The effect of layered double hydroxide on fluoride release and recharge from a commercial and an experimental resin varnish

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Abstract

Objectives: Layered Double Hydroxide (LDH) is capable of fluoride anion exchange and release. This study investigated the effects of incorporating ZnAl-LDH in commercial and experimental dental varnishes, on fluoride release and re-release after charging in NaF.

Methods: Five discs of each material (commercial ClinproXT varnish and experimental light cured resin varnish), with and without 9%ZnAl-LDH were immersed and agitated in deionised water (DW) at 37°C. A fluoride ion selective electrode measured fluoride release in DW daily over two-weeks. At 3, 6, 9 and 12 days the discs were recharged in 15ml 0.05M NaF solution (37°C) for five minutes followed by immersion in DW. Energy Dispersive X-Ray spectra (EDS), weight changes and SEMs were performed on discs, before and after each cycle.

Results: Evidence of ZnAl-LDH was confirmed by the presence of peaks for zinc and aluminium in EDS spectra. Cumulative fluoride and mean fluoride released between Clinpro, Clinpro+LDH and resin were significantly different (ANOVA, Tukey's HSD post-hoc test, $p<0.001$) except between the resin and resin+LDH. Mean fluoride concentrations differed significantly after every recharge between Clinpro, Clinpro+LDH and resin, but not between the resin and resin+LDH ($p<0.01$). The weights and SEMs of the experimental resins +/-LDH, appeared to be stable whilst the weights of Clinpro samples +/-LDH, fluctuated and fragmented.

Significance: ClinproXT and experimental resin containing ZnAl-LDH recharged with fluoride did not significantly increase fluoride release compared to the unmodified materials. However, all LDH-F incorporated materials demonstrated fluoride recharging properties which appear beneficial to dentistry and thus further work is required to improve these properties.

Key words: Layered double hydroxides, resin modified glass ionomer (RMGIC) varnish, experimental light cured resin varnish, fluoride ion selective electrode, fluoride recharge, release, energy dispersive x-ray spectroscopy, scanning electron microscopy

1.Introduction

Recurrent or secondary caries at the restoration margin is a major reason for restoration replacement which results in larger restorations and significant biological cost [1,2]. Fluoride can inhibit demineralisation and promote remineralisation and is widely available in anti-caries products such as toothpastes and mouth rinses [3]. Remineralisation requires low but constant quantities of fluoride [4]. An increase in fluoride concentration from 0.025 to 2ppm has resulted in less demineralisation in extracted teeth in-vitro and in-vivo [5,6,7]. Dental materials, acting as reservoirs, provide a constant supply of fluoride release following recharging with external products. Fluoride

release and recharge are linearly correlated indicating that materials with a high fluoride release have higher fluoride recharge capacity [8]. The ability to recharge and release fluoride may be more important than the total fluoride released, whereas a constant low level of fluoride available over prolonged periods will have superior anti-caries potential than a high fluoride release over short periods. A range of fluoride releasing restorative materials are being marketed. These include fluoride varnishes, which have demonstrated caries reduction for both permanent and primary teeth [9], releasing fluoride for a limited duration. Other examples include glass ionomer cements (GICs) and resin modified glass ionomer cements (RMGIC), with potential for fluoride release and recharge, through to the resin-based materials including compomers and composites. It cannot be assumed that all brands of one class of material behave similarly, as two glass ionomers from different manufacturers released significantly different amounts of fluoride, for example Ketac released more fluoride than Chemfil at 24hrs (5.90 versus 1.90 $\mu gF/cm^2/h$, respectively) [10]. GIC and RMGICs contain a highly permeable hydrogel matrix which allows for diffusion and thus release of fluoride ions [10]. However, this diffusion can adversely affect their mechanical properties, due to the resultant porosity [11] compared to the resin-based composites.

Dental composite resins have improved aesthetics, wear resistance and are mechanically stronger than GIC and RMGIC but are known to release a small amount of fluoride [8]. This is related to the hydrophobic polymer resin matrix in the composite, which acts as a diffusion barrier to water, resulting in low fluoride release and recharge capacity [12]. Furthermore, early composites capable of fluoride release used soluble free salts, such as NaF or SnF_2, which upon dissolution, left voids in the material resulting in inferior mechanical properties. The introduction of fluoride releasing glass fillers using fluoroaluminosilicate glass attempted to overcome the drawbacks of the salts, but fluoride release and recharge were still low. Subsequently, the inclusion of fluoride releasing dimethacrylate monomers containing tetrabutylammonium fluoride (TBAF) and a fluoride releasing filler improved the ionic exchange, fluoride release and recharge characteristics of the restorative material [13].

ClinproXT varnish (3M ESPE) is a commercial light cured resin-modified glass ionomer extended contact varnish. It has been reported to have superior cumulative fluoride release compared with conventional fluoride releasing resin varnishes, in the first hour, and over a 6-month period [14].

It has the same capacity to recharge when exposed to fluoride in both toothpaste and mouthwash formulations, and saliva, compared to glass ionomer cement products. ClinproXT manufacturer's information states it is a light cured resin modified glass ionomer material, dispensed in a clicker form as a two-part liquid-paste system. The liquid contains polyalkenoic acid, HEMA (2-hydroxethylmethacrylate), water and initiators (including camphorquinone) plus calcium glycerophosphate. The paste contains HEMA, Bis-GMA, water, initiators and fluoroaluminosilicate glass (FAS glass) [14]. Due to its fluoride recharging ability and clinical uses as an extended contact varnish and the presence of Bis GMA in Clinpro XT, it was considered as an appropriate candidate to study alongside an experimental flowable resin varnish based on Bis GMA, UDMA and TEGDMA.

Layered double hydroxides (LDH) are anionic clays comprising positively charged hydroxyl layers sandwiched between divalent (M^{2+}= Zn^{2+}, Mg^{2+} etc) and trivalent (M^{3+} eg Al^{3+}, Fe^{3+}) metal ions with transferable interlayer anions (A^{n-}, eg F^-)[15]. Their general formula is:
$[M^{2+}_{1-X}\ M^{3+}_{X}\ (OH)_2]^{x+}[A^{n-}]_{x/n}.mH_2O$ [15].

LDH structure therefore can be simply considered as positively charged scaffolds of di/trivalent metal hydroxides, which contain negatively charged interlayers, that draw in anions (F^-). LDHs were first discovered in the mid-19th Century, in Sweden, as hydrotalcite. The naturally occurring LDHs are present in two polymorphic forms, mostly rhombohedral or hexagonal [15]. LDH possesses a large surface area (20-120 m^2/g) and high anionic-exchange capacity (3.0 -4.8 meq/g). Their affinity for multivalent inorganic anions (eg SO_4^{2-}) was reported to be greater than for monovalent inorganic anions (eg F^-) [15,17,18]. Different types of LDHs can be synthesised depending on the divalent-trivalent metal chlorides used as starting materials to produce them [16]. LDH has been used to remove excess fluoride from drinking water without producing a chemical sludge [17]. It has potential applications in drug delivery systems as it has high chemical versatility, ease of laboratory synthesis, high drug transportation efficiency, high drug loading density and low cell toxicity [19].

