XIII 2025 RAD CONFERENCE

UNIVERSITY OF WARSAW

UNIWERSYTET WARSZAWSKI · WYDZIAŁ GEOLOGII · Mente et Malleo

Laboratory synthesis methods of ferromagnetic greigite for its application in cancer hyperthermia

Klaudia Tetfejer
Anna Czarnecka-Skwarek
Agnieszka Rożek

Oncological hyperthermia

is a method supporting the treatment of cancer, consisting of controlled heating of cancer tissues to a temperature of 38.5°C to 42°C. The aim is to increase the effectiveness of therapies such as radiotherapy, chemotherapy or immunotherapy, by weakening cancer cells and stimulating the patient's immune system.

The effectiveness and application of this method

Clinical studies have shown that hyperthermia combined with radiotherapy or chemotherapy can increase the effectiveness of treatment by 20–30% compared to therapy without hyperthermia!

Particularly good results have been observed in the treatment of cancers such as melanoma, head and neck cancer, brain cancer, lung cancer, esophageal cancer, breast cancer, bladder cancer, rectal cancer, liver cancer, ovary cancer, cervical cancer, and skin cancer.

Types of hyperthermia

Hyperthermia can be used in various forms:

local – heating a specific area of the body;

systemic – heating the entire body;

perfusion (HIPEC) – heated chemotherapy introduced directly into the peritoneal cavity during surgery.

How does hyperthermia work?

Heating cancer tissue leads to:

- damage to protein structures in cancer cells, which can lead to their death;
- increased blood flow to the tumor, which improves oxygenation and delivery of anticancer drugs;
- inhibition of DNA repair processes in cancer cells;
- stimulation of the immune system to fight cancer cells.

The use of this method leads to an increase in the concentration of heat shock proteins (HSPs) in cancer cells!

Heat shock proteins (HSPs)

Their main task is to protect the cell from damage caused by stress, especially high temperature, but also other factors such as toxins, radiation, hypoxia or infections.

Functions of these proteins:

- help with protein folding – newly formed proteins must take on a specific shape to function properly;
- prevent protein aggregation;
- repair damaged proteins;
- stabilize the cell under stress – they allow it to survive extreme situations;
- support immunity – they can act as alarm signals for the immune system.

Hyperthermia causes an increase in HSP70 levels in cancer cells

- on the surface of cancer cells, HSP70 can act as an alarm signal for the immune system – especially for NK (natural killer) cells;

- it stimulates the immune response when cancer cells release HSP70 as a result of stress (e.g. after hyperthermia);

- it is used in targeted therapy and cancer vaccines, as a carrier of tumor antigens (HSP70-based immunotherapy).

How minerals can help in cancer treatment?

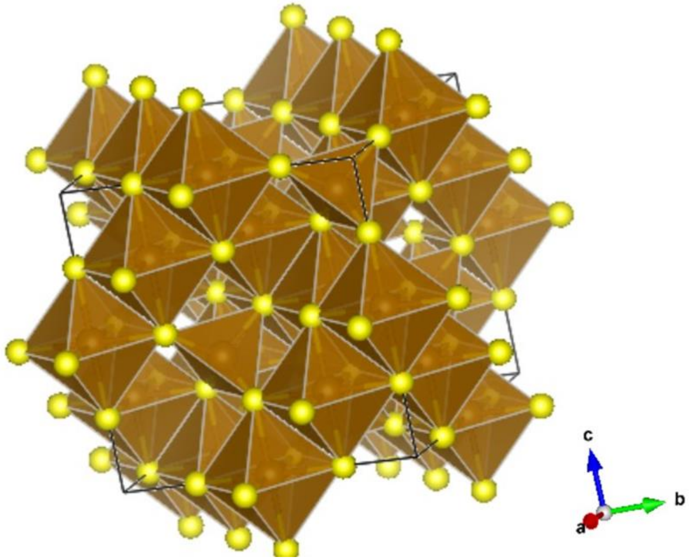
Magnetic nanoparticle hyperthermia (MNH) uses magnetic nanoparticles particles (MNPs) that are exposed to alternating magnetic field (AMF) to generate heat in local regions (tissues or cells).

