Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

for

Self-Sacrificial MOFs for Ultra-Long Controlled Release of Bisphosphonate Anti-Osteoporotic Drugs

by

M. Vassaki, K.E. Papathanasiou, C. Hadjicharalambous, D. Chandrinou, P. Turhanen, D. Choquesillo-Lazarte, and K.D. Demadis

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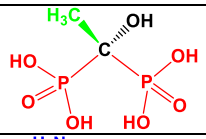
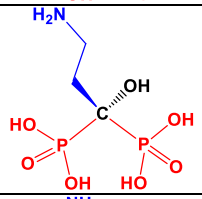
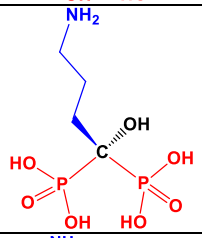
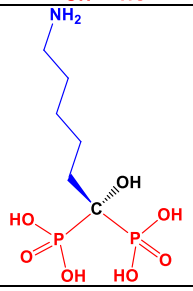
1. **Bisphosphonate description**

Table S1. Bisphosphonate notations and structures.

Bisphosphonate common name	Bisphosphonate Chemical name	Bisphosphonate abbreviation	Bisphosphonate structure	Available as "acid"	Available as "salt"
Etidronic acid	1-hydroxyethane-1,1-bisphosphonic acid	***ETID***		YES	Tetrasodium salt
Pamidronic acid	3-Amino-1-hydroxypropane-1,1-diphosphonic acid	***PAM***		YES	NO
Alendronic acid	4-Amino-1-hydroxybutane-1,1-diphosphonic acid	***ALE***		YES	Disodium salt
Neridronic acid	6-Amino-1-hydroxyhexane-1,1-bisphosphonic acid	***NER***		YES	NO

2. Experimental Section

2.1. Materials

All reagents used as the metal ion source, were from commercial sources and were used without further purification. Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, M_r = 203.3 g/mol), and calcium chloride dihydrate, ($CaCl_2 \cdot 2H_2O$, M_r = 146.99 g/mol) were purchased from Sigma-Aldrich. Tetrasodium ETID (solid) was supplied as Dequest 2016D from ThermPhos Inc. (Belgium). All other BPs were synthesized as described below. Deionized water (from a laboratory ion-exchange column) was used as solvent for all stock solution preparations, syntheses, and release experiments. When necessary, pH was adjusted by aqueous solutions of sodium hydroxide (NaOH) and hydrochloric acid (HCl), obtained from Sigma-Aldrich and Scharlau, respectively. Deuterium oxide (99.9 atom % D) containing 0.05 wt. % sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 was used for BP quantification via ^{1}H NMR, and was purchased from Sigma-Aldrich.

2.2. Instrumentation

Hydrothermal syntheses under autogenous pressure were carried out using a parallel synthesis procedure in an autoclave block made of aluminum which contains 36 reaction chambers in a 6×6 array. Teflon reactors have an inner diameter of 19 mm and a depth of 18 mm, with a total volume of about 5 mL. A thin sheet of Teflon covers the reaction vessels, which are then sealed inside a specially designed aluminum autoclave. The set-up is shown in Figure S1.

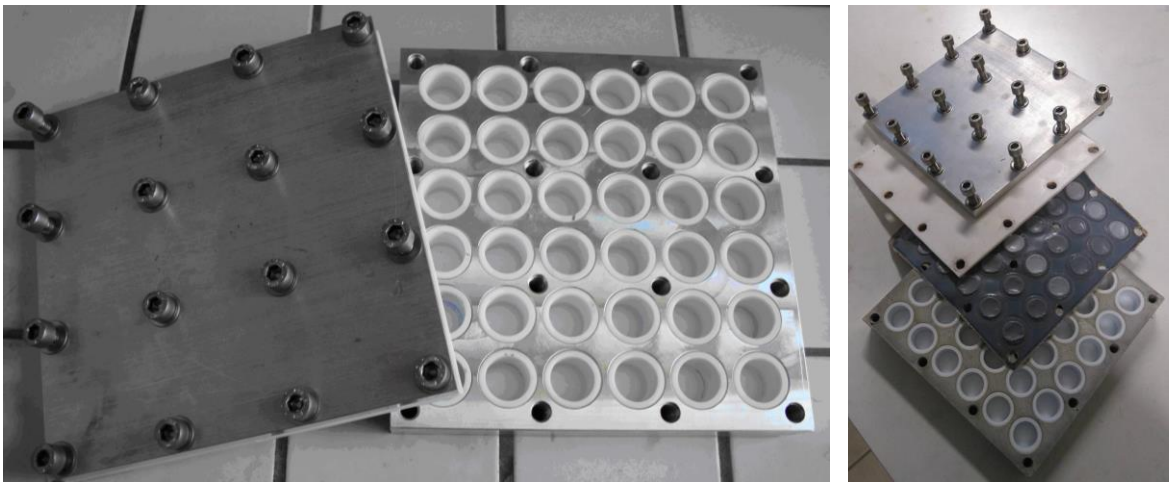


Figure S1. Image of the high-throughput system that was used for the hydrothermal syntheses.

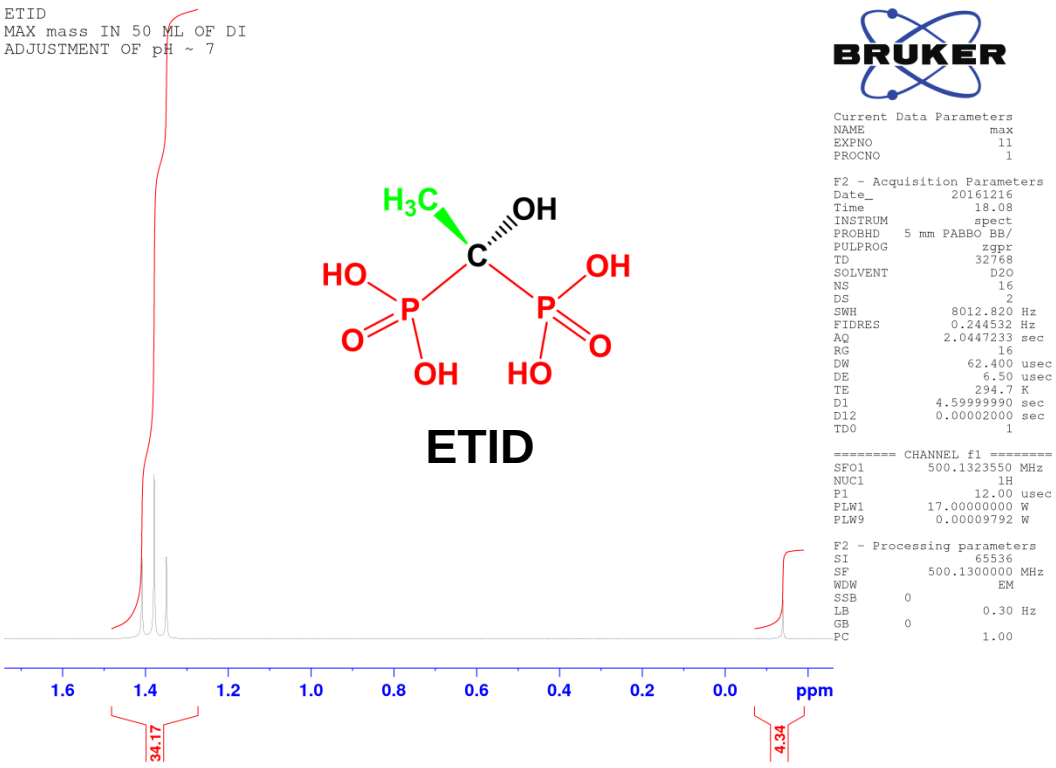
An AVANCE 300 (Bruker, Karlsruhe, Germany) spectrometer was used for the BP quantification in the release experiments.

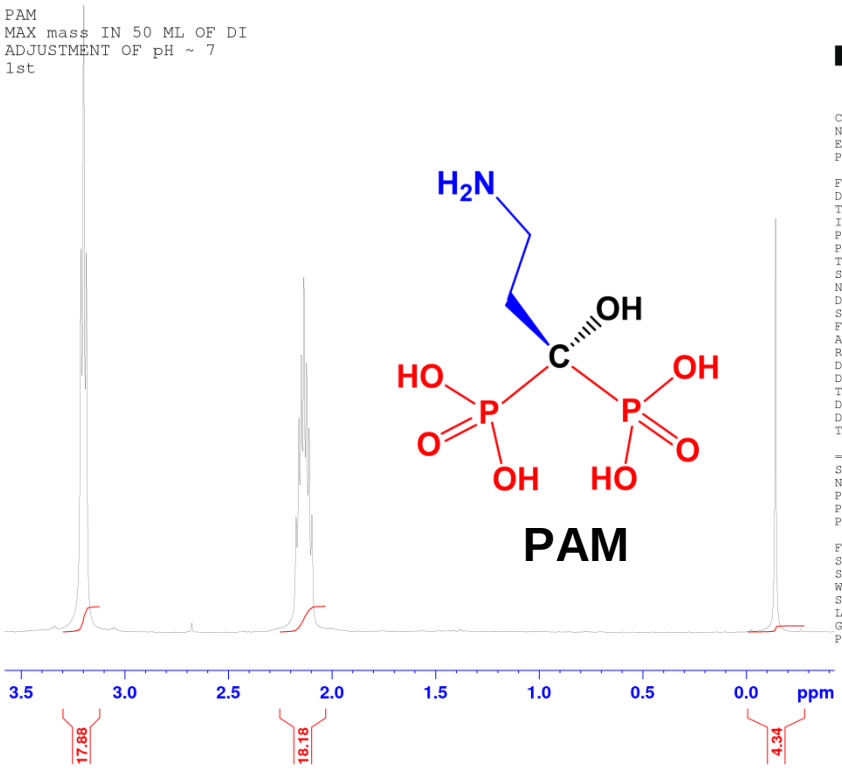
2.3. General comments on the synthesis of "free" bisphosphonic acids

Etidronic acid was synthesized according to the literature [P. Turhanen, J. Vepsäläinen, *Synthesis* **2004**, 992-994]. Alendronic, pamidronic and neridronic acids were synthesized and characterized as reported elsewhere [A.-L. Alanne, H. Hyvönen, M. Lahtinen, M. Ylisirniö, P. Turhanen, E. Kolehmainen, S. Peräniemi, J. Vepsäläinen, *Molecules* **2012**, *17*, 10928-10945]. ^{1}H and ^{31}P NMR spectra were recorded on a 600 MHz spectrometer operating at 600.2 and 243.0 MHz, respectively; ^{13}C NMR spectra were recorded on a 500 MHz spectrometer operating at 125.8 MHz. The solvent residual peak was used as a standard for ^{1}H measurements in D_2O (4.79 ppm) and in 13C measurements CD_3OD were added to be as a reference (49.00 ppm) [Gottlieb, H.E.; Kotlyar, V.; Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities. *J. Org. Chem.* **1997**, *62*, 7512-7515]. 85% H_3PO_4 was used as an external standard in the ^{31}P measurements. The $^{n}J_{HH}$ couplings were calculated from proton spectra and all J values are given in Hz. The $^{n}J_{CP}$ couplings were calculated from carbon spectra with the coupling constants given in parenthesis as Hz. Mass spectra were recorded with a Finnigan LCQ quadrupole ion trap mass spectrometer (Finnigan

MAT, San Jose, CA, USA) equipped with an electrospray ionization source. The purity of the products was determined from ^{1}H and ^{31}P NMR spectra and was typically higher than 95 % unless stated otherwise.

3. **NMR spectra of selected bisphosphonates**





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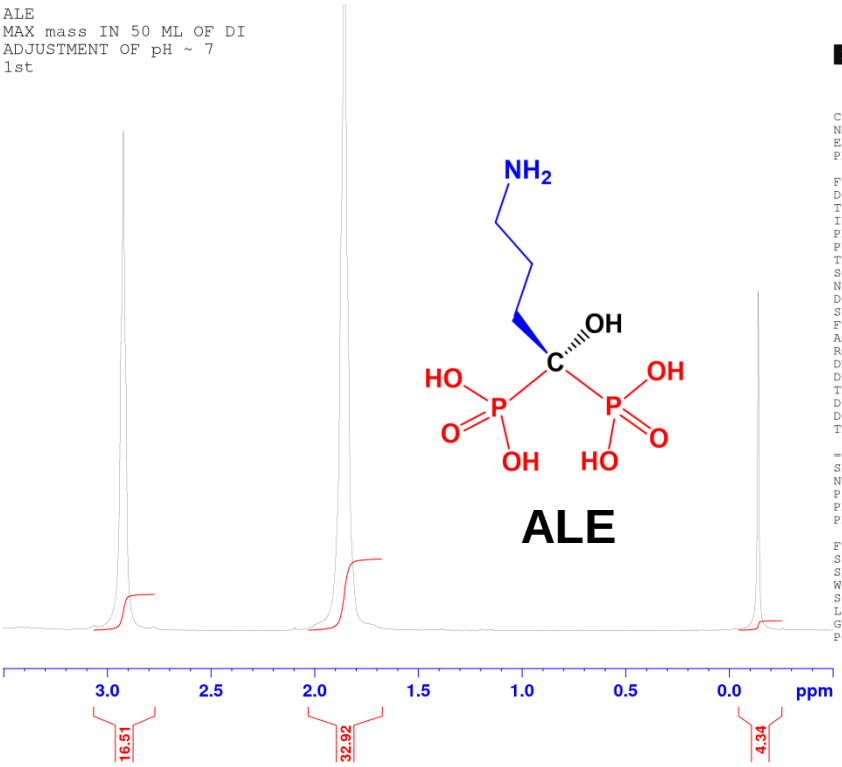