Fluoride released from BisGMA/TEGMA/ZnAl-LDH-F, light cured composites into physiological saline was studied over a 160-day period without recharge [20]. Fluoride released at all concentrations was initially rapid but plateaued and was inversely related to the fluoride concentrations within the LDH. It was postulated that the ionic exchange of fluoride by counter ions was less likely

since the anionic fluoride interlayer was better shielded by the formation of 'big tactoids' at higher LDH-F concentrations [20]. The initial concept of incorporating LDH into resin composite has been proved to be successful in terms of sustained fluoride release [21, 24] and the ability to be recharged with fluoride [23]. ZnAl-LDH, specifically has been proven to improve fluoride release and re-release after recharging in NaF solution, following incorporation into experimental composite resin (BisGMA/TEGDMA/UDMA), and room temperature cured PMMA [21].

Clearly there is a need to enhance the release of fluoride and recharge potential of experimental composite resin further in order to inhibit caries. Therefore, this study aimed to investigate and compare fluoride release and recharge potential of an experimental flowable composite varnish based on BisGMA, UDMA and TEGDMA, and a commercially available resin modified glass ionomer varnish (Clinpro XT, 3M ESPE), both with and without 9wt%ZnAl-LDH.

2.Materials and methods

This study investigated fluoride release from two materials; a commercially available resin modified glass ionomer varnish (ClinproXT [3M ESPE]) and an experimental light cured flowable resin (varnish/sealant), with and without (control) 9% ZnAl-LDH, respectively . In this contribution, the commercial varnish will also be referred to as ClinproXT and the experimental varnish will be referred to as resin.

ZnAl-LDH methodology, manufacture and incorporation were reported previously [21]. ZnAl-LDH was selected for this contribution so that the results obtained, with respect to its fluoride uptake and release properties could be compared with earlier work which used ZnAL-LDH in, for example, experimental BISGMA/UDMA based fissure sealants and a room temperature cured poly(methyl methacrylate) system. These studies utilized 2.5, 5, 7 10, 20 and 33% loadings. Eldafrawy reported that formulations above 10% LDH released similar amounts of fluoride to 20 and 33% [21]. Therefore in this study, ZnAl-LDH (9%) was incorporated into both materials.

ZnAl-LDH powder was precipitated (at room temperature; 21 ± 0.1˚C) using 0.5M $ZnCl_2$ and $AlCl_3$ solutions at pH 10 (±1) [21]. The pH was maintained by the controlled addition of 2M NaOH. The precipitate was aged for 24 hours, washed with deionised water and then centrifuged to create a

neutral solution. The supernatant was subsequently removed and dried in an oven at 80 °C for 36 h. The resulting material, ZnAl-LDH, was removed and ground using a mortar and pestle forming a powder. The particle size of the LDH powder was measured using a MasterSizer/E Particle Size Analyser (Malvern Panalytical Ltd., Enigma Business Park, Malvern, , UK) with a 45mm focal lens to analyse particles between 0.1 and 80μm in size.

The methodology for preparing test specimens and subsequent tests are described as follows: (see supplemental material; Figure A). ClinproXT (3M ESPE) was weighed on a Mettler HK balance (0.84g - approximately 7 clicks), mixed according to the manufacturer's instructions, placed into a polytetrafuoroethylene (PTFE) mould (10mm diameter by 1mm thickness) and light cured (using a 3M ESPE Elipar unit; intensity:1200mW/cm^2) for 20s on one-side (due to being only 1mm thick) to form discs. For the ClinproXT (3M ESPE) with ZnAl-LDH, 0.084g ZnAl-LDH (9%) was added to the RMGIC (0.84g) and mixed for 30s for even dispersion before curing in the moulds as above. ClinproXT and ClinproXT + ZnAl-LDH discs (n=5) were prepared in this manner. The surface area of each disc was therefore 188.5 mm^2. The moulds were supported from both sides by glass slides covered with acetate sheets to prevent adhesion of the set resin to the slides.

To form the flowable resin discs 8.75g of Bis-GMA and 7.5g of triethyleneglycol dimethacrylate (TEGDMA) were poured into a glass beaker and mixed manually, followed by the addition of 8.75g of urethane dimethacrylate (UDMA), with further stirring for 10 minutes, until a homogenous-like mix appeared. This procedure was repeated for all samples in order to standardise the mixing of the viscous components. Then camphorquinone (0.0375 g) and DMPT (0.05g) were added and stirred for a further three minutes [21]. This mix was then poured in the PTFE mould. The flowable resin discs were light cured as per the same curing criteria used for ClinproXT (20s), to create 5 discs of approximately 0.6g in weight. For the preparation of the experimental flowable composite containing ZnAl-LDH, the method described above was followed but with the addition of 0.06g ZnAl-LDH .

Initial charging of discs with fluoride

Five samples were prepared for each of the four materials and pre-weighed. The ten experimental flowable resin discs were initially charged by immersion in 15ml of 0.1M NaF solution, in an incubator shaker (IKA KS400) at 37°C and agitated at 60rpm for two days as described in earlier literature [21,23]. The experimental resin discs were removed, blotted dry and placed into 5ml of deionised water (DW) in an incubator at 37°C and agitated at 60rpm. As mentioned earlier, ClinproXT already contained fluoride, and it has been reported to release more fluoride in the first hour and over a 6-month period than conventional fluoride releasing resin varnishes [14]. Therefore the ClinproXT ± ZnAl-LDH discs were not initially charged. Instead, they were placed directly into 5ml of deionised water in an incubator shaker, at 37°C, agitated at 60rpm to release fluoride with the fluoride delivery recorded and discs placed in refreshed deionised water (5 ml) daily (every 24 hours). Fluoride ion release was measured from all discs using a calibrated fluoride ion selective electrode (ELIT 8221).

Recharging of discs with fluoride

To assess recharge and fluoride absorption, all discs were removed every 72 hours (3 days) and immersed in tubes containing 15ml 0.05M NaF solution as described in earlier literature [21, 23] in an incubator shaker at 37°C, for five minutes. This concentration was selected as most mouth rinses contain up to 250 ppm fluoride, and 0.05M is equivalent to 227ppm. After five minutes, the discs were removed, blotted dry and placed into 5ml fresh DW. A total of four fluoride recharges were performed at 3, 6, 9 and 12 days, and fluoride release was measured after 24 hours, at day 4, 7, 10 and 13 days. All data were analysed using SPSS v20 (SPSS, Chicago, IL, USA). The Shapiro-Wilk test was used to test for normal distribution of F concentration for each material (n=5) at every time point. ANOVA was applied to determine between-subject (material) differences followed by Tukey post hoc test with the level of statistical significance set at 5%.

3.Results

The particle size of ZnAl-LDH demonstrated a normal distribution and it can be assumed that the LDH particles were evenly distributed. The median particle size by volume was 4.50 μm (Dv0.5) and 90% of the particle volume was less than 22.31 μm (Dv 0.9).

