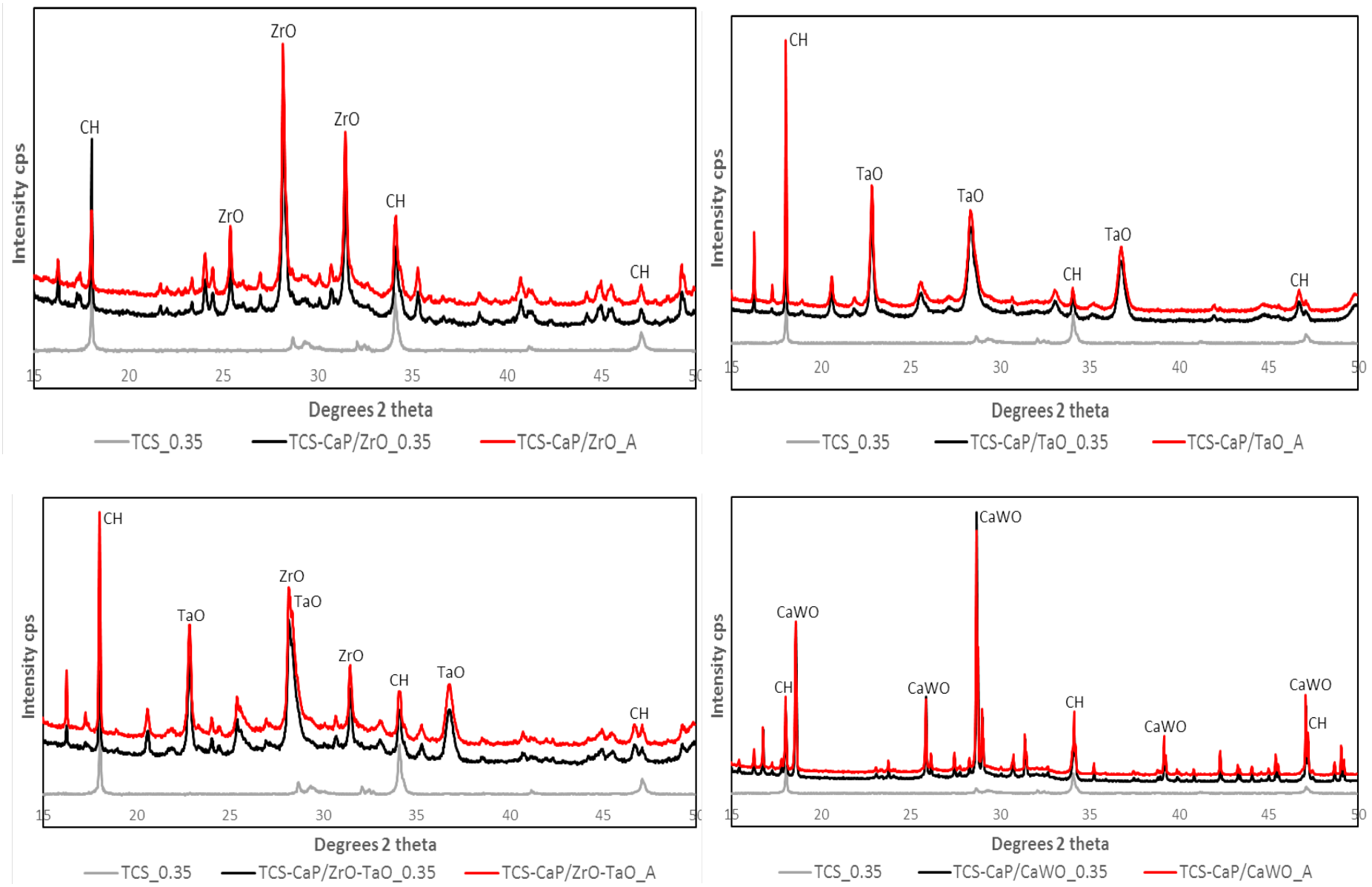


Figure 1b. X-ray diffraction plots of tricalcium silicate cement and test prototype materials with different radio-opacifiers and incorporating calcium phosphate monobasic mixed in a 0.35 water: powder ratio or an adjusted ratio after immersion in Hank's balanced salt solution for 1 week. CH: calcium hydroxide.

