Riunione di coordinamento
Progetto PRIN 2017

FIBRES: a multidisciplinary mineralogical, crystal-chemical and biological project to amend the paradigm of toxicity and cancerogenicity of mineral fibres.

Giovedì 7 Luglio 2022

Unità di Modena e Reggio Emilia

Scopi del progetto - sommario

Il 18 Ottobre 2019 il gruppo di ricerca all'unanimità ha deciso di lavorare su **due livelli**.

-livello 1: un **progetto generale** con il contributo di tutte le unità di ricerca;

-livello 2: **progetti secondari** gestiti dalle singole unità di ricerca, anche in maniera autonoma.

Scopi del progetto – livello 1

Modelli quantitativi di predizione della cancerogenicità della IARC (Cancer KCs: "*IARC in 2016 began using a new '**key characteristics (KCs) of carcinogens**' approach to evaluate the carcinogenic potency of chemicals and other agents. KCs are a set of 10 characteristics '**hallmarks of cancer**' as detailed by Hanahan and Weinberg (2011). The IARC KCs include **10 attributes**.*"

IARC's 10 key characteristics of carcinogens

Characteristic	Examples of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g. epoxide, quinone, etc.), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g. chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g. topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g. DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g. ER, PPAR, AhR) or modulation of exogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Scopi del progetto – livello 1

Modello FPTI

Parameter	Element	Major adverse effect	Major pathobiological process
Morphometric			
length L	(1,1)	frustrated phagocytosis	Inflammation and oxidative stress
diameter D	(1,2)	frustrated phagocytosis	inflammation and oxidative stress
crystal curvature	(1,3)	reduced surface adhesion of proteins/cells	inflammation and oxidative stress?
crystal habit	(1,4)	airways deposition depth	inflammation and oxidative stress
fiber density	(1,5)	airways deposition depth	inflammation and oxidative stress
hydrophobic character of the surface	(1,6)	Interaction with biopolymers, phagocytosis	inflammation and oxidative stress?
surface area	(1,7)	airways deposition depth, frustrated phagocytosis	(chronic) inflammation and oxidative stress
Chemical			
Total iron content	(1,8)	Production of ROS	DNA damage and inflammation
ferrous iron	(1,9)	Production of ROS	DNA damage and inflammation
Surface ferrous iron/iron nuclearity	(1,10)	Production of ROS	DNA damage and inflammation
content of metals other than iron	(1,11)	Production of ROS	DNA damage and inflammation
Biodurability			
dissolution rate log(R)	(1,12)	frustrated phagocytosis …	Inflammation …
velocity of iron release	(1,13)	production of ROS	inflammation
velocity of silica dissolution	(1,14)	production of ROS?	oxidative stress and inflammation?
velocity of release of metals	(1,15)	ROS production	DNA damage, inflammation, …
Surface activity			
ξ potential	(1,16)	production of ROS and hemolysis	Inflammation …
fibers' aggregation	(1,17)	frustrated phagocytosis	inflammation
Cation exchange in zeolites	(1,18)	interference with ER cross-talk?	apoptosis, necrosis?