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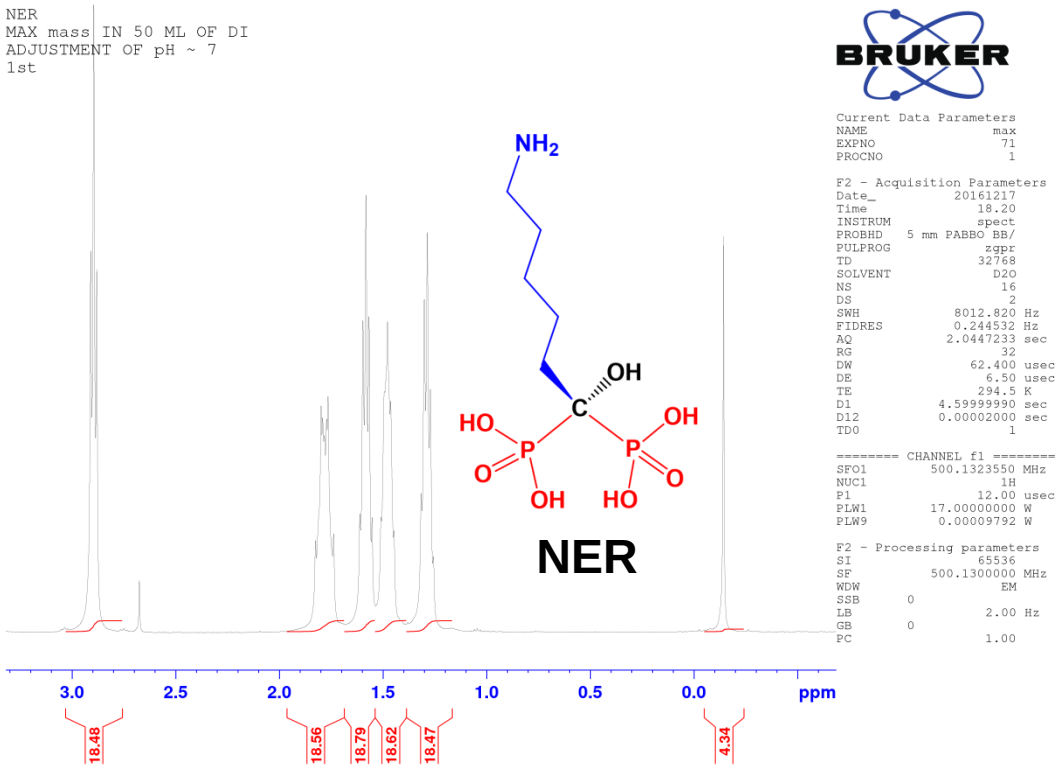
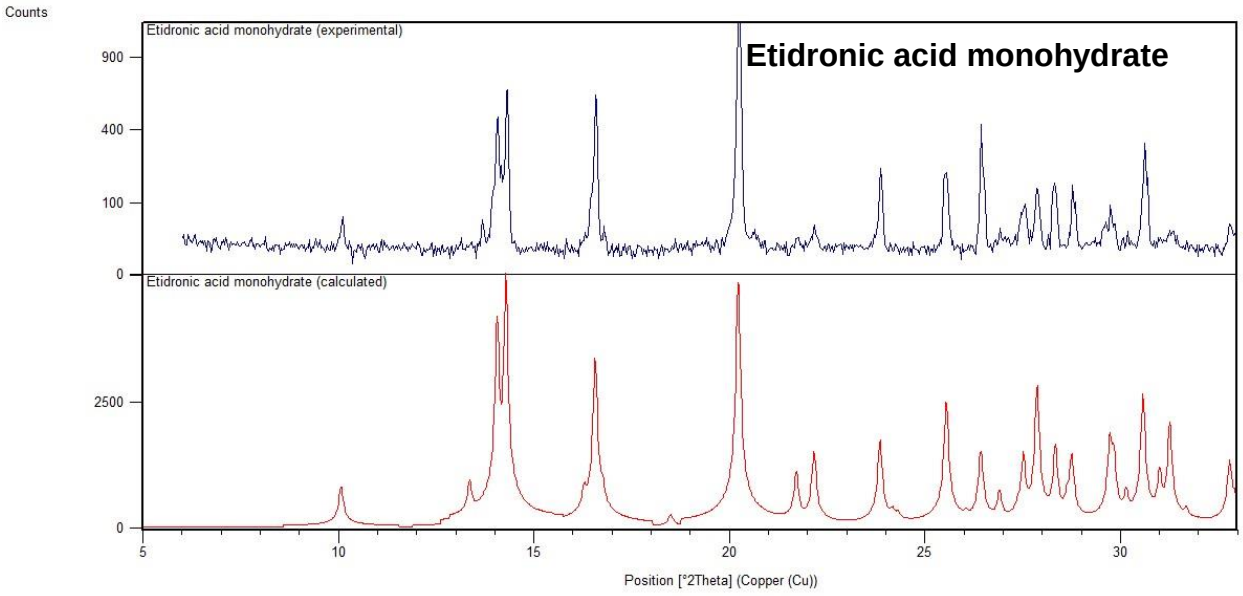
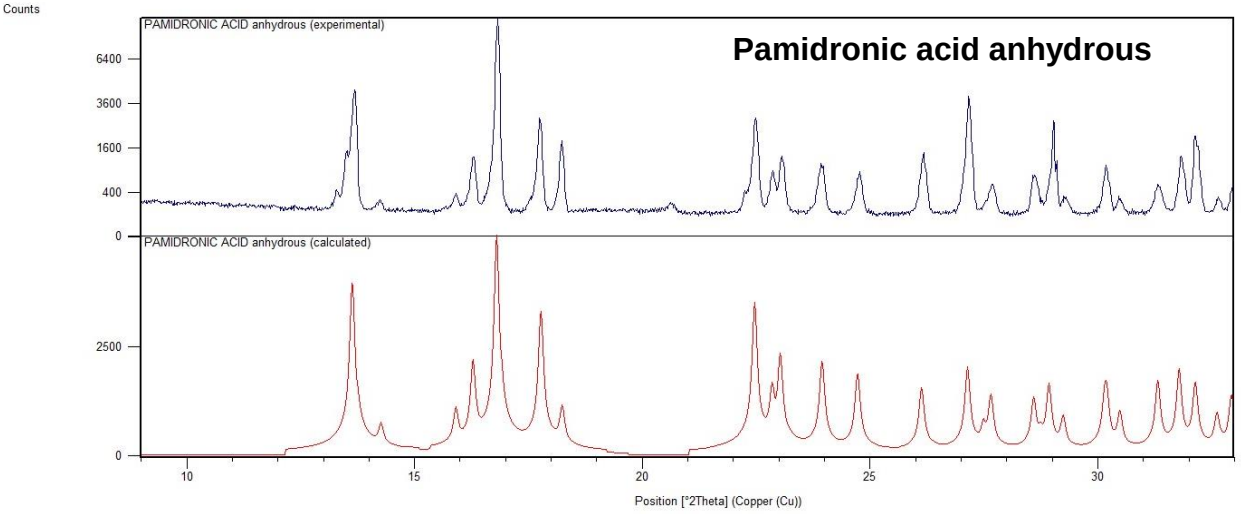


4. **Characterization of "free" bisphosphonic acids used for release studies by powder X-ray diffraction. The upper diagrams (blue) are the experimental, and the lower diagrams (red) are the calculated ones.**

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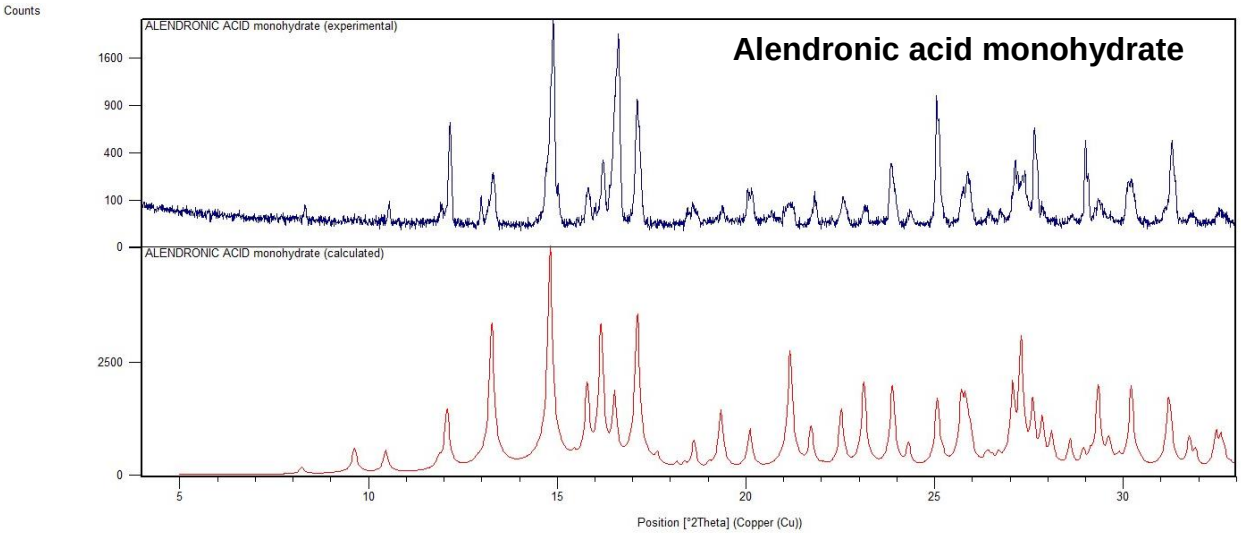
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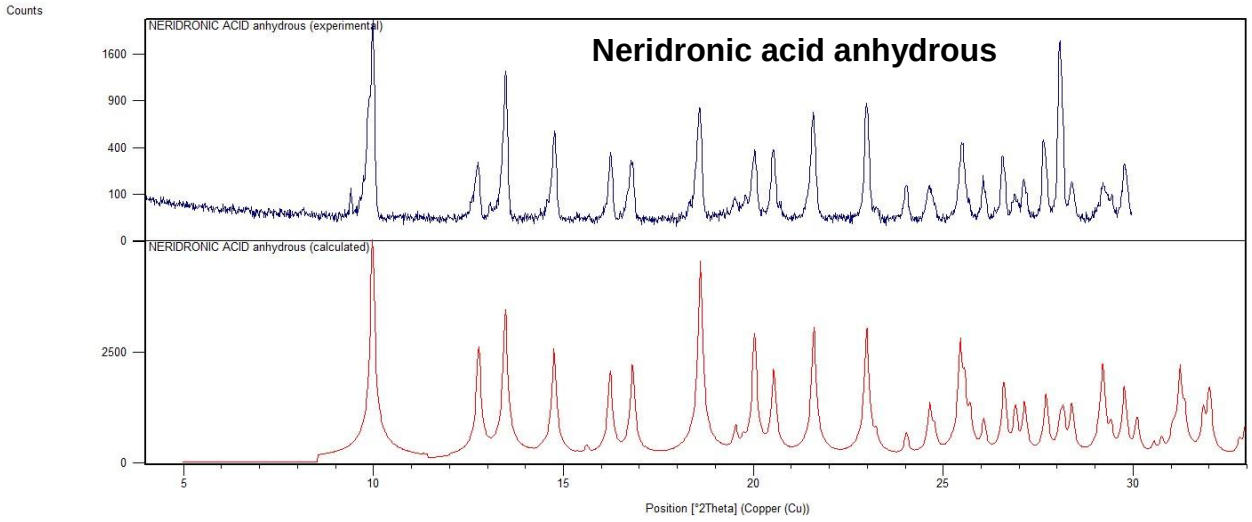
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5. **Preparation of Metal-BP MOFs**

Ca-ETID, $Ca[(HO_3P)_2C(OH)(CH_3)](H_2O)\cdot 2.5H_2O$. This compound was synthesized according to a literature procedure [Niekiel, F.; Stock, N. *Cryst. Growth Des.* **2014**, *14*, 599−606] with some modifications. Specifically, equimolar amounts of etidronic acid (ETID, Dequest 2010, 0.343 g, 1.0 mmol from a 60 % w/w aqueous solution) and $CaCl_2\cdot 2H_2O$ (0.147 g, 1.0 mmol) were dissolved in 30 mL deionized water. Solution pH was adjusted to 4.7 and immediate precipitation occurred. At this point, the pH of the reaction mixture was adjusted to 3.5 and the cloudy dispersion turned into a clear colorless solution, which was left to stand at room temperature for 10 days. A white polycrystalline powder had formed, which was isolated by simple filtration and washed with deionized water. This product was confirmed (by powder X-ray diffraction) to be the same as the Ca-ETID material reported in the literature.

Ca-PAM, $Ca[(HO_3P)_2C(OH)(CH_2CH_2NH_3)]_2\cdot 2H_2O$. Quantities of $CaCl_2\cdot 2H_2O$ (0.007 g, 0.05 mmol) and pamidronic acid (PAM, 0.024 g, 0.10 mmol) were dissolved in 3.25 mL deionized water until a clear colorless solution is obtained. The Ca:PAM molar ratio was 2:1. Then, solution pH was adjusted to 3.0 (using stock solutions of NaOH and HCl, as needed). This final solution was placed in a teflon reactor (described above, see Figure S1) and was heated under autogenous pressure at 120 °C for 3 days. The reactor was left to cool to room temperature in the turned-off oven. A white crystalline precipitate formed, which was isolated by filtration, washed with deionized water and air-dried. This product was confirmed (by powder X-ray diffraction) to be the same as the Ca-PAM material reported in the literature [Fernandez, D.; Vega, D.; Goeta, A. *Acta Cryst.* **2002**, *C58*, m494-m497]. Typical yields are > 50 %.

Ca-ALE, $Ca[(HO_3P)_2C(OH)(CH_2CH_2CH_2NH_3)]_2$. Quantities of $CaCl_2\cdot 2H_2O$ (0.007 g, 0.05 mmol) and alendronic acid (ALE, 0.025 g, 0.10 mmol) were dissolved in 3.25 mL deionized water until a clear colorless solution is obtained. The Ca:ALE molar ratio was 2:1.Then, solution pH was adjusted to 3.0 (using stock solutions of NaOH and HCl, as needed). This final solution was placed in a teflon reactor (described above, see Figure

S1) and was heated under autogenous pressure at 120 °C for 3 days. The reactor was left to cool to room temperature in the turned-off oven. A white crystalline precipitate formed, which was isolated by filtration, washed with deionized water and air-dried. This product was confirmed (by powder X-ray diffraction) to be the same as the Ca-ALE material reported in the literature [Fernandez, D.; Vega, D.; Goeta, A. *Acta Cryst.* **2003**, *C59*, m543-m545]. Typical yields are > 50 %.