Normality of fluoride concentrations was checked using the Shapiro-Wilk test on the four recharge days for all four materials, with 13 out of 16 possible distributions being normally distributed. Similarly, normality was confirmed for every 24-hours following fluoride release. The total cumulative fluoride and mean fluoride released from the four materials over the 14 days (336 hours) experimental period is shown in Table 1. The only multiple comparison for total fluoride released that was not significantly different was between the experimental resin and the experimental resin + ZnAl-LDH. All other comparisons between the material pairings were significantly different (ANOVA, Tukey's HSD post-hoc test, $p<0.001$).

Mean fluoride concentrations were significantly different ($p<0.01$), after every recharge between ClinproXT, ClinproXT + ZnAl-LDH and resin, but not between the resin and the resin + ZnAl-LDH. Although the resin + ZnAl-LDH released more fluoride after each recharge than resin alone, both tailed off within 24 hours to negligible values. The resin + ZnAl- LDH demonstrated a constant level of mean fluoride release at 0.1mmol/L after each recharge, but this was not statistically different to the mean fluoride concentration released by resin alone, at any 24 hour time point after recharge (Table 2). Fluoride release from ClinproXT and ClinproXT +ZnAl-LDH after each recharge was greater than from the experimental resin following a similar cyclic pattern. The baseline fluoride release prior to each recharge (see troughs in Figure 1) was relatively consistent for the ClinProXT+ ZnAl-LDH, but the standard ClinProXT exhibited a steady reduction in fluoride release over the two-week period, from an initial 0.7mmol/L to <0.4 mmol/l.

The cumulative fluoride release within 24 hours of each recharge at 4, 7, 10 and 13 days forms the basis for the plots in Figure 2. The gradient indicates the rate at which fluoride was released post recharge. ClinproXT released fluoride the fastest at 0.0235 mmol/l per hour, whereas ClinproXT + ZnAl-LDH discharged fluoride at 0.0179 mmol/l per hour. Resin with incorporated ZnAl-LDH released fluoride after recharge at a rate of 0.0018 mmol/l per hour, which was approximately double the rate from the resin alone, at 0.0008 mmol/l per hour.

Energy dispersive x-ray (EDS) spectra of the discs were taken before (Figure 3a) and after the experiment ended (Figure 3b). Generally, for ClinproXT before immersion, peaks for carbon and oxygen were visible identified from HEMA and BisGMA, as well for silica, identified in the silane coating and the ion-leachable glass. Peaks for calcium and phosphate were observed due to the presence of calcium glycerophosphate. Fluoride and aluminium peaks were present due to the fluoroaluminosilicate glass. After 14 days (336 hours), most of the peaks decreased in intensity, due to glass dissolution and ion release with a new peak for fluorine. Similar peaks were observed in the EDS spectra for ClinproXT + ZnAl-LDH, before and after the experiment (Figures 3c and 3d), but with an additional peak for Zn, denoting the element presence in ZnAl-LDH.

SEM images demonstrated cracks in the ClinproXT sample (at magnification x1000 and x100; Figure 4a and 4b) at the end of the experiment compared to the SEM before immersion. Figure 4c and figure 4d demonstrated the SEM image of resin + ZnAl-LDH sample before (at x1000 magnification) and after the experiment (at x100 magnification). In the before sample, the LDH powder can be observed as the white areas in the image with the particles appearing evenly dispersed and differing in size and shape. This observation agrees with the particle size analysis. In the post immersion sample, there appears to be a small pore within the sample (indicated by the white arrow).The main peaks in the EDS spectra for experimental flowable resin varnish were for carbon and oxygen (Figure 5a), which are present in Bis-GMA, UDMA and TEGMA. There was no major change identified in the before and after immersion in water spectra (Figure 5b), which would be expected since the polymerised composite matrix is hydrophobic and therefore should not dissolve. The EDS spectra for the experimental flowable resin + ZnAl-LDH before immersion showed peaks for calcium and oxygen (from Bis-GMA, UDMA and TEGMA), and aluminium, zinc and chlorine representing ZnAl-LDH (Figure 5c). At the end of the experiment the material showed an additional peak for F, indicating that the material had absorbed fluoride (Figure 5d; see section 4).

The ClinproXT (3M ESPE) samples demonstrated the greatest mean weight change over time. However, as shown in table 3, the weights for ClinproXT and ClinproXT + ZnAl-LDH fluctuated throughout the experimental period, whereas the weights of the experimental flowable resins, with and without LDH, appear to be stable (see section 4). The ClinproXT samples (with/without LDH) cracked

by day 14, whereas the experimental flowable resin varnish samples, with and without LDH remained intact.

4. Discussion

This study investigated the recharge potential of both an experimental flowable resin varnish and a commercial resin modified glass ionomer varnish (ClinProXT) incorporating ZnAl-LDH. An experimental flowable resin was selected as the carrier for LDH since it is commonly used in dentistry; but it has limited potential to release caries inhibitory ions. The particle size of ZnAl-LDH was normally distributed, similar to Eldafrawy [21], and evidence of ZnAl-LDH was confirmed by the presence of peaks for zinc and aluminium in EDS spectra.

All four materials (ClinproXT or experimental resin varnish +/-ZnAL-LDH) discharged fluoride with the highest total, mean concentration released from ClinproXT and the lowest from the experimental resin without ZnAl-LDH. This is not surprising given that glass ionomer-based materials display greater potential for post-recharge fluoride release than composites [10]. There were significant differences in the mean fluoride release after each recharge between the following materials; ClinproXT/Clinpro XT+ZnAl-LDH, ClinproXT/resin, ClinproXT/resin + ZnAl-LDH, ClinproXT + ZnAl-LDH/resin and ClinproXT+ ZnAl-LDH/resin + ZnAl-LDH, but not between the resin and resin with ZnAl-LDH.

The addition of ZnAl-LDH to ClinProXT resulted in less fluoride release compared to ClinproXT varnish alone. This was unexpected and may have been due to the cross-linking of ZnAl-LDH with the polyacrylic acid (PAA; present in the RMGIC) through electrostatic interactions, thus affecting the LDH's properties. This has previously been reported in the field of soil conditioners for water, where FTIR spectra, taken after the polymerisation process, showed evidence of grafting (cross-linking) between LDH and PAA, resulting in a composite structure. Furthermore, it has been suggested that the dissolution behaviour of the free applicable PAA decreased due to cross-linking [22]. Our

results concur with this since ClinproXT + ZnAl-LDH samples showed reduced weight changes in water compared to ClinproXT alone, thus relating to the decreased dissolution behaviour of PAA.