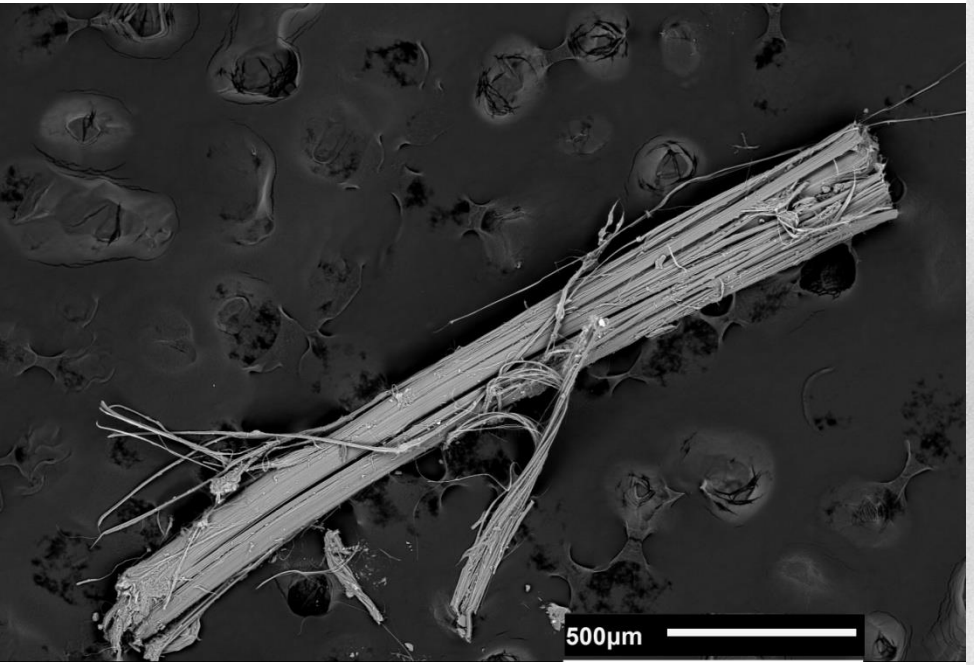
Scopi del progetto – livello 1

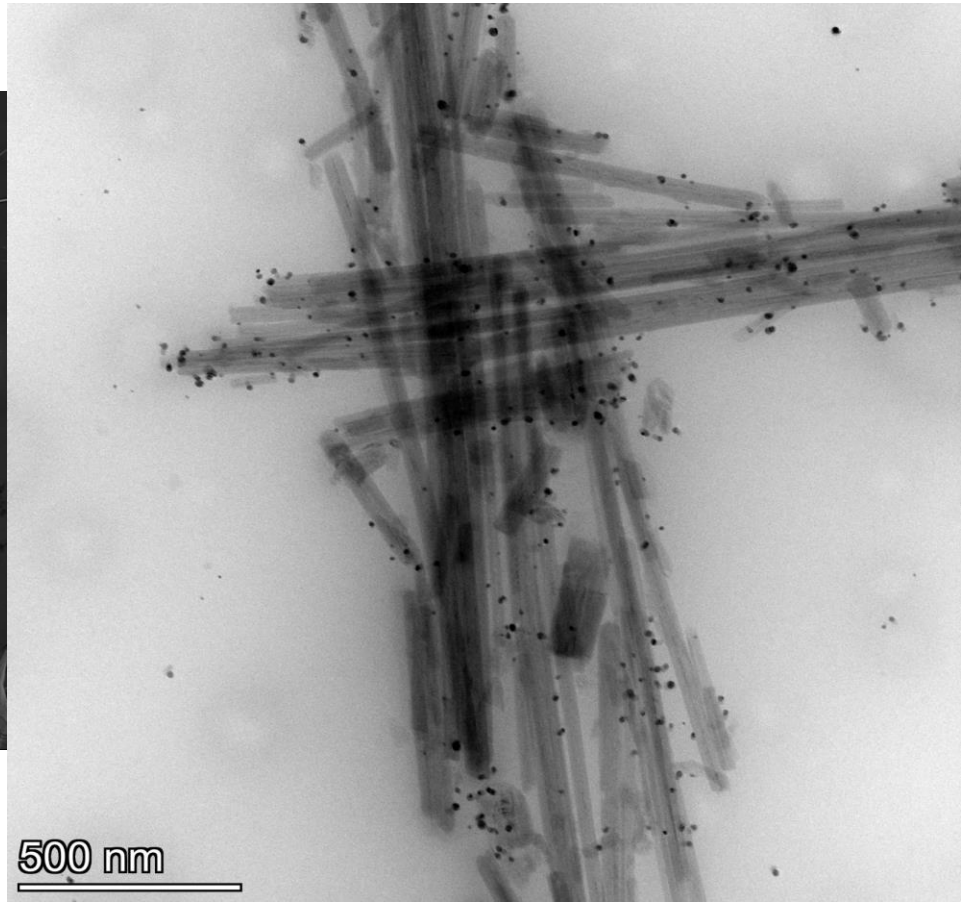
Selezione della fibra per il *case study* .

-caratterizzazione completa della fibra selezionata;

-classazione della fibra *case study* selezionata (>5 μm e <5 μm).

Russian chrysotile

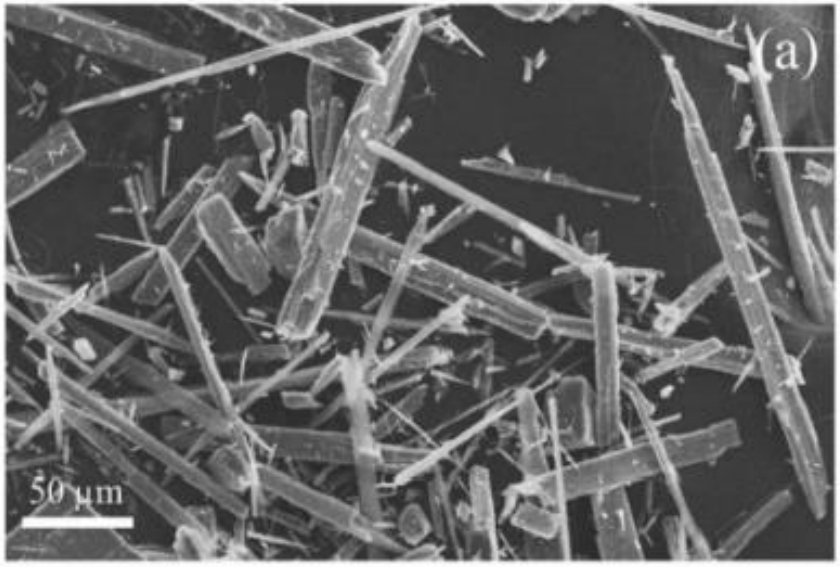




Scopi del progetto – livello 1

- Applicazione del modello FPTI sulle classi di fibre con dimensioni diverse.

- Raccolta dati sperimentali e classificazione dei tre campioni classati secondo il **modello FPTI;** aggiungere **controllo positivo** (**crocidolite**) e **negativo** (**wollastonite NYAG**).



