Research Article

"To gel or not to gel"- process variables investigation of polylactide-based nano-organogel suggests the missing link

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Abstract "To gel or not to gel" in supramolecular gel science is shroud with mixed frustration-to-mysteries even after over two decades of vigorous research. Efforts to resolve the gel puzzle came strongly when major players in this field correlated molecular gelation with solvent properties, while adopting solvent-gelator – a two-phase system as the rational way – an approach that led to the reassessment of over 100's of individual gelators by various groups using Meta analysis. Despite the several efforts, the gel puzzle still exists. It is our believe that understanding the effect of process variables in a few case-by-case studies of "privileged" scaffolds gelators using the design of experiments approach will provide a useful insight into the gelation mechanisms. Hence in this study, we have used simple 3^2 factorial designs to examine the effect of process variables of an *in-situ* formed PLA-based nano-organogel. We found that low stirring rate, the presence of tert-butyl catechol (TBC) and continuous gas flow (in this case nitrogen) are necessary experimental conditions for the nano-organogel to form. We concluded that the presence of continuous nitrogen gas flow for gel formation, suggests that gelation should also be viewed as a three-phase system (solvent-gelator-gas), rather than, entirely, the solvent-gelator two-phase system that has always been adopted.

Keywords: Gel, supramolecular gel, factorial designs, nano-organogel, process variables

Introduction

The low molecular weight gelators (LMWGs) are small organic molecules known to possess gel-forming abilities (1-6). Depending on experimental conditions, these molecules do self-assemble and immobilized organic solvents or water to form organogels or hydrogels, respectively. In the last decade, there has been increasing interest in the research of LMWGs, particularly as they now find applications in a variety of fields including biomedicine, drug delivery, material science, cosmetics, environmental sciences etc. (3-5). These molecules that self assemble to form supramolecular structures are judged to form gel-constructs through secondary interactions like H-bonding, π-stacking, metal coordination, donor-acceptor interaction, hydrophobic forces, electrostatic, or van der Waals' interaction (3-8).

While several gels have been developed with well define structure-property correlation by employing advanced synthetic methodologies (3-4), it is a common knowledge among researchers in this field that their first discoveries are usually by serendipities.

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In the last two decades, efforts have been directed toward rational design and synthesis of molecular gelators, as well as providing, at molecular level, understanding of gelation mechanisms. Partly, these efforts have been successful to the extent that it has resulted in over 1000 LMW gelators, yet the success story is tainted with dark shades, as there is still no clear path to understanding the mechanism of gel formation. Thus, to gel or not to gel is still an elusive puzzle, particularly when compounds that are structurally similar to a gelator, for unknown reasons, do not form gels. Efforts to providing some very useful insight to solving the 'gel-puzzle' continued to pursue investigations into two-phase gelling systems, namely: solvent and gelator. In this regard, various groups have conducted extensive studies and Meta analysis of over 100's individual gelators, and many insightful, but complex conclusions have been correlated, largely, to the solvent properties (6, 9-11). Till date, to the best of our knowledge, no study has rationally investigated the effect of process variables in gel formation, but rather the focus has been on molecular engineering approach of parent gelators to provide other LMWGs. It is our believe that understanding the effect of process variables in a case-by-case studies of few serendipitously found gelators will not only lead to discovering gelation potentials of 'molecules' labeled as non-gelators, yet structurally similar to a known gelator, but also help to close the gap between to gel or not to gel. Thus, we have used simple statistical design of experiments using 3^2 factorial designs to examine the effect of some process variables in *in situ* formation of PLA-based nano-organogel.

