Preparation of advanced bioceramic materials with controlled size using supercritical fluid technology

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ICMAB CSIC

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INTEMA
CONICET
UNMDP

EULANetCermat meeting
February, 28th 2013

Ultimate Objective

Preparation of scaffold materials for bone tissue engineering

↓

Development Of TiO_2/Hydroxyapatite Nanoestructured Bioceramics By Using High Pressure Methods.

OUTLINE

- **Supercritical fluids**

- **Tissue Engineering**
  - **Biomaterials**
    - **Hydroxyapatite**

- **Methods for the development of porosity**

- **Development Of TiO_2/Hydroxyapatite Nanoestructured Bioceramics**

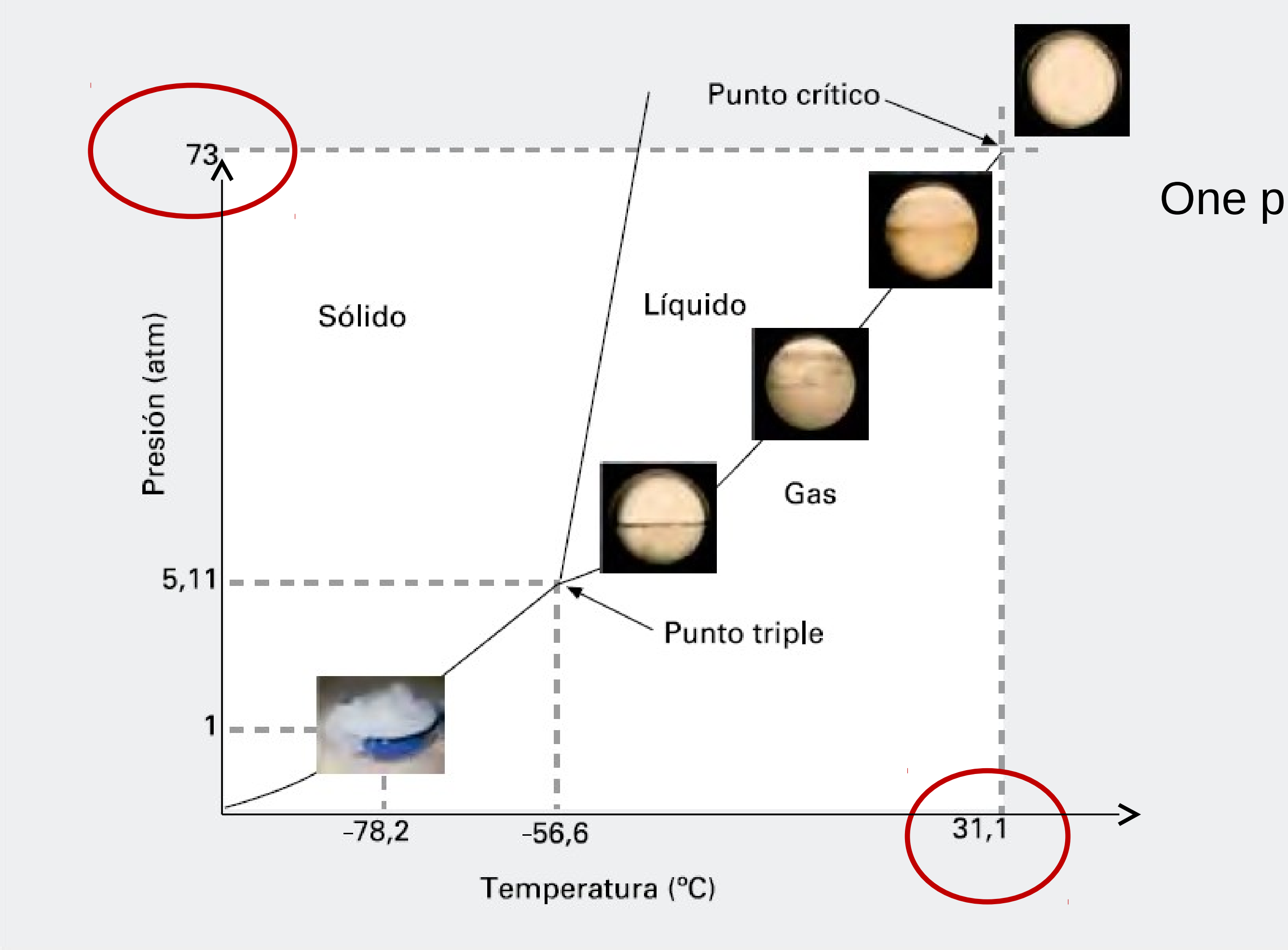
- **INTEMA-ICMAB approach**

- **Resources**

Supercritical Fluid

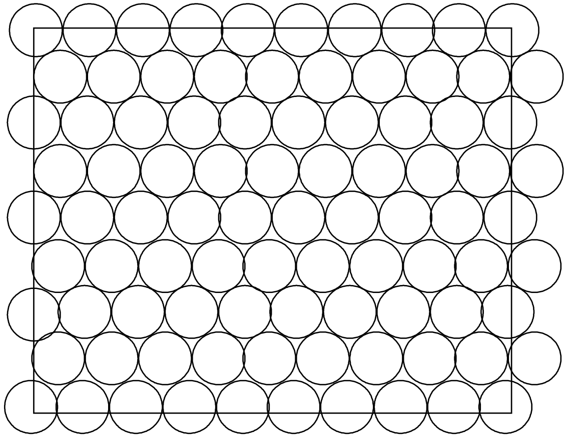
Fluid whose pressure and temperature are above the critical point

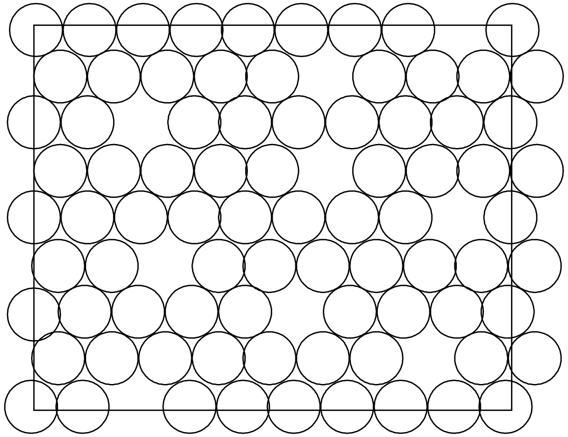
Supercritical CO_2

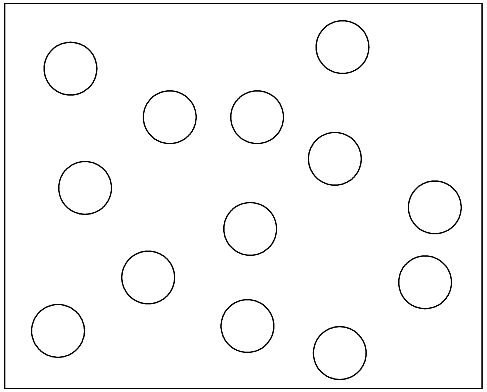


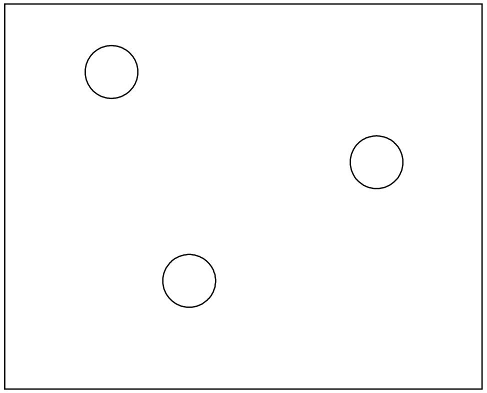
One phase only

scCO$_2$:intermediate between liquids and gases

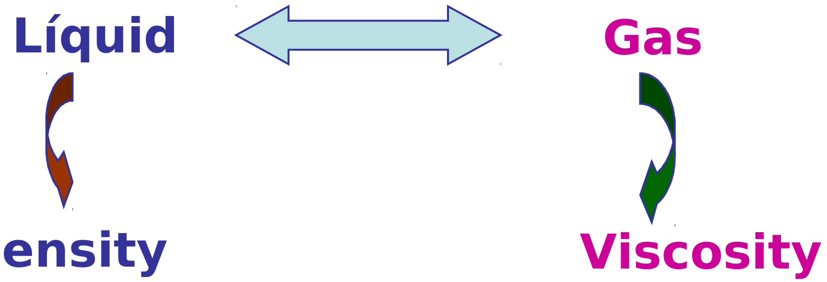

Solid


Liquid


Supercritical Fluid


Gas

The properties of the supercritical fluids are intermediate between liquid and gasses



Supercritical CO_2

- Low critical T
- High diffusivity
- Null surface tension
- Tunable density

Ideal to work in a nanometric scale

- Gas at ambient conditions

(densities 0.1-1 g/cc)

Materials processing

- Processing thermally labile materials
- Enhanced mass transfer
- Wetting of substrates with intricate geometries
- Good solvent power
- Dry and clean final products

Applications

- Extractions
- Precipitations
- Chemical reactions
- chromatography
- Impregnation agents….

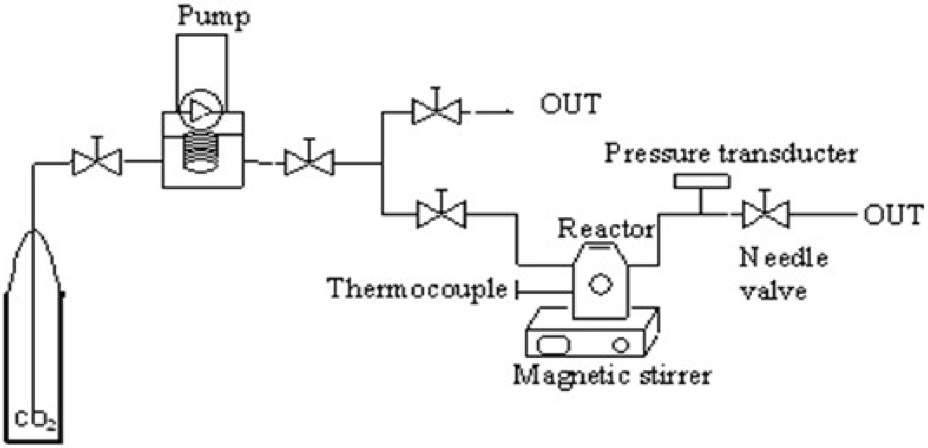
Drawbacks of $scCO_2$

•Polar molecules do not easily dissolve in CO_2.

•Heavy molecules have a very poor solubility.