Research on LDH in the dental field is limited for comparing absorption and release profiles of fluoride ions, due to the differences in the various release protocols, LDH compositions, and carrier matrices [23]. Therefore, the results obtained could not be directly compared to those reported in the literature. In the present study, the surface area of the discs (188.5 mm^2) was comparable to the Hoxha et al. study [23], whereas others have used 138.2 mm^2, 301.6 mm^2 or 691.2 mm^2 [10,13,20]. The present study incorporated 9% by weight LDH, which is within the range used by Tammaro et al. (0.7-20%) [20] and Hoxha et al. (0-45%) [23]. Tammaro et al. reported increasing LDH-F concentration in the resin, from 0.7% to 20% by weight, did not result in more fluoride release, although the 0.7% LDH-F released the most fluoride [20]. This is somewhat counter-intuitive, but the authors believe that fluoride ions in 'big tactoids', presumably large inter-lamellar layers, are less available for exchange by counter ions with less detachment and diffusion through the resin. In comparison, Hoxha et al. reported a linear increase in fluoride release with LDH loading (i.e., more fluoride was released with 45% LDH compared to 10% LDH) [23]. Another difference in experimental design was the use of LDH as the filler in this study, comparable to Hoxha et al. [23], whereas Tammaro et al. used a commercial composite resin containing a glass filler [20, 24]. The reasoning for using resin without conventional fillers was to assess the effect of LDH alone, as commercial fillers may contain other sources of fluoride [23]. Moreover, the method of incorporating LDH powder in the resin varied; Tammaro et al. intercalated fluoride anions into LDH to create LDH-F, which was added to the composite matrix prior to curing [20, 24], whereas in the present study, neat ZnAl-LDH was added to the resin, which was cured, followed by charging in a NaF solution to produce LDH-F. The current study incorporated ZnAl-LDH whereas other studies used MgAl-LDH-F [20, 24], CaAl-LDH or MgAl-LDH [23], expressing fluoride release values as $\mu g/cm^2/h$, $\mu g/cm^2/day$, or ppm [10,13,20,24]. Absorption capabilities of fluoride by LDH vary according to the type of LDH. Mg-Al-LDH has been reported to adsorb 80.12 mg/g compared to 4.14 mg/g by Zn-Al LDH [25]. The present study immersed samples in deionised water, also used by Xu and Burgess [8, 23], but artificial saliva, physiological saline and aqueous sodium bicarbonate have also been used [10,13,20,23]. Fluoride

absorption can be reduced by 50% in the presence of other ions in the bathing medium including phosphate, nitrate and, chloride [17]. However, the divalent CO_3^{2-} ion has the highest anion-LDH affinity and would therefore readily release F^-. Fluoride charging solutions include sodium fluoride, sodium monofluorophosphate and stannous fluoride whereas alternatives to solutions have been used such as foaming agents [8].

The fluoride recharge profile from this study was similar to those reported in glass ionomers, RMGICS, compomers and composites [8]. All materials exhibited an increase in fluoride release within 24 hours, but after each recharge this dropped rapidly to baseline levels, despite a longer recharge period of five minutes, compared with the much shorter recharge period of one minute used by Xu and Burgess [8]. This so-called 'burst effect' of fluoride agrees with their observation that samples were superficially recharged and longer recharge times were unnecessary in aiding greater subsequent release. A rate limiting factor in recharge kinetics may be that the fluoride deep within the material's surface is less accessible for ion exchange, particularly with resin-based materials, as rigid polymer matrices act as a diffusion barrier. This would be akin to the early caries enamel lesion which has a more mineralised surface zone than the subsurface body of the lesion.

The results presented here corroborate previous findings that less porous materials, such as resins, have little ability to store and release fluoride [12]. One may therefore speculate that the reduced level of fluoride release was due to the chemical composition of the resin. This observation concurs with Xu and Burgess [8] who reported that resin present within composite acts as a barrier preventing water and fluoride diffusion. The resin is more hydrophobic, cross-linked and less porous than ClinproXT (3M ESPE). The latter contains HEMA, a hydrogel as the resin, that is hydrophilic, and known to absorb significantly more water than, for example BisGMA. This explains the 6.1% weight gain by ClinProXT compared to the resin, which lost 1.8% weight and resin+LDH which gained `0.5% weight. On absorbing water, the ClinProXT swells and therefore it is able to easily absorb and release fluoride. However, this process results in detrimental effects on the material's structure, where the latter eventually breaks down, as observed in this study. The lower release of fluoride from the resin containing LDH can be attributed to the overall effect of the resin's hydrophobicity.

The need for constant recharge from a fluoride source, such as toothpaste, is required for any release to occur. The main clinical issue is whether the amount of fluoride released by any material modified by the incorporation of LDH is sufficiently anti-cariogenic. A therapeutically useful range has been reported as ~0.025–2 ppm [23]. Evidence also suggests that a constant supply of low levels of fluoride in the biofilm/saliva/dental interface is required for caries prevention [26,27,28]. In this study, lower levels of fluoride were released by the resin + ZnAl -LDH (0.002ppm) and the ClinproXT + ZnAl-LDH (0.012 ppm) 24 hours after recharge. However, Hoxha et al. [23] reported that fluoride released from 10% MgAl-LDH and CaAl LDH was 0.58ppm and 0.51ppm, respectively. MgAl and CaAl LDH are considered to have higher adsorption capabilities than ZnAl-LDH. It would appear that the ZnAl-LDH's lower adsorption capability, compared with 10% MgAl-LDH and CaAl-LDH, together with the observation that pre-fluoride absorbed LDH (LDH-F) that was not used in this study, could have resulted in the lower levels of fluoride release and absorption by the system.

Clearly, there is potential for the use of LDH in the field of Dentistry and therefore this research merits further work to improve the fluoride absorption and release characteristics of LDH. There is a need for additional experiments to elucidate whether ZnAl-LDH is a suitable candidate for incorporating into dental materials, with respect to fluoride absorption and release, compared to MgAl-LDH and CaAl-LDH. These include, for example increasing the amount of LDH, pre-loading the LDH with fluoride and incorporating fillers with LDH into the resin matrix thus making both the resin and the RMGIC less hydrophobic compared to BisGMa-UDMA-TEGMA alone.

5.Conclusion

Within the limitations of the present study, the addition of ZnAl-LDH to an experimental flowable resin varnish, or ClinproXT varnish, did not result in an increase in fluoride release. In the latter it is assumed this is due to the cross-linking of LDH with the polyacrylic acid. Further work is required to improve the fluoride recharge characteristics of LDH-F incorporated into dental materials.

