

Submission ID #: 71045

Scriptwriter Name: Kathyayani Sridharan

Project Page Link: <https://review.jove.com/account/file-uploader?src=21668773>

Title: Preparation of Metal-Organic Framework-Gelatin Hydrogels Through Coacervation

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 12

Number of Shots: 28

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Nathalie Steunou**: We present a novel method to synthesize and shape porous composites using metal-organic frameworks and gelatin coacervates.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Nathalie Steunou**: Conventional MOF-gelatin composites exhibit low porosity and MOF loading; this protocol yields porous composites with excellent interfacial and mechanical properties.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Irène Mangialomini**: This protocol allows gram-scale fabrication of MOF-gelatin composites into diverse macroscopic forms, including monoliths and coatings.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Irène Mangialomini** : It exploits gelatin's ability to undergo phase separation through coacervation, combined with the *in situ* formation of Zr MOFs.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

1.5. **Irène Mangialomini:** This approach extends to chemically diverse MOFs, enabling the formation of shaped composites for environmental remediation and catalysis applications.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What is the key takeaway you hope viewers remember after watching your video article?

- 1.6. **Nathalie Steunou, Professor, University Versailles St Quentin-en-Yvelines, University Paris Saclay**: (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Nathalie Steunou, Professor University Versailles St Quentin-en-Yvelines, University Paris Saclay**: (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Gelatin Solution Preparation and Zirconium Oxocluster Synthesis

Demonstrator: Irène Mangialomini

- 2.1. To begin, weigh 10 grams of commercial type A gelatin extracted from porcine skin, with a Bloom of 175 g, an average molecular weight of approximately 40,000 gram per mole, and an isoelectric point of 8 [1]. Add the weighed gelatin powder to 100 milliliters of water to prepare an aqueous gelatin gel at 10 percent weight concentration [2]. Swell the gelatin granules in ultrapure water for at least 3 hours at 5 degrees Celsius [3].
 - 2.1.1. WIDE: Talent weighing the commercial gelatin powder. *Videographer: Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent adding 100 mL water to the gelatin powder.
 - 2.1.3. Talent placing the gelatin solution at 5 degrees Celsius to allow swelling.
- 2.2. Heat the gelatin gel at 50 degrees Celsius to prepare an aqueous gelatin solution [1]. Use a magnetic stirrer to stir the gelatin solution for 30 minutes at 300 revolutions per minute [2]. Once a clear gelatin solution is obtained, keep it at 50 degrees Celsius [3].
 - 2.2.1. Talent heating the gelatin gel at 50 degrees Celsius.
 - 2.2.2. Talent placing the gelatin solution on a magnetic stirrer. **Videographer's Note: Shots 2.2.2 and 2.2.3 are combined into one shot.**
 - 2.2.3. Talent placing the clear gelatin solution at 50 degrees Celsius on a heater.
- 2.3. In a 25-milliliter scintillation vial, add 7 milliliters of dimethylformamide and 4 milliliters of acetic acid [1]. Next, add 71 microliters of a 70 percent zirconium propoxide solution in 1-propanol [2-TXT].
 - 2.3.1. Talent adding dimethylformamide, and acetic acid to a 25 mL scintillation vial.
 - 2.3.2. Talent adding the zirconium propoxide solution to the scintillation vial. **TXT: Zirconium propoxide = $[Zr(OPr^n)_4]$**
- 2.4. Heat the resulting mixture in an aluminum block on a hot plate at 130 degrees Celsius for 2 hours [1]. Observe a distinct color change from colorless to yellow, indicating the formation of Zirconium oxoclusters [2-TXT]. Leave the solution to cool at room temperature [3].
 - 2.4.1. Talent placing the scintillation vial in an aluminum block on a hot plate.

- 2.4.2. Shot of the mixture changing from colorless to yellow. **TEXT: Zirconium oxocluster = $[\text{Zr}_6\text{O}_4(\text{OH})_4(\text{OAc})_{12}]$**
- 2.4.3. Talent placing the solution on the work surface to cool at room temperature.