ICMAB

(a)

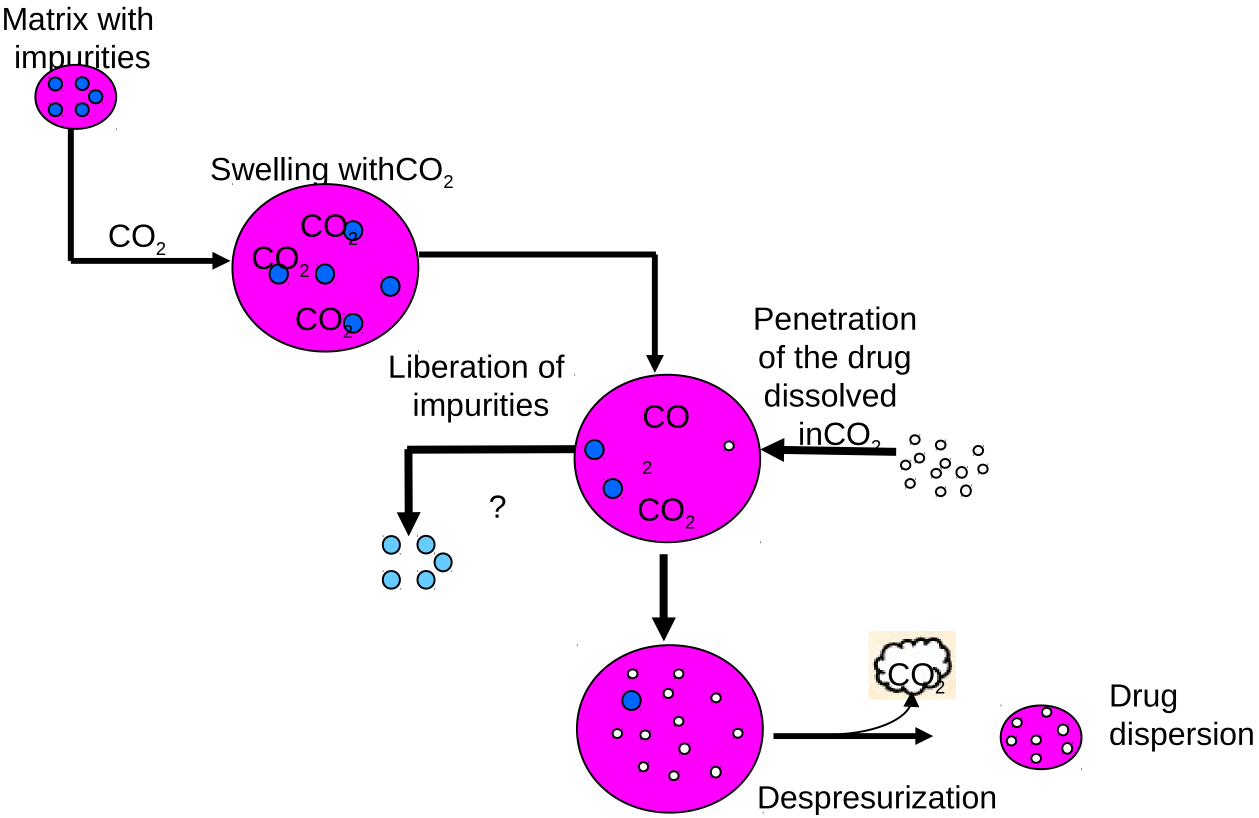

Pump
OUT
Pressure transducter
OUT
Reactor
Needle valve
Thermocouple
Magnetic stirrer
CO_2

Uses of $scCO_2$

- Supercritical CO_2 technology has significant potential for solvent replacement

Impregnation of matrices for drug delivery

ICMAB



Matrix with impurities

CO_2

Swelling with CO_2

CO_2

CO_2

CO_2

Liberation of impurities

?

Penetration of the drug dissolved in CO_2

CO_2

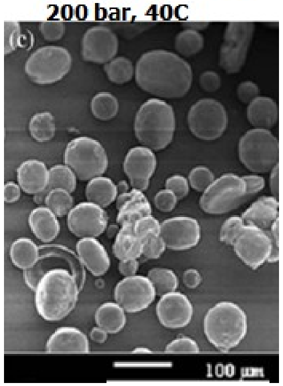
CO_2

CO_2

Despresurization

Drug dispersion

PMMA+TRF $scCO_2$
200 bar, 40C

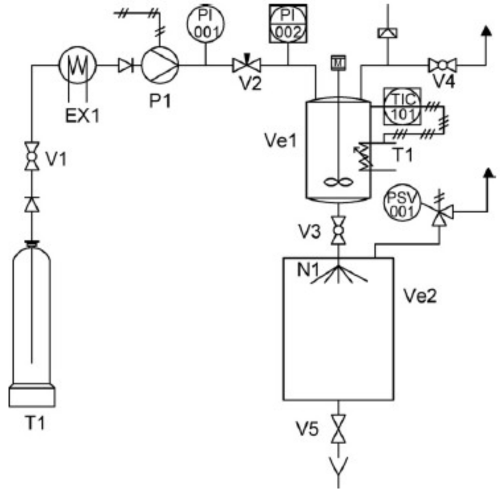


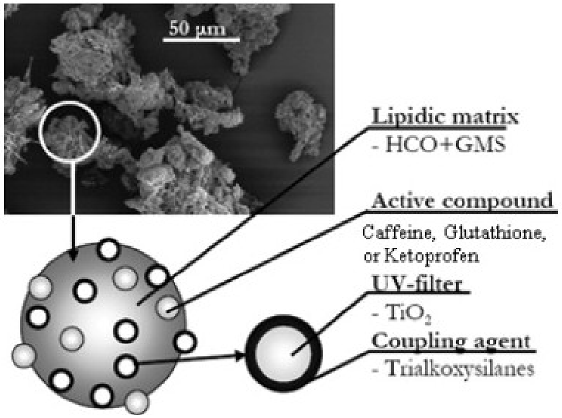
The Journal of Supercritical fluids, 48, 56-63, 2009

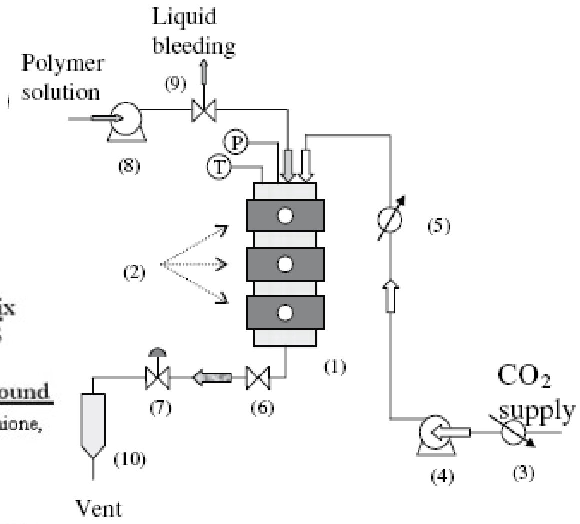
Nanoparticle and drug carrier preparation

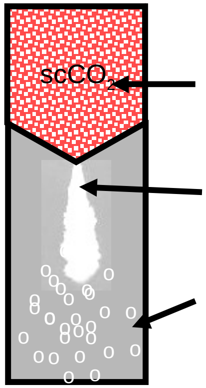
Particles from Gas saturated solutions(PGSS)

Supercritical antisolvent precipitation (SAS)









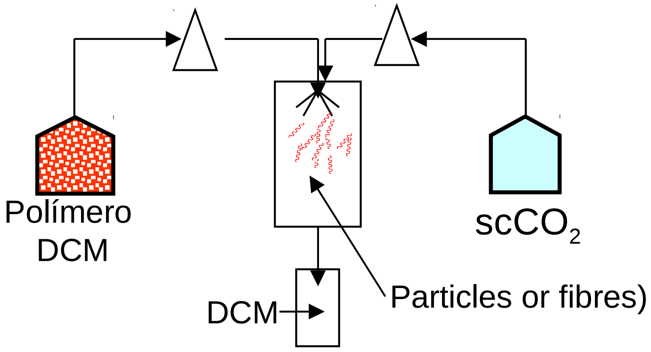
Lípido fundido a alta presión (lípido + TiO$_2$ silanizado 5% en peso)

Despresurización

Polvo seco

Pr=1 atm

International Journal of Pharmaceutics 382 (1-2) , pp. 296-304



Polímero DCM

scCO$_2$

DCM

Particles or fibres)

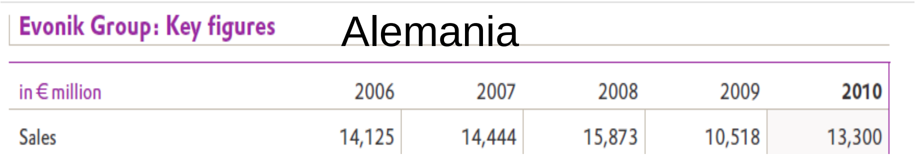
European Polymer Journal, 44,2008, 081-1094

Industrial applications

Big scale extraction

- ✓ Decaffeination of Coffee and Tea
- ✓ Extraction of yeast

EVONIK INDUSTRIES



Evonik Group: Key figures

in € million	2006	2007	2008	2009	**2010**
Sales	14,125	14,444	15,873	10,518	13,300

Alemania

TharProcess

Pittsburgh- EEUU

SEPAREX Supercritical Fluid Technology

Francia

FeyeCon

Holanda

Pharmaceutical and biotechnological Industry

phasex corporation ...the Supercritical Fluids People

aap bio implants

natex PROZESSTECHNOLOGIE

Industria Española

Altex ALTA TECNOLOGÍA EXTRACTIVA

Solutex.

DIAM

demede engineering and research

OUTLINE

- Supercritical fluids

- **Tissue Engineering**
  - **Biomaterials**
    - **Hydroxyapatite**

- Methods for the development of porosity

- Development Of TiO_2/Hydroxyapatite Nanoestructured Bioceramics

- INTEMA-ICMAB approach

- Resources

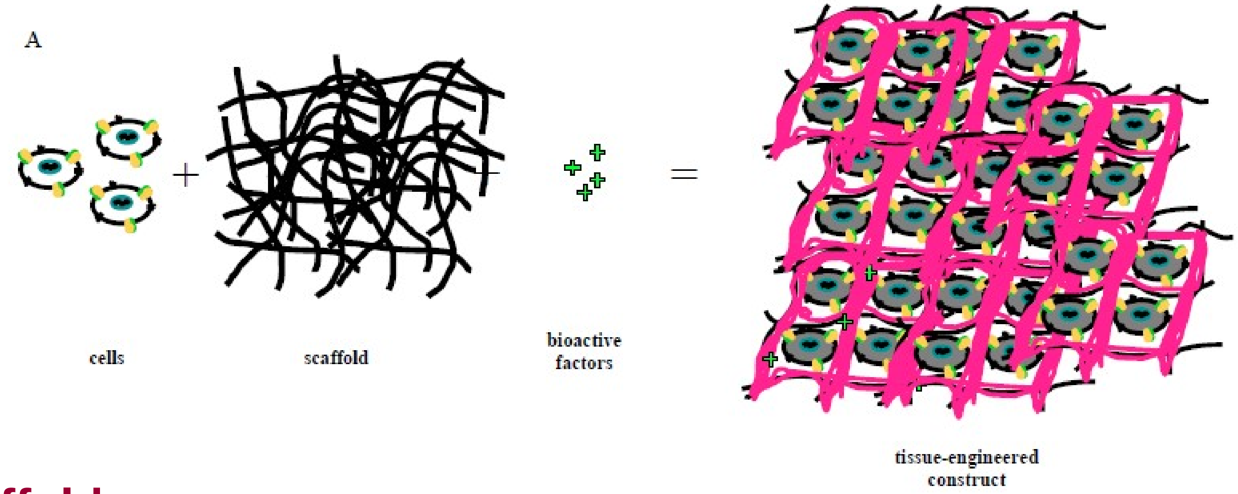
Properties of Ideal Scaffold- Bone scaffolds

- **Material Properties**
  - **Structurally strong**
  - **High porosity, high surface area to volume ratio**
  - **Uniformly distributed and interconnected pore structure**

-Pore sizes with diameters 150-300μm are recommended to promote good vascularisation and attachment of bone cells to guide their growth into all three dimensions

- **Biological Properties**
  - **Biocompatibility**
  - **Promotion of cell adhesion**
  - **Enhancement of cell growth**
  - **Retention of differentiated cell function**

Preparing a tissue engineered bone



1. Scaffold

By rapid prot)