Table 1: Total fluoride released over 14 days (336 hours) and mean fluoride released by material (mmol/L)

Material	N	Total conc F mmol/L	Mean conc F mmol/L	SD
ClinproXT	5	42.93	8.586	0.972
ClinproXT+ZnAl-LDH	5	29.54	5.908	0.487
Resin	5	4.19[a]	0.839	0.194
Resin+ZnAl-LDH	5	6.89[a]	1.379	0.545

a Not significantly different

Table 2: Mean fluoride release prior to each recharge and 24 hours later (mmol/l)

Material	N	Mean F (SD)conc mmol/L @3d	Mean F (SD)conc mmol/L @4d	Mean F (SD)conc mmol/L @6 d	Mean F (SD)conc mmol/L @7 d	Mean F (SD)conc mmol/L @9 d	Mean F (SD)conc mmol/L @10 d	Mean F (SD)conc mmol/L @12 d	Mean F (SD)conc mmol/L @13 d
ClinproXT	5	0.680 (0.078)	0.884 (0.099)	0.567 (0.095)	0.688 (0.077)	0.287 (0.057)	0.736 (0.107)	0.472 (0.054)	0.70 (0.08)
ClinproXT +ZnAl-LDH	5	0.351 (0.111)	0.654 (0.099)	0.412 (0.055)	0.616 (0.043)	0.222 (0.043)	0.578 (0.069)	0.339 (0.047)	0.586 (0.054)
Resin	5	0.007 (0.001)	0.0752 (0.040)	0.002 (0.001)	0.075 (0.030)	0.002 (0.001)	0.052 (0.016)	0.002 (0.003)	0.043 (0.023)
Resin +ZnAl-LDH	5	0.097 (0.162)	0.082 (0.037)	0.009 (0.002)	0.109 (0.07)	0.010 (0.003)	0.106 (0.054)	0.005 (0.002)	0.096 (0.032)

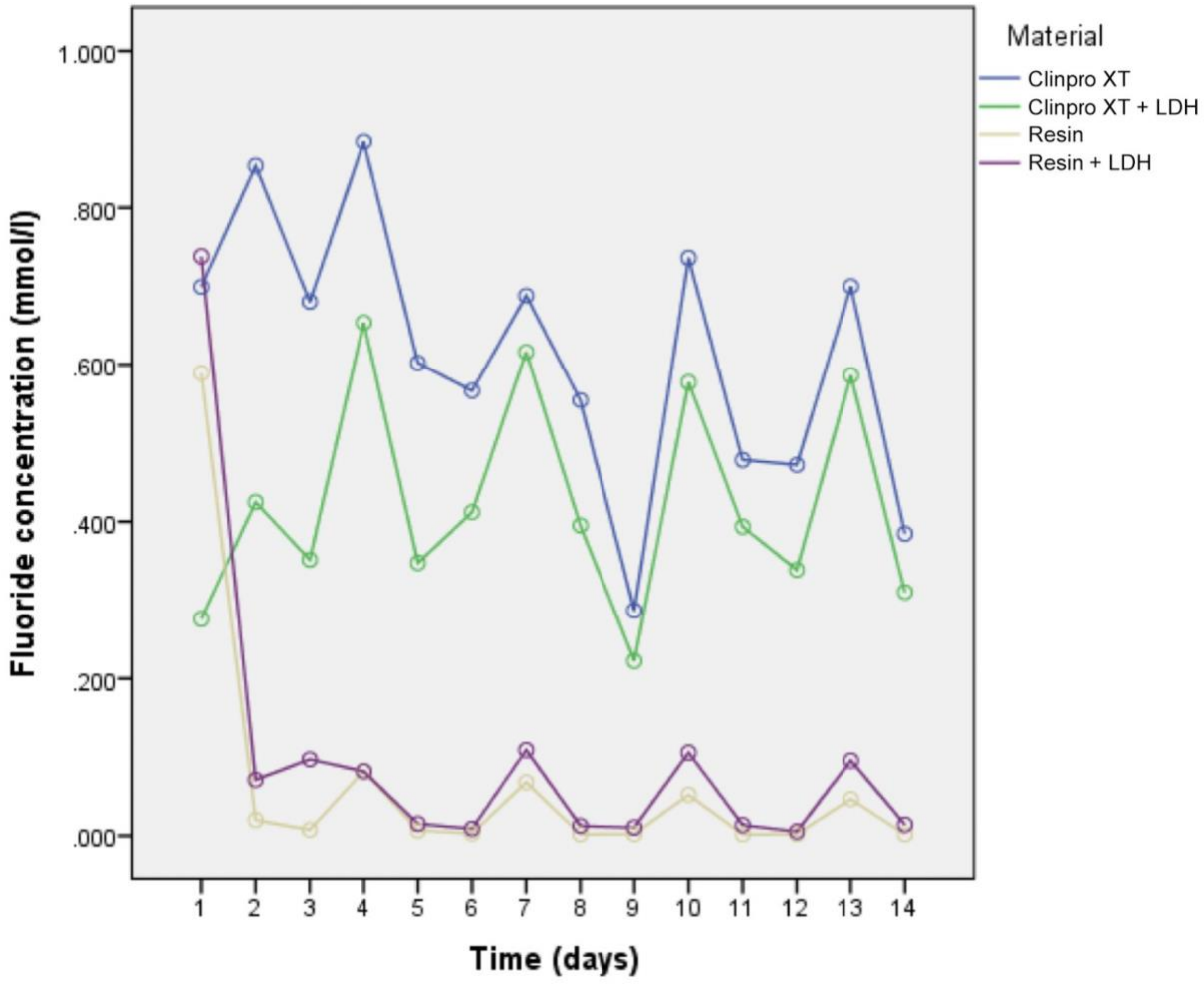


Figure 1: Mean fluoride release from the four experimental materials over 14 days (336 hours)