Wt%	EMPA	TDS NYCO Minerals
SiO_2	50.76 (0.25)	51.60
FeO	0.33 (0.09)	-
Fe_2O_3	0.17 (0.09)	0.77
MnO	0.15 (0.04)	0.15
MgO	0.03 (0.02)	0.15
CaO	48.27 (0.24)	46.36
Na_2O	* bdl	-
Al_2O_3	bdl	0.40
K_2O	bdl	0.02
TiO_2	bdl	0.05
L.o.I.	** 0.30	0.50
Tot	100.0	100.0

Doublet	δ (mm/s)	Δ (mm/s)	Γ_+ (mm/s)	A (%)	Attributions
Db1	0.41 ± 0.01	0.56 ± 0.03	0.12 ± 0.01	31 ± 2	Fe^{3+} octahedral (*M*1,*M*2,*M*3)
Db2	1.09 ± 0.04	2.26 ± 0.09	0.31 ± 0.08	46 ± 2	Fe^{2+} octahedral (*M*3)
Db3	1.26 ± 0.01	2.76 ± 0.04	0.11 ± 0.02	23 ± 2	Fe^{2+} octahedral (*M*1)

Scopi del progetto – livello 1

- Raccolta dati **IARC 10 KCs** sui **2 campioni** >5 μm e <5 μm **+ controlli.**

- Correlazione **parametri del modello FPTI** dei **2 campioni** >5 μm e <5 μm) **+ controlli** agli ***adverse effects*** e, a cascata, con i **dati IARC 10 KCs (*pathobiological effects*)**.

- Classificazione finale della *case fiber*:
  -quanti **KCs** (da 1 a 10) sono attivi?
  -Differenze tra >5 μm vs. <5 μm?
  -da quali parametri FPT vengono generati?

Scopi del progetto – livello 2

(i) nature, amount and activity of surface iron species in the different fibres. To develop a model for the iron-mediated surface production of toxic hydroxyl radicals (•OH), the project will attempt to determine the nature, amount and activity of surface iron species in the different mineral fibres, with a special attention to the nuclearity of the iron sites.

Scopi del progetto – livello 2

(ii) <u>biodurability</u>. In this project, i*n vitro* tests will measure dissolution rates of the fibres (biodurability) and use the data for a revised general classification of the fibres based on their biodurability-related effects. The new model will have to classify fibres as (a) non-biodurable (chrysotile asbestos), with fast dissolution rate accompanied by a fast rate of release of their toxic cargo; (b) biodurable (amphibole asbestos), with slow dissolution rate and minor release of their toxic cargo; (c) biodurable, but with ion exchange capacity (zeolite erionite) prompting unpredictable release or adsorption of ions/metals.

Scopi del progetto – livello 2

(iii) <u>the 'Trojan horse-type effect'</u>. The concept of the 'Trojan horse-type effect' observed for nanoparticles (NPs) will be applied to mineral fibres, and the toxicity potential of a non-biodurable fibre like chrysotile (case (a) in the classes of biodurability) will be devised so to include the contribution of this effect. In fact, when chrysotile undergoes fast dissolution, it may behave like a carrier that releases its toxic cargo of heavy metals, possibly hosted in the structure, in the lung environment. Oppositely, biodurable amphibole asbestos species slowly release their toxic cargo in the lung environment over a long time period. Fibrous erionite is a special case and requires a dedicated study because it possesses cation exchange capacity (see Ballirano and Cametti, 2015) and may release extraframework metals in the lung environment even if no dissolution occurs.

Scopi del progetto – livello 2

(iv) **Interference with the Ca cross talk prompting cell apoptosis**. This project is aimed at assessing the cancer self-defence mechanism of 'apoptosis' is influenced by the intracellular chemical reactivity of the engulfed mineral fibres. Specifically, it will be evaluated whether ion exchange is active during the phagocytosis process of the erionite fibres and if there is interference with the Ca cross talk, inhibiting cell apoptosis, so favouring the onset of the malignancies like mesothelioma.