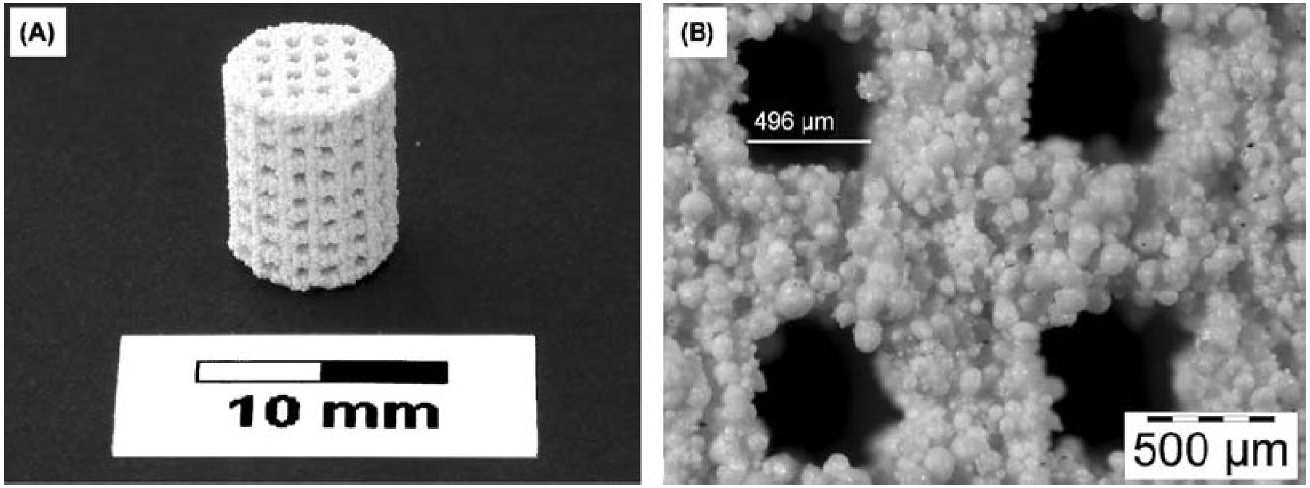
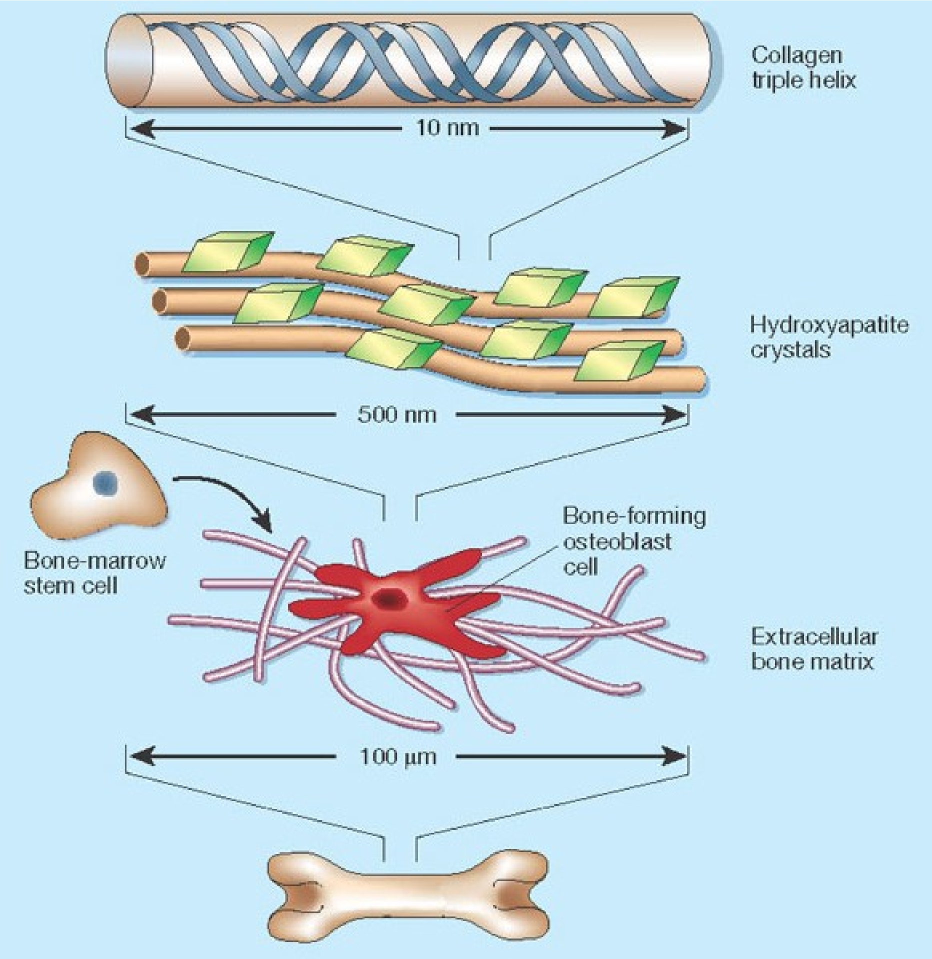


Figure 3 3D printed testpart with interconnecting channels. (a) Whole structure. (b) Detail view of the interconnecting channel structure with diameter of about 500 μm. The remaining granule structure is visible.

JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **16** (2005) 1121 – 1124

Bone is one example of a natural material whose properties depend intimately on its nanoscale structure



Nature **412**, 491-492

- ✓Bone is an inorganic–bioorganic composite material consisting mainly of collagen proteins and hydroxyapatite (a crystalline form of calcium phosphate).
- ✓Collagen spontaneously forms fibrils of aligned protein helices, on which tiny hydroxyapatite crystals (10–50 nanometres in length) can grow.
- ✓Both the size and the orientation of the crystals are dictated specifically by the collagen template, and the precise structural relationship between the collagen and hydroxyapatite is critical to bone's resilience and strength.

Biomaterials for Tissue Engineering

↓

Scaffolds composition for bone tissue engineered materials are mainly made of bioceramics (Inorganic Ca derivates)

Hydroxyapatite (HAp)

$[Ca_{10}(PO_4)_6(OH)_2]$

is the natural component of bones

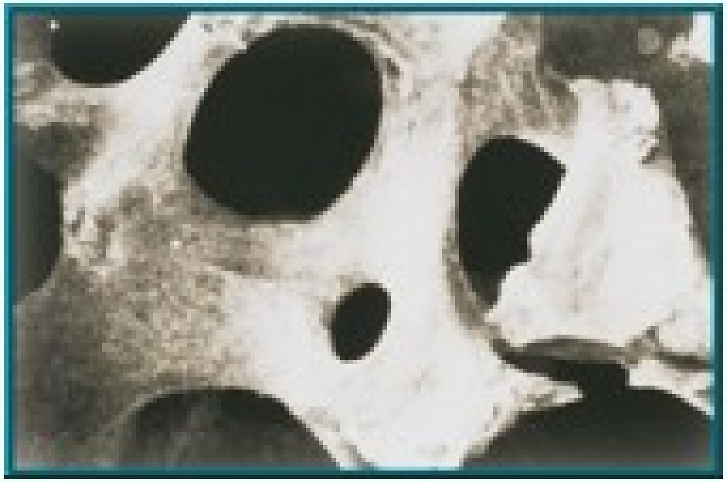
Hydroxyapatite

70% of bone is inorganic mineral hydroxyapatite [$Ca_{10}(PO_4)_6 (OH)_2$]

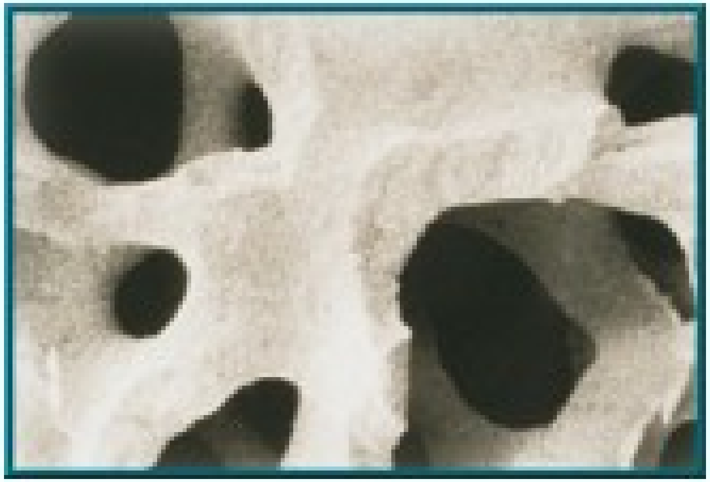
Predominantly crystalline, though may be present in amorphous forms.

The crystals are platelets or rods, about 8 to 15A thick, 20 to 40A wide and 200 to 400A long.

The substitution mechanisms that occur in the hydroxyapatite of bone include intercrystalline exchange and a recrystallisation due to dissolution and reformation of crystals, with the addition of new ions into the crystal structure replacing Ca2+ or being adsorbed on the crystal surfaces



Hydroxyapatite



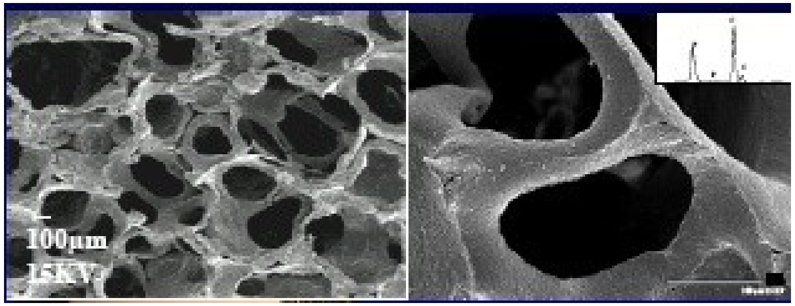
Human Bone

HAp ceramics have some weaknesses such as the poor toughness and low-bending strength.

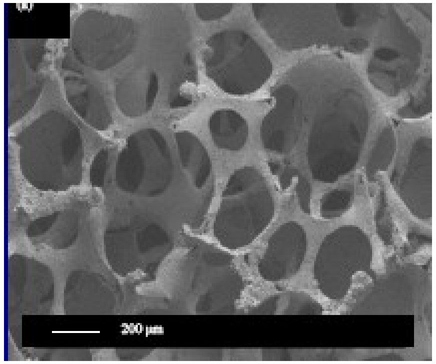
If density of HAp ceramic ↑ mechanical properties ↑ bioactivity ↓

compressive strength of a bone is about 170 MPa

scaffolds are too brittle



Compressive yield stress: 8 MPa



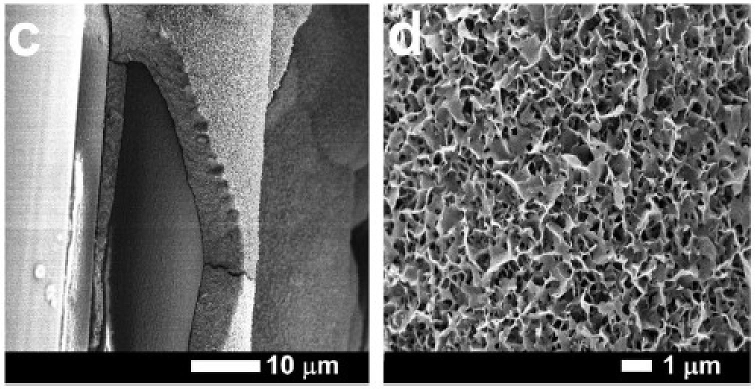
Compressive stress 9.8 MPa

Research current trends

To improve the mechanical properties

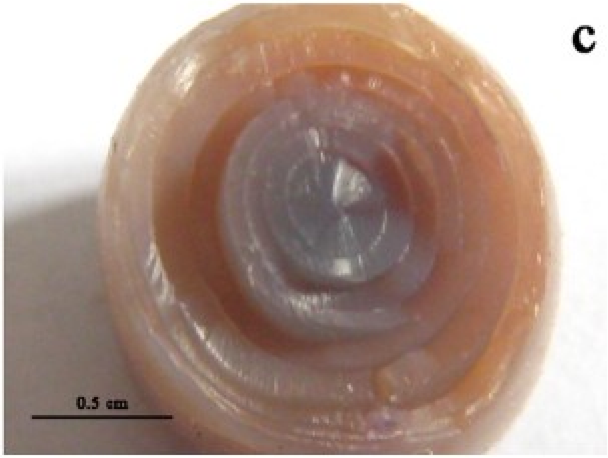
Bioabsorbable and bioactive composites have been developed combining resorbable polymers with calcium phosphates, bioactive glasses or glass-ceramics in various scaffold architectures, fibers etc

hydroxyapatite-coated PLLA fibers:



Journal of the Mechanical Behavior of Biomedical Materials, 17, 2013, 269–277

Genipin-crosslinked chitosan/hydroxyapatite composite



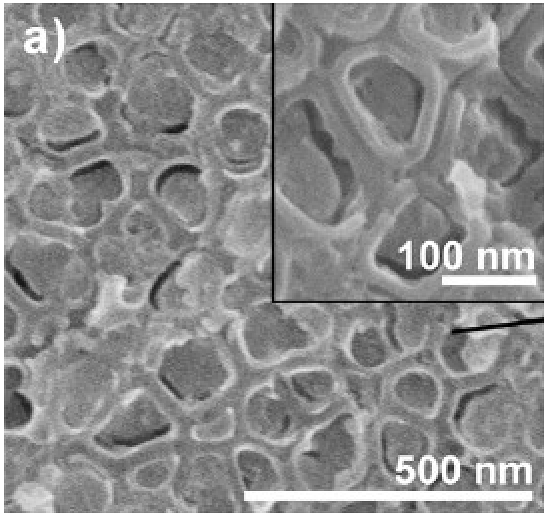
Materials Letters 94 (2013) 169–171

Design of scaffolds from HAp/Titanium

✓ **Titania composites materials are recognized as one of the best biomaterials for prostheses**

✓**The immobilization of a biocompatible metal/metal oxide on the surface of the hydroxyapatite →Would improve the cellular responses and biocompatibility of HAp along with high toughness and strength.**

TiO_2 nanotube surfaces for site-selective nucleation of hydroxyapatite



Apatite filled tubes at the sample surface

Acta Biomaterialia 7 (2011) 424–431

Deposition of HAp in TiO_2. examples

-Design of scaffolds from Titanium intermingled fibres

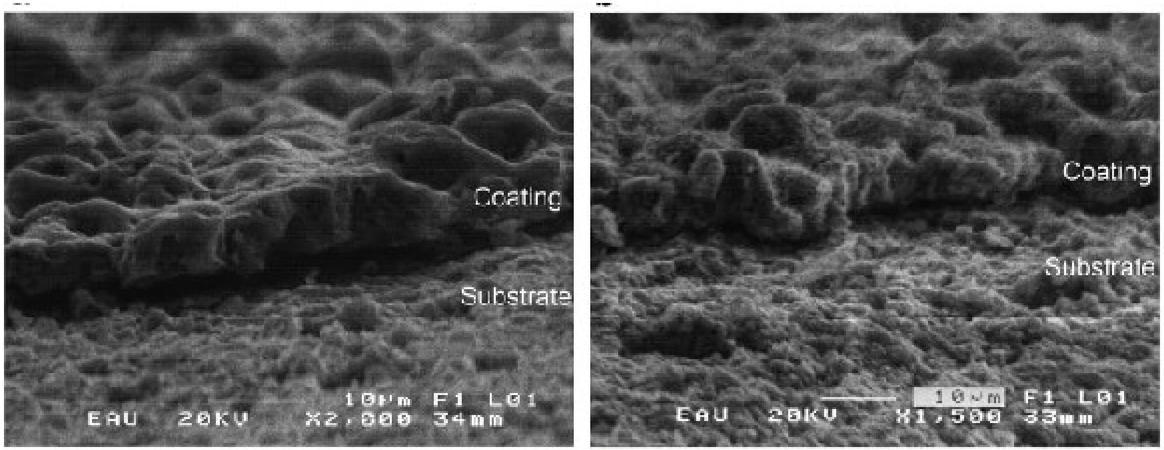
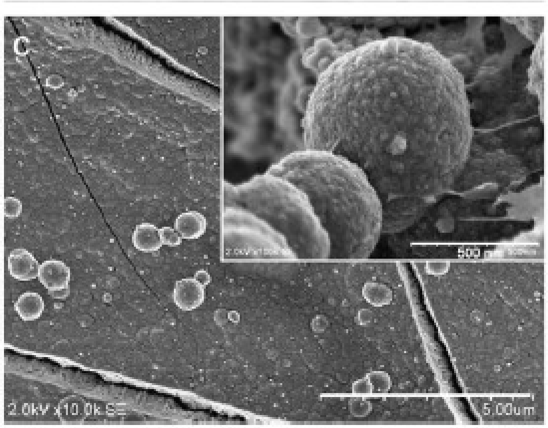


Fig. 2. SEM micrographs showing the thickness and surface morphologies of: (a) ECAE/MAO-treated sample and (b) ECAE/MAO/HT-treated sample.

-Hydroxyapatite production on ultrafine-grained pure titanium. *Surf. Coat. Technol.* (2011), doi:10.1016/j.surfcoat.2011.03.032.

-Film of TiO_2 nanotubes recovered with calcium phosphates via simply immersion



Characterization of a calcium phosphate-TiO2 nanotube composite layer for biomedical applications. *Mater. Sci. Eng. C* 31 (2011) 906-914.

OUTLINE

- Supercritical fluids

- Tissue Engineering
  - Biomaterials
    - Hydroxyapatite

- **Methods for the development of porosity**

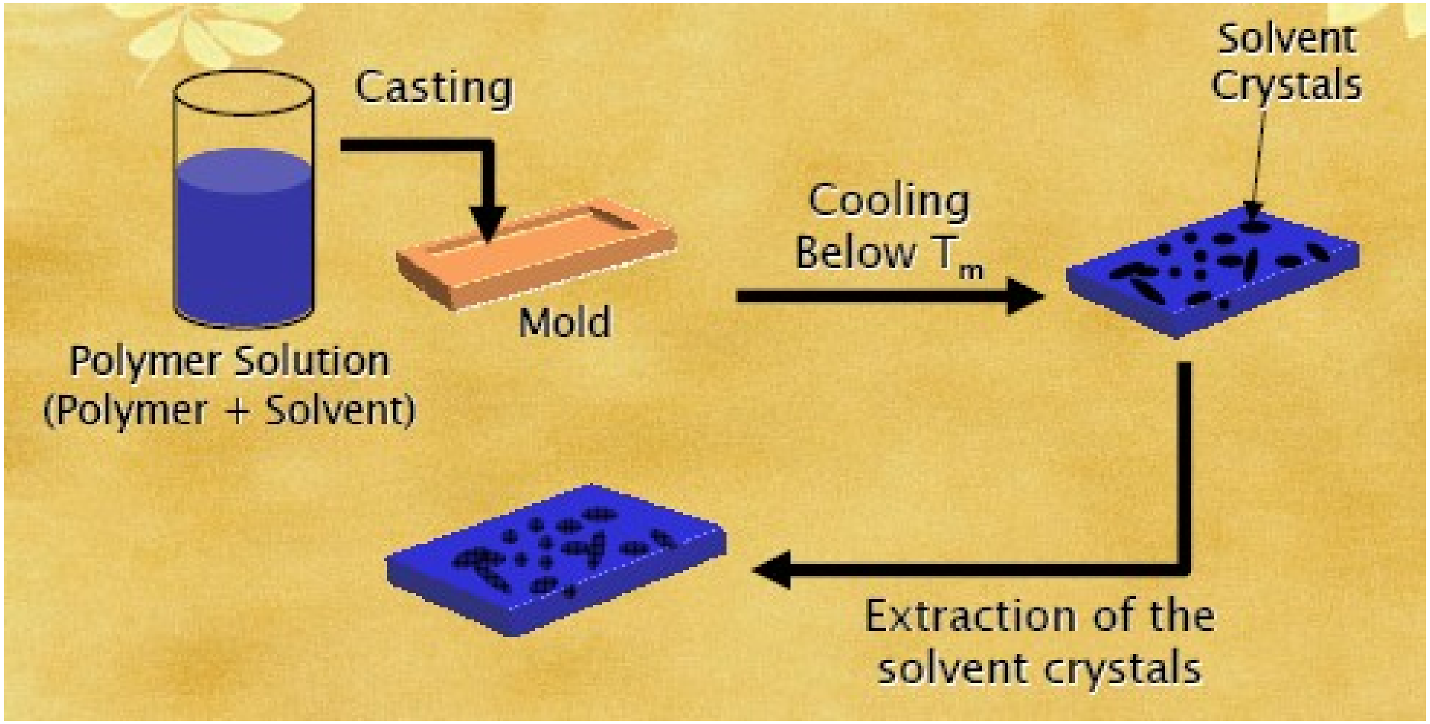
- Development Of TiO_2/Hydroxyapatite Nanoestructured Bioceramics

- INTEMA-ICMAB approach

- Resources

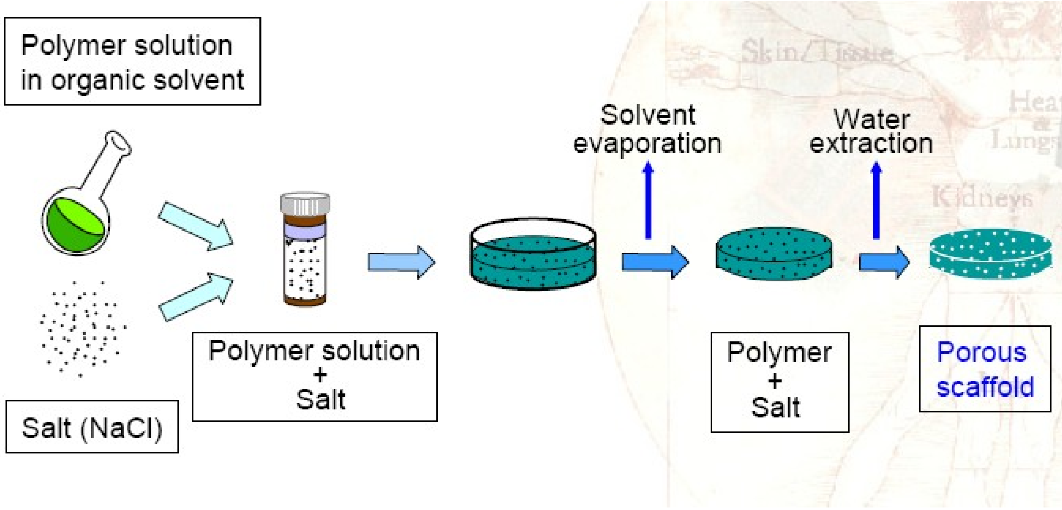
Methods for creating porosity

Thermally Induced Phase Separation



Solvent casting/Particulate leaching

1.- First the material is dissolved into a suitable organic solvent,
2.- Then the solution is cast into a mold filled with porogen particles.
(A salt like sodium chlorid, crystals of saccharose, gelatin or paraffin spheres)
4.- After the mixture solution has been cast, the solvent is allowed to fully evaporate
5.- Then the composite structure in the mold is immersed in a bath of a liquid suitable for dissolving the porogen (Water in case of sodium chloride, saccharose and gelatin, or hexane for paraffin)


Polymer solution in organic solvent

Salt (NaCl)