3. *In Situ* Synthesis and Monolithic Shaping of UiO-66-Gelatin Hydrogel Composites

Demonstrator: Navaneeth Narayan Gowda

- 3.1. Mix 11 milliliters of zirconium oxo-cluster solution with 84 milligrams of 1,4-benzene dicarboxylic acid organic linker [1]. Stir the mixture for 15 minutes until a homogeneous dispersion is obtained [2].
- 3.1.1. Talent combining the zirconium oxo-cluster solution with 1,4-benzene dicarboxylic acid.
- 3.1.2. Talent stirring the mixture until a homogeneous dispersion forms.
- 3.2. Next, add 2 milliliters of the 10 percent weight gelatin solution at 50 degrees Celsius [1]. ~~Observe the mixture becoming turbid and turning white [2].~~
- 3.2.1. Talent adding the gelatin solution to the vial.
- ~~3.2.2. Shot of the mixture becoming turbid and white.~~ **Note: The authors requested to move this shot after 3.3.2**
- 3.3. Stir the mixture at room temperature for 10 minutes [1], then add 2 milliliters of ethanol [2]. ~~Observe the mixture becoming turbid and turning white [3].~~ **Note: The VO is incorporated for the moved shot.**
- 3.3.1. Talent placing the mixture on a stirrer at room temperature.
- 3.3.2. Talent adding ethanol to the mixture.
- ~~3.2.2. Shot of mixture becoming turbid and white.~~ **Note: This shot was requested to be moved here.**
- 3.4. Stir the resulting mixture at room temperature, around 20 degrees Celsius, for 24 hours [1]. ~~Allow the microscopic liquid-liquid phase separation to occur gradually over time [2].~~
- 3.4.1. Shot of the solution on the stirrer at room temperature.
- ~~3.4.2. Shot of the mixture undergoing progressive liquid-liquid phase separation.~~ **Note: The authors requested to move this shot after 3.5.2**
- 3.5. Add 10 milliliters of ethanol to the mixture, stirring continuously for 24 hours to allow complete phase separation. [1]. ~~Stir the solution for 24 hours [2].~~ Observe the solution turn into a macroscopically biphasic sample, consisting of a dilute gelatin phase and a viscous hydrogel.[3]. **Note: The VO is incorporated for the moved shot.**

- 3.5.1. Talent adding ethanol to the mixture.
- ~~3.5.2. Shot of the solution on the stirrer.~~ **Note: This shot was removed.**
- 3.4.2. Shot of the mixture undergoing progressive liquid-liquid phase separation. **Note: This shot was requested to be moved here.**
- 3.6. Extract a small portion of the viscous phase from the supernatant solution to obtain the metal organic framework, or MOF (*M-O-F*)-hydrogel composite [1]. Leave this composite to dry at room temperature until characterization [2].
 - 3.6.1. Talent extracting the viscous phase from the supernatant solution.
 - 3.6.2. Talent leaving the hydrogel composite to dry at room temperature.
- 3.7. For monolithic shaping of hydrogel composites, heat the viscous phase with its supernatant at 70 degrees Celsius for 15 minutes [1]. Then, heat it at 90 degrees Celsius for 5 minutes to transform the MOF-gelatin hydrogel into a homogeneous, viscous liquid [2].
 - 3.7.1. Talent placing the viscous phase with its supernatant for heating at 70 degrees Celsius.
 - 3.7.2. Talent continuing the heating process at 90 degrees Celsius.
- 3.8. Pour the hydrogel without its supernatant solution into the mold thermalized at 70 degrees Celsius [1]. Form a monolith by cooling at room temperature for 15 minutes [2]. Then, keep it at 2 degrees Celsius for 1 day [3]. Confirm the formation of the final MOF-gelatin hydrogel [4]. **Note: VO is incorporated for added shot.**
 - 3.8.1. Talent pouring the hydrogel into the thermalized mold.
 - 3.8.2. Talent allowing the sample to cool at room temperature.
 - 3.8.3. Talent placing the monolith at 2 degrees Celsius.
 - Added shot: 3.8.4. Talent showing the final MOF-gelatin hydrogel.