Sotto-progetto Unità di MO e RE – livello 2

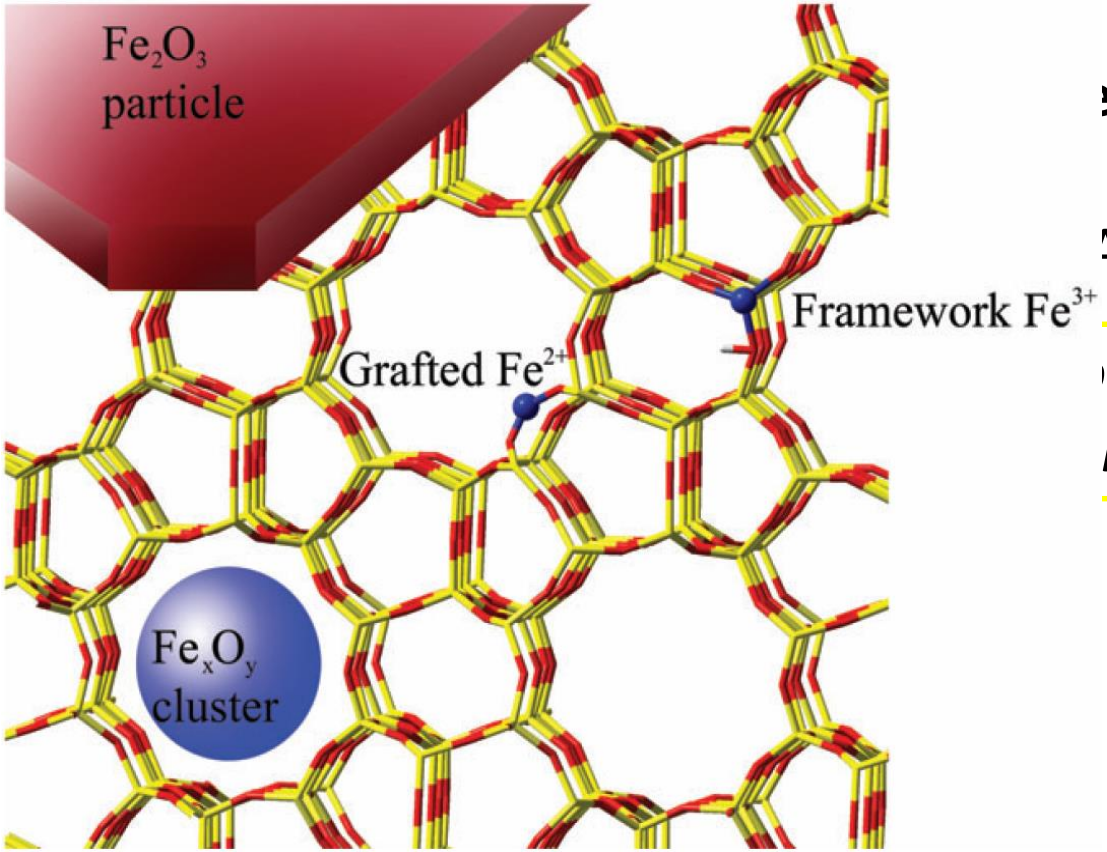
(i) nature, amount and activity of surface iron species in the different fibres. To develop a model for the iron-mediated surface production of toxic hydroxyl radicals (•OH), the project will attempt to determine the nature, amount and activity of surface iron species in the different mineral fibres, with a special attention to the nuclearity of the iron sites.

Sotto-progetto Unità Mo e RE – livello 2

fibrous erionite → partial phagocytosis and ROS (H_2O_2) generation

+

available surface iron



e hydroxyl radicals

iron species and their nuclearity (Zecchina et al., 2007).

damage

er-Weiss cycle.

-isolated species

$HO^{\bullet} + HO^-$

-dimers and small clusters

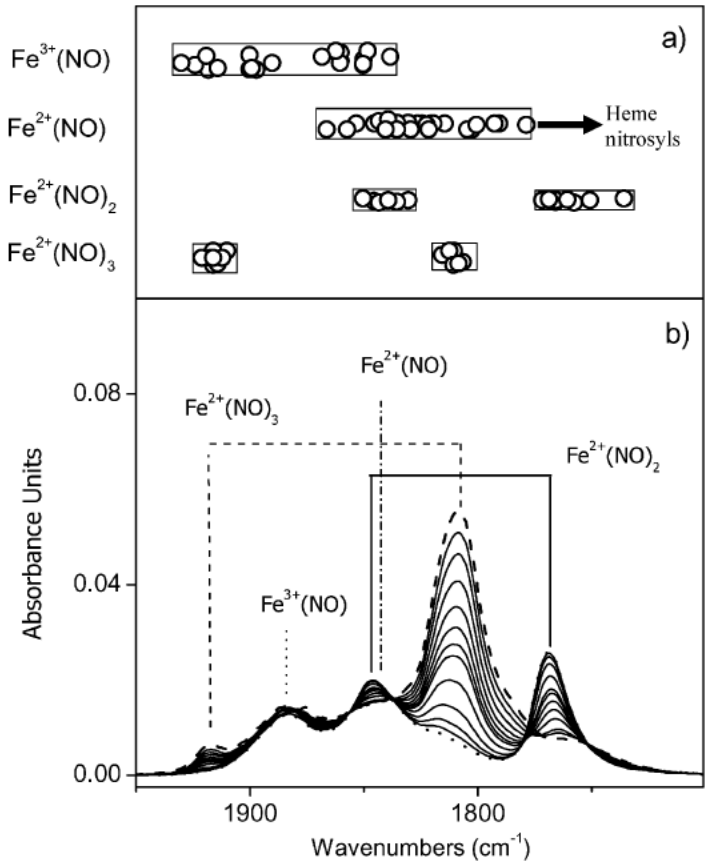
-clusters

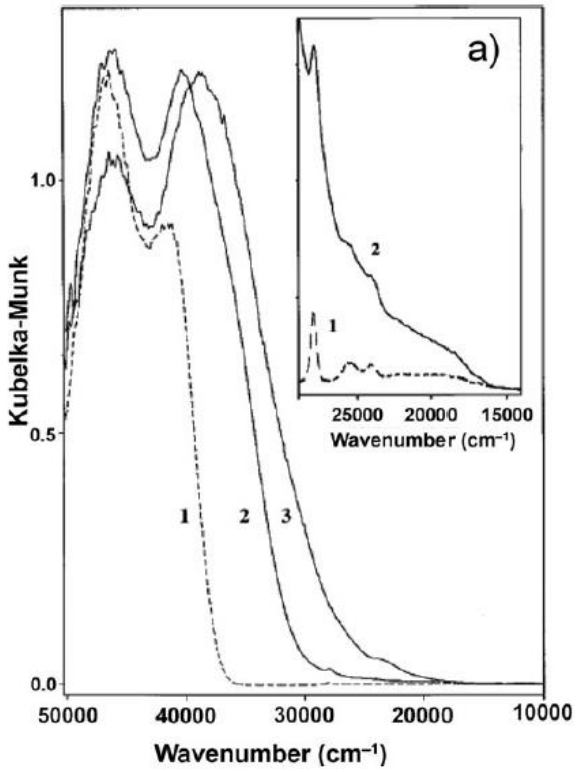
Iron nuclearity in the FPTI model (1,10)