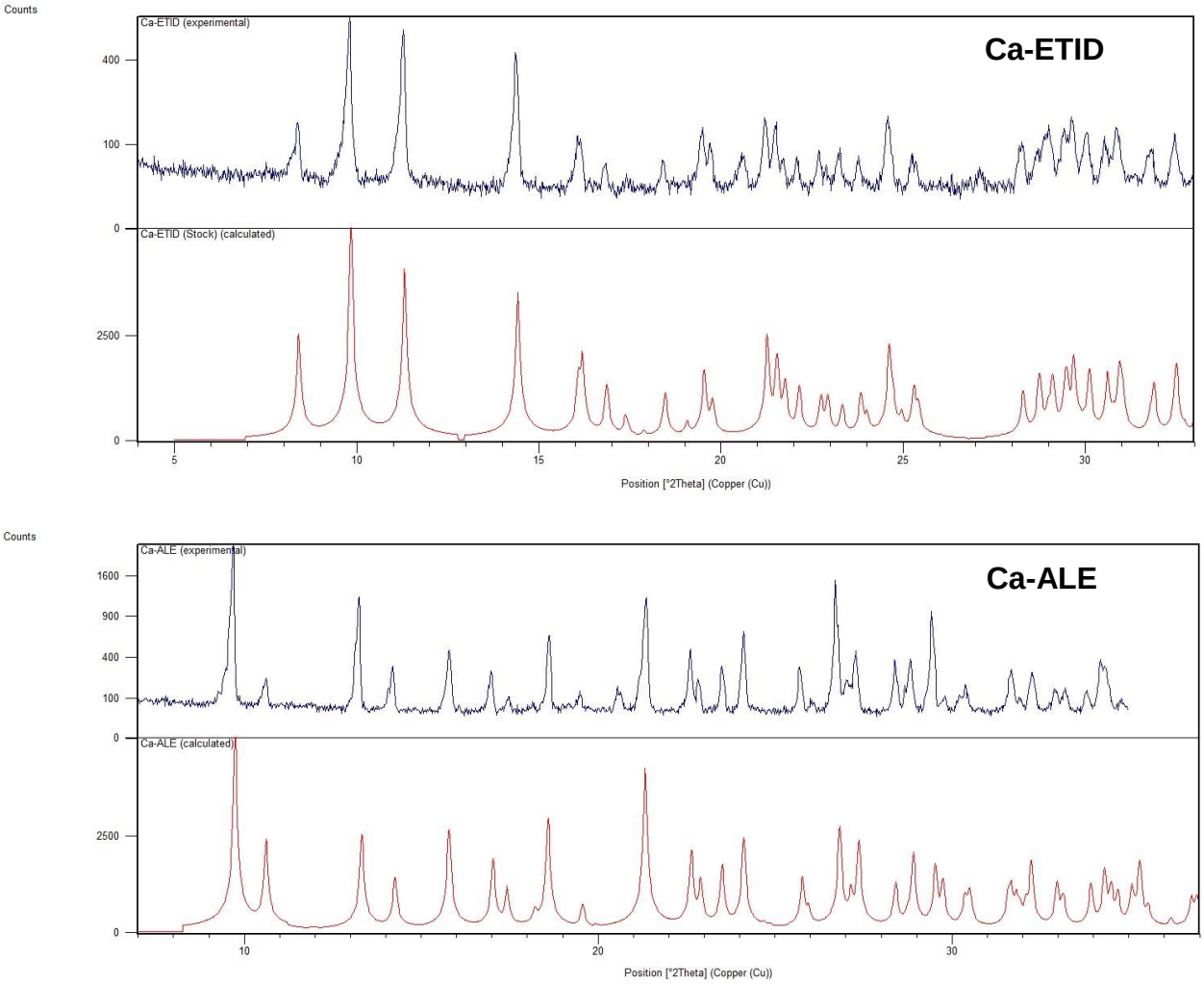
Ca-NER, $Ca[(HO_3P)_2C(OH)(CH_2CH_2CH_2CH_2CH_2NH_3)]_2 \cdot 3H_2O$. $CaCl_2 \cdot 2H_2O$ (0.015 g, 0.1 mmol) and neridronic acid (NER, 0.028 g, 0.1 mmol) were dissolved in 10.25 mL of deionized water. Then, solution pH was adjusted to 3.0 (using stock solutions of NaOH and HCl, as needed). The clear colorless solution was left to stand at room temperature. After partial water evaporation white crystals formed, which were isolated by simple filtration and washed with deionized water. Various Ca:NER molar ratios (eg. 2:1 and 1:1), pH values (eg. from 1 to 6) and synthetic methods (eg. room temperature and hydrothermal) were tested, but whenever a solid precipitate appeared, it was invariably the same Ca-NER product. Bulk product purity was confirmed by powder X-ray diffraction (comparison of the calculated and experimental patterns) and elemental analyses. Yield: 24 mg (~ 74 %, based on metal salt). Elemental analysis: Calcd for $Ca[(HO_3P)_2C(OH)(CH_2CH_2CH_2CH_2CH_2NH_3)]_2 \cdot 3H_2O$, C 22.30; H 5.93; N 4.33. Found: C 22.01; H 6.12; N 4.35. The synthesis can be scaled-up to 10-fold, without compromise in product purity.

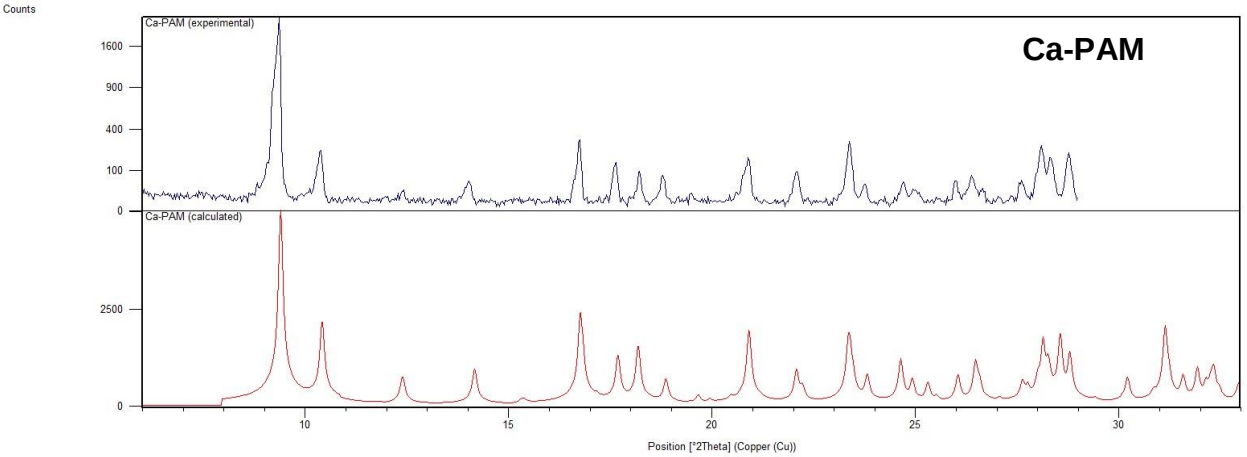
Mg-ALE, $\{Mg[(HO_3P)_2C(OH)(CH_2CH_2CH_2NH_3)]_2(H_2O)\} \cdot 2H_2O$. $MgCl_2 \cdot 6H_2O$ (0.020 g, 0.05 mmol) and alendronic acid (ALE, 0.024 g, 0.1 mmol) were dissolved in 10.25 mL of deionized water. Then, solution pH was adjusted to 2.0 (using stock solutions of NaOH and HCl, as needed). This final solution was placed in a teflon reactor (described above, see Figure S1) and was heated under autogenous pressure at 120 °C for 3 days. The reactor was left to cool to room temperature in the turned-off oven. A white crystalline precipitate formed, which was isolated by filtration, washed with deionized water and air-dried. Bulk product purity was confirmed by powder X-ray diffraction (comparison of the calculated and experimental patterns) and elemental analyses. Yield: 1 mg (~ 40 %,

based on metal salt). Elemental analysis: Calcd for $\{Mg[(HO_3P)_2C(OH)(CH_2CH_2CH_2NH_3)]_2(H_2O)\}\cdot 2H_2O$, C 17.27; H 5.07; N 5.03. Found: C 17.01; H 5.29; N 5.09.

Mg-NER, $\{Mg[(HO_3P)_2C(OH)(CH_2CH_2CH_2CH_2CH_2NH_3]_2(H_2O)_2\}\cdot 2H_2O$. $MgCl_2\cdot 6H_2O$ (0.020 g, 0.05 mmol) and neridronic acid (NER, 0.028 g, 0.1 mmol) were dissolved in 10.25 mL of deionized water. Then, solution pH was adjusted to 4.0 (using stock solutions of NaOH and HCl, as needed). The clear colorless solution was left to stand at room temperature. After partial water evaporation white crystals formed, which were isolated by simple filtration and washed with deionized water. Various Mg:NER molar ratios (eg. 2:1 and 1:1) and pH values (eg. from 1 to 6) were tested, but whenever a solid precipitate appeared, it was invariably the same Mg-NER product. Bulk product purity was confirmed by powder X-ray diffraction (comparison of the calculated and experimental patterns) and elemental analyses. Yield: 20.8 mg (~ 64 %, based on metal salt). Elemental analysis: Calcd for $\{Mg[(HO_3P)_2C(OH)(CH_2CH_2CH_2CH_2CH_2NH_3]_2(H_2O)_2\}\cdot 2H_2O$, C 22.22; H 6.21; N 4.32. Found: C 21.96; H 6.47; N 4.33. The synthesis can be scaled-up to 10-fold, without compromise in product purity.

6. **Characterization of metal-BP MOFs used for release studies by powder X-ray diffraction. The upper diagrams (blue) are the experimental, and the lower diagrams (red) are the calculated ones.**

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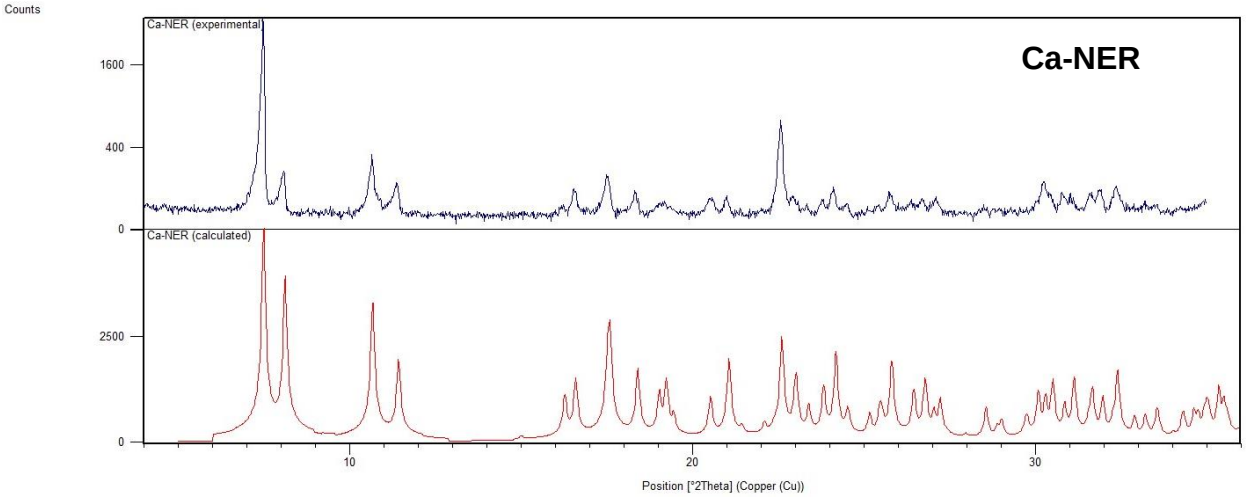
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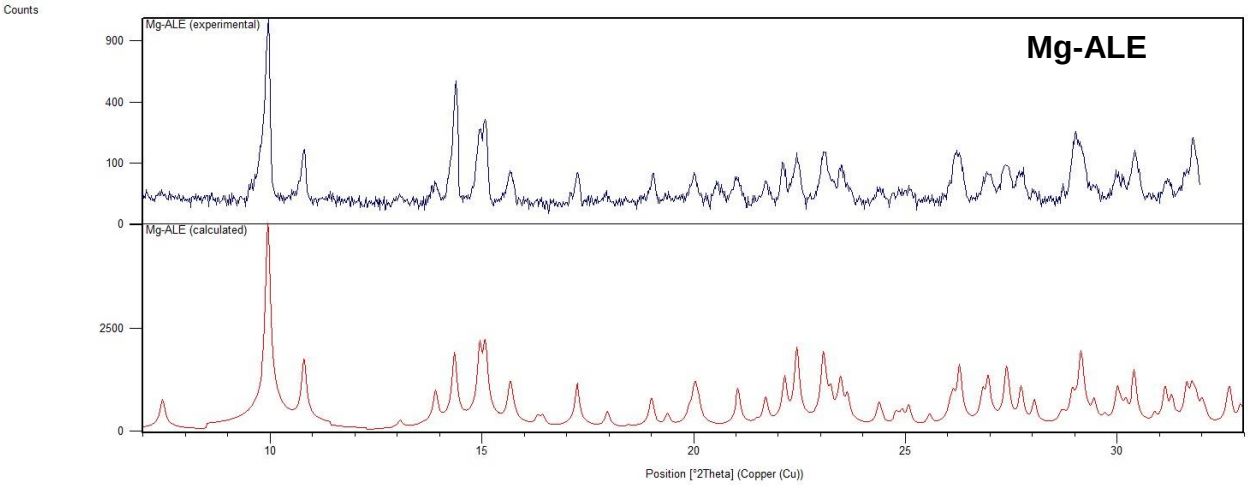
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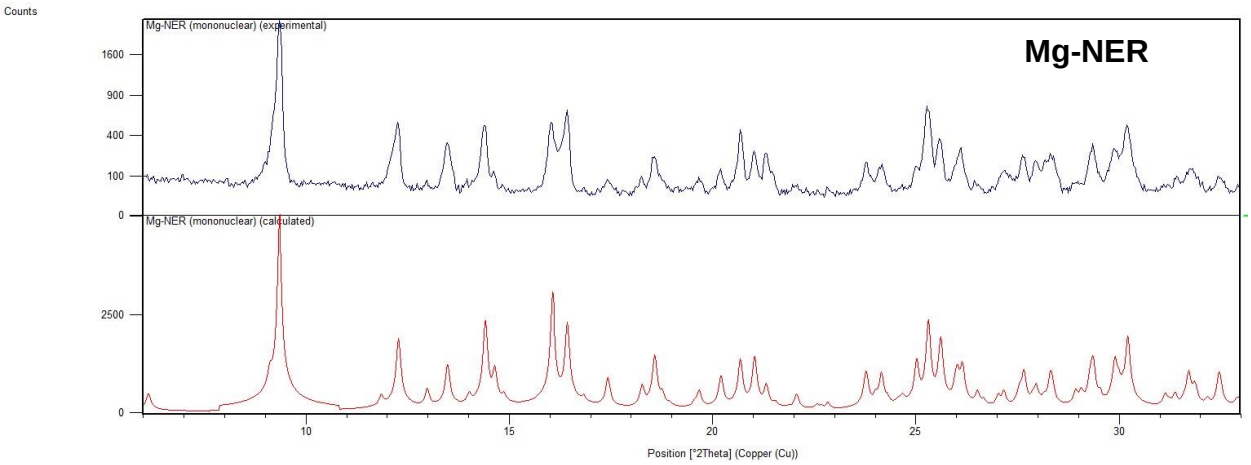


7. Controlled release studies

7.1. Tablet preparation for controlled release

Tablets were prepared that contained the ETID drug (as acid monohydrate) as the active agent (0.190 g, 850.5 μmol) and three common excipients, cellulose (0.270 g), lactose (0.270 g) and silica (0.270 g). The solid ingredients were mechanically ground in a mortar and pestle, until a free-flowing powdery solid sample was obtained. The powder was then transferred to a press that produced the tablet (10 tons pressure). Such tablets containing the “free” BPs (ETID, PAM, ALE and NER) are referred to as the “control” curves in the release diagrams. Also, tablets were prepared that contained the metal-BPs (Ca-ETID, Ca-PAM, Ca-ALE, Ca-NER, Mg-ALE and Mg-NER) as the active agents in equimolar quantity as the corresponding “control” tablet. As an example, Ca-ETID (0.261 g, 850.5 μmol of Ca-ETID) and the same excipients cellulose (0.246 g), lactose (0.246 g) and silica (0.246 g) were combined to prepare the tablet in the same way as before. The total weight of each tablet was 1.000 g. The amounts of each active BP or metal-BP agent and excipients used in the tablet preparation are presented in Table S2.

Table S2. Amounts of each active BP or metal-BP agent and excipients used in the tablet preparation.