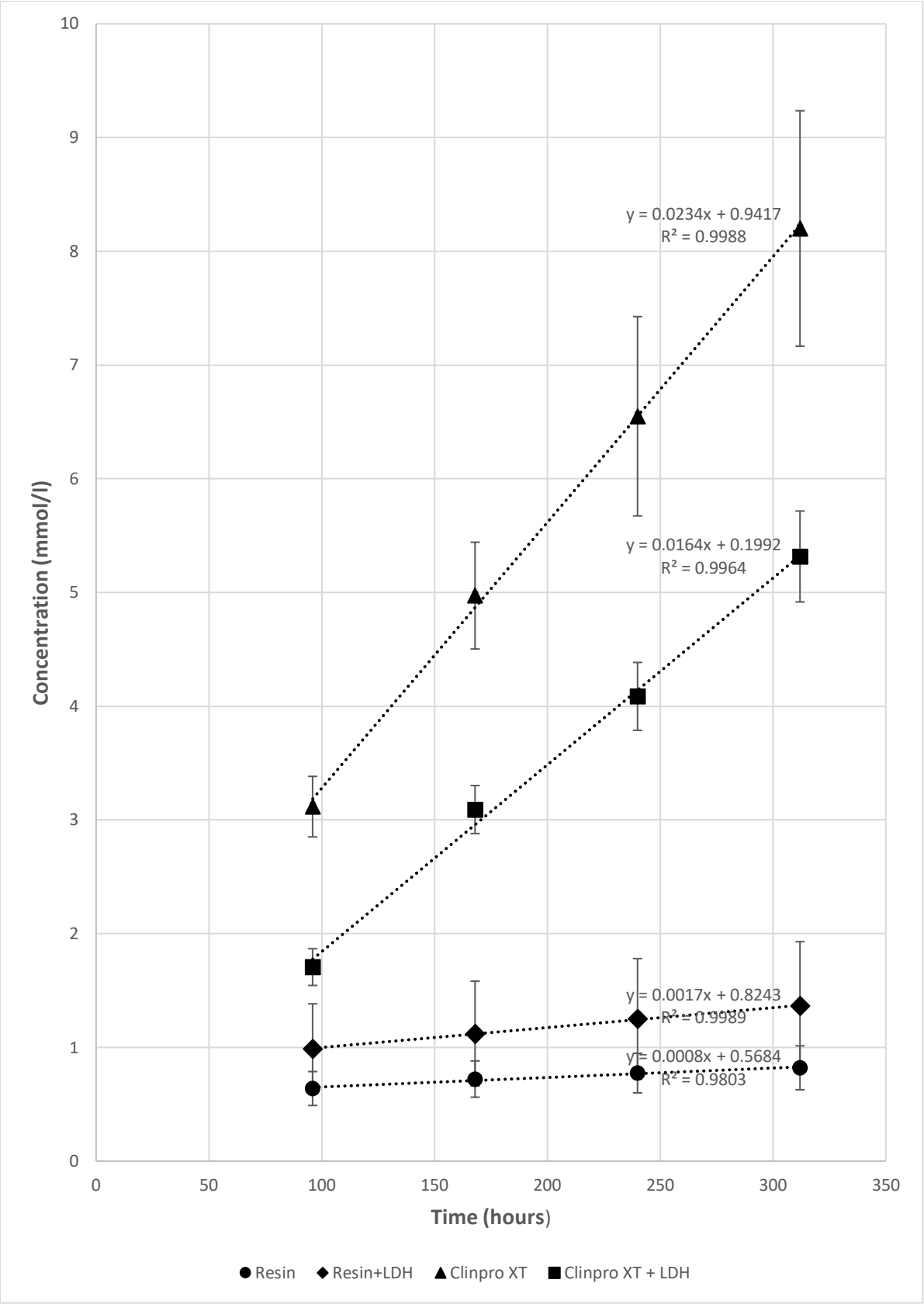


Figure 2: Rate of fluoride release after each 24-hour recharge/discharge perio

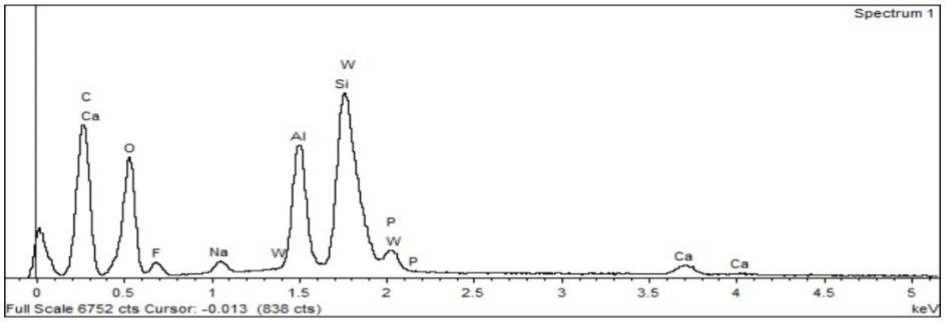
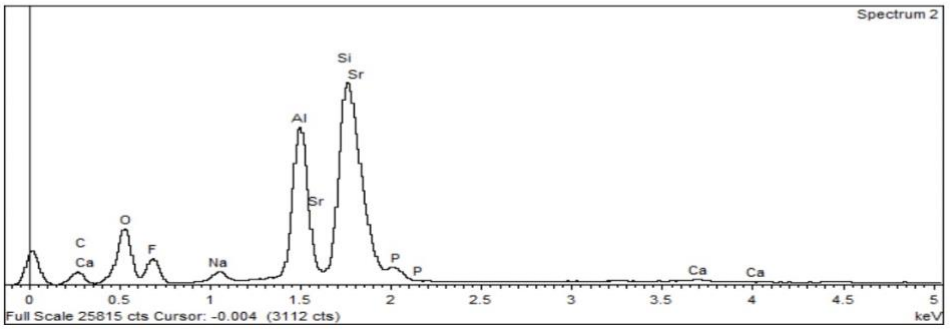


Figure 3a



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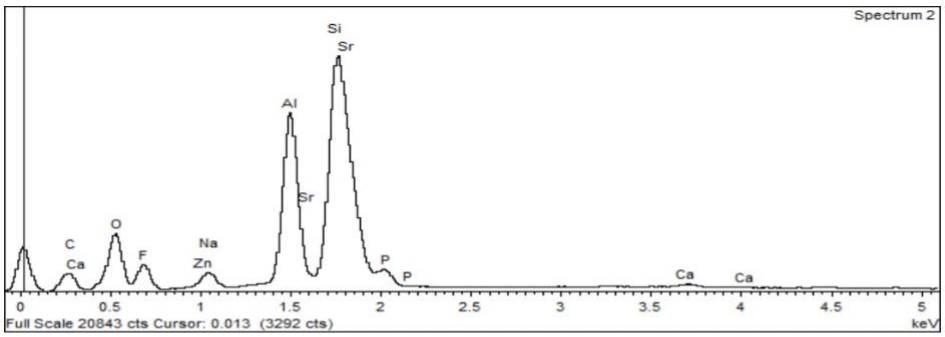


Figure 3c

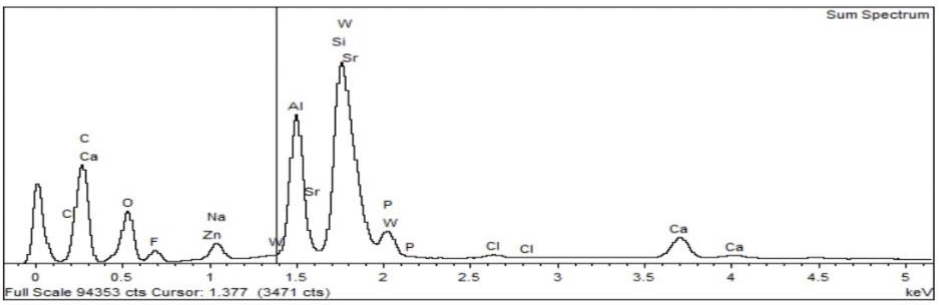


Figure 3d

Figure 3a: Energy dispersive x-ray spectroscopy represents the spectrum of ClinproXT (3M Espe) sample before immersion experiment

Figure 3b: Energy dispersive x-ray spectroscopy represents the spectrum of ClinproXT (3M Espe) following experiment

Figure 3c: Energy dispersive x-ray spectroscopy represents spectrum of ClinproXT (3M ESPE) + LDH before experiment

Figure 3d: Energy dispersive x-ray spectroscopy represents spectrum of ClinproXT (3M ESPE) + LDH EDS after experiment

