

**A Reversibly Self-Assembling Hydrated  
Matrix  
Based on Hydroxylated Fullerenes and  
Calcium:  
Formation, Persistence, and  
Physicochemical Characterization.**

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## Abstract

Hydroxylated fullerenes (fullerenols) are water-compatible fullerene derivatives studied for their distinctive redox, supramolecular, and functional properties. Although aggregation and self-assembly of fullerene derivatives in aqueous media are established, the formation and persistence of macroscopically organized, highly hydrated fullerene-derived states remain comparatively less explored [1-4].

Here we describe a hydrated matrix containing a fullerene-derived oxygen-functionalized component, water, and calcium. The study focuses on the reproducibility, physical-state reversibility, washing persistence, morphology, and physicochemical signatures of the resulting material. Preparation parameters that are part of ongoing intellectual-property development are intentionally not disclosed; downstream characterization procedures and observations are reported independently of those proprietary parameters.

The matrix demonstrated reproducible bulk self-assembly, reversible transitions among hydrated, dispersed, and dry states, recovery after dehydration, persistence through sequential washing, freeze-thaw cycling, and moderate heating. Optical and electron microscopy revealed anisotropic plate-like and lamellar morphology. SEM/EDS and XPS showed predominantly C, O, and Ca; ATR-FTIR was consistent with a strongly oxygen-functionalized and highly hydrated environment; and Raman spectroscopy provided a reproducible fullerene-derived carbon fingerprint.

Published work has shown that water-soluble fullerene derivatives can self-assemble in aqueous media and, in some systems, form supramolecular hydrogels without an external polymeric matrix [3,4]. The present work therefore does not claim fullerene self-assembly itself as novel. Rather, it documents a distinct experimentally observed property set in a hydroxylated-fullerene/calcium/water system, including macroscopic phase formation, sequential-wash persistence, dehydration/rehydration recovery, and calcium retention within the structured phase.

The purpose of this study is to establish the observed material behavior and analytical characteristics of this matrix, not to establish a biological effect, application-specific performance, or a definitive molecular assembly mechanism.

## 1. Introduction

Fullerenes are closed-cage carbon allotropes whose hydrophobicity limits their direct use in aqueous systems. Covalent functionalization with hydrophilic groups provides access to water-compatible derivatives; among the most widely studied are polyhydroxylated fullerenes, commonly described as fullerenols, C<sub>60</sub>(OH)<sub>n</sub> [1,2]. Their fullerene carbon framework combined with a hydroxylated surface has motivated research in redox

chemistry, nanobiomedicine, drug-delivery concepts, radiobiology, agriculture, sensing, and other functional-material applications [1,2].

Aqueous compatibility does not imply that fullerenols behave as simple molecular solutes. Water-soluble fullerene derivatives can aggregate and form supramolecular structures, and their organization depends on functionalization, concentration, solvent conditions, pH, temperature, and ionic environment [3,4]. Fullerenol C60(OH)<sub>36</sub>, for example, has been reported to self-assemble in aqueous solutions and cell-culture media, while broader fullerene chemistry has produced a variety of nanoscale and hierarchical assemblies [3].

Importantly, fullerene-derived hydrogels are not unprecedented. Rašović et al. recently reported hierarchical self-assembly of covalently functionalized water-soluble C60 and higher-fullerene derivatives into macroscale supramolecular hydrogels without an external polymeric matrix [4]. That work establishes a particularly relevant precedent and shows that fullerene building blocks can themselves generate extended hydrated soft-matter structures.

The present study addresses a related but distinct material system. During experimental work with a hydroxylated fullerene-derived component in water and a calcium-containing component, we repeatedly observed a cooperative macroscopic transition into a highly hydrated organized phase. The resulting material could be diluted, washed, dehydrated, rehydrated, frozen and thawed, and mechanically redistributed while retaining or recovering characteristic macroscopic organization.

Accordingly, the contribution examined here is not the general proposition that fullerene derivatives can self-assemble. Instead, we ask whether this particular hydroxylated-fullerene/calcium/water system exhibits a reproducible combination of properties: (i) macroscopic bulk phase formation, (ii) persistence through repeated washing, (iii) reversible transitions between hydrated and dry states, (iv) recovery after freeze-thaw and moderate heating, (v) anisotropic plate-like and lamellar morphology, and (vi) persistent association of calcium with the structured phase.

The system is also of technological interest because a single material can be handled in concentrated hydrated, diluted aqueous, and dehydrated forms. This observation suggests possible future utility as a controllable material state for fullerene-derived components. Such technological and application-specific uses, however, are outside the evidentiary scope of the present study and are treated only as future research directions.