Hyperthermia leads to the induction of heat-shock proteins (HSPs).

Iron sulfide
– **greigite** –
can be use in
magnetic nanoparticle hyperthermia (MNH)

Characteristics of greigite found in nature

Structure



Greigite ($Fe^{2+}Fe^{3+}{}_2S_4$)

Exhibits ferromagnetic properties

Li et al., 2015

Greigit is a natural sulphide mineral with the chemical formula Fe_3S_4, which is a mixture of iron(II) and iron(III) sulphides.

- appearance: dark, blue-black mineral;
- magnetic properties: has strong magnetic properties; crystal structure: similar to spinel.

Possible applications:

- in geology: palaeomagnetic analyses, interpretation of fossil sedimentary environments
- in technology: precursors for the synthesis of superconductors
- in medicine: cancer treatment therapy.

Synthesis of greigite nanoparticles in the laboratory

Ferromagnetic nanoparticle used in hyperthermia:

- should be biocompatible, biodegradable, with good colloidal stability
- should be obtained by a reproducible synthesis method yielding only ferromagnetic greigite nanoparticles with a uniform size distribution
- should have large heat generation capabilities

Developing a method for synthesising greigite nanoparticles that meets the above criteria is key to further progress in cancer treatment

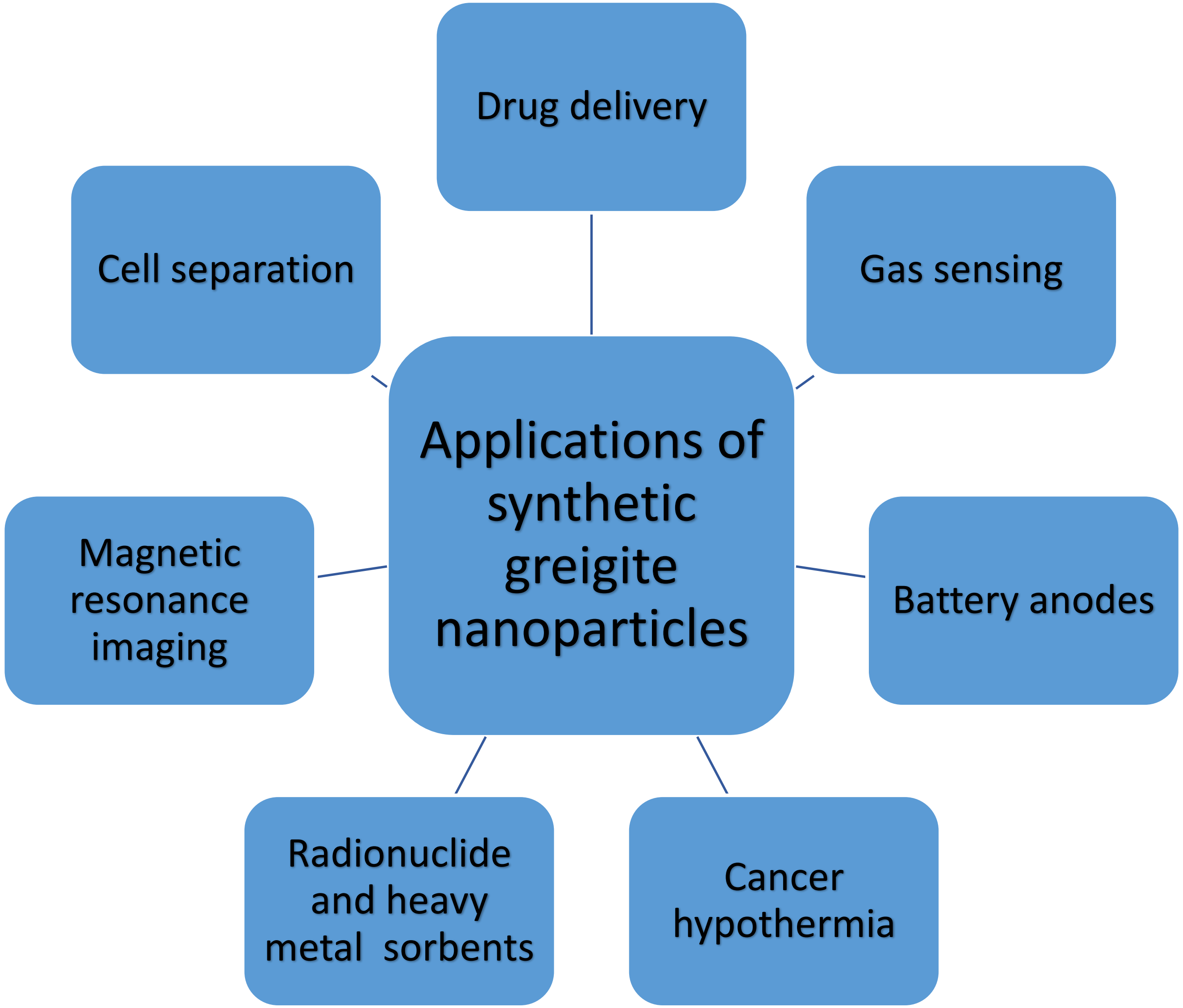
Advantages of greigite nanoparticles

- strong magnetic properties: enables efficient heat generation under the influence of a magnetic field;
- low toxicity: studies have shown low cytotoxicity at concentrations up to 1 mg/ml;
- functionalization possibilities: greigite nanoparticles can be modified to deliver drugs or increase selectivity for cancer cells;
- synergy with other therapies: combining magnetic hyperthermia with photothermal therapy increases the effectiveness of treatment, especially in the case of inflammation and cancer.