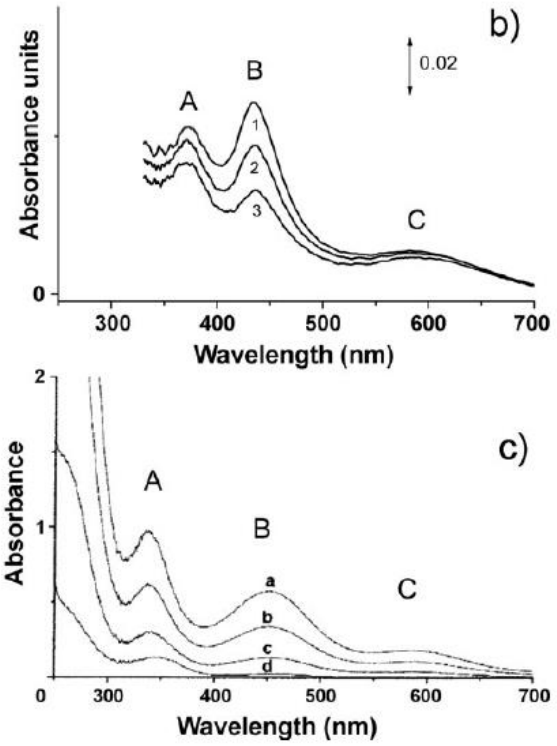
Sotto-progetto Unità Mo e RE – livello 2

INVITED ARTICLE www.rsc.org/pccp | Physical Chemistry Chemical Physics

Structure and nuclearity of active sites in Fe-zeolites: comparison with iron sites in enzymes and homogeneous catalysts







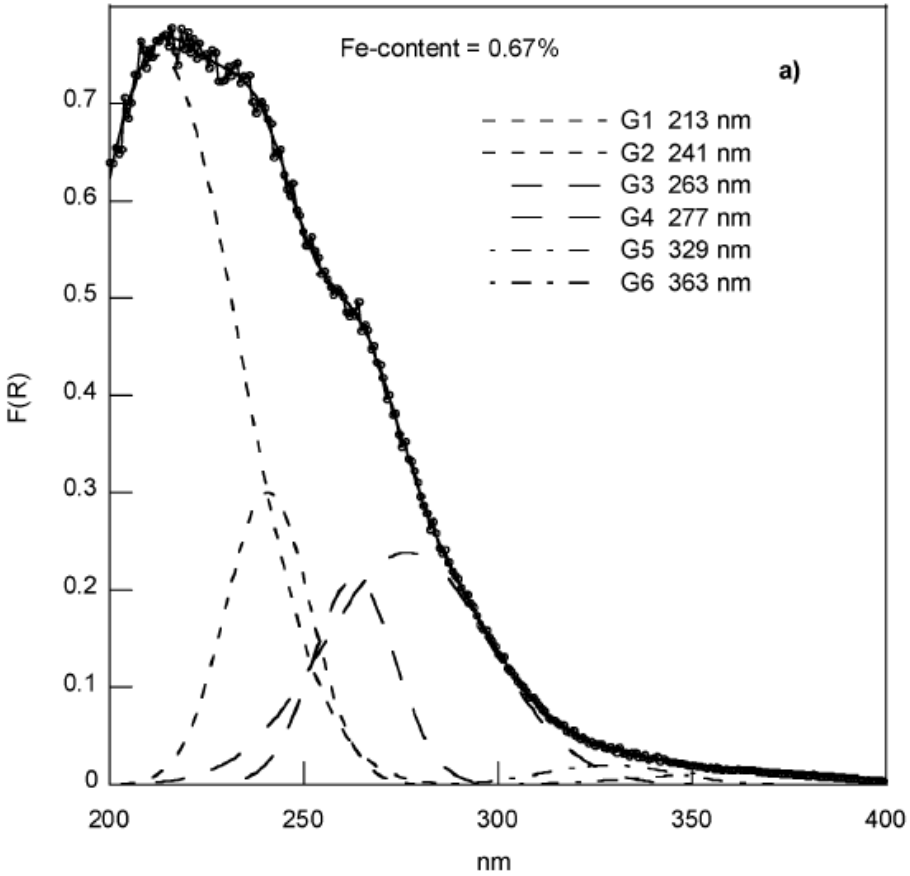
La tecnica FTIR in sito è ideale ma test eseguiti dal gruppo di Torino (Dr. Turci) hanno evidenziato che a basse concentrazioni di ferro, il segnale è troppo basso per permettere una corretta interpretazione.