The objective of this study was therefore to document the formation, reproducibility, reversibility, persistence, morphology, and physicochemical characteristics of the hydrated matrix. We further examined its response to sequential washing, dehydration and rehydration, storage, freeze-thaw cycling, moderate heating, and substitution of

magnesium for the calcium-containing component. The study does not attempt to establish a complete molecular assembly mechanism or biological performance.

## 2. Materials and Methods

### 2.1 Materials and matrix preparation

The investigated system was prepared from a hydroxylated fullerene-derived aqueous component, water, and a calcium-containing component. Following controlled aqueous processing, the system underwent spontaneous macroscopic phase organization into a hydrated matrix and a supernatant phase. Exact precursor concentrations, component ratios, order of addition, and selected processing conditions are proprietary parameters associated with ongoing intellectual-property protection and are not disclosed in this manuscript. These withheld preparation parameters do not alter the downstream analytical methods or the reported observations.

### 2.2 Reproducibility and macroscopic observation

Independent preparations were evaluated for macroscopic phase formation, approximate gel-to-liquid phase ratio, densification, transparency, reassembly behavior, and recovery after physical-state changes. Bulk self-assembly was observed in more than 50 independent experiments at preparation scales of approximately 0.1-1.5 L.

### 2.3 Sequential washing

Sequential washing was used as a stress test of matrix persistence and re-formation. After each cycle, the material was evaluated for recovery of the gel phase and changes in pH, electrical conductivity (EC), total dissolved solids (TDS), water uptake, transparency, and macroscopic reassembly. One series was quantitatively followed for 11 cycles. The manuscript reports the measured progression of pH, EC, and TDS without disclosing proprietary upstream preparation parameters.

### 2.4 Dehydration, rehydration, freeze-thaw, and thermal observations

Hydrated material was subjected to dehydration and subsequent water addition to assess recovery of a hydrated state. Reversible behavior was observed across more than 30 complete state-transition cycles. Freeze-thaw behavior was examined for at least three cycles. Moderate-heating observations were made at approximately 70-80 °C. Additional procedural detail should be reported in a submission version to the extent that it does not disclose protected preparation know-how.

### 2.5 Storage observations

More than 50 prepared samples were observed during storage for gel densification, supernatant formation, visible volume change, and retention of rehydration capability. The exact storage duration, container conditions, and observation schedule should be inserted

from contemporaneous laboratory records before journal submission; these details describe the stability test and need not disclose the proprietary synthesis procedure.

## 2.6 Microscopy and spectroscopic characterization

Optical microscopy was used to examine hydrated matrix and filtrate fractions. SEM was used to examine dehydrated material, with EDS used for elemental assessment of selected structured regions. XPS was performed on dehydrated samples after 2, 5, and 11 washes; three areas were analyzed per sample for the surface-composition comparison shown in Figure 1. ATR-FTIR was used to compare hydrated/original and dried states. Raman spectra were acquired at 785 nm to obtain a vibrational fingerprint of the fullerene-derived carbon component.

## 2.7 Mg/Ca substitution experiment

A comparative experiment evaluated whether replacement of the calcium-containing component with  $\text{MgCO}_3$  produced the characteristic macroscopic transition.  $\text{MgCO}_3$  alone did not trigger the characteristic collapse; subsequent addition of  $\text{CaCO}_3$  produced a hydrated matrix with macroscopic properties distinct from the standard material.

## 3. Results: Main Reproducible Properties

| Observed phenomenon                      | Reproducibility             | Experimental observation                                                                  |
|------------------------------------------|-----------------------------|-------------------------------------------------------------------------------------------|
| Bulk self-assembly with phase separation | >50 independent experiments | Collapse across the bulk volume, forming approximately 2/3 gel phase and 1/3 liquid phase |
| Reversible liquid-gel-solid transitions  | >30 complete cycles         | Dilution, dehydration, rehydration, and mechanical redispersion                           |
| Recovery after drying                    | Repeatedly observed         | Dry films, blocks, and powder returned to a hydrated state after water addition           |
| Storage stability                        | >50 samples                 | Densification without visible volume loss; rehydration capability retained                |
| Stability after sequential washing       | >20 series                  | Gel phase re-formed after each cycle                                                      |
| Property evolution during washing        | >20 cycles                  | pH, EC, TDS, water uptake, transparency, and self-assembly                                |