Results

4. Results

- 4.1. Powdered X-ray diffraction studies, or PXRD (*P-X-R-D*) studies demonstrate that the UiO-66(Zr)/gelatin (*U-I-O-sixty-six-Z-R-Gelatin*) composite maintains high crystallinity [1], matching that of pure UiO-66(Zr) (*U-I-O-sixty-six-Z-R*) metal organic framework, or MOF (*M-O-F*) nanoparticles [2].
 - 4.1.1. LAB MEDIA: Figure 6A. *Video Editor: Please emphasize the black line labelled UiO-66/gelatin*
 - 4.1.2. LAB MEDIA: Figure 6A. *Video Editor: Please emphasize the grey line labelled calculated UiO-66*
- 4.2. Nitrogen porosimetry shows that the UiO-66/gelatin (*U-I-O-sixty six-gelatin*) composite remains highly porous, retaining approximately 79% of the surface area [1] of the parent MOF, suggesting that the gelatin chains are unable to penetrate the micropores of UiO-66 [2].
 - 4.2.1. LAB MEDIA: Figure 6B. *Video Editor: Please emphasize the bottom curve labelled UiO-66/Gelatin*
 - 4.2.2. LAB MEDIA: Figure 6B. *Video Editor: Please emphasize the top curve labelled UiO-66*
- 4.3. Transmission Electron Microscopy, or TEM (*tem*) analysis of ultramicrotomed slices reveals that UiO-66(Zr) nanoparticles are highly dispersed throughout the gelatin matrix without aggregation or phase separation [1]. Furthermore, scanning electron microscopy, or SEM (*sem*) imaging of water-washed samples confirms that the embedded nanocrystals maintain a well-defined octahedral morphology [2].
 - 4.3.1. LAB MEDIA: Figure 6C
 - 4.3.2. LAB MEDIA: Figure 6D. *Video Editor: Please emphasize the zoomed-in inset showing the crystal structure*

Pronunciation Guide:

- Gelatin
Pronunciation link: <https://www.merriam-webster.com/dictionary/gelatin>
IPA: /'dʒelətɪn/
Phonetic Spelling: jel·uh·tin
- Porcine
Pronunciation link: <https://www.merriam-webster.com/dictionary/porcine>