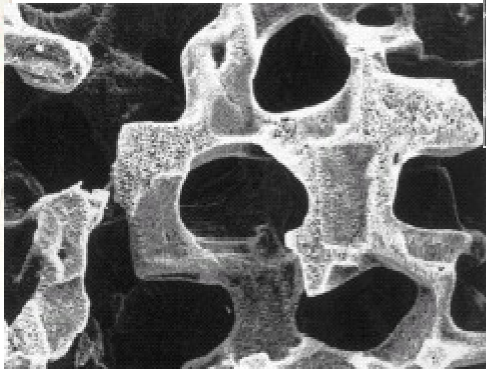
Polymer solution + Salt

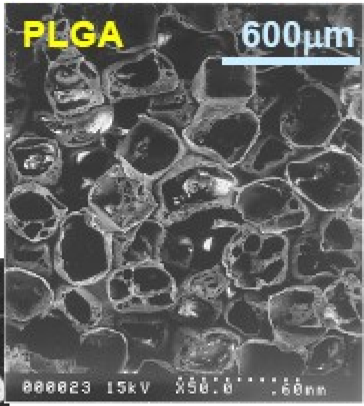
Solvent evaporation

Water extraction

Polymer + Salt

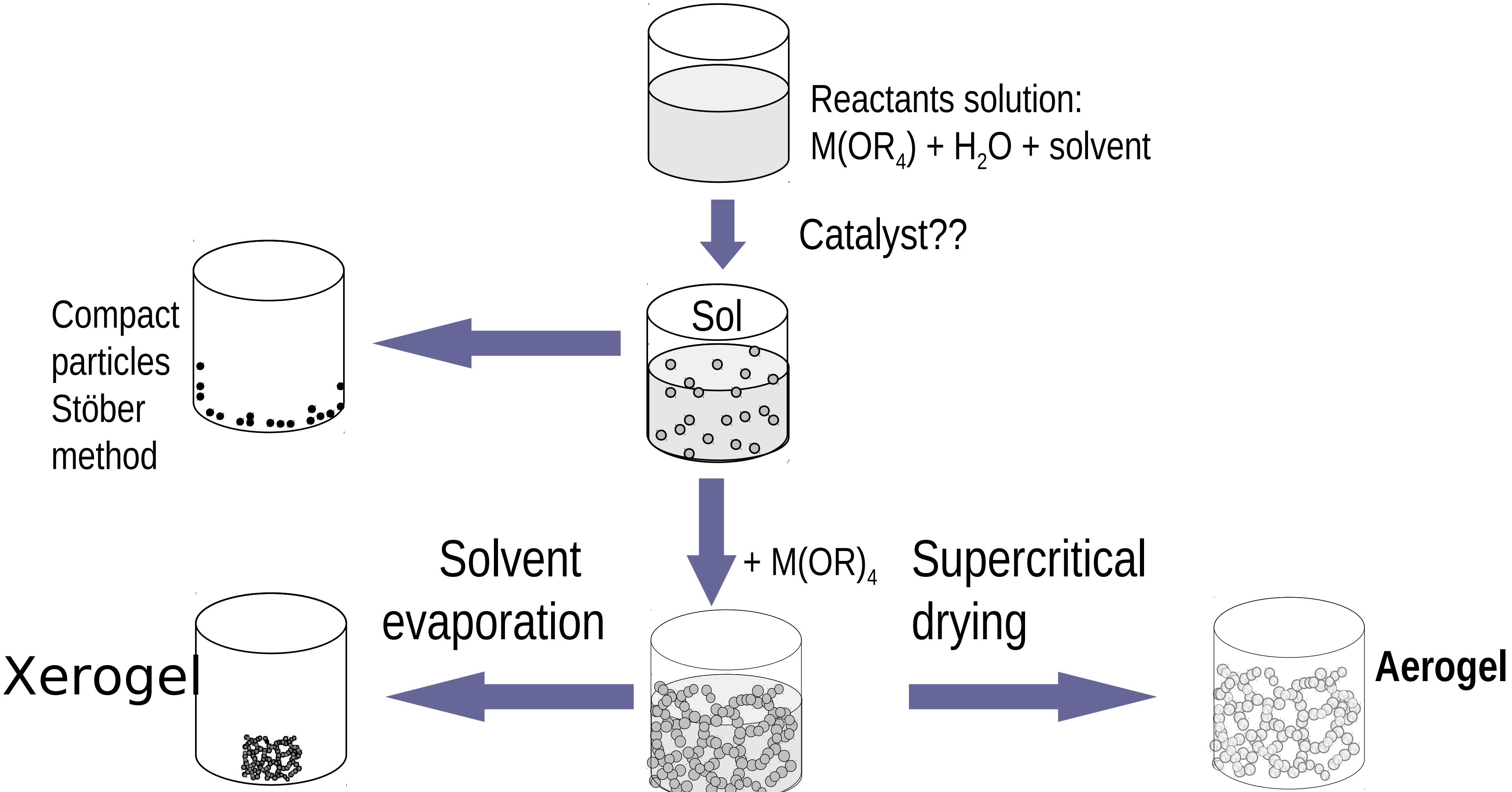
Porous scaffold





Salt leaching, Kohn, as described in Lhommeau, et al., 1997

Supercritical drying with CO_2



Reactants solution:
$M(OR_4) + H_2O$ + solvent

Catalyst??

Sol

Compact
particles
Stöber
method

$+ M(OR)_4$

Solvent
evaporation

Supercritical
drying

Xerogel

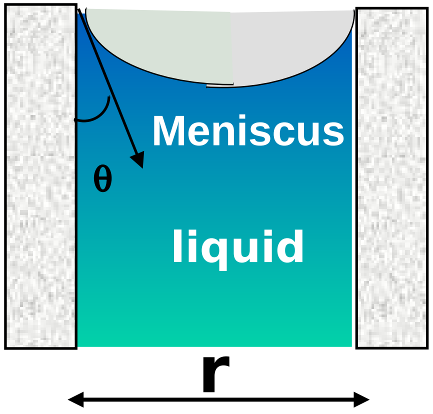
Aerogel

Wet gel

Supercritical Drying

This is where the liquid within the gel is removed, leaving only the linked aerogel network.

vapour-liquid interphase in the pores

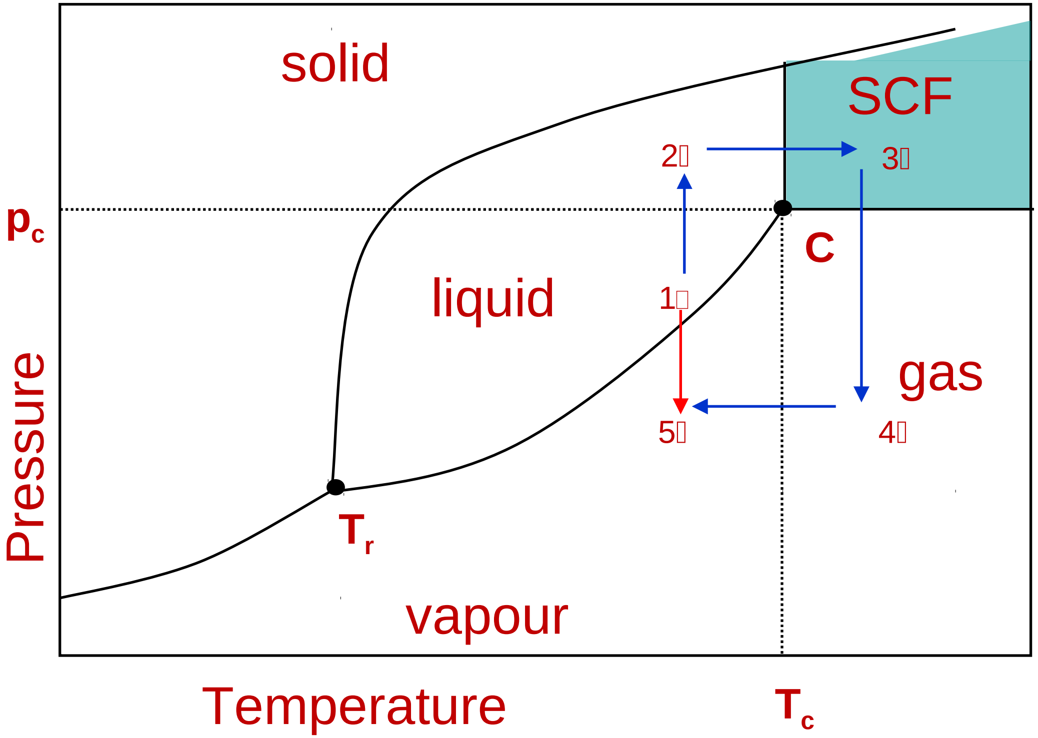


Capilar pressure

$$\Delta p = \frac{2 \cdot \gamma \cdot \cos\theta}{r}$$

γ surface tension of liquid

supercritical extraction of the solvent



INTEMA-ICMAB approach

Development of porous nanostructured materials of using high pressure technologies

-Hydrothermal synthesis of HAp nanoparticles

-Synthesis of TiO_2 precursor gels

-Synthesis of TiO_2-HAp opened structures through supercritical drying

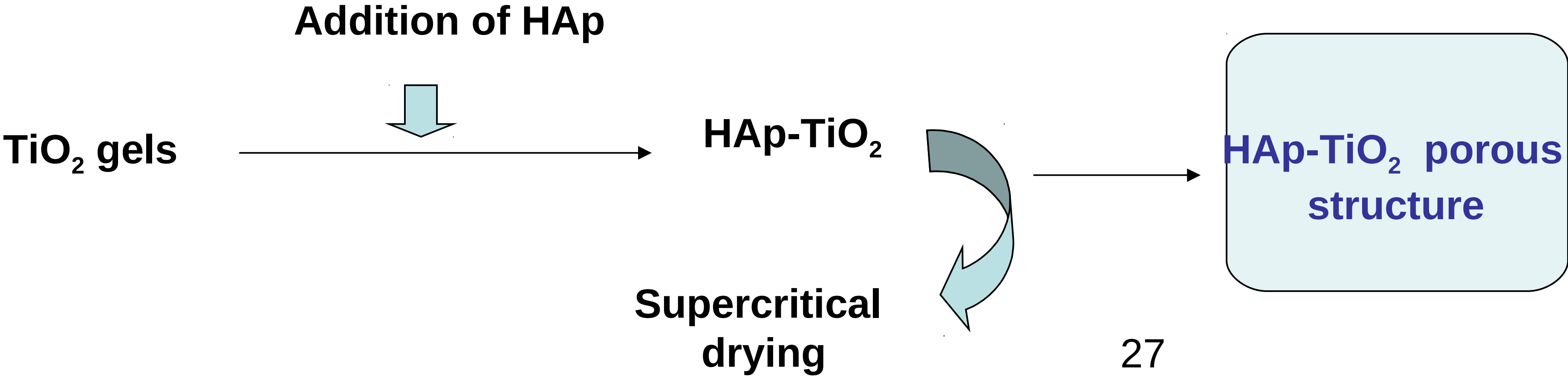
I-Hydrothermal synthesis of HAp nanoparticles

Morphology control of HAp nanoparticles- (nanorods)

II-Synthesis of TiO_2 porous structures

Sol-gel synthesis

III- Synthesis of TiO_2-HAp opened structures



Addition of HAp

TiO_2 gels

HAp-TiO_2

Supercritical drying

HAp-TiO_2 porous structure

I- Hydrothermal synthesis of HAp nanoparticles

-Hydrothermal synthesis (subcritical conditions in batch reactors) **is generally defined as crystal synthesis or crystal growth under high temperature and high pressure water conditions from substances which are insoluble in ordinary temperature and pressure**

-Tc(water)=374 °C Pc(Water)= 22.1 MPa

-Hydrothermal synthesis is usually carried out below 300 °C.

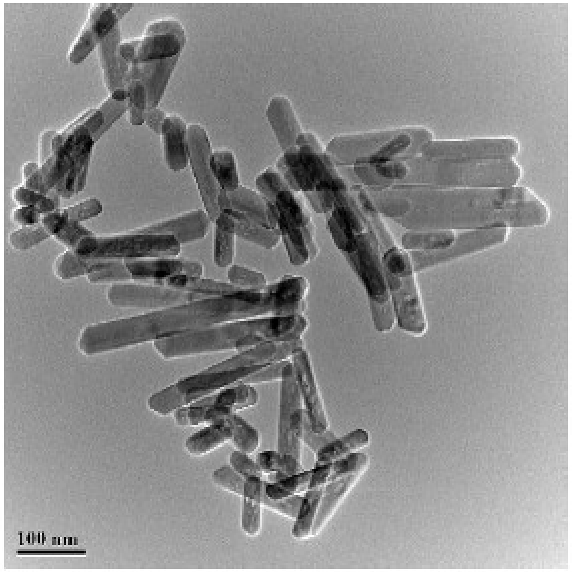


Fig. 2. FETEM image of the HAp nanorods.

K.Ling et al Materials Letters 61 (2007) 1683–1687

Hydrothermal microemulsion synthesis of stoichiometric single crystal hydroxyapatite nanorods

Hydrothermal synthesis of HA nanoparticles

OBJECTIVE: Synthesis of Nanoparticles of (HAp) with particle size control and monodispersity.