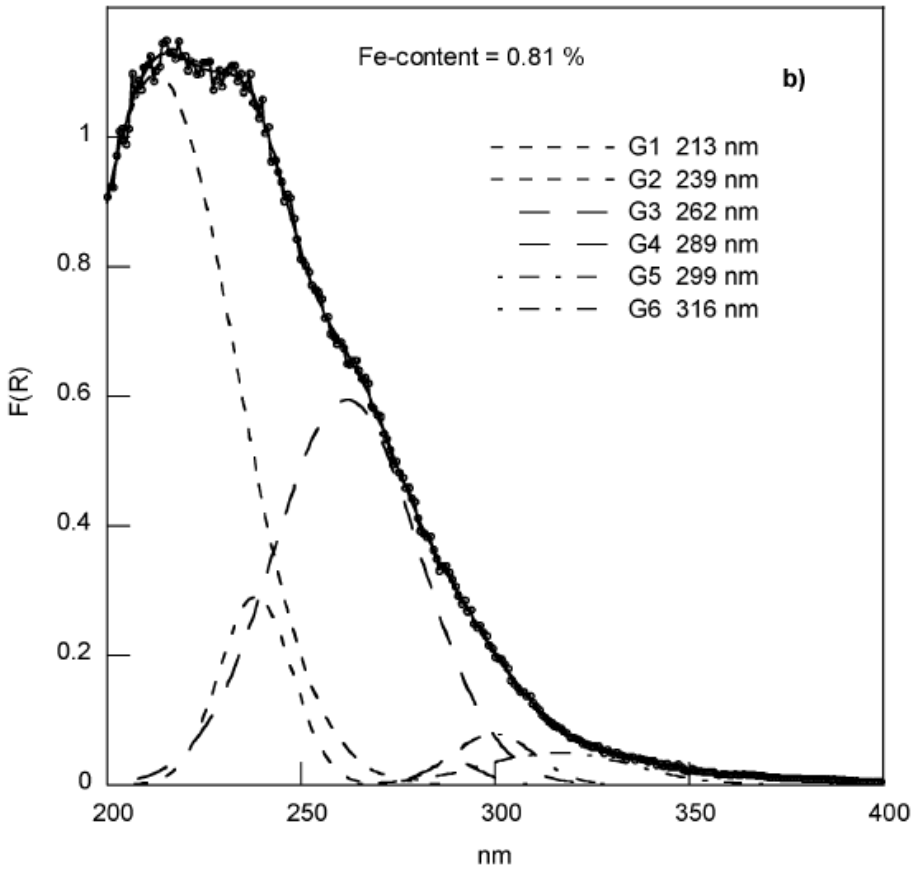
Sotto-progetto Unità Mo e RE – livello 2

PAPER www.rsc.org/pccp | Physical Chemistry Chemical Physics

Spectroscopic characterization of Fe-doped synthetic chrysotile by EPR, DRS and magnetic susceptibility measurements†

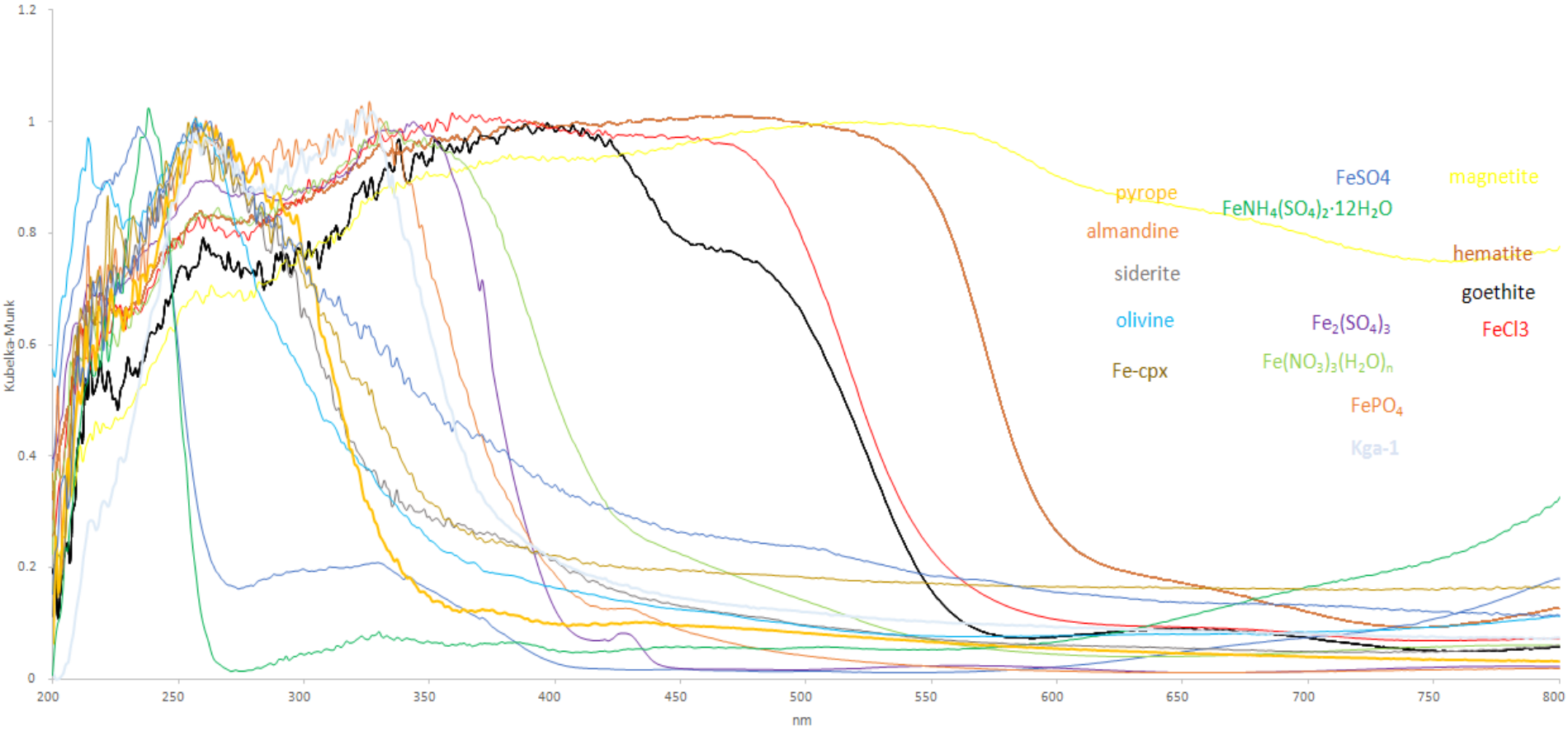
CT bands for isolated [FeO_4] tetrahedral group have been observed in the 215–240 nm range, while bands between 270–290 nm have been detected for isolated octahedral [FeO_6] group and CT bands between 300 and 400 nm are assigned to octahedral Fe^{3+} in cluster-like Fe_xO_y species, which can be small oligomeric clusters and larger species.