|                                                           |                         |                                                                                     |
|-----------------------------------------------------------|-------------------------|-------------------------------------------------------------------------------------|
|                                                           |                         | behavior changed progressively                                                      |
| <b>Parameter plateau</b>                                  | 11-cycle series         | pH approached ~5.3 while EC and TDS decreased                                       |
| <b>Self-assembly from filtrates</b>                       | >50 observations        | Re-formation of organized phases from filtrate fractions                            |
| <b>Lamellar and plate-like microstructure</b>             | >20 samples             | Observed by optical microscopy and SEM                                              |
| <b>Filament-like structures in selected fractions</b>     | Repeatedly observed     | Observed by optical microscopy of filtrates                                         |
| <b>Freeze-thaw stability</b>                              | ≥3 cycles               | Ability to self-assemble was retained                                               |
| <b>Stability at 70-80 °C</b>                              | Reproducible            | Characteristic behavior was retained                                                |
| <b>Self-organization in saline media</b>                  | Reproducible            | Structured phase formation persisted                                                |
| <b>Mg did not trigger macroscopic self-assembly</b>       | Experimentally observed | Characteristic collapse was absent after MgCO <sub>3</sub> addition                 |
| <b>Ca triggered self-assembly in Mg-containing system</b> | Experimentally observed | Addition of CaCO <sub>3</sub> produced a matrix distinct from the standard material |

#### 4. Bulk Self-Assembly

Once the formation conditions are reached, the system collapses across nearly the entire volume. Two phases emerge: approximately 2/3 hydrated gel matrix and 1/3 transparent or relatively transparent liquid phase. After the initial transition, the matrix continues to densify and stabilize over the following hours. Bulk self-assembly was repeatedly observed at scales of approximately 0.1-1.5 L.

#### 5. Phase Reversibility

Experimentally observed transitions include:

- dispersed liquid state → hydrated matrix
- hydrated matrix → dispersed state
- hydrated matrix → dry solid
- dry solid → hydrated matrix.

Changing water content allows the material to be moved repeatedly between these states.

After drying, the material may form films, dense blocks, or powder. Upon addition of water, these forms return to a hydrated state. During prolonged dehydration of larger volumes, internal layers may remain plastic or paste-like.

## 6. Sequential Washing

Sequential washing was used as an experimental stress test of matrix persistence and of its ability to re-form the gel phase after partial removal of the surrounding liquid. The gel phase re-formed after each cycle.

In one quantitatively tracked 11-cycle series:

- pH changed from approximately 4.08 to 5.35,
- electrical conductivity decreased from approximately 1860 to 334  $\mu\text{S}/\text{cm}$ ,
- TDS decreased from approximately 926 to 168 ppm.

After roughly 7-9 cycles, the measured parameters approached a relatively stable region.

The observations are consistent with early cycles preferentially removing more mobile and weakly associated components while the principal organized architecture remains. As washing progressed, the system showed increased water capacity, greater transparency of the liquid phase, and changes in the character of reassembly.