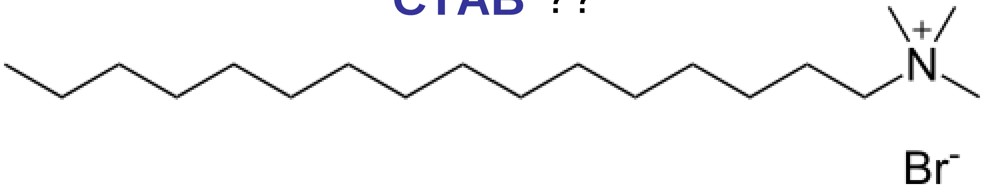
HAp will be prepared from

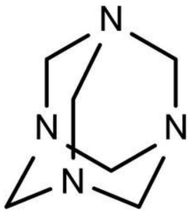
CaHPO4.2H2O / NaOH / distilled water

Evaluation of the use of different templates:

Hexametilenetetraamine

CTAB ??





Evaluation of pH control and Temperature 110-200 C

-Activity under development-

Dr. A Fanovich

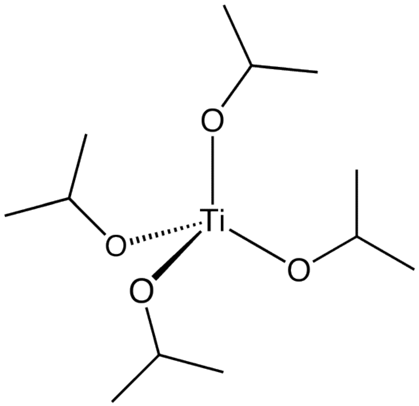
II- Sol-Gel synthesis of TiO_2

Precursors

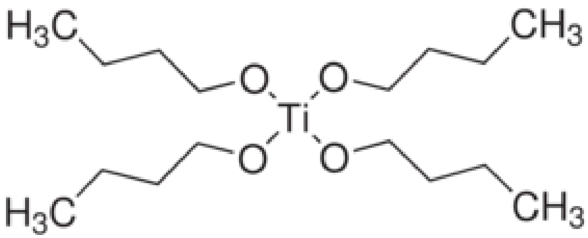
EtOH

Dry box and N2

Titanium isopropoxide



Titanium butoxide



TiOR + H_2O -→ TiOH + EtOH (hydrolysis)

TiOH + HO-Ti -→ Ti-O-Ti + EtOH (condensation)

TiOR + TiOH → Ti-O-Ti

-Activity under development-

-Activity under development-

-To delay the polycondensation and prevent the precipitation of titanium Oxi / hydroxydes, The alcoxydes will be modified with chelant ligands or beta-diketones

-Ti(OR)$_4$ reacts vigorously with water producing titanium-oxo/hydroxy precipitates.

In contrast, when chemical additives are employed, transparent titania-based sols and gels can be obtained.

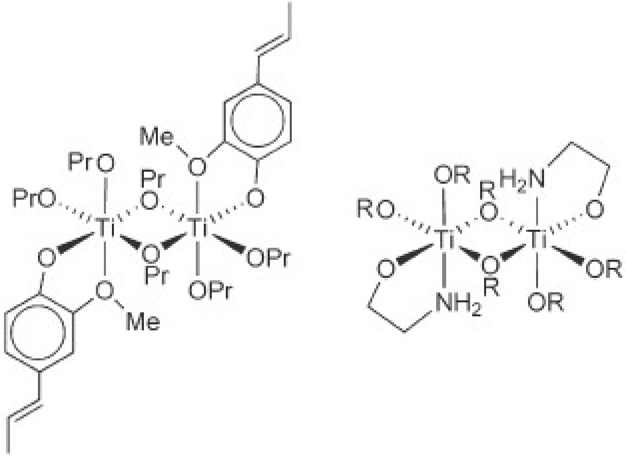


Fig. 4 The structures of $Ti_2(OPr)_6(isoeugenolate)_2$ (left) and $Ti_2(OR)_6(OCH_2CH_2NH_2)_2$ (R = ^iPr, Et) (right).[30]

Addition of water to promote the hydrolysis and condensation to lead to the formation of Ti-O-Ti bonds and the growing of a TiO_2 3D-network

J. Mater. Chem., 2005, 15, 3701–3715 | 3701

Dr. A Fanovich

-Activity under development-

Addition of porous generator agents to the "sol" to allow an open porous micro structure of 150-300 microns

PEG 400-600
Ethylcelulose,
Starch granules etc..

?

Ageing of the gel (hermetically closed) → Maximise polycondensation

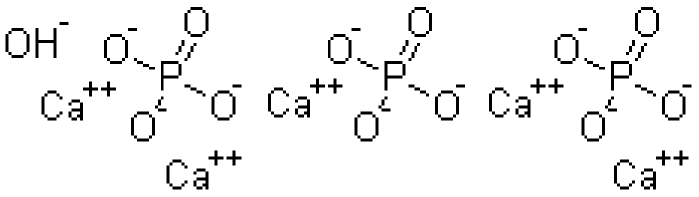
Ageing of the gel (hermetically closed) → Avoid evaporation

Solvent and additive extraction → **TiO_2 Porous structure**

II- Synthesis of HAp-TiO2

Objective: prepara TiO_2/HAp with HAp nanoparticles homogeneously dispersed in a TiO_2

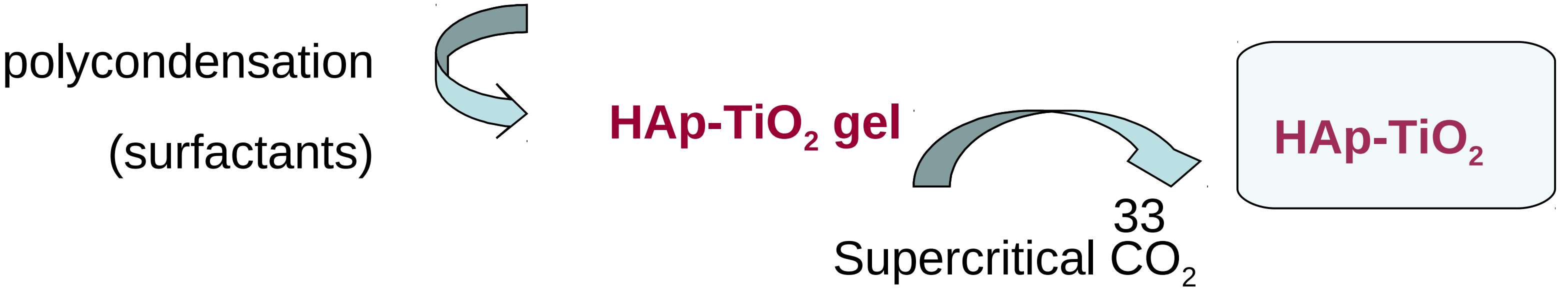
HAp contains –OH groups that can be linked to TiO_2



Ti alcoxyde “sol” $\xrightarrow{\text{HAp}}$ HAp-TiO2

TiOR + H_2O -→ TiOH + EtOH (hydrolysis)

TiO- + “HO-HAp”→ Ti-O-HAp + EtOH (condensation)



polycondensation

(surfactants)

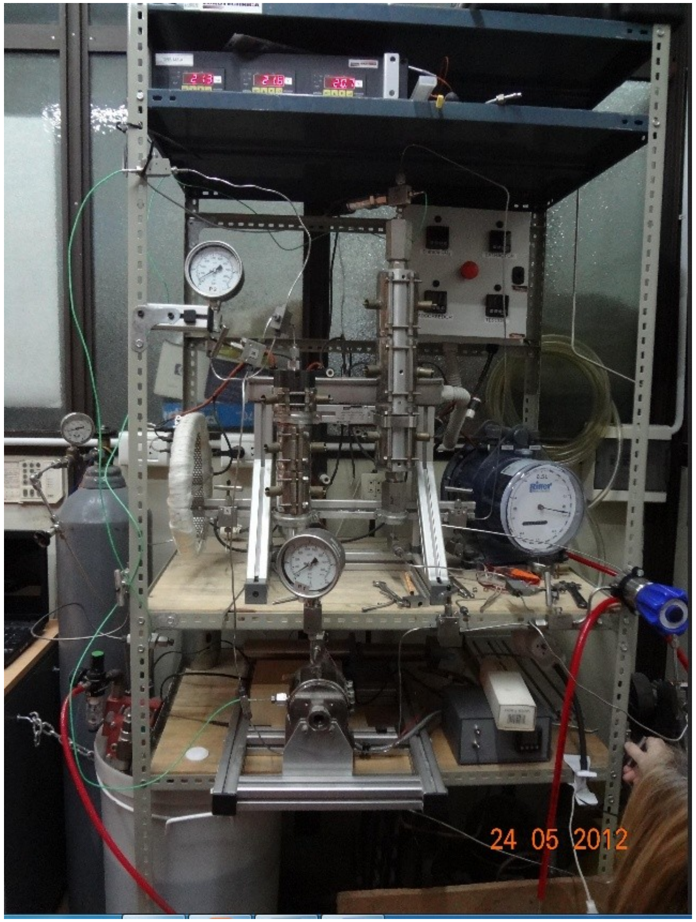
HAp-TiO_2 gel

Supercritical CO_2

HAp-TiO_2

Resources

INTEMA
CONICET
UNMDP


24 05 2012

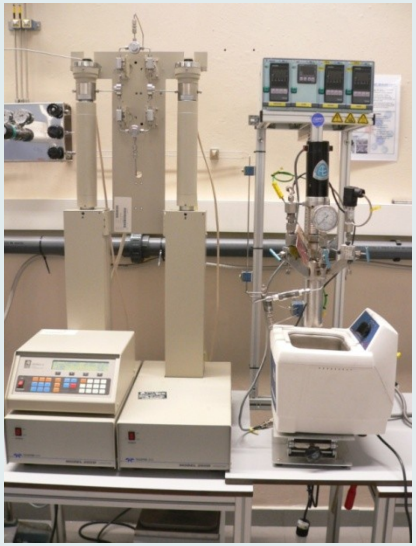
Resources

Continuous flow

60/350bar,-10/-15ºC,0.2-2lh^{-1} **35/100ºC, 150-250bar** **80-120ºC,**

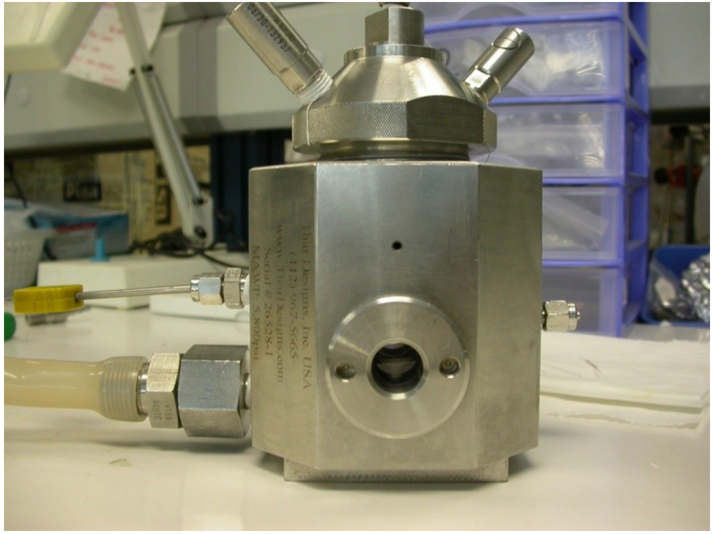
Compresión del gas **Procesamiento de muestras** **Expansión del gas**