Figure 4a

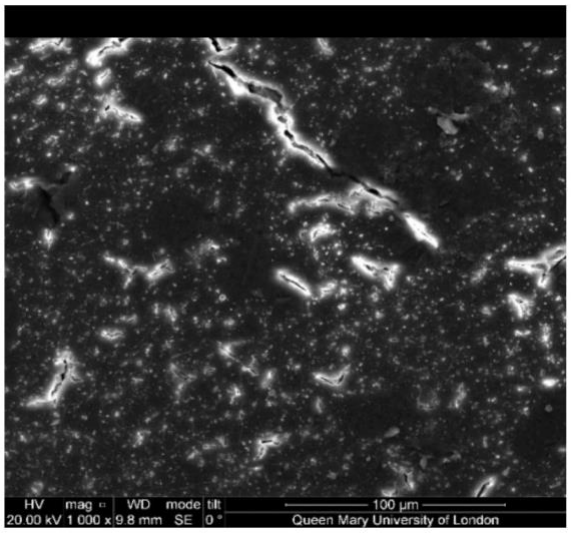
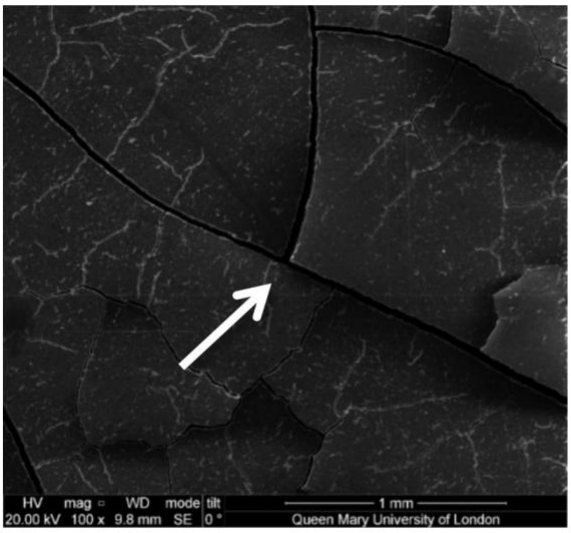
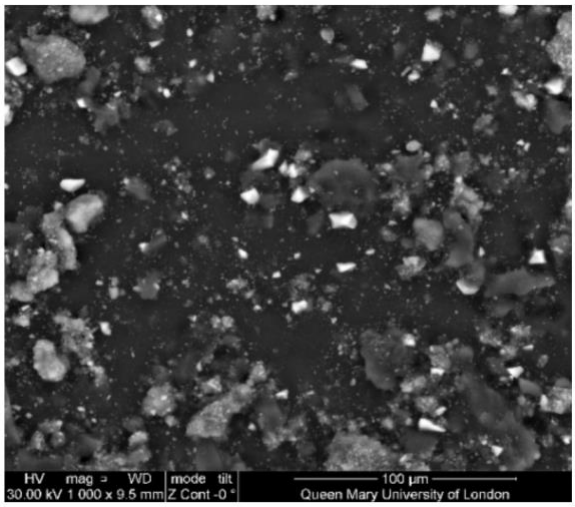


Figure 4b





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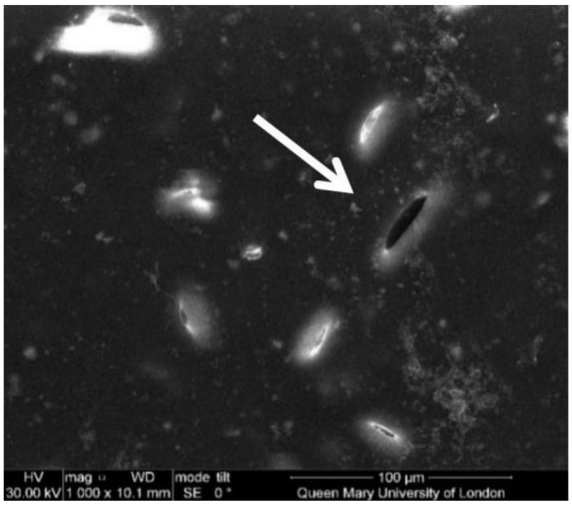

Figure 4d

Figure 4a: S.E.M image of ClinproXT (3M ESPE) sample after experiment with magnification X 1000 showing small scale cracks

Figure 4b: S.E.M image of ClinproXT with magnification X 100 showing large scale cracks as shown by white arrows

Figure 4c: S.E.M images of resin + ZnAl-LDH sample before experiment with a magnification of x1000

Figure 4d: S.E.M images of a resin + ZnAl-LDH sample after magnification of X100

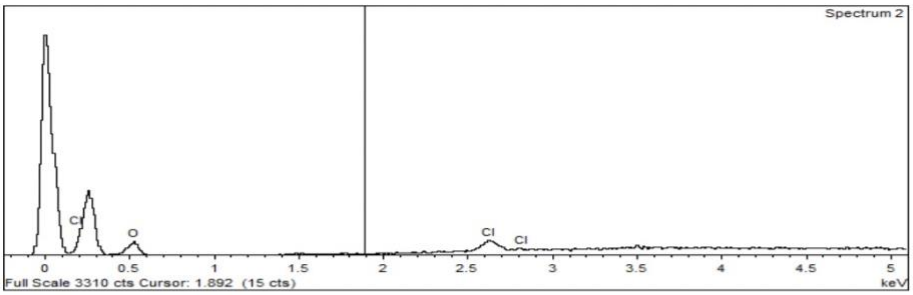

Figure 5a

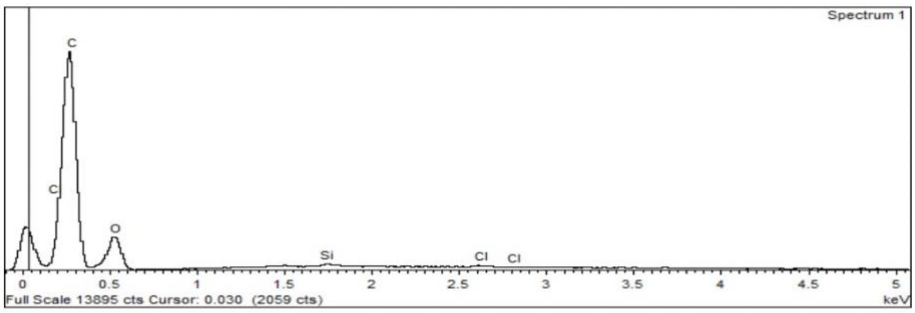

Figure 5b

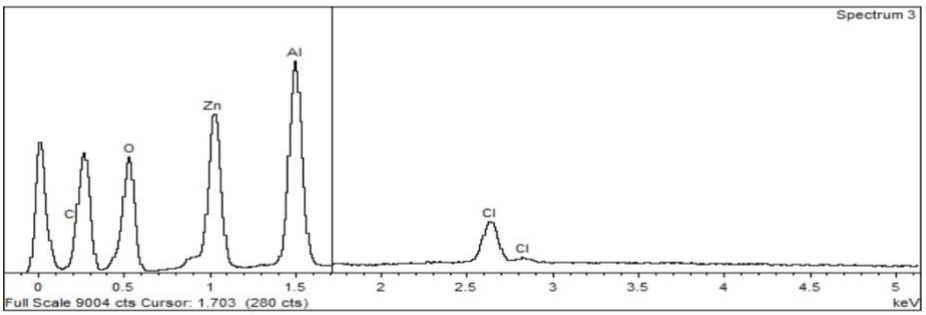

Figure 5c

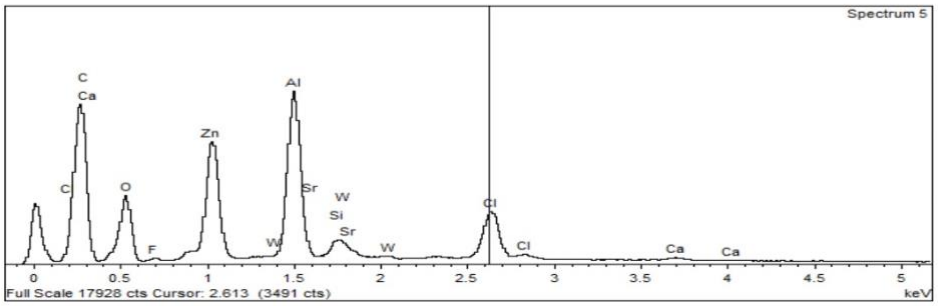

Figure 5d

Figure 5a: Energy dispersive x-ray spectroscopy represents spectrum of resin sample before experiment