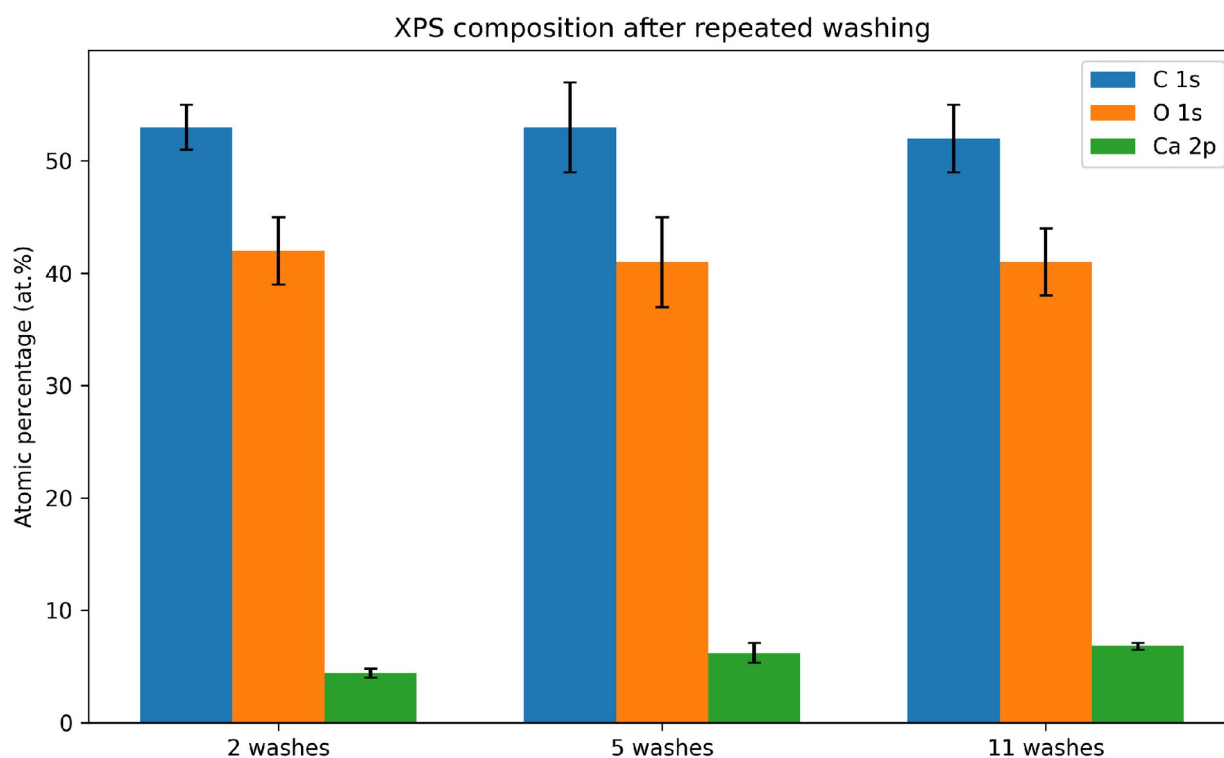


Figure 1. XPS composition after repeated washing. Average surface atomic percentages for C, O, and Ca are shown for dehydrated samples identified as 2, 5, and 11 washes; error bars represent the standard deviation across three analyzed areas per sample.

## 7. Storage and Densification

During storage, the gel phase gradually densifies **without a visually obvious decrease in total volume**, while a transparent supernatant develops above it. After mixing, the matrix returns to a more homogeneous gel-like state. Under comparable conditions, the supernatant was also observed **to dry more slowly than ordinary water**.

## 8. Morphology

Optical microscopy reveals:

- plate-like structures,
- lamellae,
- rectangular and trapezoidal elements,

- elongated structures,
- and filament-like formations in selected filtrate fractions.

SEM of dried material shows a plate-like and lamellar organization with pronounced anisotropy.

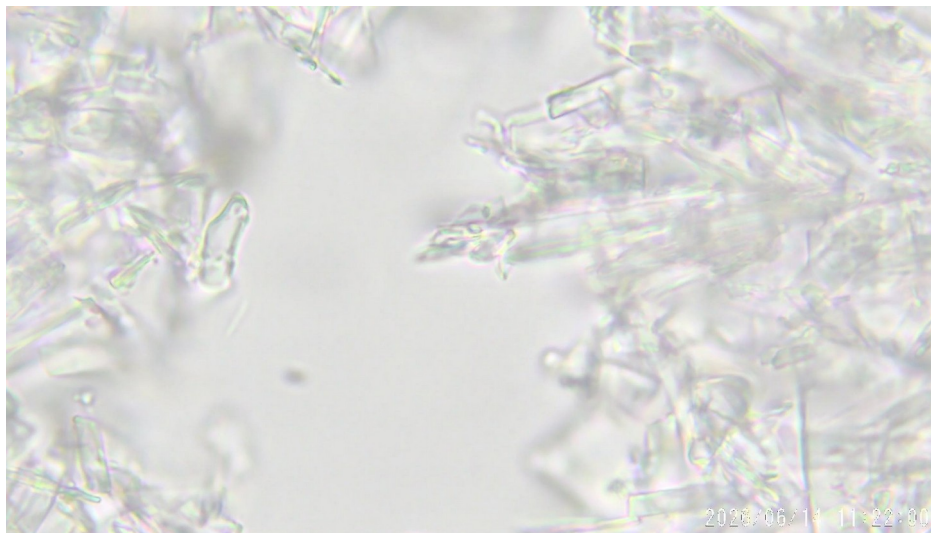


Figure 2. Representative optical micrograph of the 11-wash gel matrix at 1000× magnification, showing anisotropic plate-like and elongated structures.