Funcionalization Equipment- Batch

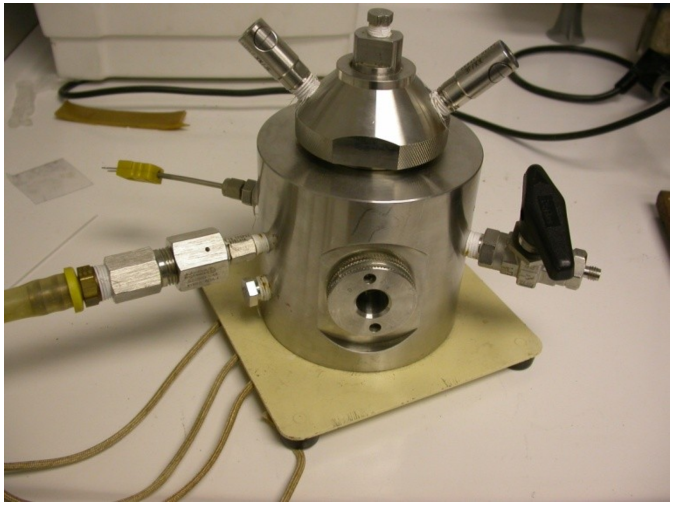


Celda de volumen variable

100 ml



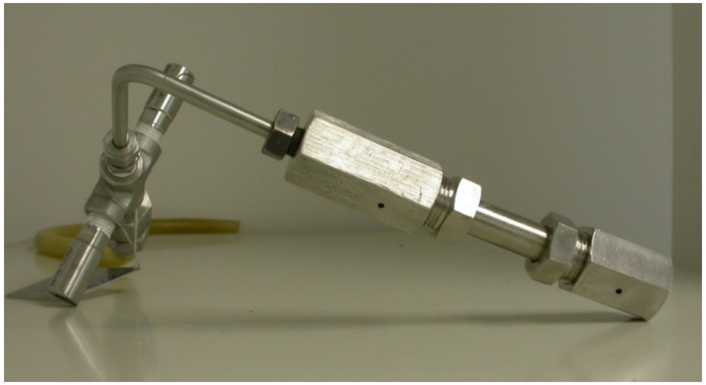
100 ml

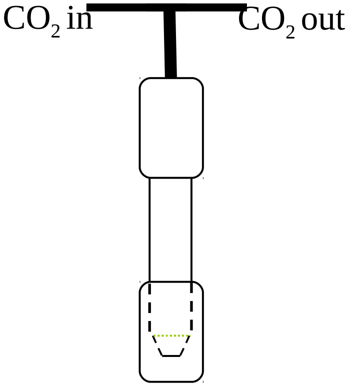


200 ml

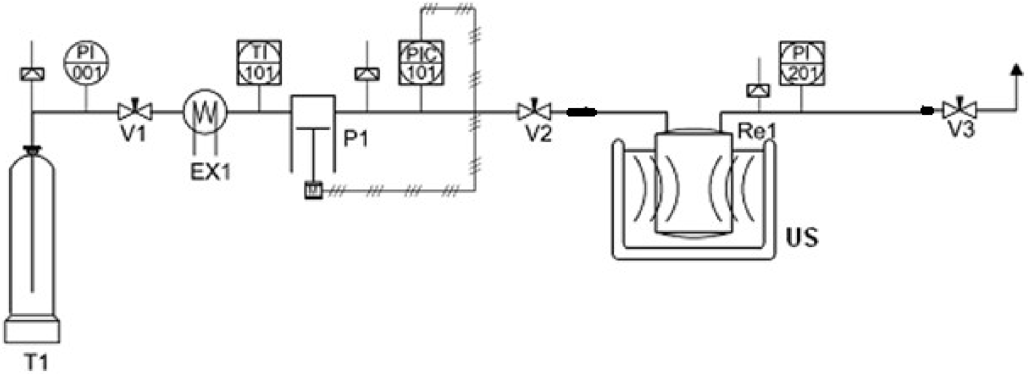
Designed for supercritical-ultrasound research

5 ml

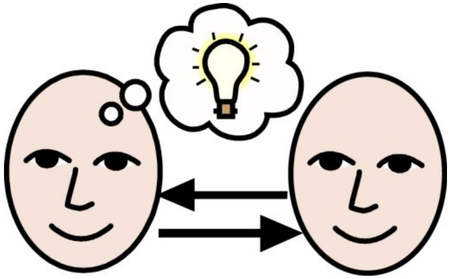




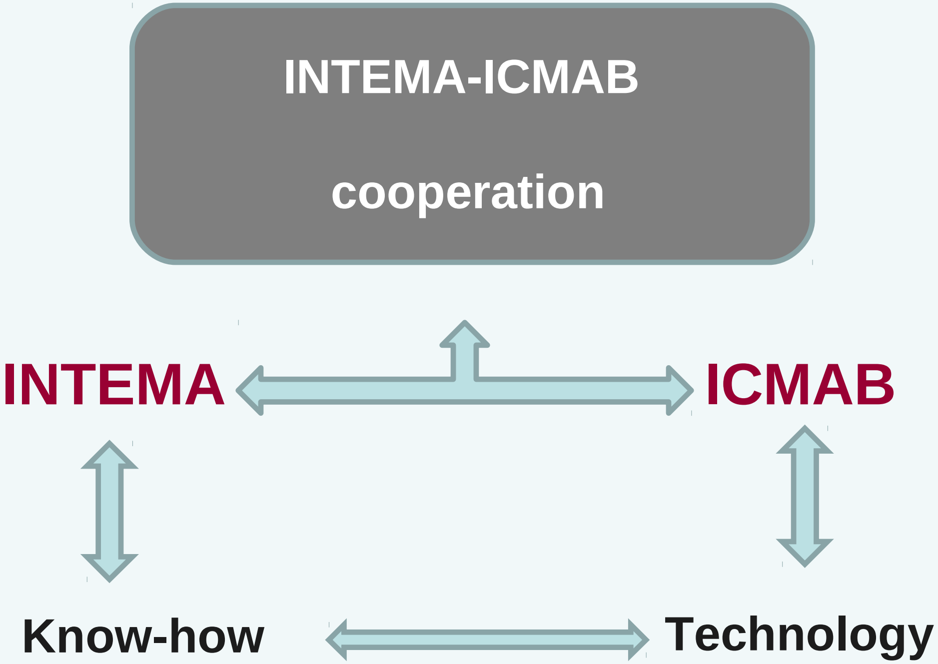
10 ml



HAp-TiO_2 gels using ultrasonic cavitation??



ICMAB **INTEMA**



INTEMA-ICMAB

cooperation

INTEMA

ICMAB

Know-how

Technology

Characterisation

-To evaluate homogeneity of aerogels and morphology SEM

-Raman and DRX to analyse crystallisation and phases

- IR to check purity of the samples

-BET. Fo size and pore distribution

-Size distribution- Malvern Nanosizer

I-Hydrothermal synthesis of HAp nanoparticles

The hydrothermal synthesis allows morphology control of the hydroxyapatite particles.

-Halide precursors and organic templates will be used to promote the growth of HAp nanorods. We will study the effect of the main synthetic parameters on the characteristics of the material obtained.

II-Synthesis of TiO2 gels

Sol-gel synthesis of TiO2 will allow the introduction of porous generator agents.

-The chemicals that will be used are: Ti alcoxydes, EtOH, surfactants, and porous generator agents.

-Precursors condensation will be promoted my the addition of water

- Gels will be aged within their original pot, to avoid evaporation

III- Synthesis of TiO2-HAp opened structures

The HO- groups of the hydroxyapatite particles can condensate with the Titanium alcoxydes to lead to HAp-O-Ti

-polycondensation will result in a a¡gel composed of HAp nanoparticles anchores in the TiO2 network

Sol-Gel synthesis process

Step 1: Formation of solutionos of alkoxide or solvated metal precursor (the sol).

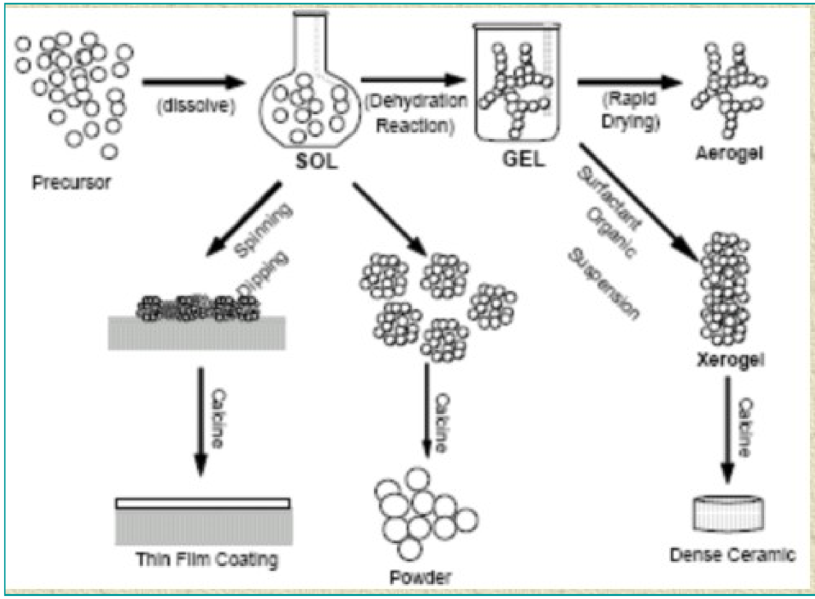
Step 2: Gelation resulting from the formation of an oxide- or alcohol- bridged network (the gel) by a polycondensation or polyesterification reaction that results in a dramatic increase in the viscocity of the solution.

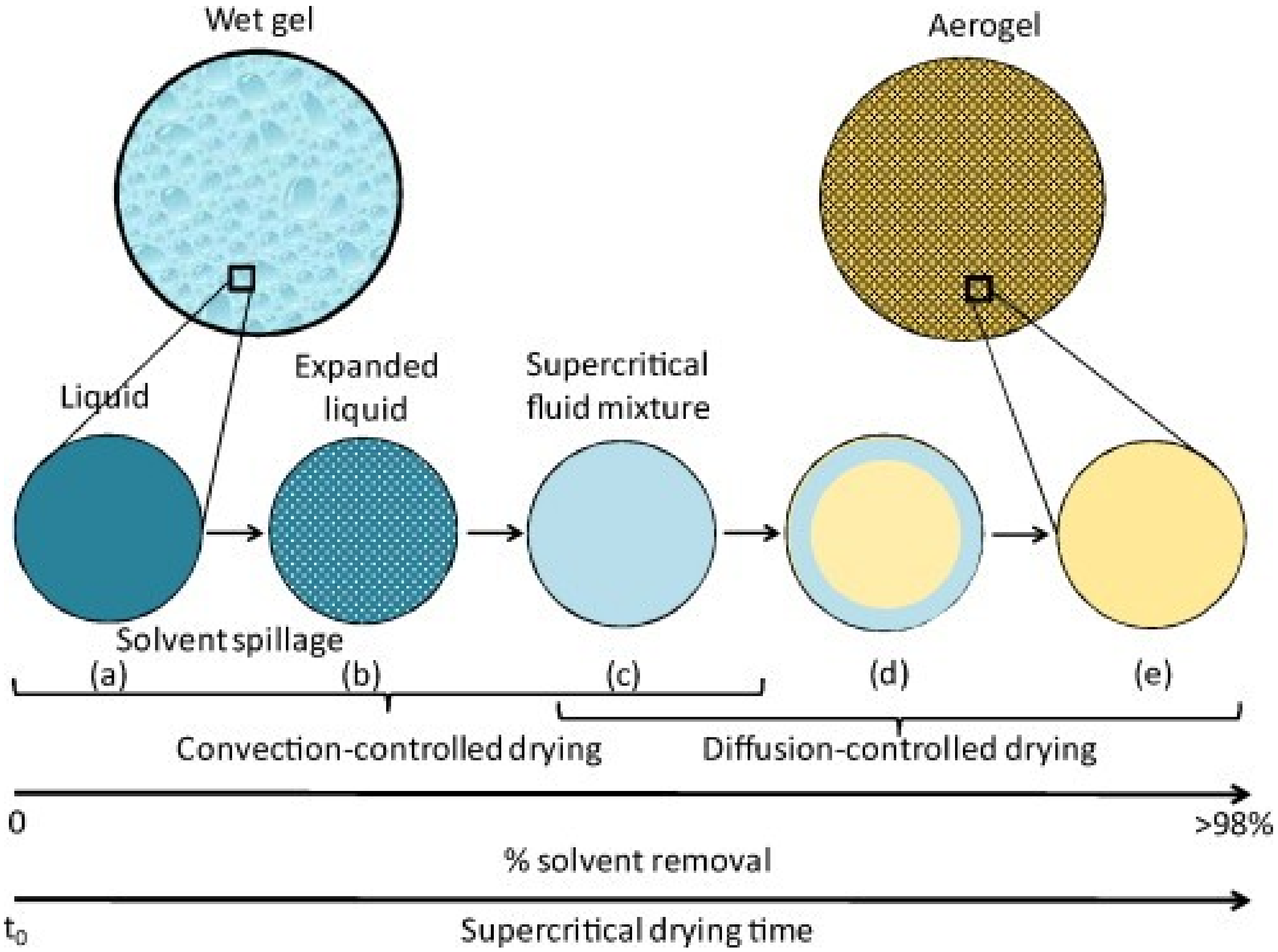
Step 3: Aging of the gel (Syneresis).- polycondensation reactions continue until the gel transforms into a solid mass, -expulsion of solvent from gel pores.