Design of experiments (DOE)

Design of experiments (DOE), otherwise known as experimental design, is a systematic method to determine the relationship between factors affecting a process and the output of that process. DOE is often used in four main engineering problem areas, namely: comparative, characterizing, modeling, and optimizing. In optimization evaluations, the interest is to determine optimal setting of the process variables (12). In DOE, two main design approaches are often used to evaluate, simultaneously, the effects of participating variables in a process, namely: factorial and mixture experimental designs (13). Factorial design is a technique introduced by Fisher in 1926, and it provides a means whereby the factors that may have influence on a reaction or process can be evaluated simultaneously and their relative importance assessed. Consequently, it is a means of separating those factors that are important from those that are not. It is often used to find optimum conditions for a process (13). Factorial designs have been used to investigate the effect of processing parameters on the size of PLGA nanoparticles (14), and on characteristics of poly(lactic acid) nanoparticles prepared by the double emulsion method (15).

This work shows, among other things, that the perceived several mysteries behind molecular gelation phenomena could be due to the overlooked role of gas phase. In other words, to solve the puzzle of to gel or not to gel, there is need to explore also the solvent-gelator-gas flow landscape as a three-phase system, rather than, entirely, the solvent-gelator two-phase system model, as the rational way to go.

Materials and Methods

Materials

L-lactide (Polyscience Inc.) was recrystallized twice with toluene before use. Toluene (Acros, 99%) was purified by drying over CaH_2 to remove moisture and oxygen and distilled under reduced nitrogen atmosphere. Hydroxyethyl methacrylate (HEMA) (Aldrich, 97%) was dried over molecular sieves (4 A°) for 24 hours and distilled under negative pressure before use. Stannous octoate (Sigma, 95%) and phosphorous pentaoxide (Aldrich, 97%) were used as received. All other reagents were of analytical grade and used without further purification.

Synthesis and Purification of Nano-organogel

These were carried out as previously reported (16). Briefly, it involves the novel ring-opening-dispersion polymerization of monomer of L-lactide and HEMA, as initiator. The reaction was carried out using stannous octoate as a catalyst.

The mole ratio of monomer/initiator, as L-lactide/HEMA (1:1) was used after preliminary experiment showed that that mole ratio gave a low molecular weight product.

Effects of Process Variables on the Synthesis of Nano-organogel

The effects of process variables on the reaction synthesis were investigated. Such variables as nitrogen gas flow, presence or absence of tert-butyl catechol (TBC) and stirring rate on the ring-opening-dispersion polymerization reaction were varied, while temperature, monomer: initiator feed ratio and catalyst concentration were kept constant. The 3^2 factorial design was adopted. In this design, the following factors and levels were selected.

Factor X1: Nitrogen purging (Absent = -1; present = +1)

Factor X2: Tert-butyl Catechol (TBC) (Absent = -1; present = +1)

Factor X3: Stirring rate (Low = -1; high = +1)

The response to be measured is the formation of nano-organogel (or supramolecular gel), which is a solid product that self assembles in the reaction vessels. The gel products, after purification, were then assessed for presence of characteristic functional groups (FT-IR), thermal property using TGA, average molecular weight using MALDI-MS, size (DLS), and morphology (SEM). Table 1 shows how the experiments were set up.

Table 1. Three-Factor, Two-level Factorial Design to Investigate the Formation of PLA-based Nano-organogel

Runs	Experiment	Factor X1 Nitrogen gas flow	Factor X2 TBC	Factor X3 Stirring Rate	Formed Product
1.	(-1, -1, -1)	Absent	Absent	Low	Liquid
2.	(+1, -1, -1)	Present	Absent	Low	Supramolecular gel formed
3.	(-1, +1, -1)	Absent	Present	Low	Liquid
4.	(+1, +1, -1)	Present	Present	Low	Nano-organogel formed

5.	(-1, -1, +1)	Absent	Absent	High	Liquid
6.	(+1, -1, +1)	Present	Absent	High	Liquid
7.	(-1, +1, +1)	Absent	Present	High	Liquid
8.	(+1, +1, +1)	Present	Present	High	Liquid

Note: Liquid product formed, means no gel was formed; TBC is Tert-butyl Catechol.

Supramolecular gel and nano-organogel are both gel products; the supramolecular gel is used going forward for gel whose particles are greater than 1000 nm.