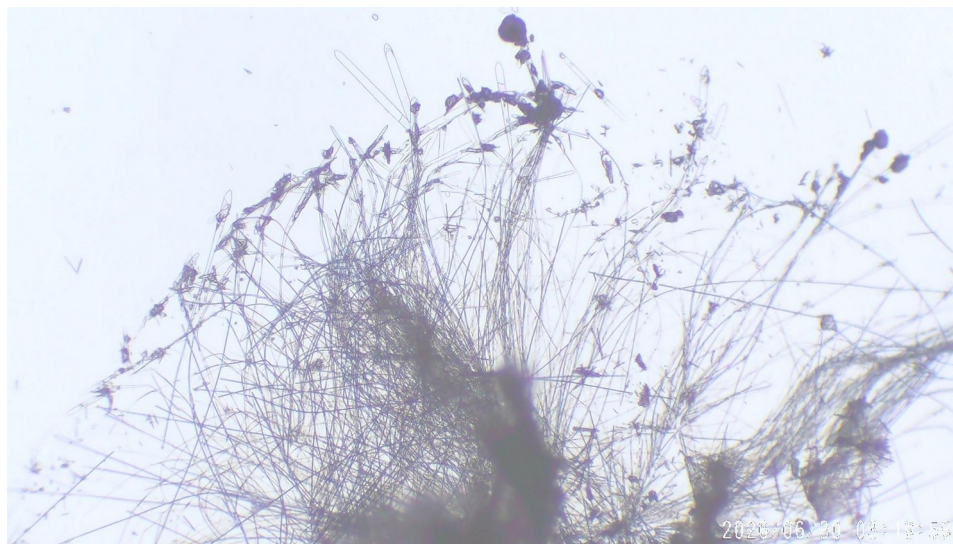


Figure 3. Representative optical micrograph of a filtrate fraction showing filament-like structures together with ribbon-like fragments.

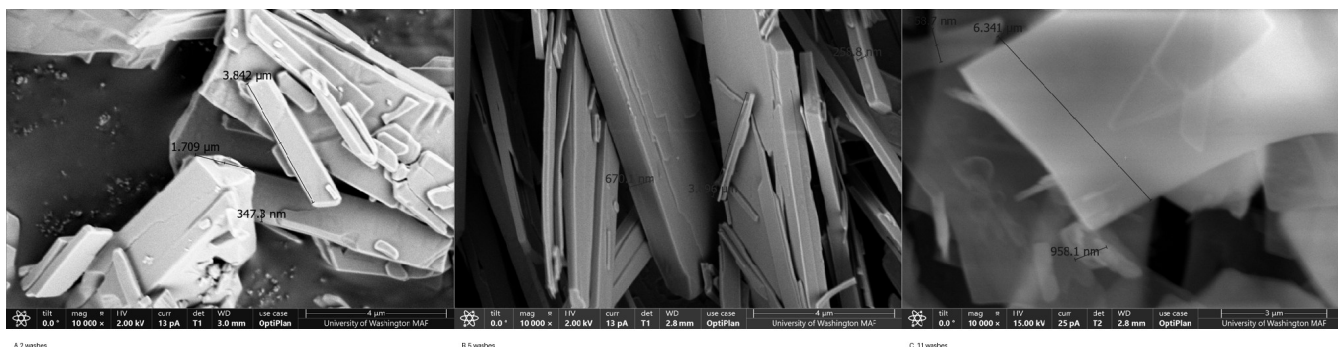


Figure 4. Representative SEM micrographs of dehydrated matrix material after (A) 2 washes, (B) 5 washes, and (C) 11 washes. The images show persistent micron-scale plate-like, elongated, ribbon-like, and lamellar morphologies.

## 9. SEM/EDS

SEM/EDS measurements indicate that the structured plate-like and lamellar regions examined are composed predominantly of **C**, **O**, and **Ca**. This elemental picture is consistent with the XPS results.

## 10. XPS

The principal elements detected at the surface of the analyzed dehydrated samples were C, O, and Ca. Calcium remained present at several atomic percent. The relatively high oxygen fraction compared with carbon is consistent with a strongly oxygen-functionalized carbon system.

Persistence of Ca after matrix formation and subsequent washing is consistent with its association with the structured phase.

## 11. ATR-FTIR

ATR-FTIR spectra of the original hydrated matrix and dried material show a pronounced OH-related region and substantial changes in the spectral profile between hydrated and

dried states. The spectra are consistent with a strongly oxygen-functionalized and highly hydrated system.