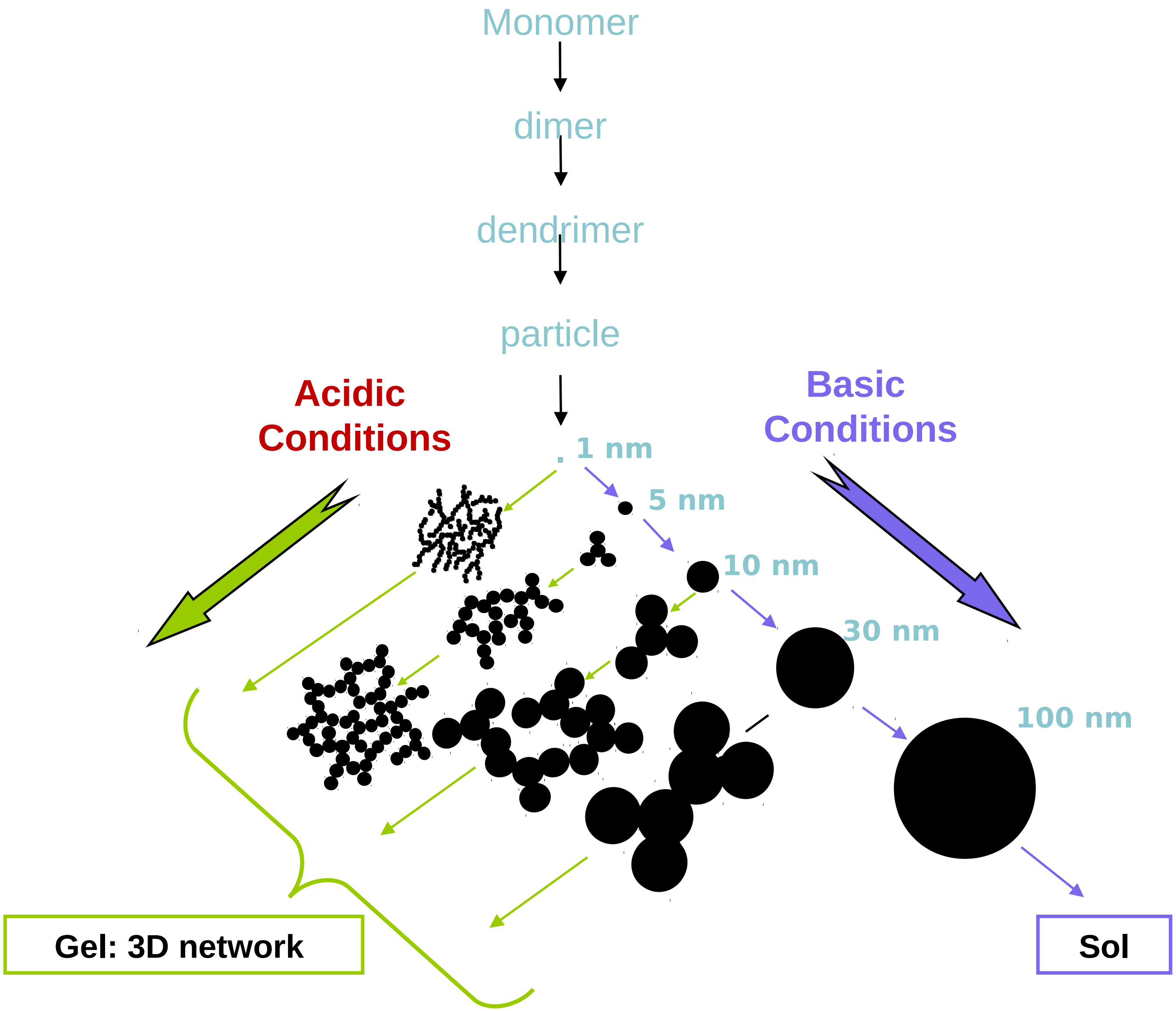
Step 4: Drying of the gel, when water and other volatile liquids are removed from the gel network.

Step 5: Dehydration- Ti-OH groups are removed, there by stabilizing the gel against rehydration. This is normally achieved by calcining the monolith at temperatures up to 8000C.

Step 6: Densification and decomposition of the gels at high temperatures (T>8000C).
The pores of the gel network are collapsed, and remaining organic species are volatilized. The typical steps that are involved in sol-gel processing are shown in the schematic diagram below.




Wet gel
Aerogel
Liquid
Expanded liquid
Supercritical fluid mixture
Solvent spillage
(a)
(b)
(c)
(d)
(e)
Convection-controlled drying
Diffusion-controlled drying
0
>98%
% solvent removal
t0
Supercritical drying time



Monomer

dimer

dendrimer

particle

Acidic Conditions

Basic Conditions

1 nm

5 nm

10 nm

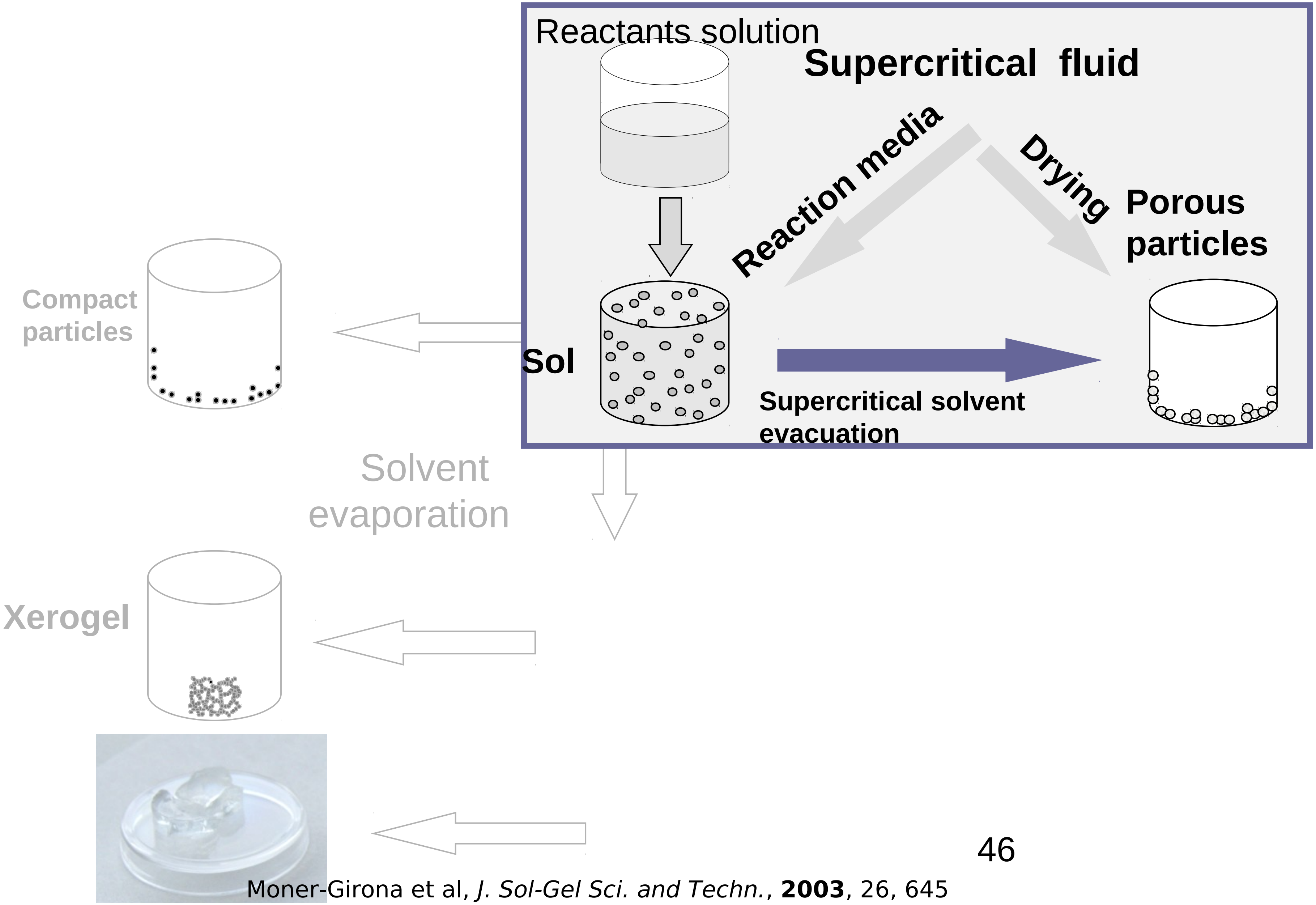
30 nm

100 nm

Gel: 3D network

Sol

Pierre & Pajonk, *Chem. Rev.* 2002, **102**, 42



Reactants solution

Supercritical fluid

Reaction media

Drying

Porous particles

Sol

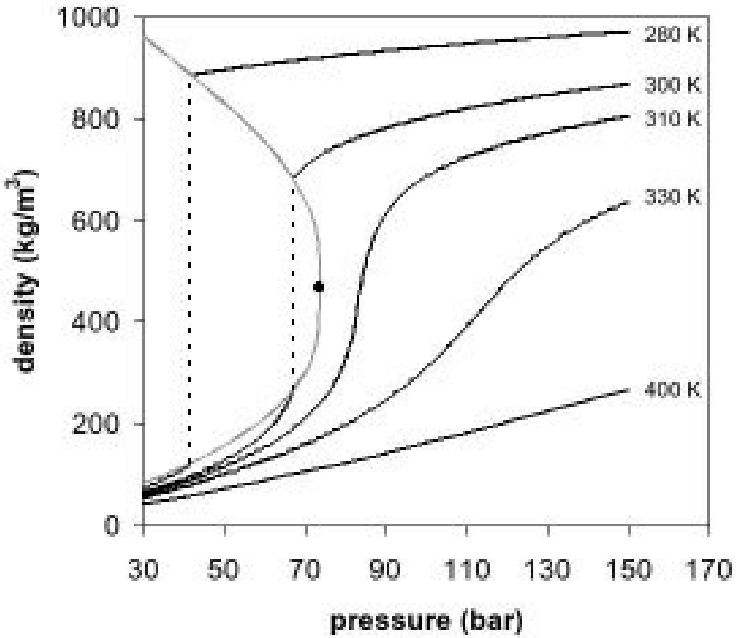
Supercritical solvent evacuation

Compact particles

Solvent evaporation

Xerogel

Moner-Girona et al, *J. Sol-Gel Sci. and Techn.*, **2003**, 26, 645


1000
800
600
400
200
0
density (kg/m^3)
30
50
70
90
110
130
150
170
pressure (bar)
280 K
300 K
310 K
330 K
400 K