Tablet	ETID "free"	PAM "free"	ALE "free"		NER "free"	
MW (g/mol)	**224.04**	**235.07**	**267.11**		**277.15**	
CDS* mass (g)	*0.190*	*0.200*	*0.227*		*0.236*	
Excipient (g)	*0.270*	*0.267*	*0.258*		*0.254*	
Tablet	**Ca-ETID**	**Ca-PAM**	**Mg-ALE**	**Ca-ALE**	**Mg-NER**	**Ca-NER**
MW (g/mol)	**307.14**	**544.24**	**556.51**	**536.26**	**648.65**	**646.62**
CDS* mass (g)	*0.261*	*0.231*	*0.237*	*0.228*	*0.276*	*0.275*
Excipient (g)	*0.246*	*0.256*	*0.255*	*0.257*	*0.240*	*0.241*

* CDS = Controlled Delivery System, either "free" BP, or metal-BP.

7.2. Quantification of BP released from tablets

The quantification of BP release vs. time was carried out with ^{1}H NMR spectroscopy. As an example, the procedure for an ETID-containing tablet is described. In a 100 mL glass beaker, 50 mL of deionized water were added and the pH was adjusted to 1.3 with hydrochloric acid. The ETID-containing tablet (as prepared above) was placed in a plastic net and was immersed into the solution just above the stirring bar. Mild stirring was applied to ensure solution homogeneity. The solution was sampled (sample volume 350 μL) every hour for the first six hours, and every three hours until the 12th hour, and every twelve hours until the 48th hour of the release experiment. After the 48th hour, samples were withdrawn every 24 hours or every 48 hours or longer, if necessary. Each sample was placed in a NMR tube, and then the D_2O standard solution (150 μL) was added. The concentration of the D_2O TSP standard solution was 4.337 μmol. Quantification of ETID concentration in each sample was achieved by peak ($-CH_3$) integration in the ^{1}H NMR spectrum and its comparison to the peak of the TSP standard solution peak [$-Si(CH_3)_3$].

8. **Various release graphs**

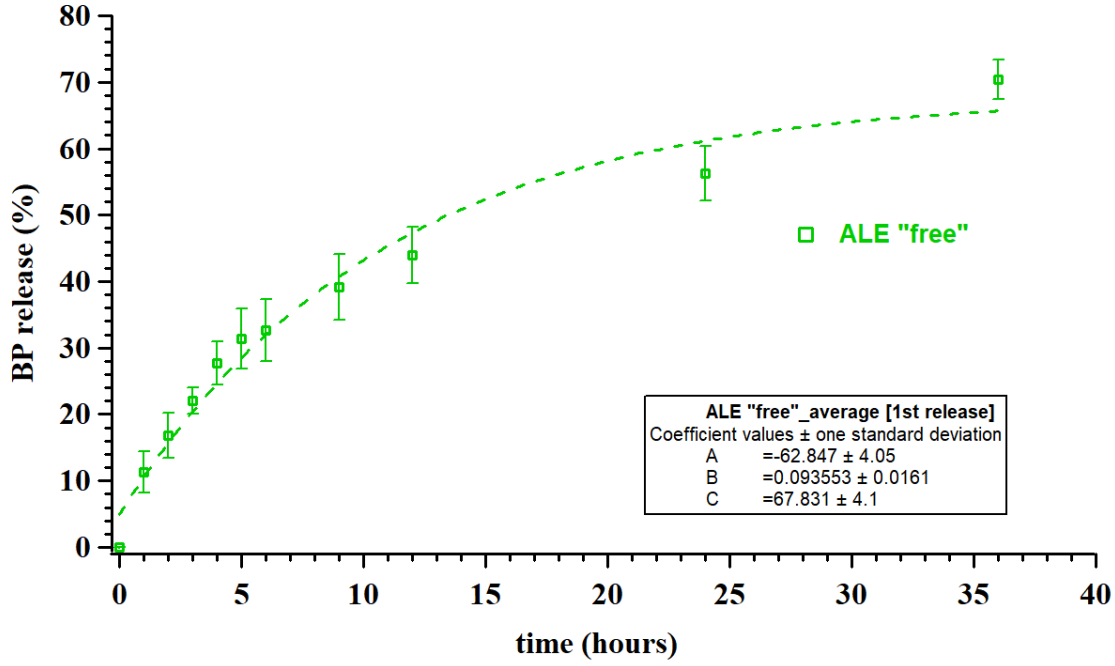


Figure S2. Statistics for the controlled release measurements for four separate experiments in the “free” ALE system. The error is < 7 %.

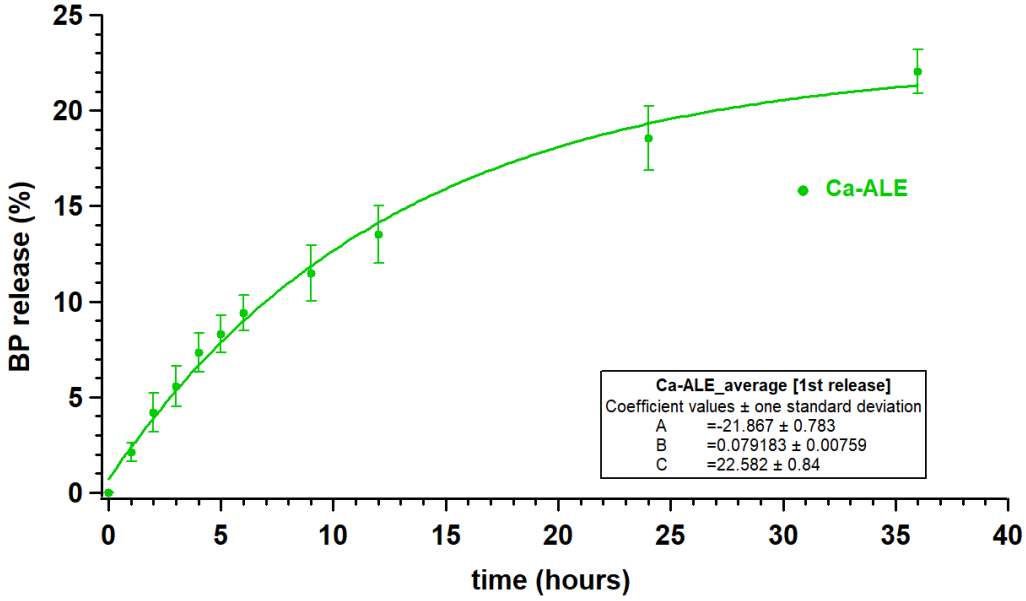


Figure S3. Statistics for the controlled release measurements for four separate experiments in the Ca-ALE system. The error is < 4 %.

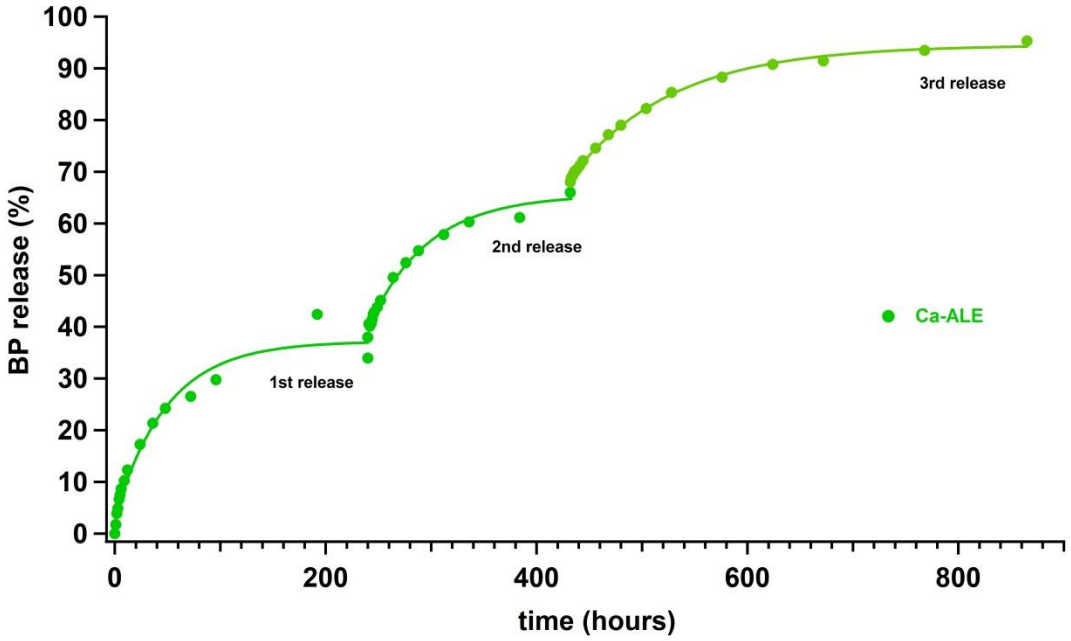


Figure S4. Three-step sustained controlled release of ALE from the Ca-ALE CDS for 840 hours.

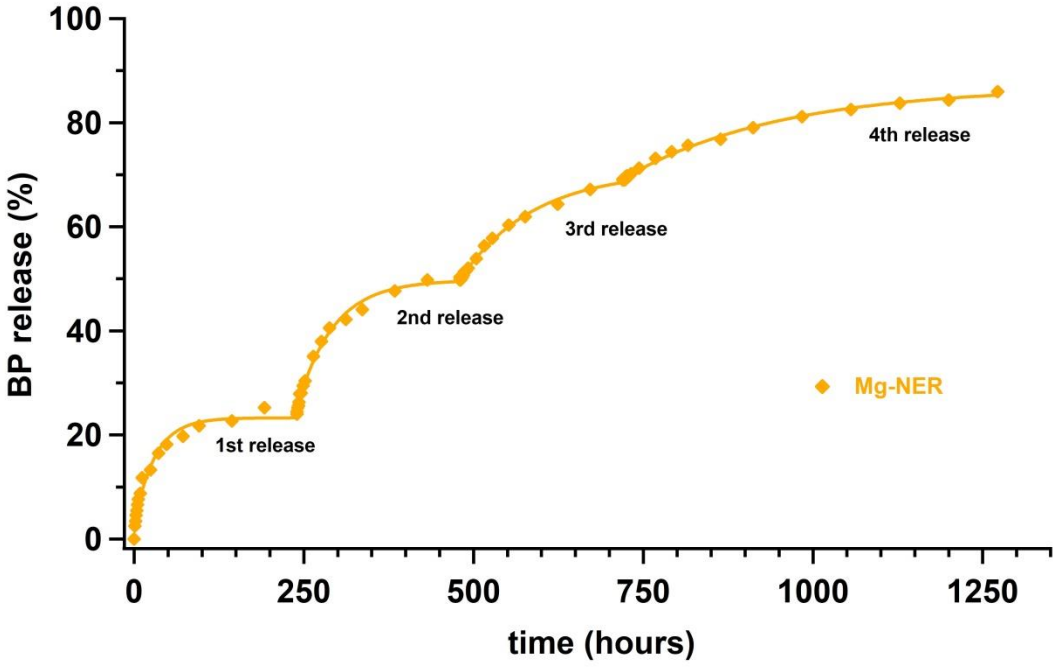


Figure S5. Four-step sustained controlled release of NER from the Mg-NER CDS for 1260 hours.

9. **Various structural views**

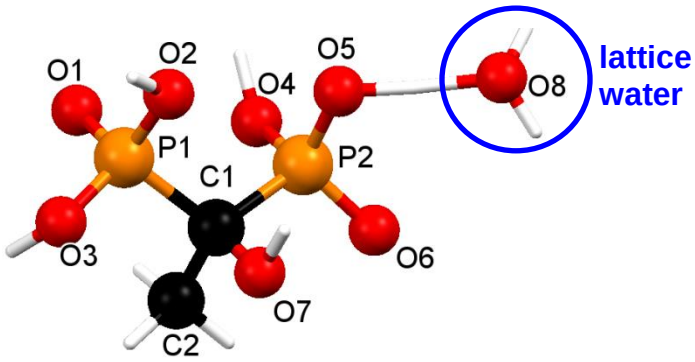


Figure S6. Structure of ETID·H_2O.

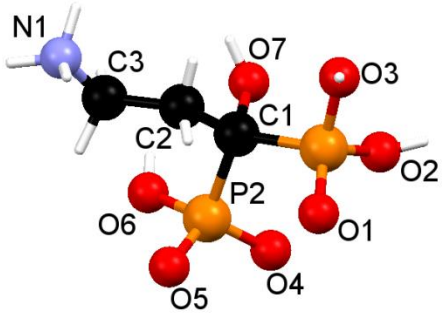


Figure S7. Structure of PAM (anhydrous).

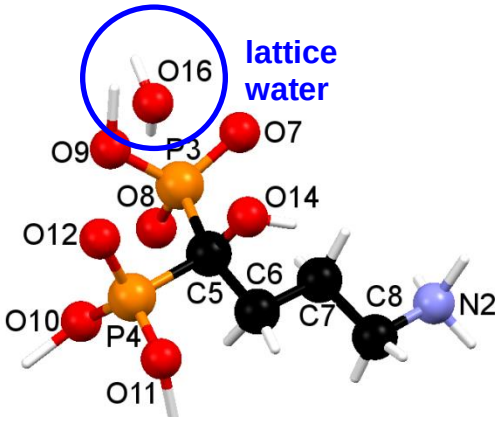


Figure S8. Structure of ALE·H_2O.

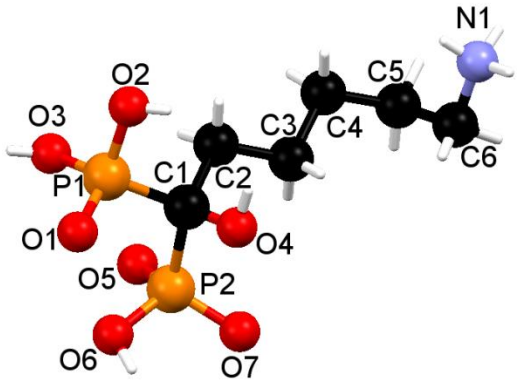


Figure S9. Structure of NER (anhydrous).

Table S3. Total number of hydrogen bonds in the structures of "free" bisphosphonic acids.

"free" BP	lattice H_2O molecules	P1	P2	N	OH	total
ETID·H_2O	1	2	1	-	2	5
PAM (anhydrous)	0	4	6	3	2	15
ALE·H_2O	1	5	5	3	1	15
NER (anhydrous)	0	5	4	3	2	14

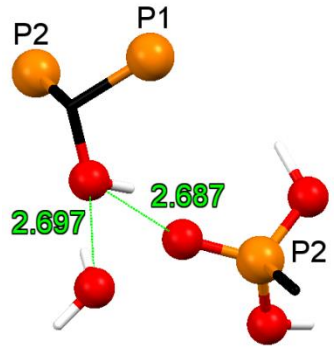


Figure S10. Hydrogen bonds involving the C-OH moiety in the structure of ETID·H_2O.