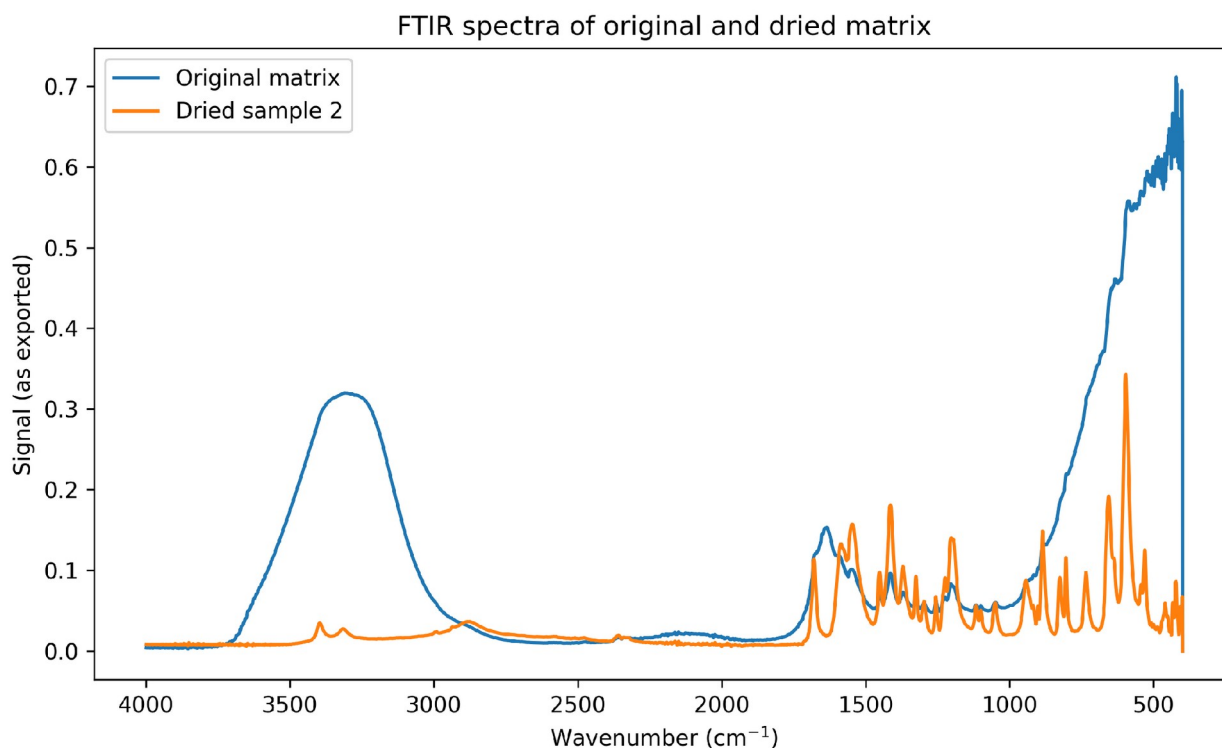


Figure 5. ATR-FTIR spectra of the original matrix and dried Sample 2. The hydrated/original state shows a strong broad OH-associated region and a spectral profile distinct from the dried material.

## 12. Raman Spectroscopy

Raman spectra acquired at 785 nm provide a reproducible vibrational fingerprint of the fullerene-derived carbon component in the analyzed matrix.

In combination with XPS and ATR-FTIR, the data support characterization of the material as **a hydrated fullerene-derived, oxygen-functionalized, calcium-associated matrix.**