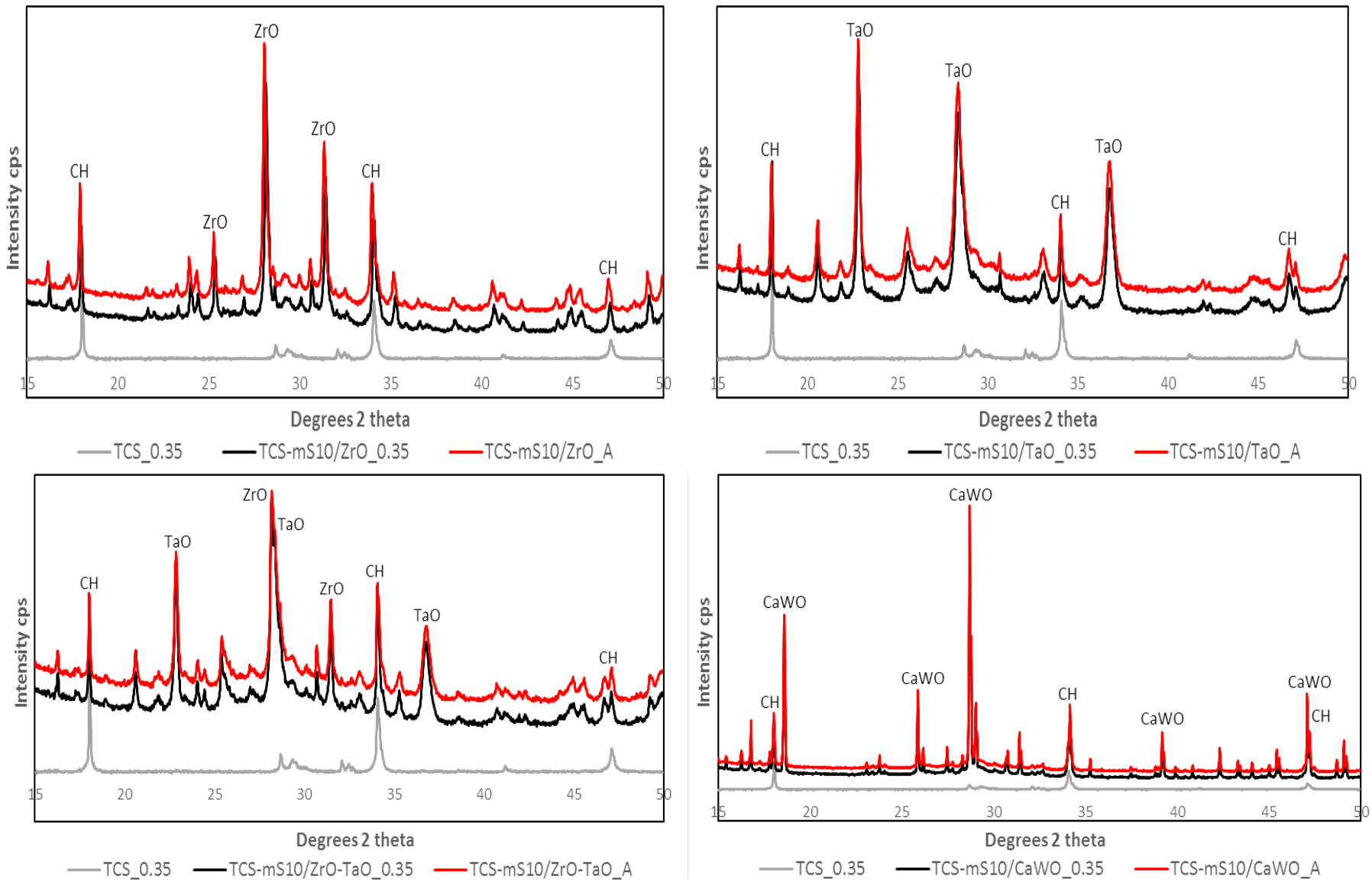


Figure 1c. X-ray diffraction plots of tricalcium silicate cement and test prototype materials with different radio-opacifiers and incorporating 10% microsilica mixed in a 0.35 water: powder ratio or an adjusted ratio after immersion in Hank's balanced salt solution for 1 week. CH: calcium hydroxide.