Figure 5b: Energy dispersive x-ray spectroscopy represents spectrum of resin sample after experiment

Figure 5c: Energy dispersive x-ray spectroscopy represents spectrum of resin + ZnAl-LDH sample before experiment

Figure 5d: Energy dispersive x-ray spectroscopy represents spectrum of resin + ZnAl-LDH sample after experiment

Table 3: Mean weight changes of all samples over 14 days

Time (hours)	Mean weight Resin (SD) g	Mean weight of Resin + ZnAl-LDH (SD) g	Mean weight of ClinproXT (SD) g	Mean weight of ClinproXT + ZnAl-LDH (SD) g
0	0.231 (0.036)	0.285 (0.042)	0.393 (0.058)	0.471 (0.032)
24	0.231 (0.036)	0.290 (0.042)	0.421 (0.060)	0.493 (0.032)
48	0.231 (0.036)	0.290 (0.042)	0.407 (0.057)	0.472(0.028)
72	0.230 (0.035)	0.288 (0.042)	0.422 (0.060)	0.488 (0.027)
96	0.229 (0.035)	0.287 (0.042)	0.422 (0.060)	0.455 (0.075)
120	0.229(0.036)	0.288 (0.042)	0.408 (0.057)	0.478 (0.029)
144	0.228 (0.035)	0.288 (0.042)	0.420 (0.059)	0.493 (0.030)
168	0.228 (0.035)	0.288 (0.042)	0.420 (0.059)	0.492 (0.030)
192	0.227 (0.035)	0.286 (0.042)	0.416 (0.058)	0.483 (0.031)
216	0.227 (0.035)	0.286 (0.042)	0.395 (0.057)	0.475 (0.025)
240	0.227(0.035)	0.287 (0.042)	0.418 (0.059)	0.487 (0.027)
264	0.227 (0.035)	0.286 (0.042)	0.423 (0.059)	0.497(0.030)
288	0.226 (0.035)	0.285 (0.042)	0.419 (0.059)	0.490(0.030)
312	0.227 (0.036)	0.289 (0.042)	0.424 (0.060)	0.498(0.029)
336	0.227 (0.036)	0.287 (0.042)	0.417 (0.058)	0.490(0.032)

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Supplemental Material

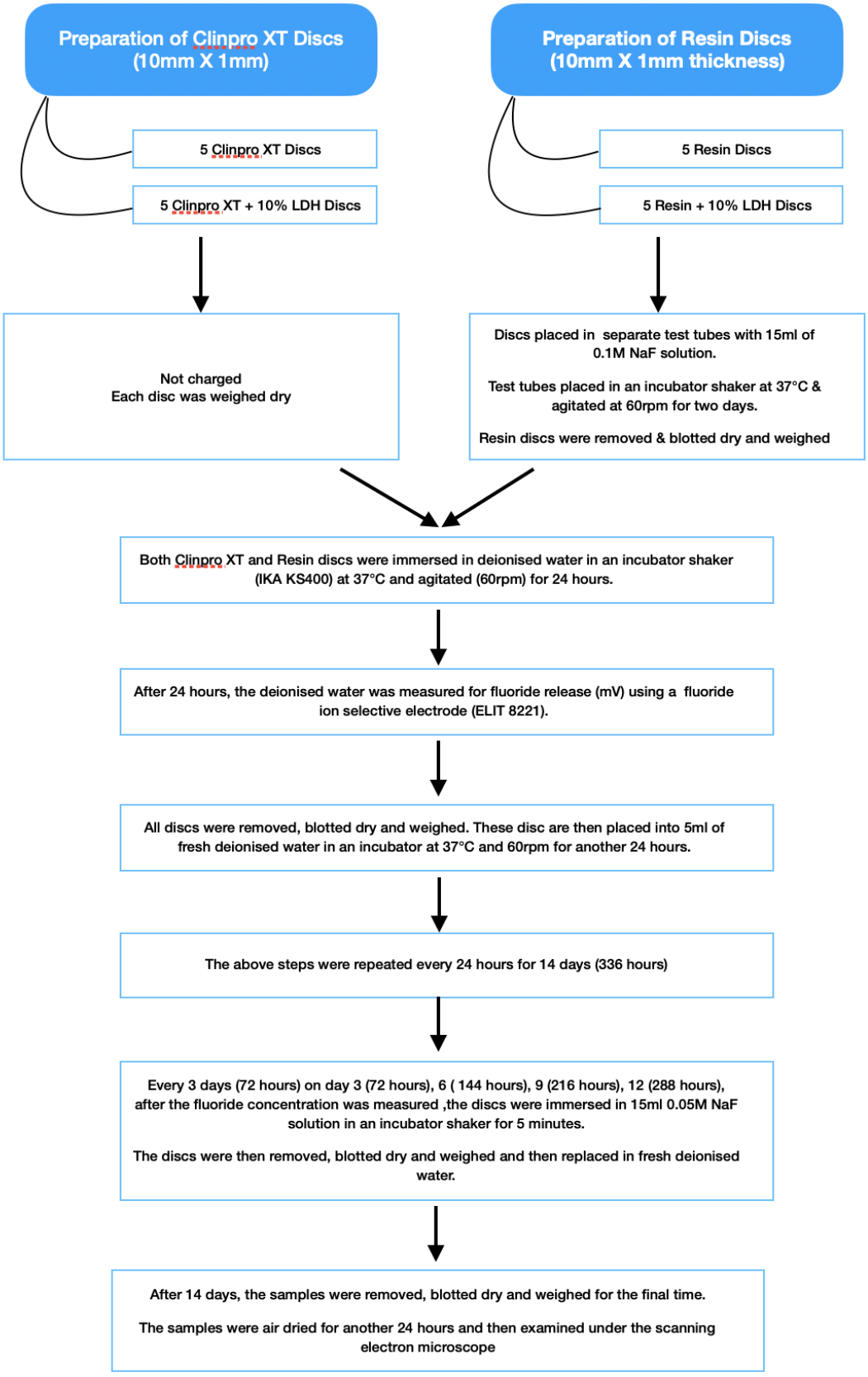

Figure A: Flow Diagram illustrating method