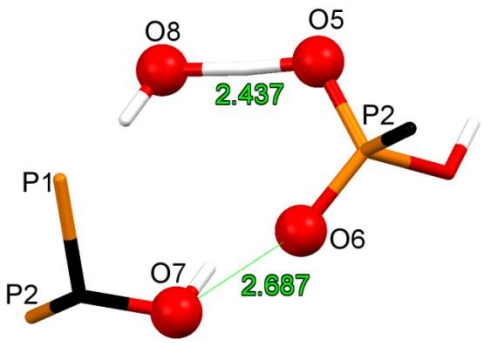


Figure S11. Hydrogen bonds involving the P1 phosphonate moiety in the structure of ETID·H_2O.

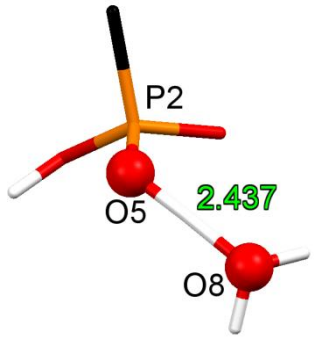


Figure S12. Hydrogen bonds involving the P2 phosphonate moiety in the structure of ETID·H_2O.

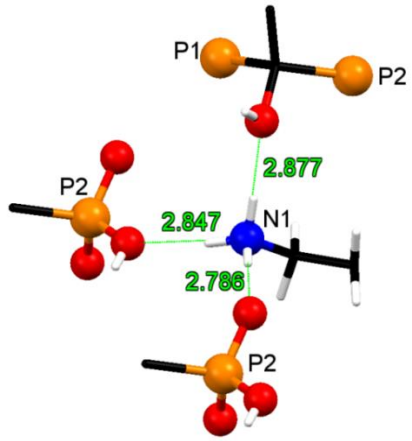


Figure S13. Hydrogen bonds involving the $–NH_3^+$ moiety in the structure of PAM (anhydrous).

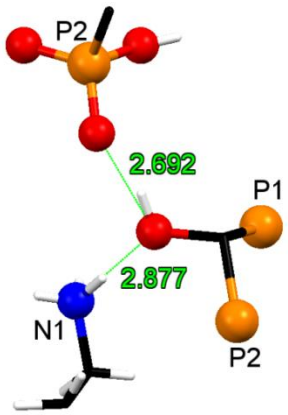


Figure S14. Hydrogen bonds involving the C-OH moiety in the structure of PAM (anhydrous).

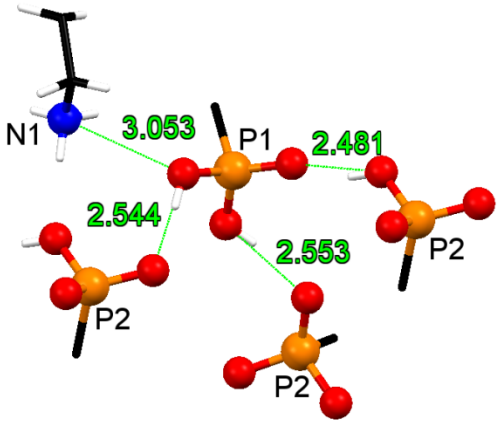


Figure S15. Hydrogen bonds involving the P1 phosphonate moiety in the structure of PAM (anhydrous).

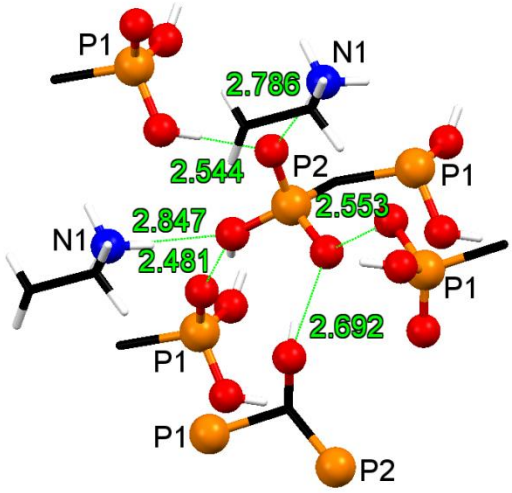


Figure S16. Hydrogen bonds involving the P2 phosphonate moiety in the structure of PAM (anhydrous).

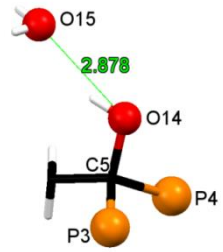


Figure S17. Hydrogen bonds involving the C-OH moiety in the structure of ALE·H_2O.

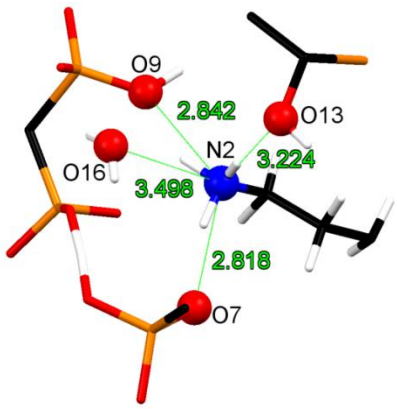


Figure S18. Hydrogen bonds involving the $–NH_3^+$ moiety in the structure of ALE·H_2O.

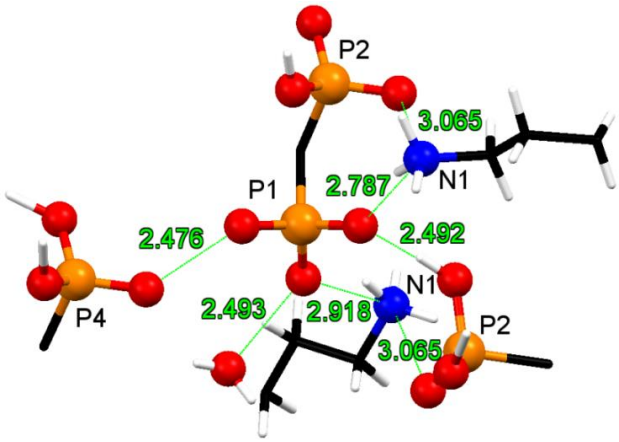


Figure S19. Hydrogen bonds involving the P1 phosphonate moiety in the structure of ALE·H_2O.

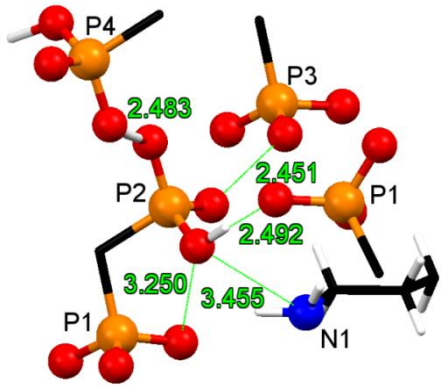


Figure S20. Hydrogen bonds involving the P2 phosphonate moiety in the structure of ALE·H_2O.

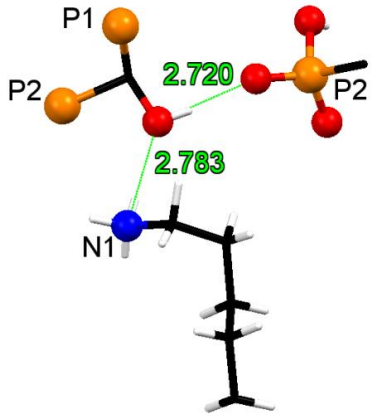


Figure S21. Hydrogen bonds involving the C-OH moiety in the structure of NER (anhydrous).

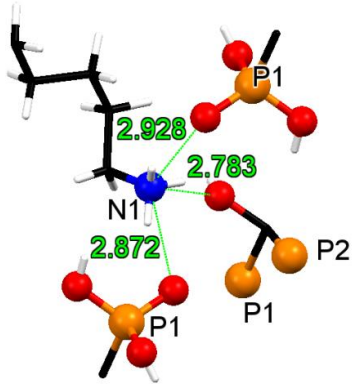


Figure S22. Hydrogen bonds involving the $–NH_3^+$ moiety in the structure of NER (anhydrous).

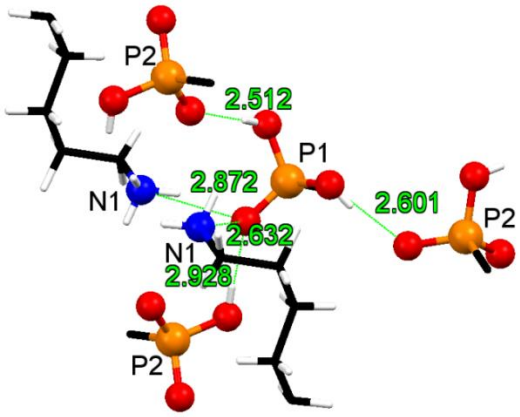


Figure S23. Hydrogen bonds involving the P1 phosphonate moiety in the structure of NER (anhydrous).

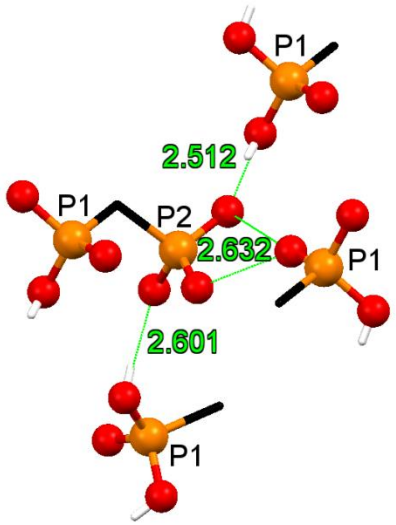
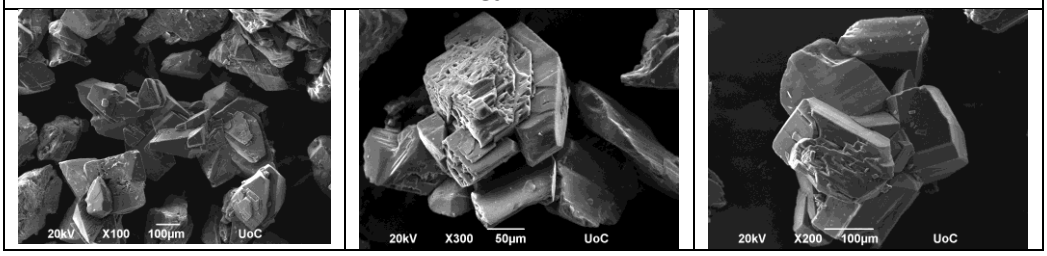


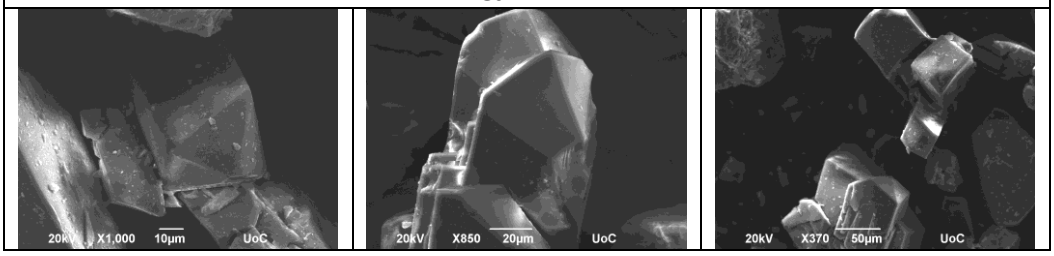
Figure S24. Hydrogen bonds involving the P2 phosphonate moiety in the structure of NER (anhydrous).

10. **SEM characterization of metal-BP MOFs**

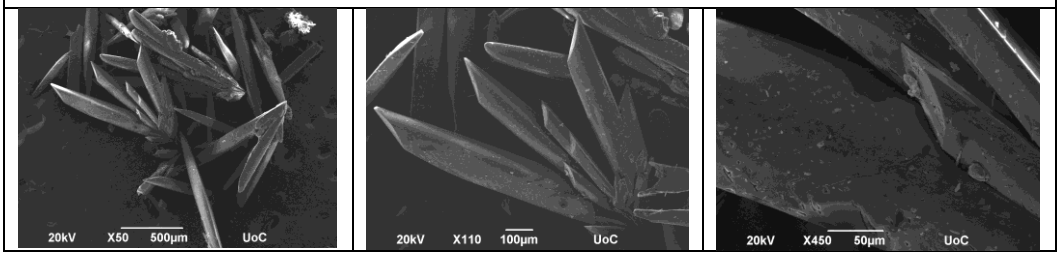
Ca-ETID



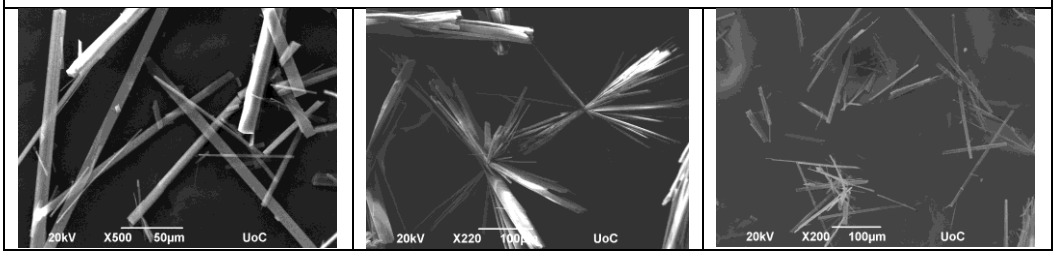
Ca-PAM

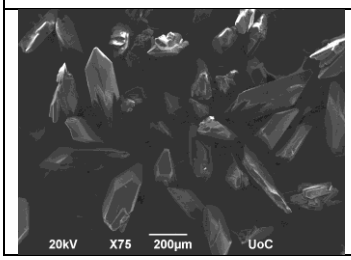
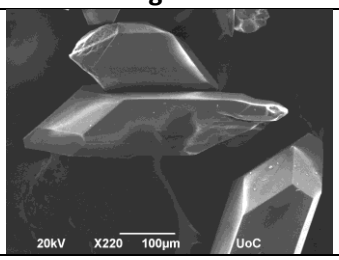
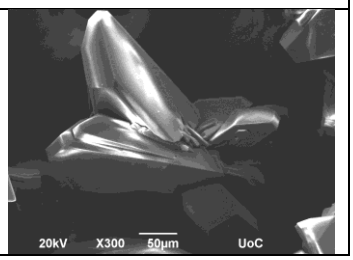


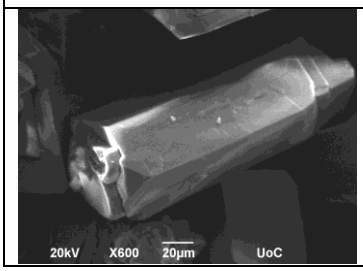
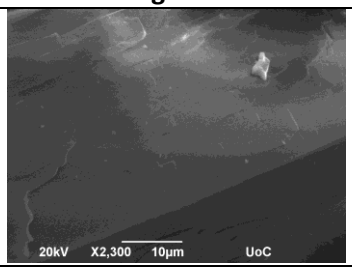
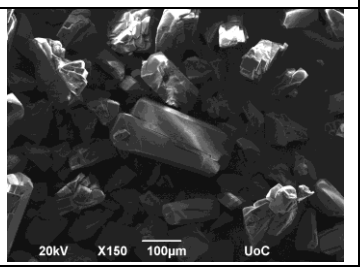
Ca-ALE