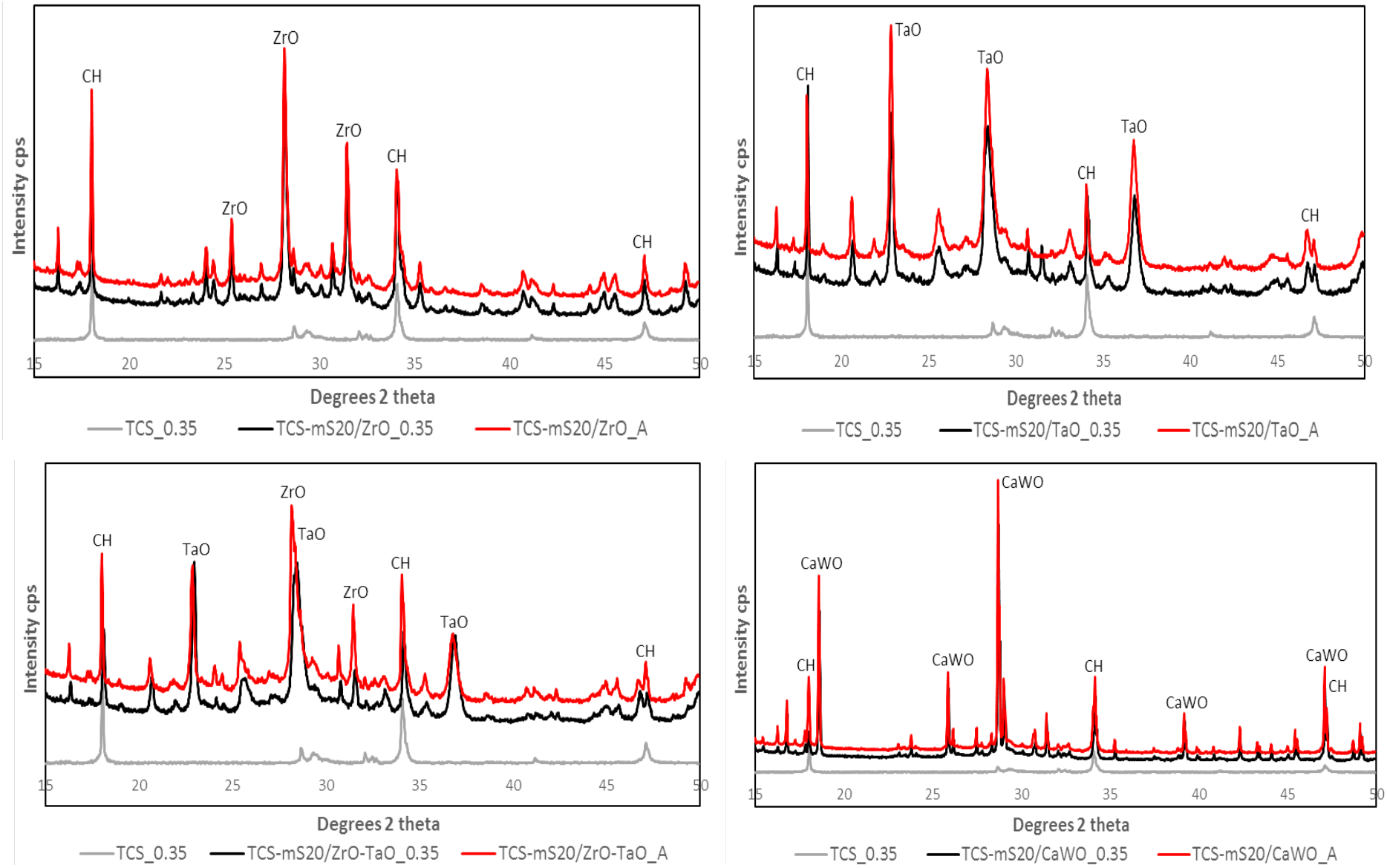


Figure 1d. X-ray diffraction plots of tricalcium silicate cement and test prototype materials with different radio-opacifiers and also incorporating 20%microsilica mixed in a 0.35 water: powder ratio or an adjusted ratio after immersion in Hank's balanced salt solution for 1 week. CH: calcium hydroxide.