Results and discussion

In our previous paper (16) we reported the synthesis of a low molecular weight gelator (LMWG) that self assembles in excess organic solvent HEMA, which acted as the initiator in the reaction. The novel ring-opening-dispersion polymerization reaction that generated the LMWG is presented as scheme 1. A closer look at the LMWG shows it as an amphiphile, with both polar head and a non-polar tail – a perquisite for supramolecular structure.
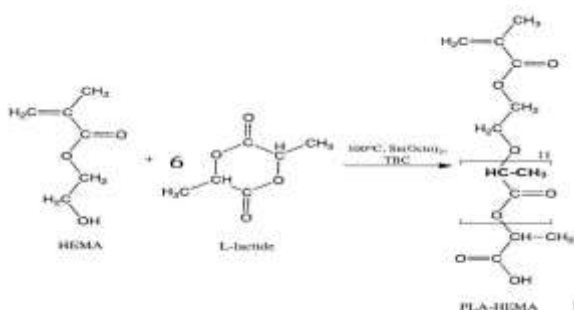
Scheme 1: Reaction scheme for the synthesis of polylactide-based low molecular weight gelator, PLA-HEMA.

Effect of process variables

The results of the investigation of process variables indicate that low stirring rate, nitrogen gas flow, and presence of TBC are important for optimizing conditions for the synthesis of mono-disperse discrete nano-organogel.

Effect of stirring rate: We found out, as evidenced in Table 1, that in the *in-situ* formation of supramolecular gel (nano-organogel), stirring rate is an important factor. It was observed that the gel products are formed only at a low stirring rate (see experimental runs 2 and 4 in Table 1). The effect of stirring rate could be explained as follows: the high stirring rate does not allow sufficient time for the required equilibrium condition to be attained – possibly due to none formation of necessary intermediate product (as previous report (16) identified) that could drive the reaction to the formation of a gelator, which could have self-assembled in excess initiator, HEMA, to form the nano-organogel.

Effect of nitrogen gas flow: On the use of gases in flow synthesis, Mallia and Baxendale (17) noted that flow of gases affects: the yield; presence of intermediate product; the resident time in which the reaction is carried to completion, such as shorter reaction times; and gas could be used as quencher to suppress the formation of some byproducts e.g. dimerization. In this *in situ* formation of polylactide-based gel, the nitrogen gas flow is essential to keep the reaction chamber inert – free of oxygen so that the polymerization could proceeds to completion. This is consistent with the general use of nitrogen gas purging for chemical reactions that require an inert environment. Additionally, we found out that continuous nitrogen gas flow is required for the amphiphilic molecule to aggregate and self-assemble to form the gel-construct (see

experimental runs 2 and 4 in Table 1). This inference was first made when it was observed in a preliminary investigation aimed at establishing the optimum ratio of the monomer: initiator that after overnight experimental run, no gel was formed because the nitrogen gas supply had run out, but when nitrogen gas was restored, gel was formed. To further validate this observation, nitrogen gas supply was intentionally removed 4 h into another experimental set up and the reaction allowed to continue for another 20 h, but no gel product was formed. And on re-introduction of nitrogen gas flow, the gel began to form.

The explanation for the additional role of nitrogen in this reaction is as follows: The formation of nanoaggregates, like micelle formation, is an air (gas)-liquid (solvent) interface phenomenon. The synthesized amphiphilic PLA-HEMA gelator on the interface assumes an orientation to form a monolayer film, such that the double bond end remains in the solvent (excess HEMA), and the polar end (i.e. -COOH group) remains in the air (in this case, nitrogen gas). Beyond a certain concentration of the amphiphile in the liquid (solvent), monolayer formed film begins to self-assemble to form nanoaggregates, which then move from the interface into the liquid (see Scheme 2). Gas (air)-solvent interface is essential for the formation of the monolayer state (18-19), which is a perquisite for the formation of nano-organogel or gel. In the absence of nitrogen gas (serving like the air interface) there is no formation of the monolayer state; and consequently, there is no formation of nanoaggregates. It should be noted that the amphiphilic property is required for aggregation (19-20).
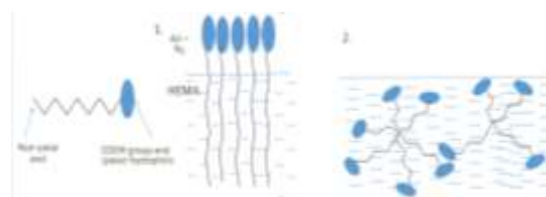