IPA: /'pɔːrsɪn/

Phonetic Spelling: por·sine

- Bloom

Pronunciation link: <https://www.merriam-webster.com/dictionary/bloom>

IPA: /'bluːm/

Phonetic Spelling: bloom

- Molecular

Pronunciation link: <https://www.merriam-webster.com/dictionary/molecular>

IPA: /mə'lekjələr/

Phonetic Spelling: muh·lek·yuh·ler

- Isoelectric

Pronunciation link: <https://www.merriam-webster.com/dictionary/isoelectric>

IPA: /ˌaɪsoʊɪ'lektrɪk/

Phonetic Spelling: eye·soh·ee·lek·trik

- Aqueous

Pronunciation link: <https://www.merriam-webster.com/dictionary/aqueous>

IPA: /'eɪkwɪəs/

Phonetic Spelling: ay·kwee·uhs

- Ultrapure

Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrapure>

IPA: /ˌʌltrə'pjʊr/

Phonetic Spelling: ul·truh·pyur

- Celsius

Pronunciation link: <https://www.merriam-webster.com/dictionary/Celsius>

IPA: /'selsiəs/

Phonetic Spelling: sel·see·uhs

- Magnetic

Pronunciation link: <https://www.merriam-webster.com/dictionary/magnetic>

IPA: /mæg'netɪk/

Phonetic Spelling: mag·net·ik

- Scintillation

Pronunciation link: <https://www.merriam-webster.com/dictionary/scintillation>

IPA: /ˌsɪntə'leɪʃən/

Phonetic Spelling: sin·tuh·lay·shuhn

- Dimethylformamide

Pronunciation link: <https://www.howtopronounce.com/dimethylformamide>

IPA: /daɪˌmɛθəl'fɔːrməˌmaɪd/

Phonetic Spelling: dye·meth·uhl·for·muh·mide

- Acetic

Pronunciation link: <https://www.merriam-webster.com/dictionary/acetic>

IPA: /ə'siːtɪk/

Phonetic Spelling: uh·see·tik

- Zirconium
Pronunciation link: <https://www.merriam-webster.com/dictionary/zirconium>
IPA: /zər'koʊniəm/
Phonetic Spelling: zer·koh·nee·uhm
- Propoxide
Pronunciation link: <https://www.howtopronounce.com/propoxide>
IPA: /proʊ'pɑːksaɪd/
Phonetic Spelling: proh·pok·side
- Propanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/propanol>
IPA: /'proʊpə,nɔːl/
Phonetic Spelling: proh·puh·nol
- Oxocluster
Pronunciation link: <https://www.howtopronounce.com/oxocluster>
IPA: /ˌɑːksəʊ'klʌstər/
Phonetic Spelling: ok·soh·klus·ter
- Aluminum
Pronunciation link: <https://www.merriam-webster.com/dictionary/aluminum>
IPA: /ə'luːmənəm/
Phonetic Spelling: uh·loo·muh·nuhm
- Dicarboxylic
Pronunciation link: <https://www.howtopronounce.com/dicarboxylic>
IPA: /daɪ,kɑːrbaːk'sɪlɪk/
Phonetic Spelling: dye·kar·bok·sil·ik
- Homogeneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogeneous>
IPA: /ˌhoʊmə'dʒiːniəs/
Phonetic Spelling: hoh·muh·jee·nee·uhs
- Dispersion
Pronunciation link: <https://www.merriam-webster.com/dictionary/dispersion>
IPA: /dɪ'spɜːrʒən/
Phonetic Spelling: dih·spur·zhuhn
- Turbid
Pronunciation link: <https://www.merriam-webster.com/dictionary/turbid>
IPA: /'tɜːrbɪd/
Phonetic Spelling: tur·bid
- Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /'eθə,nɔːl/
Phonetic Spelling: eth·uh·nol
- Microscopic
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopic>

IPA: /ˌmaɪkrəˈskaːpɪk/

Phonetic Spelling: my·kruh·skop·ik

- Biphasic

Pronunciation link: <https://www.merriam-webster.com/dictionary/biphasic>

IPA: /baɪˈfeɪzɪk/

Phonetic Spelling: by·fay·zik

- Viscous

Pronunciation link: <https://www.merriam-webster.com/dictionary/viscous>

IPA: /ˈvɪskəs/

Phonetic Spelling: vis·kuhs

- Supernatant

Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>

IPA: /suːˈpɜːnətənt/

Phonetic Spelling: soo·pur·nuh·tuhnt

- Characterization

Pronunciation link: <https://www.merriam-webster.com/dictionary/characterization>

IPA: /ˌkerəktərəˈzeɪʃən/

Phonetic Spelling: kar·ik·ter·uh·zay·shuhn

- Monolithic

Pronunciation link: <https://www.merriam-webster.com/dictionary/monolithic>

IPA: /ˌmɑːnəˈlɪθɪk/

Phonetic Spelling: mon·uh·lith·ik

- Hydrogel

Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrogel>

IPA: /ˈhaɪdroʊˌdʒel/

Phonetic Spelling: hy·droh·jel

- Thermalized

Pronunciation link: <https://www.howtopronounce.com/thermalized>

IPA: /ˈθɜːrməlaɪzd/

Phonetic Spelling: thur·muh·lized

- Monolith

Pronunciation link: <https://www.merriam-webster.com/dictionary/monolith>

IPA: /ˈmɑːnəlɪθ/

Phonetic Spelling: mon·uh·lith

- MOF

Pronunciation link: <https://www.howtopronounce.com/mof>

IPA: /ˌɛm.ʊʃˈɛf/

Phonetic Spelling: em·oh·ef

- Composite

Pronunciation link: <https://www.merriam-webster.com/dictionary/composite>

IPA: /kəmˈpɑːzɪt/, /ˈkɑːmpəzɪt/

Phonetic Spelling: kuhm·poz·it; kom·puh·zit