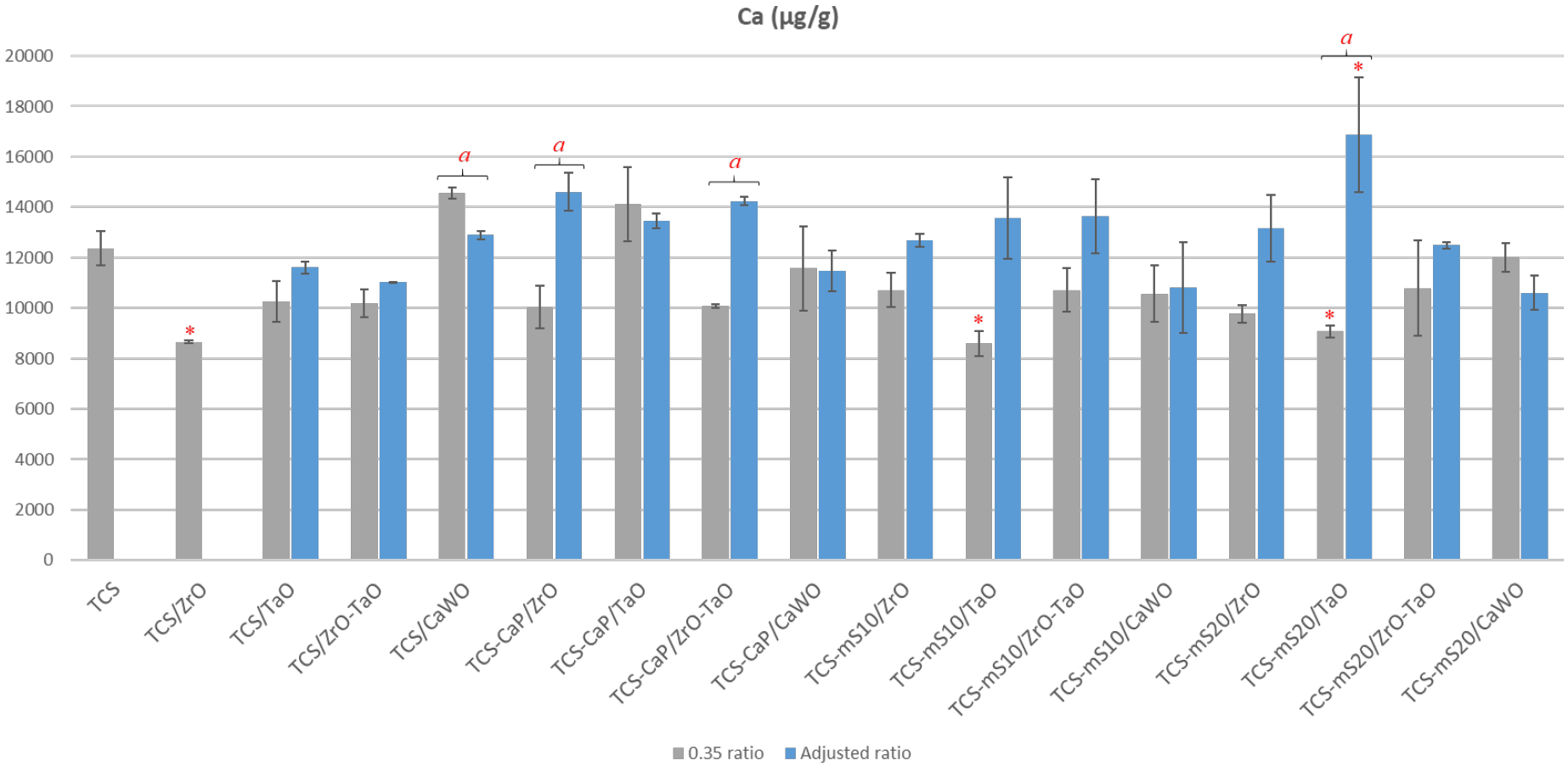
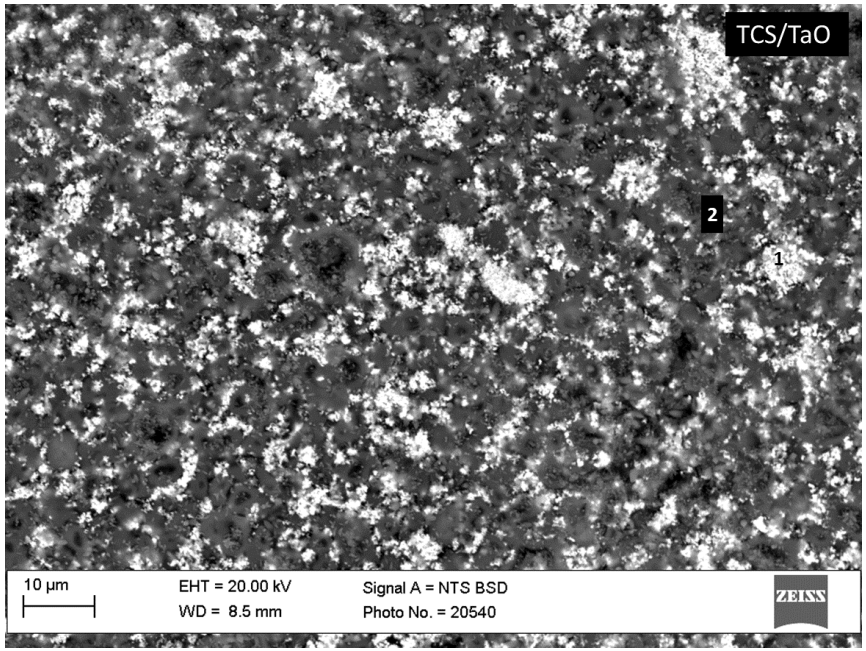
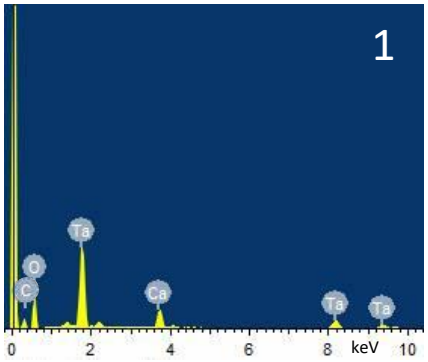
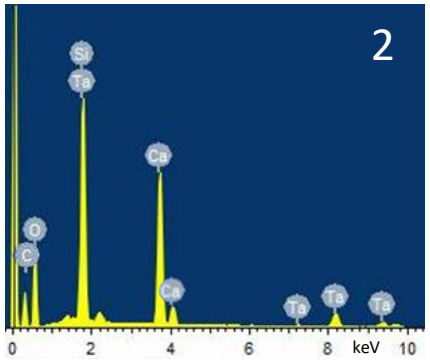