Scheme 2: Schematic diagram showing the effect of nitrogen in the formation of supramolecular gel or nano-organogel. In the presence of nitrogen there are: (1) the formation of monolayer film, (2) the formation of nanoaggregates

Effect of TBC: Previous investigators (21-23) have indicated that a suitable inhibitor was required in the ring-opening copolymerization reaction to prevent competitive reactivity between the end capping double bonds and the hydroxyl group of HEMA. In our experiment, TBC was to function as a suitable inhibitor. However, we observed that in the absence of TBC, the *supramolecular gel* product was still formed (see experimental run 2 in Table 1). This indicates that the hydroxyl functional group is more reactive and precludes the participation of the double bonds.

In order to assess the characteristics of the supramolecular gel/nano-organogel formed in the absence/presence of TBC, the gels formed from experimental runs 2 and 4 (Table 1) were evaluated for the presence of characteristic functional groups (FT-IR), thermal behavior (TGA), average molecular weight (MALDI-MS), particle size, and morphology (SEM). The results are compared in Figures 1-5.

The FTIR spectrum of the product (*supramolecular gel*) formed in absence of TBC is the same in all-characteristic peaks with that of nano-organogel synthesized in the presence of TBC (see Figure 1). The characteristic peaks are 3486, 2990, 1726, and 1640 cm^{-1}, which correspond to

hydroxyl, C-H stretch, an organic acid carbonyl, and alkene groups, respectively.
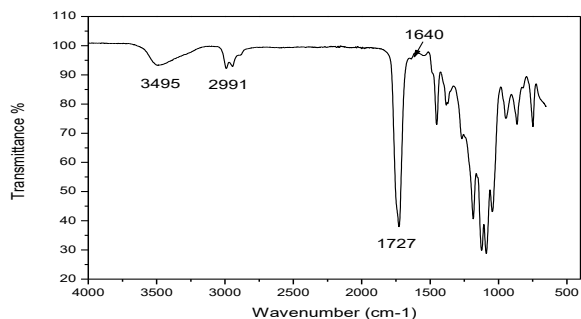
Figure1. FT-IR spectrum of the nano-organogel, representing the characteristic functional groups of the gel

The TGA curves for gel materials are similar. The onsets of thermal degradation of the gel products are 203.85°C and 201.04°C in the presence and absence of TBC, respectively (see Figure 2).
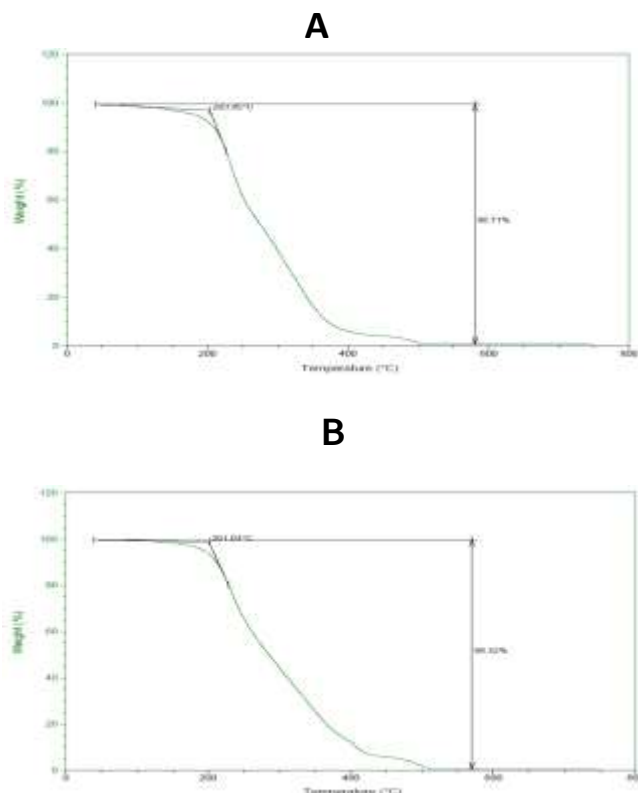
Figure 2: TGA curve of gels; (A) nano-organogel synthesized in presence of TBC, (B) supramolecular gel synthesized in the absence of TBC