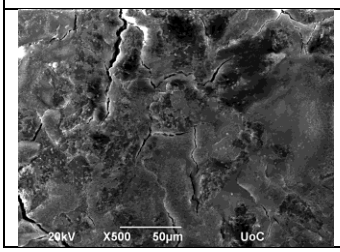
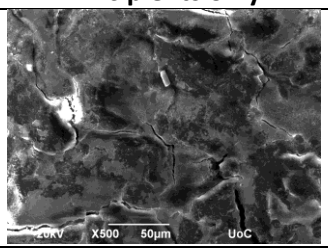
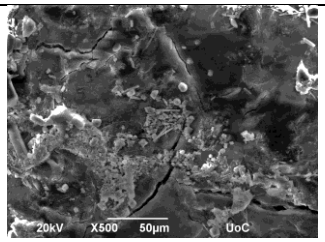
Ca-NER

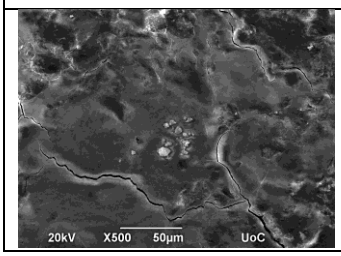
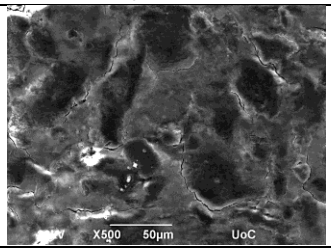


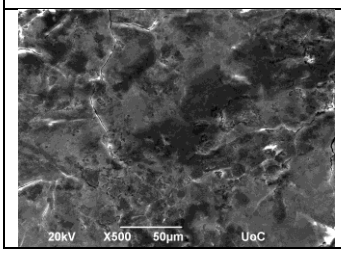
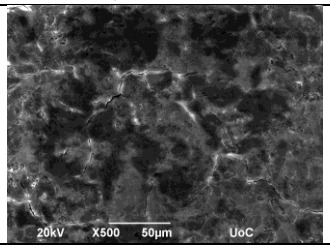
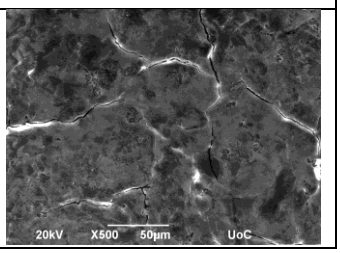
Mg-ALE		
 20kV X75 200µm UoC	 20kV X220 100µm UoC	 20kV X300 50µm UoC

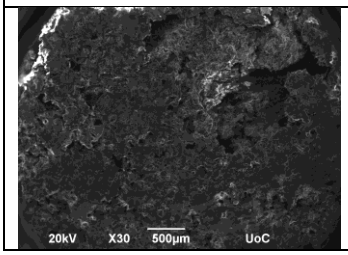
Mg-NER		
 20kV X600 20µm UoC	 20kV X2,300 10µm UoC	 20kV X150 100µm UoC

11. SEM characterization of BP-containing tablets

Excipients only		
		

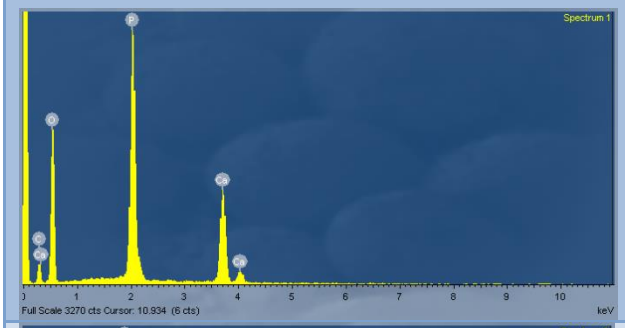
"free" NER, before release		
		

Ca-NER, before release		
		

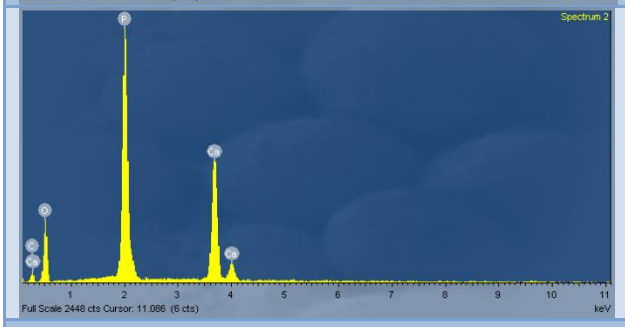
Ca-NER, after release		
		

12. **EDS characterization of crystalline metal-BP MOFs**

Crystalline Ca-ETID MOF



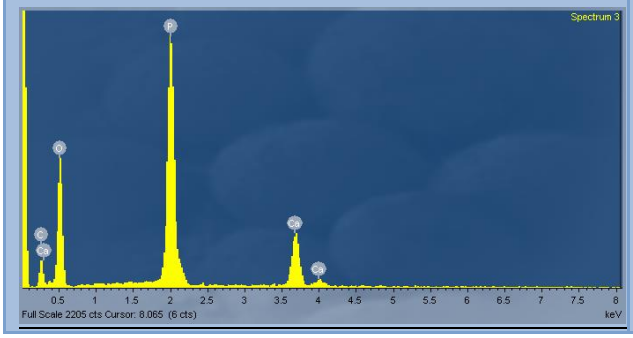
Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	15.21	0.3598	42.28	2.60	23.13
O K	94.05	0.6274	149.90	3.04	61.57
P K	68.21	1.3364	51.04	0.81	10.83
Ca K	26.36	0.9663	27.28	0.57	4.47
Totals			270.50		



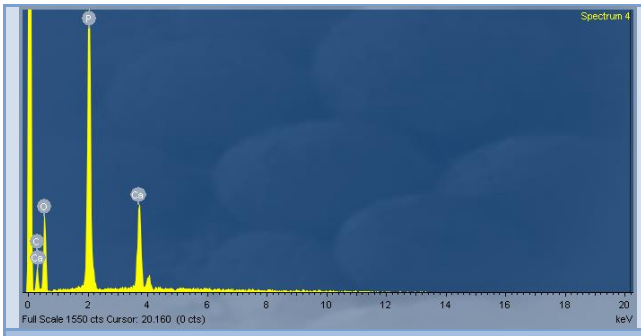
Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	5.47	0.3005	18.22	1.93	21.30
O K	27.54	0.4580	60.12	2.30	52.76
P K	49.47	1.3771	35.92	0.68	16.28
Ca K	26.75	0.9694	27.60	0.57	9.67
Totals			141.87		

Ca:P expected 1:2, found 1:2.05

Crystalline Ca-PAM MOF



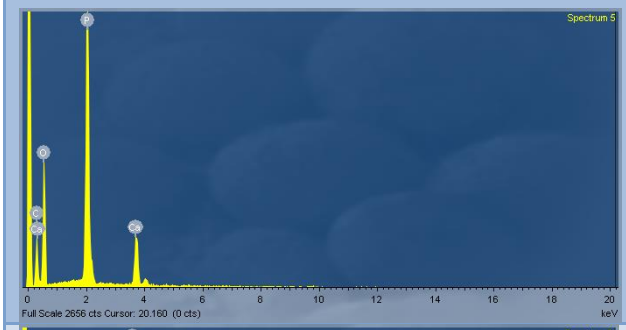
Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	13.03	0.3474	37.50	2.47	31.89
O K	52.34	0.6154	85.05	2.37	54.31
P K	45.39	1.3484	33.66	0.65	11.10
Ca K	10.14	0.9583	10.58	0.38	2.70
Totals			166.79		



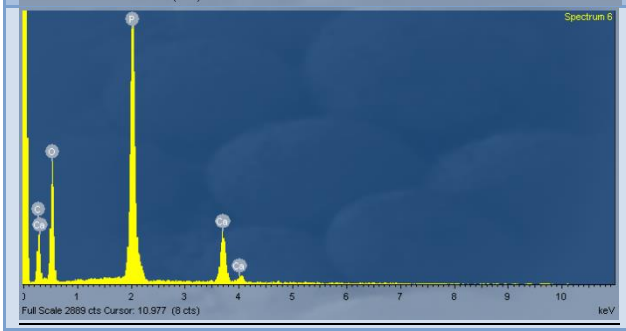
Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C K	7.96	0.3145	25.29	2.13	35.60
O K	21.35	0.4926	43.33	1.89	45.79
P K	34.61	1.3773	25.13	0.56	13.72
Ca K	11.13	0.9588	11.61	0.39	4.90
Totals			105.35		

Ca:P expected 1:4, found 1:3.05

Crystalline Ca-ALE MOF



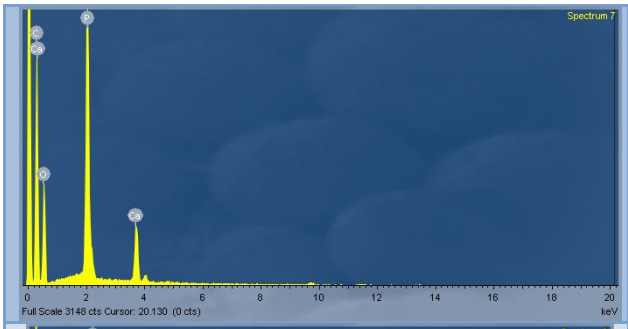
Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C K	27.20	0.3797	71.64	3.28	41.55
O K	59.87	0.5590	107.11	2.80	46.64
P K	58.10	1.3540	42.91	0.74	9.65
Ca K	11.91	0.9585	12.42	0.41	2.16
Totals			234.08		



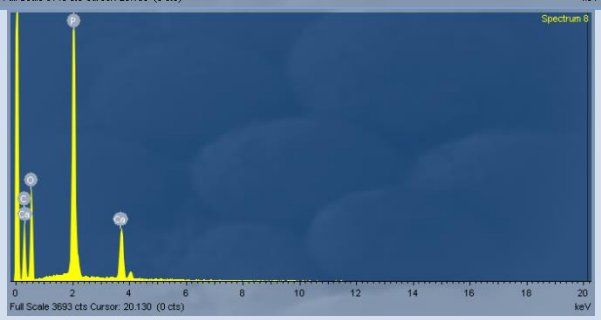
Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C K	32.42	0.3863	83.91	3.48	42.93
O K	65.67	0.5527	118.81	2.98	45.63
P K	64.11	1.3544	47.33	0.77	9.39
Ca K	12.84	0.9585	13.39	0.43	2.05
Totals			263.45		

Ca:P expected 1:4, found 1:4.4

Crystalline Ca-NER MOF



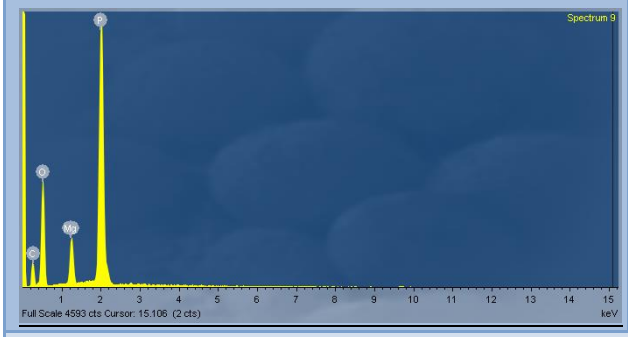
Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	159.90	0.5994	266.77	4.57	67.29
O K	58.06	0.4139	140.27	3.83	26.56
P K	68.45	1.3664	50.09	0.83	4.90
Ca K	15.99	0.9640	16.59	0.48	1.25
Totals			473.73		



Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	48.38	0.3941	122.75	3.92	49.61
O K	63.39	0.4939	128.35	3.26	38.94
P K	80.72	1.3665	59.07	0.87	9.26
Ca K	17.30	0.9582	18.05	0.50	2.19
Totals			328.23		

Ca:P expected 1:4, found 1:4.07

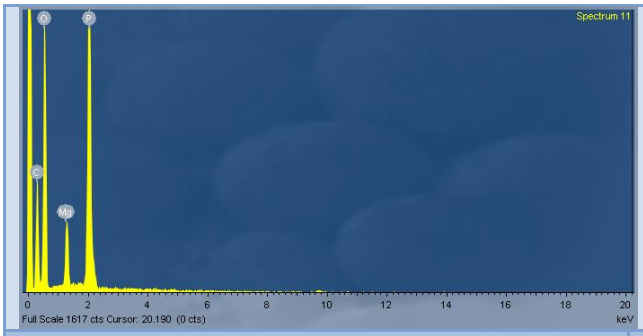
Crystalline Mg-ALE MOF



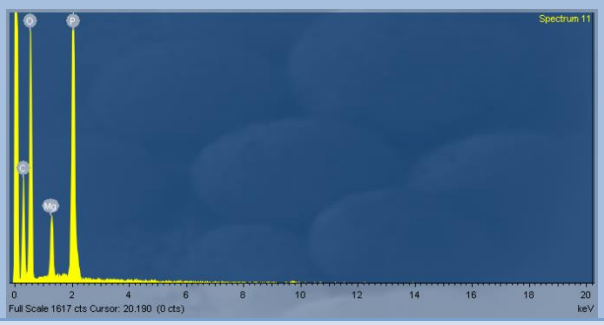
Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	17.73	0.3313	53.53	3.05	36.42
O K	69.53	0.7035	98.82	2.33	50.48
Mg K	6.34	0.7664	8.28	0.36	2.78
P K	51.26	1.3113	39.09	0.72	10.31
Totals			199.71		

Mg:P expected 1:4, found 1:3.71

Crystalline Mg-NER MOF



Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C K	38.78	0.4693	82.62	3.02	44.76
O K	77.42	0.6617	117.02	2.64	47.59
Mg K	5.00	0.7440	6.72	0.34	1.80
P K	36.26	1.3021	27.85	0.61	5.85
Totals			234.21		

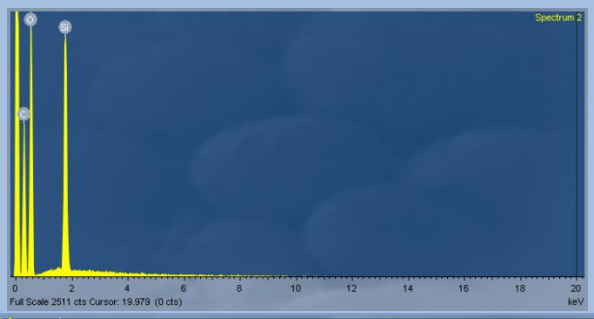


Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C K	38.78	0.4693	82.62	3.02	44.76
O K	77.42	0.6617	117.02	2.64	47.59
Mg K	5.00	0.7440	6.72	0.34	1.80
P K	36.26	1.3021	27.85	0.61	5.85
Totals			234.21		

Mg:P expected 1:4, found 1:3.25

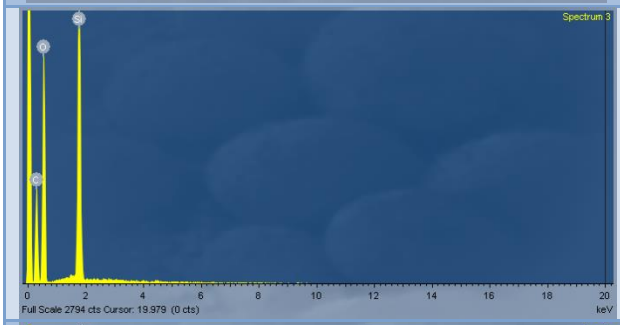
13. EDS characterization of BP-containing tablets (before and after release)