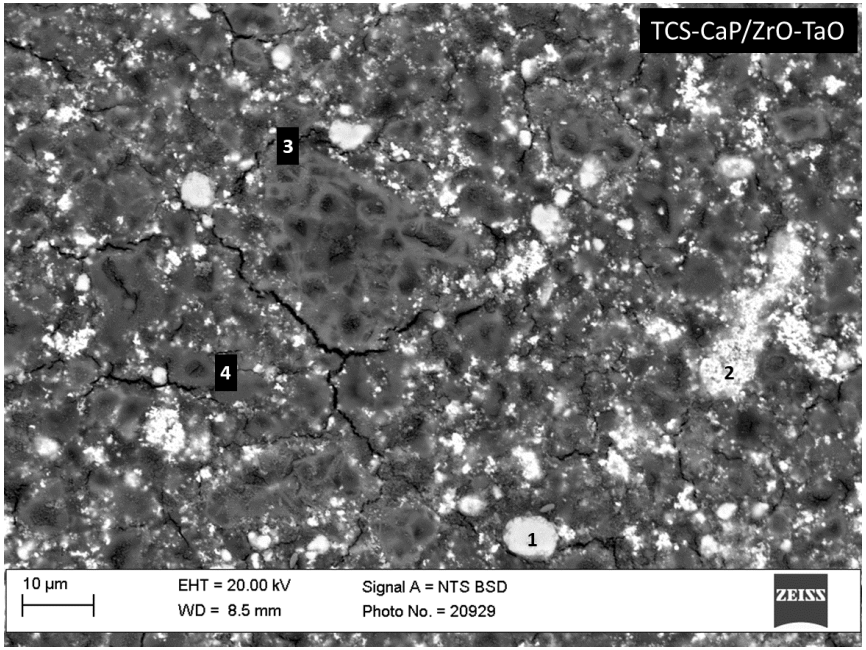
Figure 2. Calcium release of prototype materials mixed in a 0.35 water: powder ratio or an adjusted ratio after immersion in Hank's balanced salt solution for 1week. Asterisk indicates significant difference with the TCS_0.35 ($p<0.05$); the latin letter '*a*' represents statistical difference between materials with the same components and different water: powder ratios ($p<0.05$).

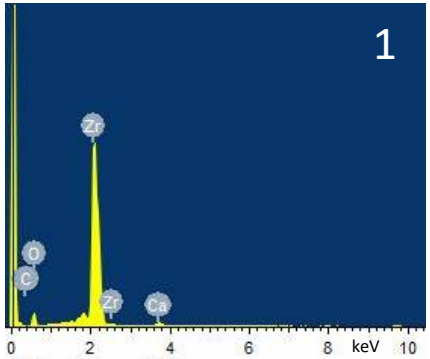

TCS/TaO
2
1
10 µm
EHT = 20.00 kV
WD = 8.5 mm
Signal A = NTS BSD
Photo No. = 20540
ZEISS