Sotto-progetto Unità Mo e RE – livello 2

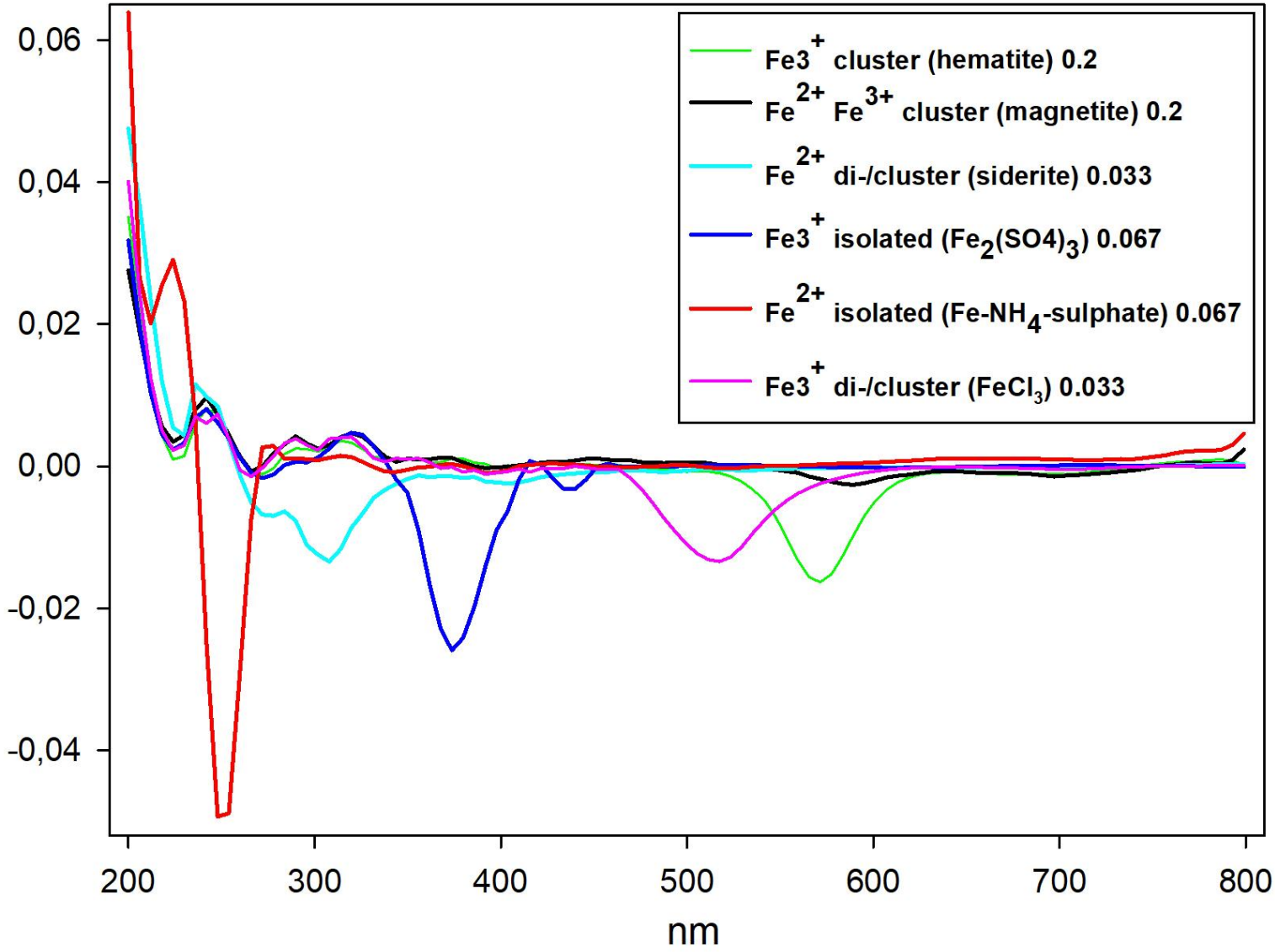
Spettri UV-Vis degli standard e di alcuni campioni, normalizzati nella regione 200-800 nm.