The average molecular weights for both products are comparable (see Figures 3a and b). The supramolecular gel synthesized in the absence of TBC has an average molecular weight of 854, while it is 994 for nano-organogel obtained in the presence of TBC. It should be noted that to account for Na+ cationization, 23 is subtracted from the molecular peak values (875 and 1017) in both spectra. The molecular peak is the last peak observed on the spectrum (24).
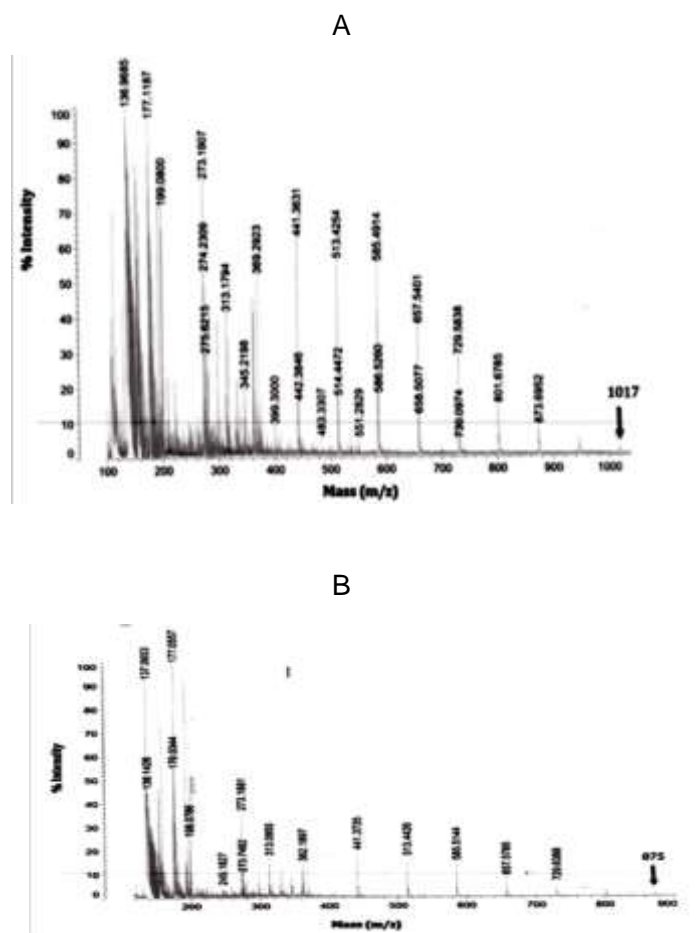
Figure 3: MALDI-MS spectrum of (a) nano-organogel synthesized in the presence of TBC, and (b) supramolecular gel synthesized in the absence of TBC

The above comparable results of the FT-IR, TGA and the MALDI–MS (Figure 1-3) assessments of the gels synthesized in the absence and presence of TBC, almost

suggest that TBC is not an important factor in this *in-situ* gelation. However, the SEM, the particle size and particle size distribution results clearly demonstrated that the TBC plays a very significant role (see Figures 4 and 5).