Greigite nanoparticles are a promising alternative to traditional materials used in magnetic hyperthermia, such as iron oxides!

Their unique magnetic properties, low toxicity, and potential for functionalization make them an attractive material for further research in the context of cancer therapy.

Synthesis of greigite nanoparticles in the laboratory



Synthesis methods

No.	Author, year	Synthesis method	T [°C]	Atmosphere	pH	Medium	Fe source	S source	t	Synthesis product
1	Uda, 1965	co-precipitation + hydrothermal	**25 + 190**	O_2	-	H_2O	$Fe(SO_4)(NH_4)_2(SO_4)$	SO_4^{2-}, Na_2S	**5-6 days + 1 h**	Grg
2	Sweeney and Kaplan, 1973	co-precipitation + elevated temperature transformations	**25 + 60 (additionally 85)**	O_2 + N_2	-	H_2O + H_2O (±)	$FeCl_2 \cdot 4H_2O$ + FeS	H_2S, S^0 + FeS, S^0	**4 days + 5 days (additionally 6 days)**	FeS **Grg** (Py)
3	Chang et al., 2011	co-precipitation	**25**	N_2	3, 4, 5	H_2O	$FeSO_4 \cdot 7H_2O$	SO_4^{2-}, Na_2S	**5, 10, 15, 20 min**	**Grg** (50-100nm, high crystallinity)
4	Moore et al., 2019	co-precipitation/ hydrothermal	**25**/ 180	Ar/ **O_2, Ar**	3-5/ -	H_2O/ **ethylene glycol + H_2O** (2:1), PVP	$FeSO_4 \cdot 7H_2O$/ $FeCl_3 \cdot 6H_2O$	SO_4^{2-}, Na_2S/ thiourea (CH_4N_2S)	**5 min/ 6, 12, 18, 24 h**	FeS_{am}, Mkw/ **Grg** (700 nm), Py + (Mt w O_2)
5	Naser et al., 2024	hydrothermal	180, 210, 230	**O_2**	**-**	**ethylene glycol + H_2O** (2:1)	$FeCl_3 \cdot 6H_2O$	thiourea (CH_4N_2S)	**18 h**	**Grg** (tens/hundreds nm), Mgh
6	Zhang and Chen, 2009	solvothermal	120, 140, 160, 180, 200	**O_2**	**-**	**ethylene glycol + H_2O:** 4:0 **3:1** 2:2 1:3 0:4	$FeCl_3 \cdot 6H_2O$	thiourea (CH_4N_2S)	**12 h**	**Grg** (hundreds nm)
7	Liao et al., 2015	hydrothermal	100, 125, 150, 175	**Ar, O_2**	-	H_2O + **HTMA**	$FeSO_4 \cdot 7H_2O$	SO_4^{2-}, thioacetamide (CH_3CSNH_2)	**24 h**	**Grg** (tens/hundreds nm)