Tablet A (only excipients, no drug)



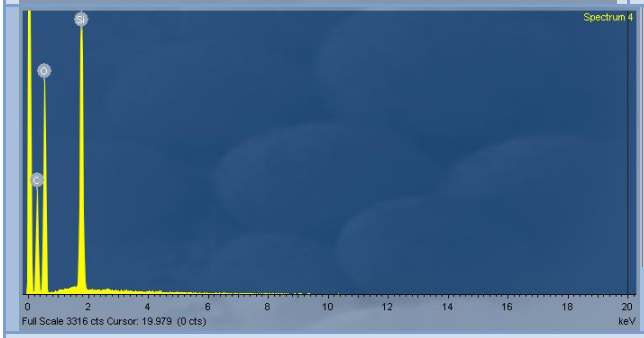
Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	91.70	0.6378	143.78	3.27	46.89
O K	131.41	0.6659	197.35	3.29	48.32
Si K	31.22	0.9077	34.39	0.60	4.80
Totals			375.52		

No P



Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	60.97	0.5391	113.08	3.16	43.31
O K	120.40	0.6925	173.88	3.04	50.00
Si K	37.33	0.9135	40.86	0.63	6.69
Totals			327.83		

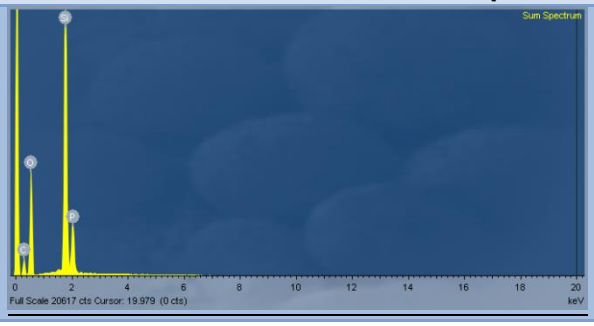
No P



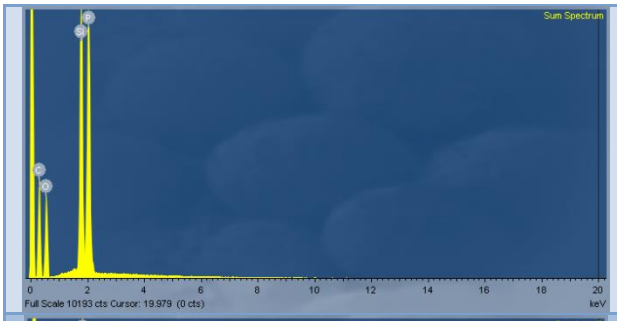
Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	75.39	0.5363	140.56	3.52	45.52
O K	129.39	0.6607	195.86	3.32	47.62
Si K	45.45	0.9190	49.46	0.69	6.85
Totals			385.87		

No P

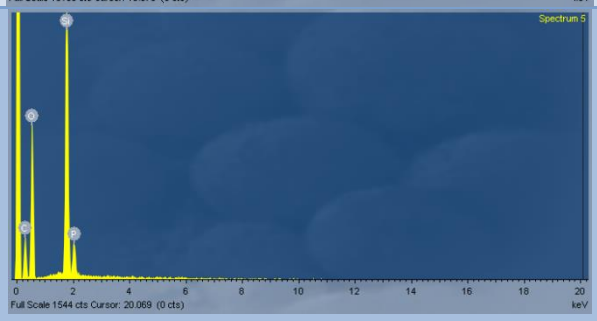
Tablet B ("free" NER before release)



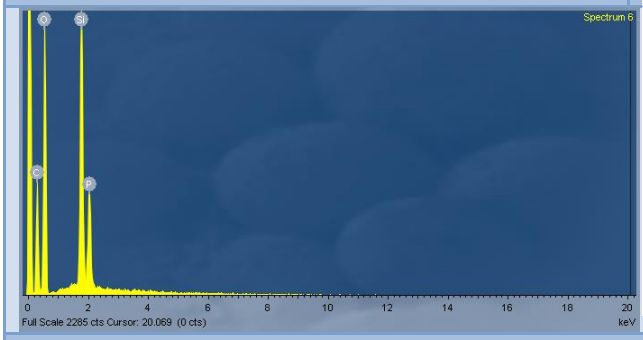
Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	20.50	0.2866	71.56	1.82	32.01
O K	104.50	0.7001	149.27	1.43	50.13
Si K	72.72	0.9641	75.43	0.42	14.43
P K	20.47	1.0366	19.74	0.32	3.43
Totals			316.00		



Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C K	59.26	0.3525	168.09	2.35	62.58
O K	40.78	0.4499	90.66	1.43	25.34
Si K	35.89	1.0015	35.83	0.30	5.71
P K	54.19	1.2281	44.12	0.41	6.37
Totals			338.71		

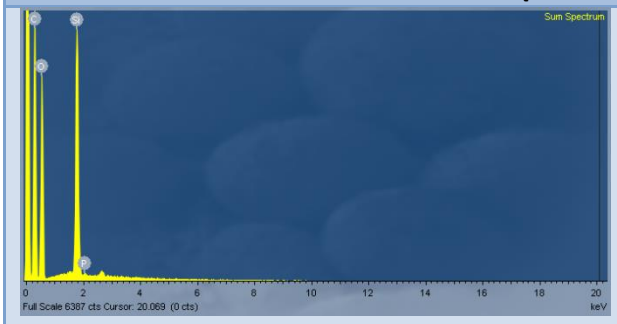


Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C K	15.33	0.3931	39.01	2.27	39.13
O K	45.66	0.6915	66.03	1.88	49.73
Si K	21.26	0.9357	22.72	0.47	9.75
P K	3.92	1.0906	3.59	0.29	1.40
Totals			131.36		



Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C K	57.18	0.4856	117.75	3.37	44.42
O K	112.58	0.6628	169.88	3.11	48.11
Si K	30.52	0.9237	33.04	0.59	5.33
P K	17.15	1.1770	14.57	0.52	2.13
Totals			335.25		

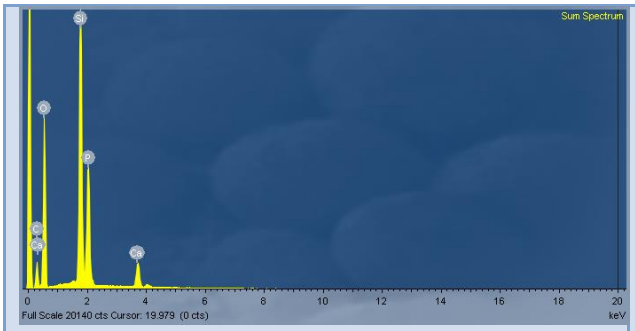
Tablet C (“free” NER after release)



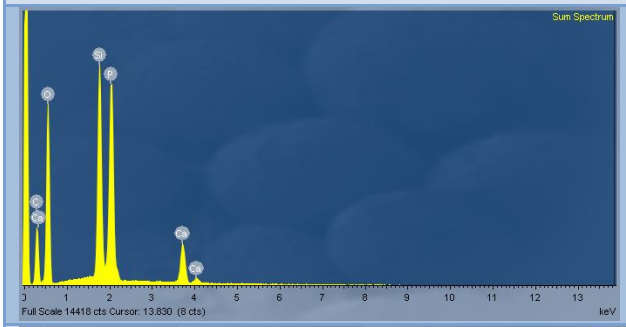
Element	App	Intensity	Weight%	Weight%	Atomic%
	Conc.	Corrn.		Sigma	
C K	81.22	0.7197	112.85	2.70	51.84
O K	78.86	0.6127	128.72	2.78	44.39
Si K	17.48	0.9105	19.20	0.44	3.77
Totals			260.77		

No P

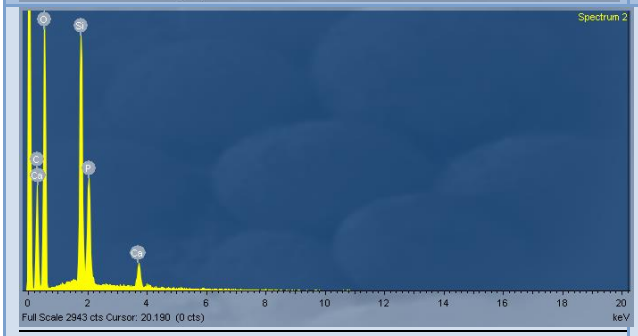
Tablet D (Ca-NER before release)



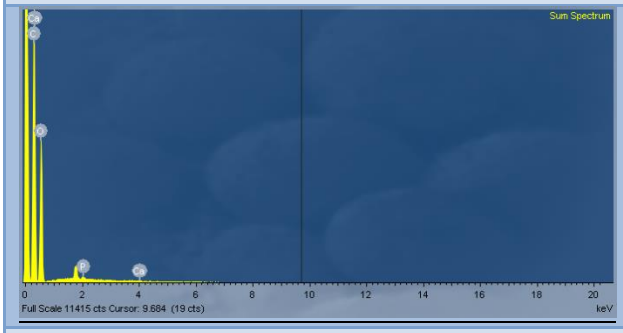
Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	29.38	0.3111	94.45	2.07	29.56
O K	159.10	0.6920	229.91	1.81	54.02
Si K	71.15	0.9476	75.08	0.44	10.05
P K	48.61	1.1150	43.59	0.44	5.29
Ca K	10.98	0.9469	11.60	0.22	1.09
Totals			454.63		



Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	47.09	0.3626	129.89	2.16	40.10
O K	122.24	0.6020	203.06	1.84	47.06
Si K	40.28	0.9475	42.50	0.34	5.61
P K	60.22	1.2043	50.01	0.44	5.99
Ca K	12.80	0.9528	13.44	0.22	1.24
Totals			438.90		

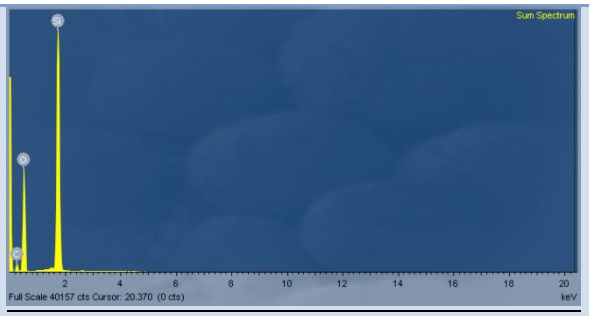


Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	68.54	0.4860	141.02	3.76	41.79
O K	150.28	0.6653	225.91	3.61	50.26
Si K	36.72	0.9191	39.95	0.66	5.06
P K	24.10	1.1838	20.36	0.62	2.34
Ca K	5.88	0.9608	6.12	0.33	0.54
Totals			433.36		



Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	166.10	1.2090	137.39	1.10	59.01
O K	70.83	0.5589	126.74	1.44	40.87
P K	0.67	1.3016	0.51	0.08	0.09
Ca K	0.22	0.9768	0.22	0.07	0.03
Totals			264.87		

Pill E (Ca-NER after release)

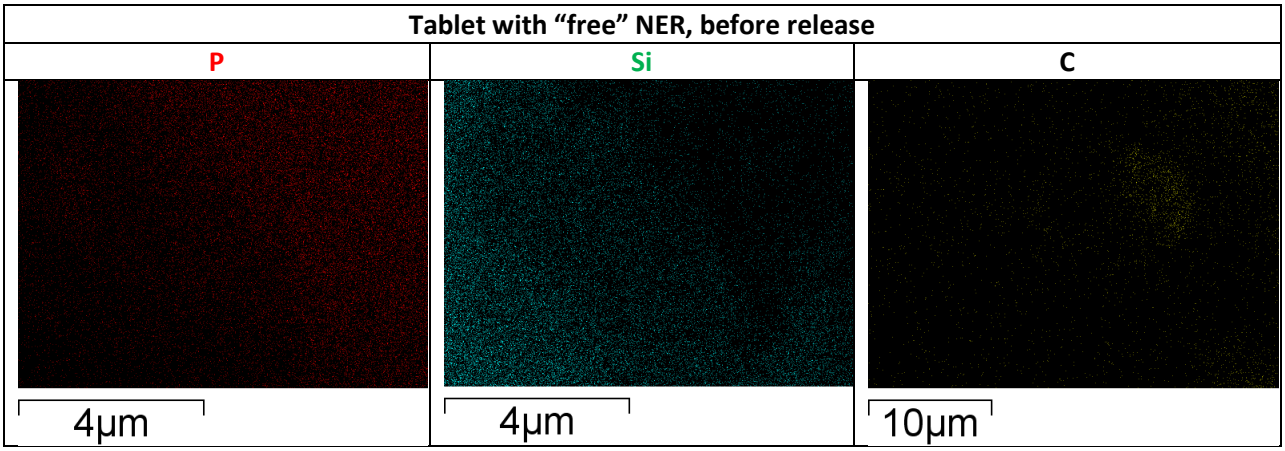


Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	20.21	0.2887	70.01	1.93	22.32
O K	204.30	0.8306	245.97	1.69	58.87
Si K	130.73	0.9475	137.96	0.56	18.81
Totals			453.95		

No P

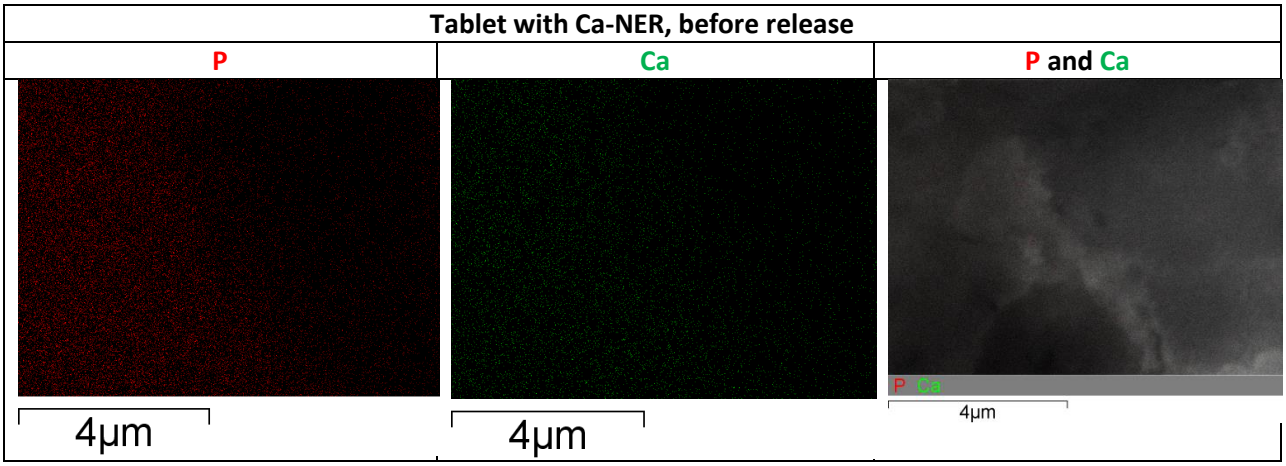
14. Elemental mapping of BP-containing tablets (before and after release)