A

Size in μm

B

Size in μm
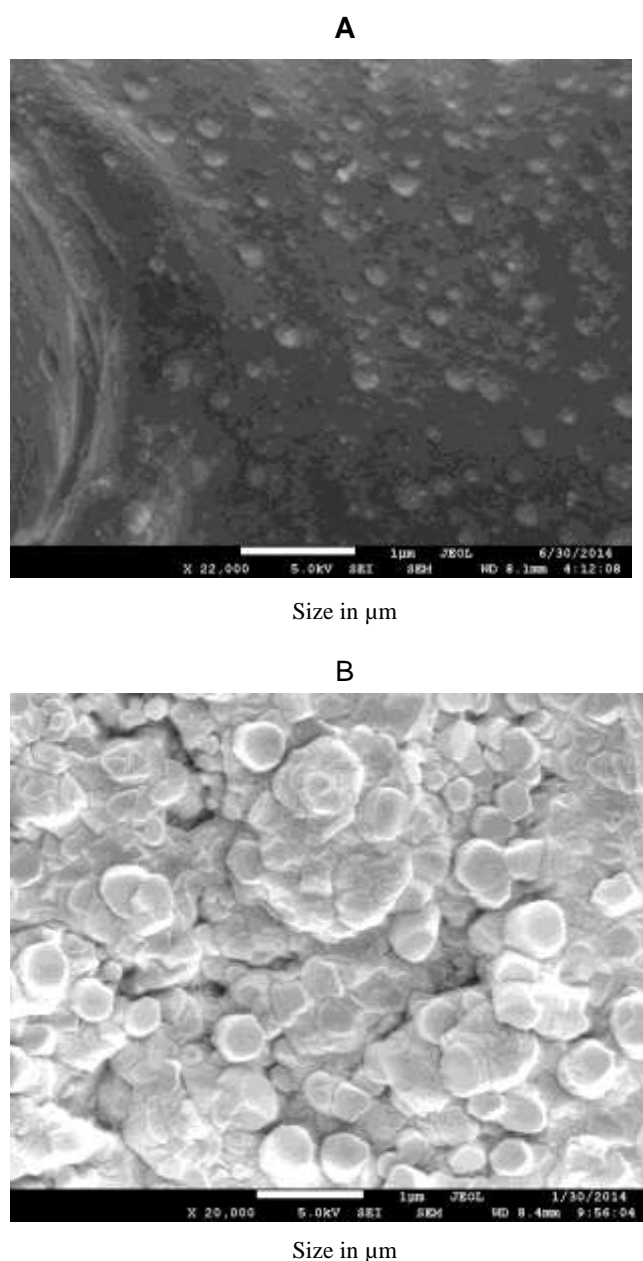
Figure 4: SEM of synthesized gels, showing the effect of TBC: (A) absence of TBC, (B) presence of TBC (nano-organogel is formed))

Figure 4 shows the effect of TBC on the morphology of the gels. The figure shows that in the absence of TBC, the gel particles are not discrete and mono-dispersed, as well as not in nanometer size. In contrast, nanomeric size,

mono-dispersed discrete particles are observed in gel formed in the presence of TBC – hence the name nano-organogel. Kawaguchi and Ito (25) reported that the inclusion of a stabilizer in a dispersion polymerization reaction is an important factor for preparing mono-dispersed nanoparticles/microparticles. Since in this experiment, mono-dispersed nanoparticles are formed in the presence of TBC, it can be concluded that TBC functioned as a stabilizer rather than an inhibitor, as reported previously (21, 23).