Table part 1

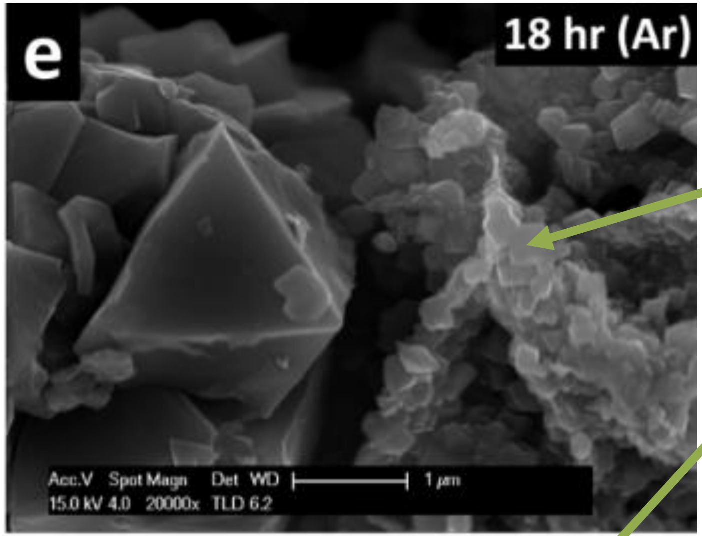
Abiotic synthesis

No.	Author, year	Synthesis method	T [°C]	Atmosphere	pH	Medium	Fe source	S source	t	Synthesis product
8	Dekkers and Schoonen, 1996	hydrothermal	**~140**	N_2	5,7-10	-	$Fe(SO_4)(NH_4)_2(SO_4)$	**SO_4^{2-}, Na_2S, S^0**	32-960 min	**Grg** (hundreds nm)
9	Dekkers et al., 2000	hydrothermal + heating	**140 + 250, 350, 450, 600**	N_2 + O_2	-	-	$Fe(SO_4)(NH_4)_2(SO_4)$	**SO_4^{2-}, Na_2S, S^0**	-	**Grg +** Py, Mrc, **Grg**, Mgh, Mt, Po
10	Nie et al., 2019	hydrothermal/ hydrothermal*	**100/ 200***	Ar	-	H_2O	$FeSO_4·7H_2O$/ Fe_3S_4*	**SO_4^{2-}, Na_2S/ thiourea (CH_4N_2S) lub S^0, Na_2S***	3 h/ 24, 72, 168 h*	Grg/ Py*
11	Chang et al., 2007 Chang et al., 2008 Chang et al., 2009	hydrothermal	170	N_2	<4	H_2O + formic acid (HCOOH)	$FeCl_3·6H_2O$	**SO_4^{2-}, thiourea**	8 h	**Grg (several μm)**
12	Li et al., 2015	hot injection	180, 190, 200, 210, 220	N_2	-	**diphenyl ether + octadecylamine**: 1:1 2:1 5:1	Fe(acac)$_3$, $FeCl_3·6H_2O$	**S^0 (Fe:S 1:8, 1:7, 1:6, 1:5, 1:4)**	3 h	**Grg (30-50 nm)**
13	Shi et al., 2022	hot injection	240	N_2	-	**oleylamine**	Fe(acac)$_3$	**S^0 (Fe:S 1:6, 1:8, 1:10, 1:12)**	4 h	**Grg (~90 nm) Py**
14	Liu et al., 2014	solvothermal	200	O_2	-	H_2O	$FeSO_4·7H_2O$	**SO_4^{2-}, L-cysteine (C_3H_7NS)**	24 h	**Grg** (~50nm)