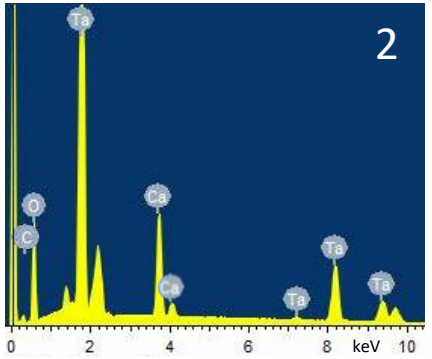

1
O
C
Ta
Ca
Ta
Ta
0
2
4
6
8
keV
10

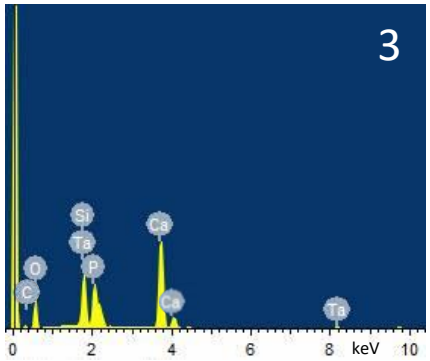

2
Si
Ta
Ca
O
C
Ca
Ta
Ta
Ta
0
2
4
6
8
keV
10

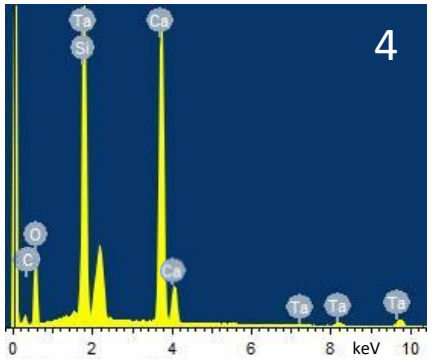
a)


TCS-CaP/ZrO-TaO
3
4
2
1
10 µm
EHT = 20.00 kV
WD = 8.5 mm
Signal A = NTS BSD
Photo No. = 20929
ZEISS