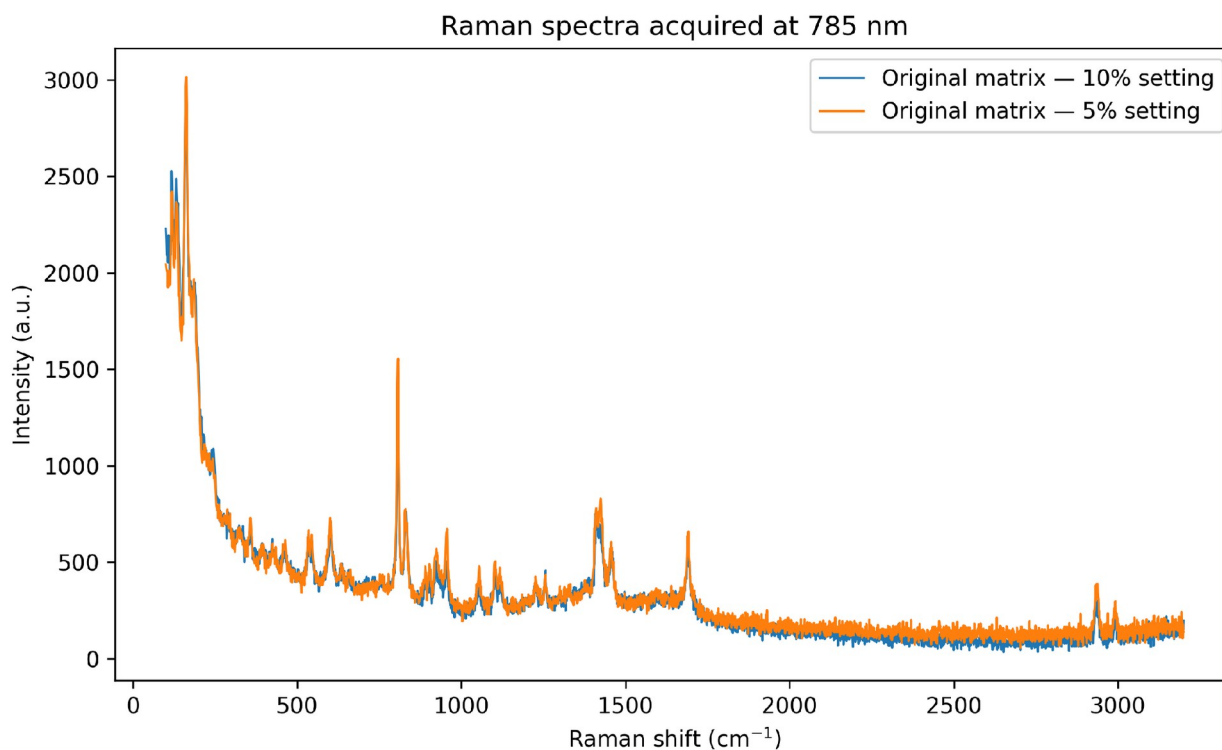


Figure 6. Representative Raman spectra of the original matrix acquired at 785 nm using two acquisition settings. The spectra provide a reproducible matrix fingerprint.

### 13. Mg/Ca Experiment

When MgCO<sub>3</sub> was used instead of the calcium-containing component, the characteristic macroscopic self-assembly was not triggered.

After subsequent addition of CaCO<sub>3</sub>, the system collapsed throughout the bulk volume and formed a hydrated matrix.

The resulting Mg/Ca-containing matrix differed from the standard material in growth behavior, density, and macroscopic appearance.

### 14. Discussion and Working Model

The current analytical data likewise define the material at the level supported by the measurements. XPS and SEM/EDS support the presence of C, O, and Ca in the analyzed dehydrated structured regions; ATR-FTIR supports a strongly hydroxylated/oxygen-functionalized environment; and Raman provides a reproducible fullerene-derived carbon

fingerprint. These measurements do not by themselves establish a unique molecular structure for the matrix.

The Mg/Ca comparison provides preliminary evidence that calcium is relevant to the observed macroscopic transition:  $\text{MgCO}_3$  did not trigger the characteristic collapse under the tested conditions, whereas subsequent  $\text{CaCO}_3$  addition did. This result should not be interpreted as proof of a specific calcium-binding geometry or molecular mechanism. Determining the role of calcium will require controlled compositional studies and additional structural characterization.

The most notable feature of the present observations is therefore the combined property set rather than any single behavior in isolation. Repeated bulk phase formation, persistence after washing, recovery from dry forms, retention of calcium after washing, and anisotropic morphology were observed within the same material system. Whether these behaviors arise from a single hierarchical assembly mechanism or from coupled processes at different length scales remains unresolved.

The closest published comparison identified in the literature search is the 2024 report by Rašović et al., in which covalently functionalized water-soluble fullerene derivatives formed hierarchical supramolecular hydrogels without an external polymer matrix [4]. Their system used fullerene derivatives functionalized to generate self-assembling hydrogel-forming building blocks and emphasized hierarchical structure and mechanical properties. The present system differs in composition and experimental emphasis: it contains a hydroxylated fullerene-derived component together with calcium and is characterized here primarily through macroscopic phase behavior, sequential-wash persistence, reversible dehydration/rehydration, elemental and spectroscopic analysis, and the Mg/Ca comparison.

The present observations should be interpreted in the context of established fullerene aggregation and self-assembly. Functionalized fullerenes are known to form supramolecular assemblies in water, and fullerenol self-assembly has been directly examined in aqueous and biological media [3]. Thus, aggregation or self-organization of a hydroxylated fullerene component alone is not a sufficient novelty claim.

The current working model is a multilevel hydrated architecture in which a fullerene-derived oxygen-functionalized component, calcium, and water form a spatially organized state.

Self-assembly develops as a cooperative bulk transition.

At the local level, the dried and optically observed structures are strongly anisotropic and plate-like/lamellar.

The matrix retains the capacity to recover after changes in hydration, sequential washing, and mechanical disturbance.