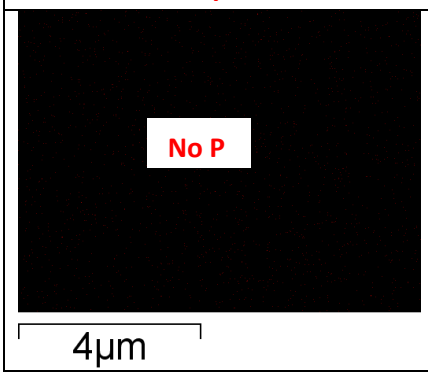
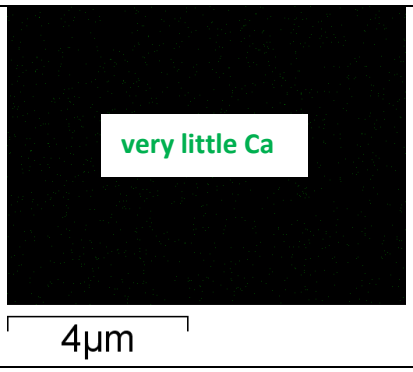
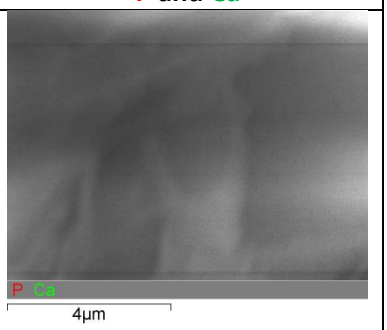
Tablet with "free" NER, before release



Tablet with "free" NER, after release

Tablet with Ca-NER, before release



Tablet with Ca-NER, after release		
P	Ca	P and Ca
		

15. Cell viability studies

15.1. Cell culture

The NIH3T3 mouse embryonic cell line is the standard cell line of fibroblasts, which display versatile functions in the body and are frequently used in drug discovery. NIH3T3 cells were cultured in D-MEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and subcultured twice per week using trypsin/EDTA. Cultures were maintained at 37 ^{0}C in a humidified atmosphere of 5% CO_2 in air. Prior to seeding on tissue culture polystyrene plates (TCPS), confluent cells (80%) were harvested using trypsin/EDTA and counted on a haemocytometer. Stock solutions (5 mM) of both modified and unmodified bisphosphonate drugs were prepared in distilled water (acidified with HCI 5M) and passed through 0.22 μm filters. Before addition to the cells, drugs were diluted in culture medium to reach 1, 10 and 100 μM final concentrations.

15.2. Cell viability assay

The effect of the bisphosphonate drugs on cellular proliferation was assessed using the PrestoBlue® live cell assay (Invitrogen, CA). The assay is dependent on the cellular reduction of a resazurin-based, non-toxic metabolic indicator to a red product, which can be detected by absorbance and provides a measure of cell viability (Hadjicharalambous, C., Alexaki, V.I., Alpantaki, K. and Chatzinikolaidou, M., Effects of NSAIDs on the osteogenic differentiation of human adipose tissue-derived stromal cells. J. Pharm. Pharmacol. 2016, 68, 1403-1408. doi:10.1111/jphp.12595). NIH3T3 fibroblasts were seeded in 96-well plates at 5000 cells/well in culture medium and cellular adhesion was allowed to take place without the influence of drugs. After 24 h, culture media were replaced with drug-containing media or drug-free media (control sample) and cell growth was monitored at 1 and 3 days. At each time point, the Presto Blue reagent was added to the wells (1:10) and the microplates were allowed to incubate at 37 ^{0}C for 60 min. Absorbance at 570 nm/600 nm was measured using a spectrophotometer (Multiskan Sky, Thermo Scientific). Each drug dilution was tested in triplicate samples in at least three independent experiments and culture medium was used as blank. Results were expressed as mean values ± standard error (SE) of % cell viability of control (untreated) cultures (100%). Statistical analysis was performed using two-way ANOVA (GraphPad Prism 8.0.2 software) followed by Tukey's multiple comparisons test, to evaluate the differences among control and drug-treated cultures in cell viability. A p value < 0.05 was considered significant.

15.3. Effects of bisphosphonate drugs on cell viability

Our results showed that the bisphosphonate drugs were well tolerated by the cells. Changes in NIH3T3 cell viability were drug-specific and influenced by dose and treatment time. Overall, the behavior of all six Ca- or Mg- modified bisphosphonate drugs was similar to their unmodified counterparts; significant differences in cell viability were not observed between these formulations at any dose or duration of treatment tested. An exception was treatment with Ca-PAM, which decreased cell viability compared to treatment with PAM (approx. 20% vs 80% relative to control), but this effect was observed only at the dose of 10 μM and after 3 days.

In comparison to control, cultures treated with NER- and EDIT- modified or unmodified drugs, displayed similar cell viability at both time points and all concentrations. Significant impact was observed following a 3 day treatment with ALE or PAM drugs at doses of 10 μM and 100 μM respectively, which reduced cell viability to < 10 %, irrespective of drug modification.

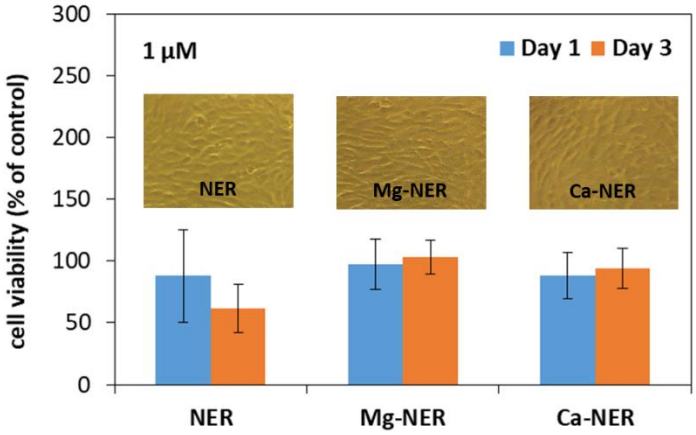


Figure S25. Neridronate drug effects on cell viability of NIH3T3 cells after 1 and 3 days of treatment. At each time point data are expressed as % cell viability based on control cultures without drug treatment (100%). Each bar represents the mean ± SE of triplicate samples from three independent experiments (n=9). Inset pictures are optical microscopy images of NIH3T3 cells after 3 days of treatment.

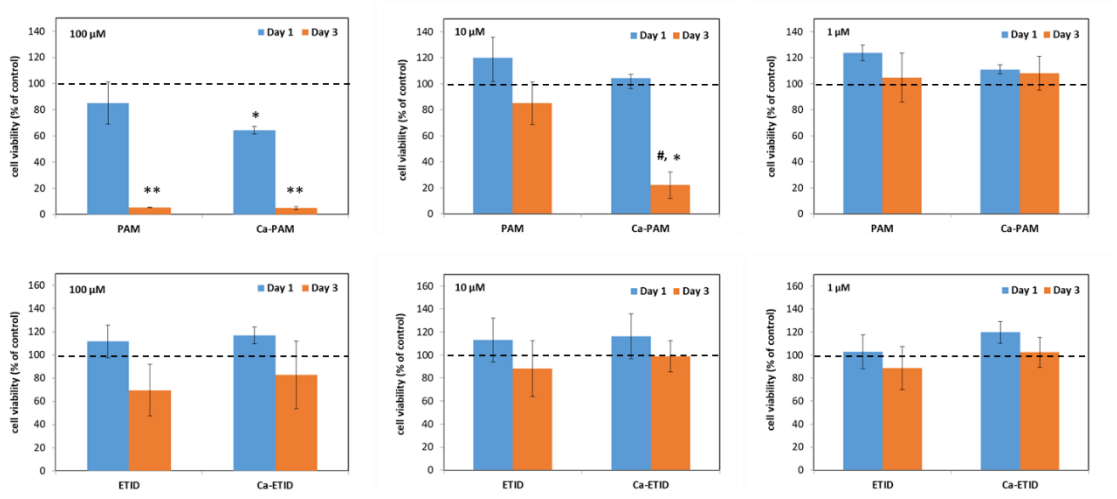


Figure S26. Bisphosphonate drug effects on cell viability of NIH3T3 cells after 1 and 3 days of treatment. At each time point data are expressed as % cell viability based on control cultures without drug treatment (100%). Each bar represents the mean ± SE of triplicate samples from three independent experiments (n=9). $^{\#}p < 0.05$ vs. PAM ; $*p < 0.01$ vs. control ; $**p < 0.001$ vs. control

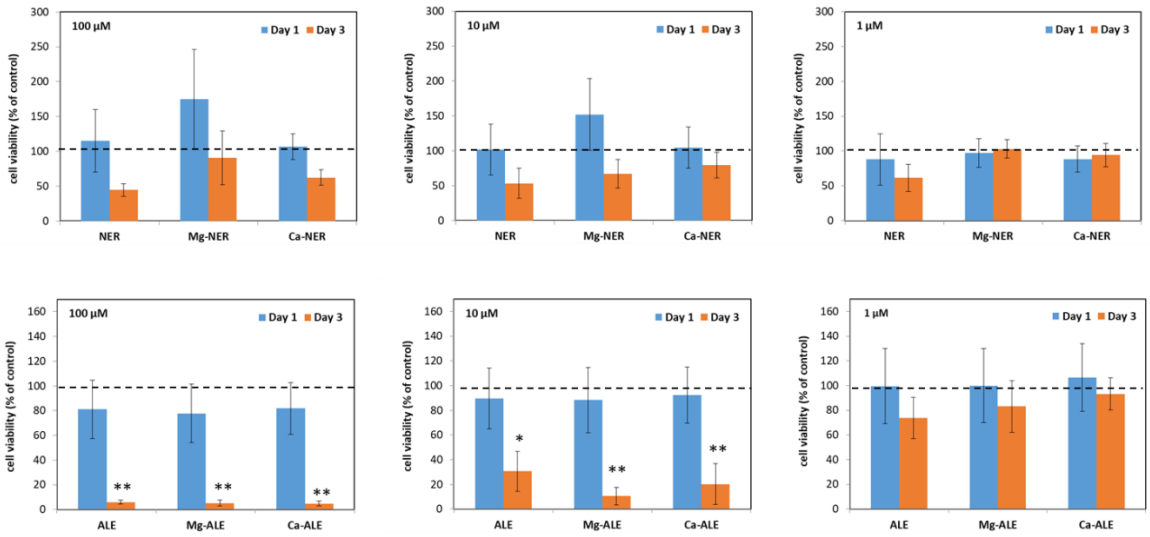


Figure S27. Bisphosphonate drug effects on cell viability of NIH3T3 cells after 1 and 3 days of treatment. At each time point data are expressed as % cell viability based on control cultures without drug treatment (100%). Each bar represents the mean ± SE of triplicate samples from three independent experiments (n=9). $*p < 0.01$ vs. control; $**p < 0.001$ vs. control

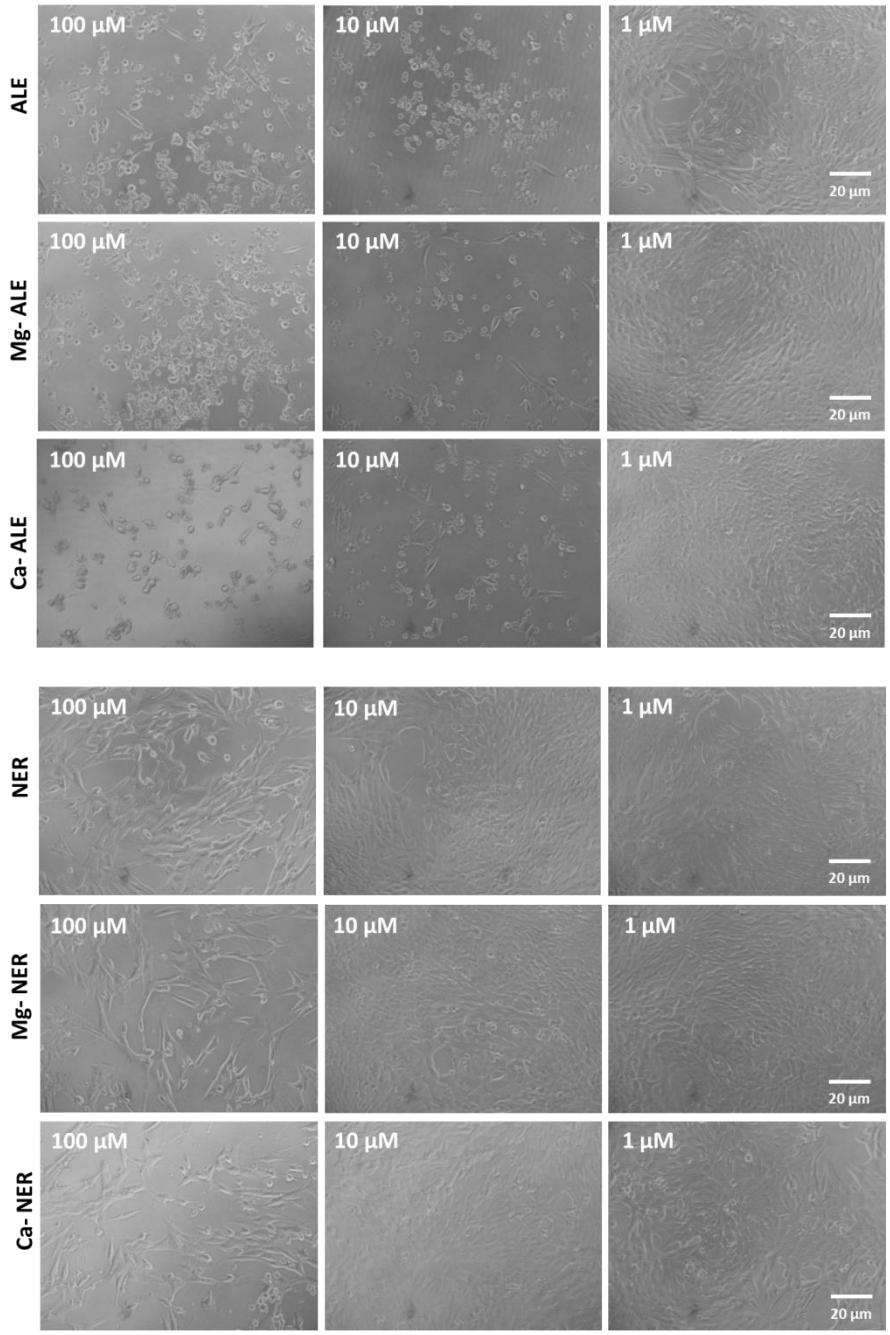


Figure S28. Bisphosphonate drug effects on cell viability of NIH3T3 cells after 3 days of treatment. Representative optical microscopy images of NIH3T3 cells after 3 days of treatment with 1,10 and 100 µM ALE or NER drugs. Scale bars indicate 20 µm in length.