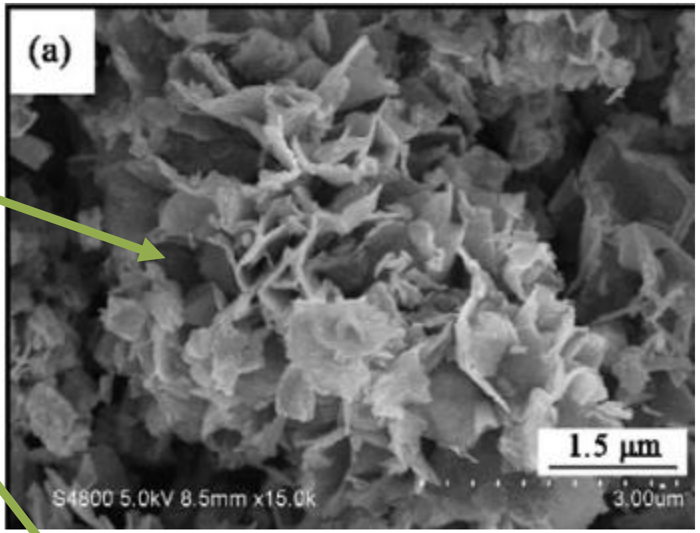
Table part 2

Abiotic synthesis

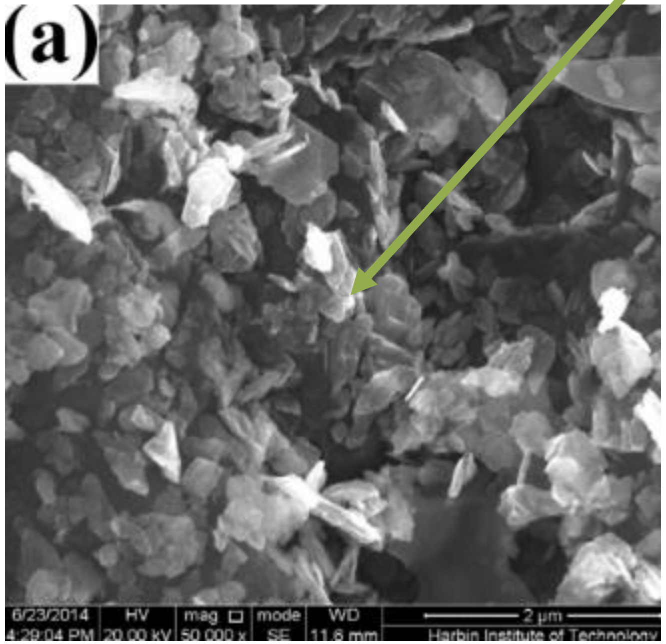
Greigit - varying morphology and size of particles synthesised in the laboratory