## 15. Combined Property Set

The system combines:

- self-assembly,
- high hydration,
- phase separation,
- anisotropy,
- reversibility,
- rehydration,
- washing persistence,
- densification without obvious macroscopic volume loss,
- slow evaporation of the supernatant,
- freeze-thaw stability,
- moderate thermal stability,
- fullerene-derived carbon,
- calcium association,
- fractionation,
- reassembly, and
- compositional tunability.

This combination supports viewing the material as an **adaptive matrix platform** rather than only as an isolated hydrogel.

## 16. Matrix as a Technological Form for Hydroxylated Fullerenes

The matrix integrates multiple states of fullerene-derived material:

- concentrated hydrated matrix  $\rightleftharpoons$  diluted structured matrix  $\rightleftharpoons$  highly diluted aqueous state,
- and hydrated matrix  $\rightleftharpoons$  dried matrix-derived material.

In the concentrated hydrated state, the fullerene-derived component is retained within a structured aqueous environment. Fractionation can yield gel, supernatant, and filtrate fractions. Dilution produces aqueous states with different concentrations of matrix-derived material, while dehydration provides a transportable dry form that can later be rehydrated.

In this sense, **one system can potentially integrate preparation, fractionation, storage, transport, rehydration, controlled dilution, and functional use.**

## 17. Platform for Studying Hydroxylated Fullerenes

The matrix may also be considered both a **reservoir** and a **controllable source** of fullerene-derived components. Fractionation enables separate investigation of concentrated gel, supernatant, filtrates, and highly diluted states.

This creates a platform for examining relationships among fullerene-derived species, hydration, calcium, morphology, and macroscopic self-assembly.

## 18. Potential Application Directions

The combination of high hydration, reversible state transitions, fractionation, and recovery after dehydration suggests that the matrix may warrant future investigation as a functional aqueous material or carrier platform. Potential directions include cosmetic and post-procedure formulations, hydrated interfaces, loading/release systems, composites, and other functional-material applications. These possibilities are hypotheses rather than demonstrated performance claims and require separate evaluation of safety, compatibility, biological activity, transport behavior, and application-specific performance.

## 19. Key Scientific Questions

- What is the minimum self-assembling unit?
- What role does Ca play in initiating bulk collapse?
- Why does Mg fail to initiate macroscopic self-assembly yet alter matrix properties after Ca is introduced?
- How is water organized within the system?
- What determines the anisotropy?
- Why do sequential washes change material properties without eliminating the ability to self-assemble?
- Why does the matrix densify without an obvious decrease in total volume?
- Why does the supernatant evaporate more slowly than ordinary water?
- Which fullerene-derived species are present in different fractions and material states?
- How does solution composition alter matrix properties?
- Can loading and release of additional components be controlled?

## 20. References

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## 21. Conclusion

A reproducible hydrated fullerene-derived matrix was obtained through a bulk collapse process that typically produced approximately 2/3 gel phase and 1/3 liquid phase.

The system demonstrated phase reversibility, recovery after dehydration, resistance to repeated washing, freeze-thaw cycling, and moderate heating.

During storage, the gel phase densified without obvious macroscopic volume loss, while a transparent supernatant formed.

Optical microscopy and SEM showed anisotropic plate-like and lamellar morphology. SEM/EDS and XPS showed predominantly C/O/Ca composition, ATR-FTIR characterized a strongly oxygen-functionalized and hydrated environment, and Raman spectroscopy provided a reproducible fullerene-derived carbon fingerprint.

Magnesium did not trigger the characteristic macroscopic self-assembly, whereas subsequent introduction of Ca produced matrix formation with altered properties.

Taken together, the results support describing the system as an **adaptive hydrated fullerene-derived, oxygen-functionalized, calcium-associated matrix**.

The broader significance of the system lies in the experimentally observed combination of macroscopic self-organization, reversible hydration-state changes, washing persistence, anisotropic morphology, and calcium association. Possible use as a technological



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fullerene-handling or functional aqueous platform remains a future research direction rather than a demonstrated application.

### **Invitation for Independent Verification**

We welcome independent verification of these observations and collaboration with research groups working in aqueous self-assembly, fullerene-derived materials, soft matter, hydrated interfaces, and functional materials. Samples of the system may be made available for collaborative research and comparative analysis.

### **Intellectual Property and Disclosure Note**

Specific preparation parameters are intentionally omitted because they are the subject of ongoing intellectual-property protection. The manuscript is intended to provide sufficient detail to evaluate the reported material behavior and analytical characterization without disclosing proprietary manufacturing know-how. A journal submission should be reviewed for patent strategy before public disclosure.

### **Acknowledgement**

Part of the analytical work shown here was conducted at the University of Washington Molecular Analysis Facility (MAF).