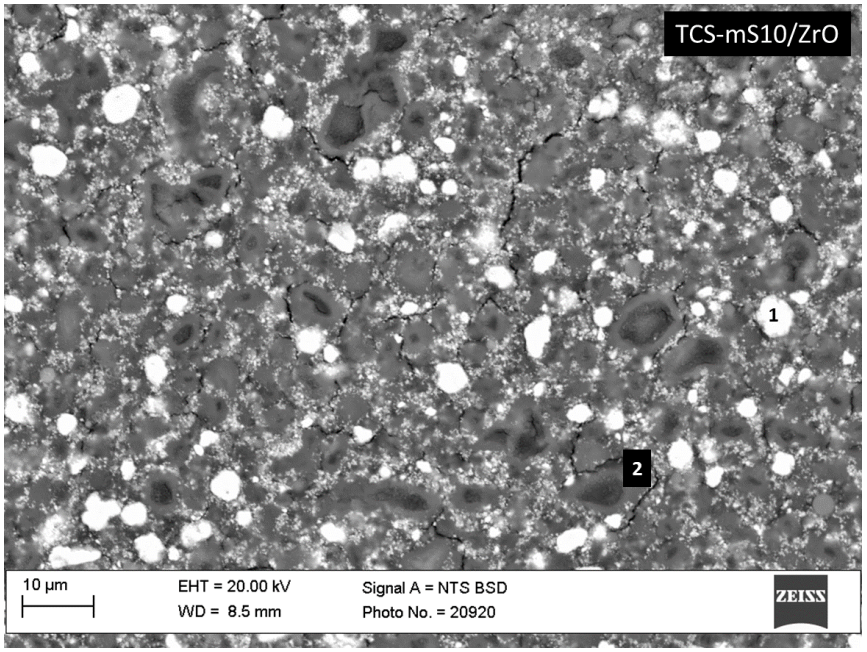

1
Zr
O
C
Zr
Ca
0
2
4
6
8
keV
10

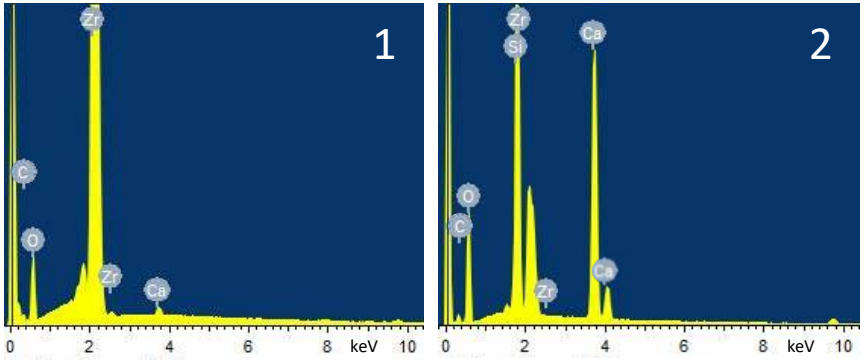

2
Ta
O
C
Ca
Ca
Ta
Ta
0
2
4
6
8
keV
10


3
Si
Ta
P
O
C
Ca
Ca
Ta
0
2
4
6
8
keV
10

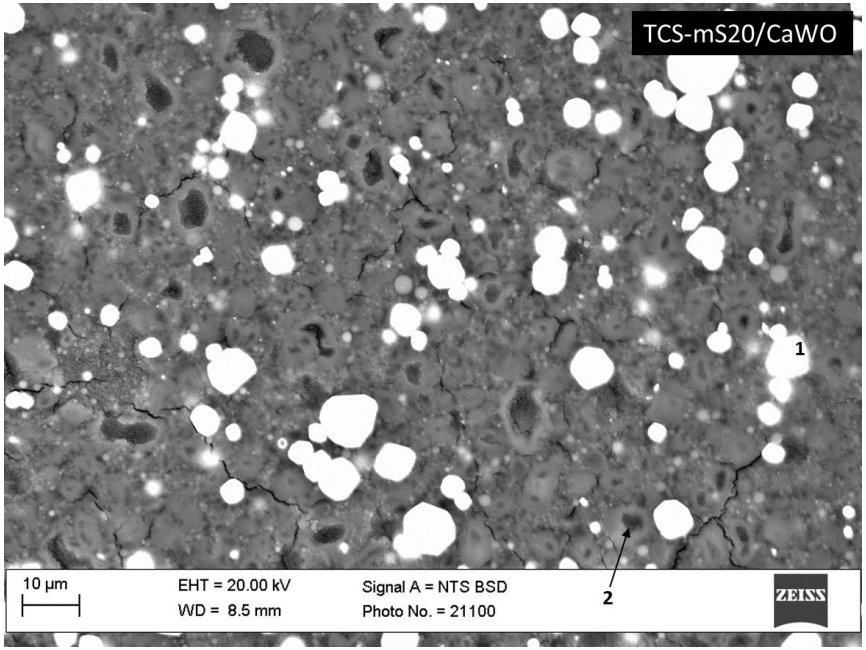

4
Ta
Si
Ca
O
C
Ca
Ta
Ta
Ta
0
2
4
6
8
keV
10

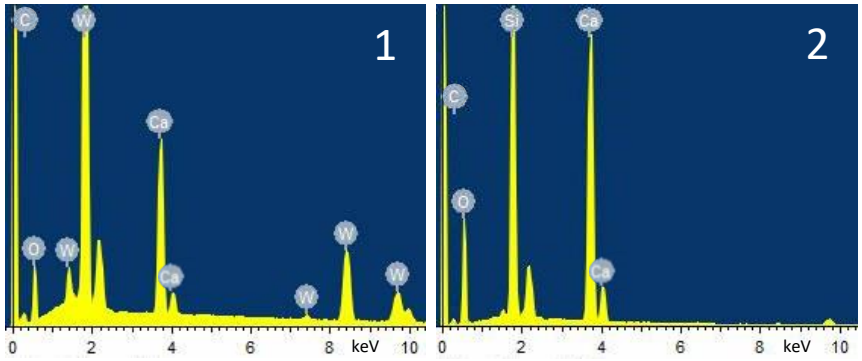
b)





c)





d)

Figure 3. Representative back-scatter scanning electron micrographs of polished sections of prototypes with different additives and radio-opacifiers (2500X magnification) showing microstructural components and energy-dispersive spectroscopic scans of selected spectrums (a-d).