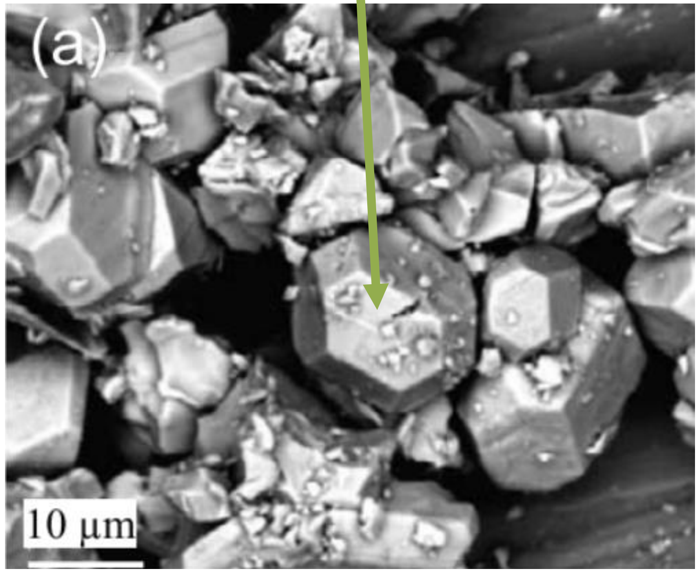
Moore et al., 2019



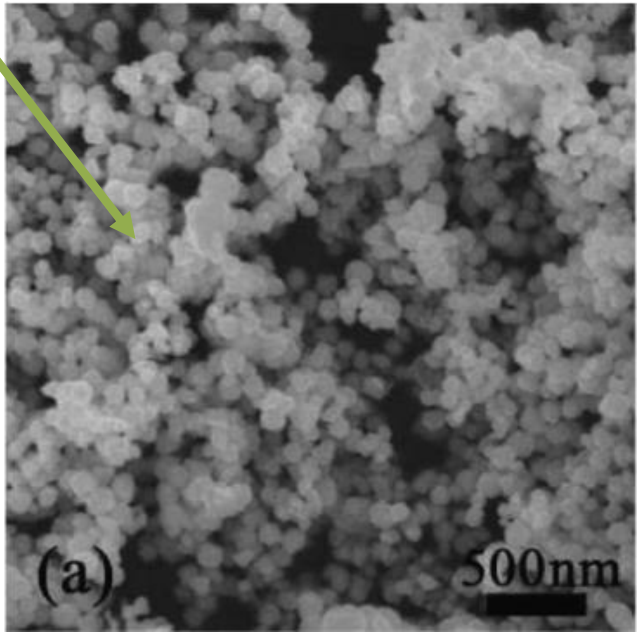
Zhang and Chen, 2009



Liao et al., 2015



Chang et al., 2008



Liu et al., 2014

Summary of laboratory syntheses

Greigite exhibits antioxidant activity and cytotoxicity, can be use in magnetic nanoparticle hyperthermia;

in experimental laboratory syntheses it is possible to obtain a monomineral phase, sometimes with pyrite or maghemite;

abiotic, laboratory syntheses require high temperatures, sometimes a two-step synthesis process, and an inert gas atmosphere;

under abiotic conditions greigite can be fast synthesised (h), is characterised by a high crystallinity;

abiotic syntheses of greigite carried out under laboratory conditions confirm the possibility of obtaining monomineral phases using organic compounds such as hexamethylenetetramine (HTMA), octadecylamine (ODA) or oleylamie (OLA) or ethylene glycol.

Challenges and prospects for the use of greigite in hyperthermia method

chemical stability: greigite may be less stable than iron oxides, which may affect its long-term efficacy;

control over size and shape is crucial to optimizing their magnetic properties and bioavailability;

comparable or lower toxicity than magnetite (Fe_3O_4);

greigite has strong magnetic properties and a potentially higher heating effect than magnetite, but is less stable and less well-studied, especially in terms of long-term effects on the body;

lack of clinical studies: research to date is mostly laboratory-based; further preclinical and clinical studies are needed.