Assunzione: bulk rappresentativo della superficie!?!

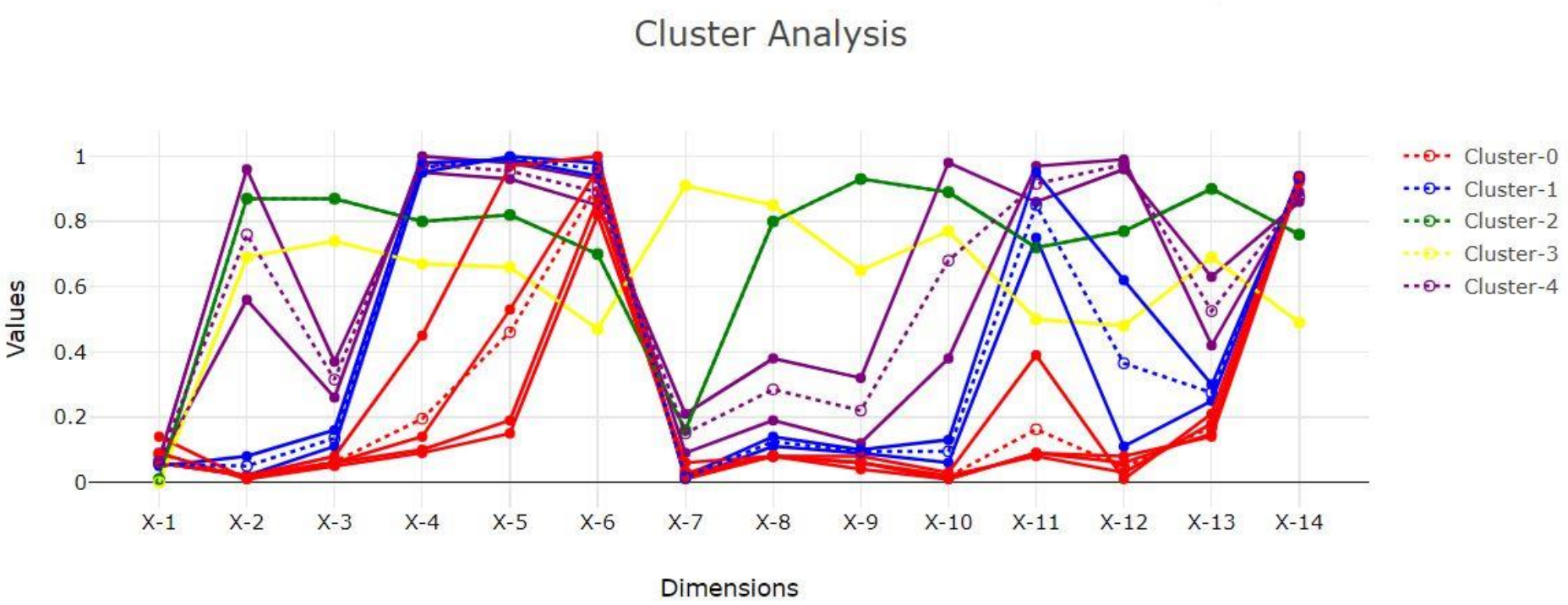
Sotto-progetto Unità Mo e RE – livello 2

Derivata prima spettri UV-Vis normalizzati degli standard nella regione 200-800 nm.



Sotto-progetto Unità Mo e RE – livello 2

Cluster analysis con k-means (software test *Statistics Kingdom*)



Come continua l'analisi statistica?

K-means clustering and PCA clustering utilizzando il software:

- **XLSTAT**
- **DATAtab online**
- **SIMCA 13.0.3**

Unità di Mo e RE - Attività di ricerca 2022-23

Livello 1

- finire studio nuclearità Fe con UV-Vis
- correlazione **parametri del modello FPTI** con i **IARC 10 KCs**
  + classificazione finale dei campioni della *case fiber*: quanti **KCs** (da 1 a 10) sono attivi per ogni campione?
  + definire per ogni campione quali sono i parametri FPTI che attivano i KCs
- review generale "*Toxicity/pathogenicity of mineral fibres*" dal punto di vista mineralogico?
- collaborazione con gruppi UNIPI e UNIGE per studiare la cito- e geno-tossicità di fibre grezze vs. fibre "denaturate" (lisciviate) per misurare l'effetto del rilascio di metalli (***Trojan horse effect***). **Esperimenti ad ELETTRA ed ESRF!**

Livello 2

- struttura ERI turca
- collaborazione alla sperimentazione per la determinazione della tossicità *in vitro* 2D THP1 vs. 3D con UNIGE e sistemi misti organoidi

- studio di magnesiti naturali contaminate da amianto