Functionalization of hydroxyapatite nanocrystals modified with polyethylenimine, as drug delivery system for risedronate.

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Aim of the work



Bisphosphonate – Risedronate-



	R ,	R ₂		Relative Potency
Etidronate	ОН	- CH ₃)	1
Clodronate	CI	- CI	► no N	10
Tiludronate	н	– S – 🔿 – CI	J	10
Pamidronate	ОН	-(CH2)2 - NH2	7	100
Alendronate	он	-(CH2)3 - NH2		1000
Risedronate	н	$-CH_2 - O^N$		5000
Ibandronate	OH	(CH ₂) ₂ -N-(CH ₂) ₄ -CH ₃	×≺	10,000
Zoledronic acid	он		J	100,000

Polyethylenimine (PEI)



PEI is a polyelectrolyte with branched chain, containing

- Primary amine groups
- Secondary amine groups
- Tertiary amine groups In the ratio 1:2:1

High charge density

High reactive groups

Direct Synthesis of HA-PEI



HA-PEI Adsorption



X-Ray Diffraction - Direct Synthesis-



HA as unique crystalline phase Increase of crystallinity with the increase of PEI concentration

X-Ray Diffraction – Adsorption of PEI on HA-



Cell Parameters

Sample	a (Å)	b (Å)	c (Å)
Ha_Ref	9.4406(4)	9.4406(4)	6.8859(3)
HAPEI1M	9.4305(3)	9.4305(3)	6.8840(2)
HAPEI2M	9.4348(4)	9.4348(4)	6.8873(2)
HAPEI4M	9.4261(3)	9.4261(3)	6.8823(2)
HAPEI2M_abs	9.4395(4)	9.4395(4)	6.8786(3)
HAPEI4M_abs	9.4373(4)	9.4373(4)	6.8788(3)

TGA and Zeta Potential

Samples	TGA (wt%)	ZetaPotential(mV)
HA Ref	-	-7.2 (5)
HAPEI1M	1.7	+19.5 (5)
HAPEI2M	2.4	+24.2 (5)
HAPEI4M	2.4	+26.2 (5)
HAPEI2M_ads	0.6	+19.8(5)
HAPEI4M_ads	1	+20.5(5)

SEM



SEM



FT-IR



HA		



 $v_{3}PO_{4}$

cm⁻¹	Attribution
2950	Asymmetric vibration of CH2
2849	Symmetric vibration of CH2
1647	N-H deformation
1585	C-N stretching
1465	Bending in plane of CH2
1110	N-H stretching vibration
1043	C-N stretching
910-665	N-H wag

cm⁻¹	Attribution
3000-3400	H ₂ O stretching asym/sym
3570	OH stretching asym
1630	H ₂ O bending
1087/1046	PO ₄ ³⁻ stretching asym V ₃
962	PO_4^{3-} stretching sym V_1
875/633	Stretching P-OH libration OH
571/601	PO_4^{3-} bending asym V_2
474	PO_4^{3-} bending sym V_4

FT-IR -Direct Synthesis-



Decomposition



FT-IR -HA+PEI-



Raman PEI Raman (cm⁻¹) Attribution [Eichert07] v_2PO_4 v_4PO_4 P-OH, HPO4 HA $\nu_1 PO_4$ v_3PO_4 v_1CO_3 type B type A

Attribution	Raman (cm⁻¹)
CH ₂ wagging	1310
CH deformation	1460
NH deformation	1603
CH ₂ stretching sym	2861
CH ₂ 2stretching asym	2954
NH stretching sym	3309
NH stretching asym	3373 17



Raman

3572 cm⁻¹ OH stretching vibration

Release of PEI



Sample Preparation



Absorption Kinetic Test

Calibration curve Risedronate

Std	Abs
0,086mM	0,411316
0,129mM	0,60086
0,172mM	0,769276
0,215mM	0,944015
0,258mM	1,103744
0,301mM	1,261133



 $Qads = \frac{Ci - Cf}{m} x V$

MS Risedronate 0,3gr/L

LANGMUIR ISOTHERM

$$Q_{ads} = N \times \frac{\left(K \times C_{eq}\right)}{1 + \left(K \times C_{eq}\right)}$$

N (μmol/m²) : amount adsorbed at saturation K (L/mM) : affinity constant

•Equivalent adsorption sites •Energetically homogeneous surface •No interaction between adsorbate molecules •Formation of monolayer

FREUNDLICH ISOTHERM

$$Q_{ads} = K \times C_{eq}^{n}$$

n <1 K (μmol/m²) : constant

 Non equivalent adsorption sites
 Low interaction Adsorbate/Surface (low quantity adsorbed)

SIPS or LANGMUIR-FREUNDLICH ISOTHERM



N(L/mM), K (μmol/m²), n : surface heterogeneity and lateral interactions parameters

Non equivalent adsorption sites
Low interaction Adsorbate/Surface
Formation of multilayer



Isotherm HA+Risedronate



Isotherm HAPEI + Risedronate





Raman HA + Risedronate



Raman HA + Risedronate



Conclusions

This work clarifies the interaction of risedronate with two different apatitic supports, a well-crystallized hydroxyapatite (HA) and HA with PEI.



But further experiments and full characterization of the solids and solution after adsorption have to be performed to understand the adsorption mechanism and if there is an ion exchange between the support and the risedronate.

Future plan:

Release test of risedronate from HA and HAPEI samples after adsorption
Characterization of the solids samples by RAMAN, FT-IR, XRD, SEM, and of the solution after adsorption : Ca and P titration.



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Thank you for your kind attention

I have not failed. I've successfully discovered 10,000 things that won't work. Thomas Edison