References

- A. P. Roberts, “Magnetic mineral diagenesis,” Earth Sci Rev, vol. 151, pp. 1–47, 2015.
- W. Qin et al., “Microbe-mediated extracellular and intracellular mineralization: environmental, industrial, and biotechnological applications,” Advanced Materials, vol. 32, no. 22, p. 1907833, 2020.
- Lefèvre CT, Bazylinski DA. 2013. Ecology, Diversity, and Evolution of Magnetotactic Bacteria. Microbiol Mol Biol Rev 77:.https://doi.org/10.1128/mmbr.00021-13
- D. Rickard, “The chemistry of iron sulphide formation at low temperatures,” *Stockhom Contributions to Geology*, vol. 20, pp. 67–95, 1969.
- Y.-S. Chang, S. Savitha, S. Sadhasivam, C.-K. Hsu, and F.-H. Lin, “Fabrication, characterization, and application of greigite nanoparticles for cancer hyperthermia,” *J Colloid Interface Sci*, vol. 363, no. 1, pp. 314–319, 2011.
- J. Moore, E. Nienhuis, M. Ahmadzadeh, and J. McCloy, “Synthesis of greigite (Fe3S4) particles via a hydrothermal method,” *AIP Adv*, vol. 9, no. 3, 2019.
- T. Liao, W. Wang, Y. Song, X. Wang, Y. Yang, and X. Liu, “HMTA-assisted one-pot synthesis of greigite nano-platelet and its magnetic properties,” *J Mater Sci Technol*, vol. 31, no. 9, pp. 895–900, 2015.
- D. M. Naser, S. H. Lafta, and M. S. Hashim, “Antioxidant activity and cytotoxicity of greigite nanoparticles synthesized by hydrothermal technique,” *Biotechnol Appl Biochem*, 2024.
- Z. J. Zhang and X. Y. Chen, “Magnetic greigite (Fe3S4) nanomaterials: Shape-controlled solvothermal synthesis and their calcination conversion into hematite (α-Fe2O3) nanomaterials,” *J Alloys Compd*, vol. 488, no. 1, pp. 339–345, 2009.
- X. Liu *et al.*, “Synthesis and electromagnetic properties of Fe3S4 nanoparticles,” *Ceram Int*, vol. 40, no. 7, pp. 9917–9922, 2014.
- Uda, M. (1965). On the synthesis of greigite. American Mineralogist: Journal of Earth and Planetary Materials, 50(9), 1487-1489.
- Dekkers, M. J., & Schoonen, M. A. (1996). Magnetic properties of hydrothermally synthesized greigite (Fe3S4)—I. Rock magnetic parameters at room temperature. Geophysical Journal International, 126(2), 360-368.
- Dekkers, M. J., Passier, H. F., & Schoonen, M. A. (2000). Magnetic properties of hydrothermally synthesized greigite (Fe 3 S 4)—II. High-and low-temperature characteristics. Geophysical Journal International, 141(3), 809-819.
- Li, T., Li, H., Wu, Z., Hao, H., Liu, J., Huang, T., ... & Guo, Z. (2015). Colloidal synthesis of greigite nanoplates with controlled lateral size for electrochemical applications. Nanoscale, 7(9), 4171-4178.
- Shi, X., Xu, Y., Zhang, Y., Si, J., Zhang, P., Li, W., ... & Miao, S. (2022). Stoichiometry-controlled synthesis of pyrite and greigite particles for photo-Fenton degradation catalysis. New Journal of Chemistry, 46(29), 14205-14213.
- Sweeney, R. E., & Kaplan, I. R. (1973). Pyrite framboid formation; laboratory synthesis and marine sediments. Economic Geology, 68(5), 618-634.
- Chang, L., Roberts, A. P., Muxworthy, A. R., Tang, Y., Chen, Q., Rowan, C. J., ... & Pruner, P. (2007). Magnetic characteristics of synthetic pseudo-single-domain and multi-domain greigite (Fe3S4). Geophysical Research Letters, 34(24).
- Chang, L., Roberts, A. P., Tang, Y., Rainford, B. D., Muxworthy, A. R., & Chen, Q. (2008). Fundamental magnetic parameters from pure synthetic greigite (Fe3S4). Journal of Geophysical Research: Solid Earth, 113(B6).
- Chang, L., Rainford, B. D., Stewart, J. R., Ritter, C., Roberts, A. P., Tang, Y., & Chen, Q. (2009). Magnetic structure of greigite (Fe3S4) probed by neutron powder diffraction and polarized neutron diffraction. Journal of Geophysical Research: Solid Earth, 114(B7).
- Nie, X., Luo, S., Yang, M., Zeng, P., Qin, Z., Yu, W., & Wan, Q. (2019). Facile hydrothermal synthesis of nanocubic pyrite crystals using greigite Fe3S4 and thiourea as precursors. Minerals, 9(5), 273.
- Obaidat, I. M., Narayanaswamy, V., Alaabed, S., Sambasivam, S., & Muralee Gopi, C. V. (2019). Principles of magnetic hyperthermia: a focus on using multifunctional hybrid magnetic nanoparticles. Magnetochemistry, 5(4), 67