A

B
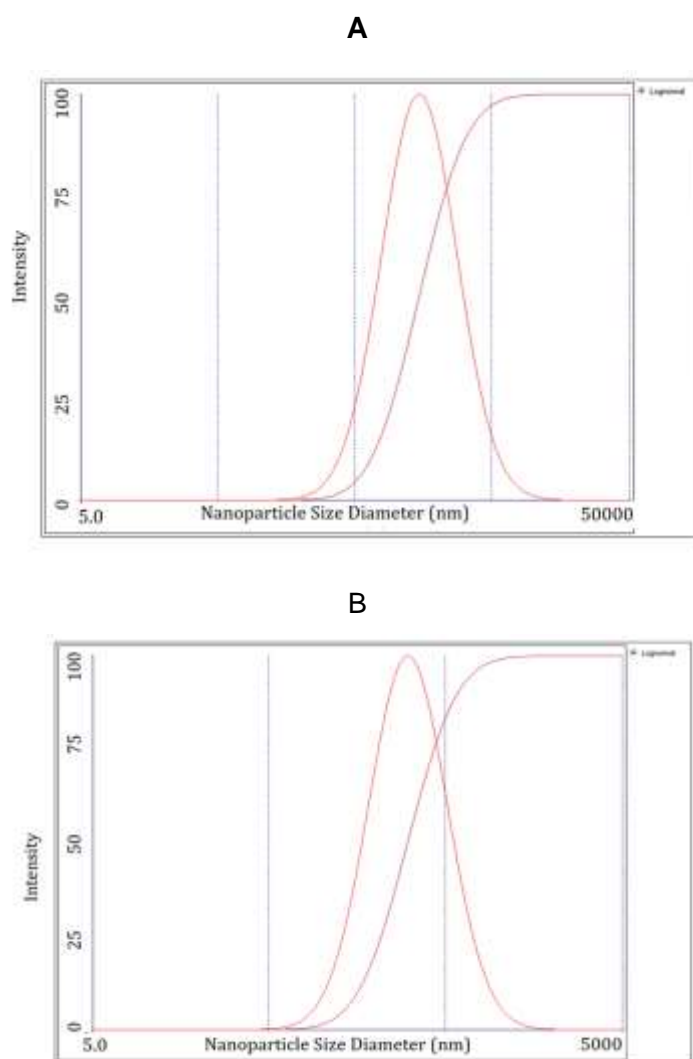
Figure 5: Particle size distribution of synthesized gels, showing the effects of TBC: (A) absence of TBC (particle size = 1474 nm, PD = 0.5), (B) presence of TBC (particle size = 307 nm, PD = 0.3)**.**

Finally, Figure 5 shows the size characteristics of the gels: (a) in the absence of TBC and (b) in the presence of TBC. The size averaged 1474 nm with a polydispersity index (PI)

of 0.5 for *supramolecular gel* synthesized in the absence of TBC, while the size is 307 nm with a PI of 0.3 for nano-organogel synthesized in the presence of TBC. PI values of ≤ 0.3 indicate a fairly mono-dispersed narrow size distribution, and PI values ≥ 0.5 indicate broad size distributions (26). On the basis of the foregoing, it can be seen very clearly that while gels are formed in both the absence and presence of TBC. However, nanomeric particles gel (nano-organogel) is formed only in the presence of TBC - the implication of this finding is that the addition of a stabilizer (such as TBC) to previous chemical reactions that investigated supramolecular gels syntheses or formations could result in the formation of nano-organogels.

Conclusion

In summary, the studies on the effects of process variables demonstrated that low stirring rate, continuous flow of nitrogen gas, and the presence of TBC contribute to the optimization of conditions for synthesizing mono-dispersed discrete nano-organogel in this facile reaction. In particular, the findings are:

 i. Tert-butyl Catechol (TBC) is not a necessary requirement for the formation of gels.

 ii. However, for the formation of discrete nanomeric size gel particles (nano-organogel), TBC is required.

 iii. Continuous nitrogen gas flow and low stirring rate are required for the formation of gels.

 iv. And more importantly, at least in this study, that the presence of continuous nitrogen gas flow is needed for gel formation, suggests that gelation as a process of forming gel should also be viewed as a three-phase system (solvent-gelator-gas), rather than, entirely, the

solvent-gelator two-phase system that has always been adopted. This approach, we believe, could provide information to closing the gap between 'to gel or not to gel' puzzle and consequently change our views on molecules which are structurally so similar to gelator molecules (the "privileged" scaffold gelators, as some authors would prefer to call them), yet do not form gels.

Author Contributions

*Frank O. Ohwoavworhua (corresponding author: **frankohwo@gmail.com)** carried out the experimental synthesis, characterizations and investigation of the effects of process variables, as well as wrote the main manuscript. James W. Mitchell supervised all aspects of the work. Both authors reviewed the manuscript.

Competing interests

The authors declare no competing interests

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