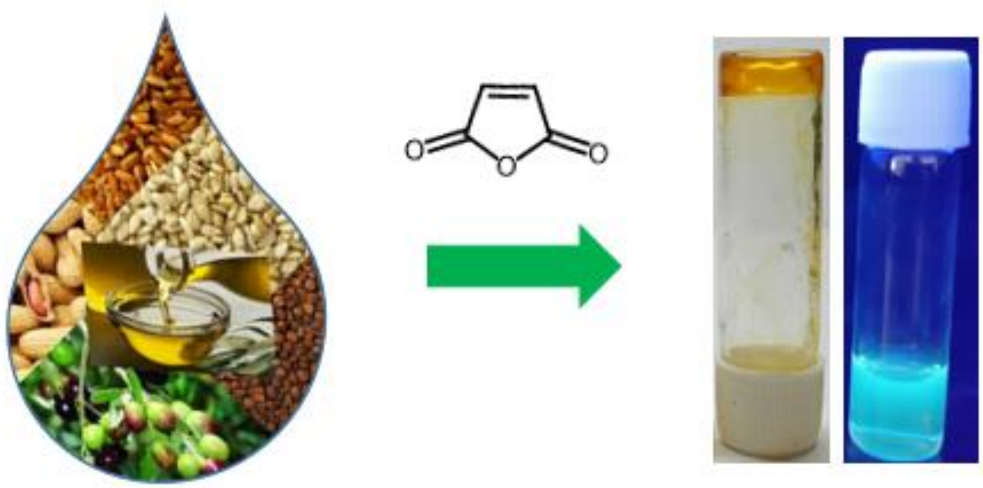
Chapter 5

Unusual Fluorescence compounds from Oleylamine :



Fluorescence and its Potential Applications

5.1 Introduction

Fluorescent biomaterials have become a focus of research in biology due to their broad applications in cellular imaging, bio-sensing and drug delivery. In the fluorescent materials studies include fluorescent organic dyes, fluorescent proteins, and quantum dots. Some aliphatic biodegradable synthetic polymers, which show intriguing photoluminescence phenomena.[1] New fluorescent materials with special photo- and/or bio-active features are very attractive for both scientific and applicative reasons. For fluorescent organic compounds, existence of some units with large π-conjugated systems and rigid planar structures is a necessary prerequisite according to the classical theory.[2] However, in recent years, strong blue fluorescence from several types of amine-containing polymers without conventional fluorophores was observed, for example, poly (amido amines) (PAMAM), polyurea dendrimer, poly ethylene imine, poly (amino esters), which have been successfully applied in biological imaging. Obviously, the photoluminescence mechanism and properties of amine-containing compounds are quite different from those of the current fluorescent materials, and amine groups are thought to be responsible for the fluorescence emission, owing to the lone-pair electrons on their nitrogen atom. Importantly, amine-containing compounds are expected to be good candidates for optical and biological materials, because of their outstanding features such as high polarity, biocompatibility and water-solubility relative to conventional chromophores. To date, research studies in this field raise several questions. Firstly, although fluorescence emitted from tertiary amines has been extensively studied, the fluorescence from primary and secondary amines is seldom observed, which has been explained in terms of loss of the hydrogen atom via predissociation.[2] Tertiary aliphatic amines of different structures display different fluorescence behaviours due to the formation of different excimers, such as α, ω-diaminoalkanes form various intramolecular excimers. The quantum yields of tertiary amines measured in various solvents are very low generally due to quenching of the solvents, which is explained by possible energy transfer and electron (or charge) transfer. So, the photoluminescence behaviours of tertiary amine chromophores are obviously different from the current fluorescent materials, such as fluorescent organic dyes and quantum dots. Synthesis of the biodegradable, biocompatible, and water-soluble fluorescent polymers containing tertiary amine is relatively easy. Drawbacks of the current chromophores, such as cytotoxicity and photo-bleaching, might be avoided.[1] Secondly, studies on fluorescent amine-containing polymers have been reported by many groups. However, there are only a few reports on amine-containing small molecules, due to their weak emission in the visible spectral region. Thirdly, there is no reliable and facile method to fabricate fluorescent amine-containing compounds; although some methods such as acidification, oxidation and synchrotron radiation have been proposed, these methods are cumbersome and are effective only in the case of amine containing polymers.[2] One-step heat strategy with outstanding characteristics of versatility, rapidity and facility to obtain fluorescent amine-containing compounds. In a heating process, fluorescence peaks of amine-containing compounds shift gradually from the ultraviolet spectral region to the visible region. They reported two important features for amines: (a) many kinds of amine-containing compounds such as small molecules, oligomers and polymers are found to emit strong fluorescence after heat treatment and (b) not only tertiary amines but also primary and secondary amines can act as fluorescent moieties. Hence, a broad class of fluorescent amine-containing compounds without any conjugated unit is revealed. Upon heating at 90 ºC under ambient condition, colourless pristine HP-PAMAM gradually turned pale yellow and could emit luminescence. A gradual increase in the red shift and intensity of fluorescence emission was observed. This variation is likely a consequence of formation of a new N→O chromophores, as a result of oxidation by oxygen in air. By this way, primary, secondary and tertiary amine groups can act as all-round building blocks for the design of a wide range of fluorescent compounds. Significantly, many amine-containing compounds have a close relationship to biological activity.[2] Poly (amido amine) (PAMAM)-type dendrimers are actually the most tested dendrimers as drug vectors; however, their cytotoxicity is a major drawback. The construction of biocompatible and biodegradable water-soluble dendrimers with urea within the interior and amino groups on the periphery. The dendrimers show a pH-dependent intrinsic blue fluorescence at very low concentrations (Fig. 1). They also present an absence of acute cytotoxic effect for Human Fibroblast cells. Endocytic uptake (including nucleus) was observed without disruption of the cell membrane, thus showing a high potential for biomedical applications.[4] A variety of N-alkyl carboxylic acid amides was prepared from the reaction of cyclic dicarboxylic acid anhydrides and various amines and screened for anti-rust properties and antimicrobial activity in spent coolants of water-based cutting fluids. The triethanolamine salts, adduct of maleic anhydride with octylamine, decylamine, dodecylamine and oleylamine. Phthalic anhydride with octylamine showed both good anti-rust and antimicrobial activity. However, fluorescence property of these kind of adducts were not reported.[3]

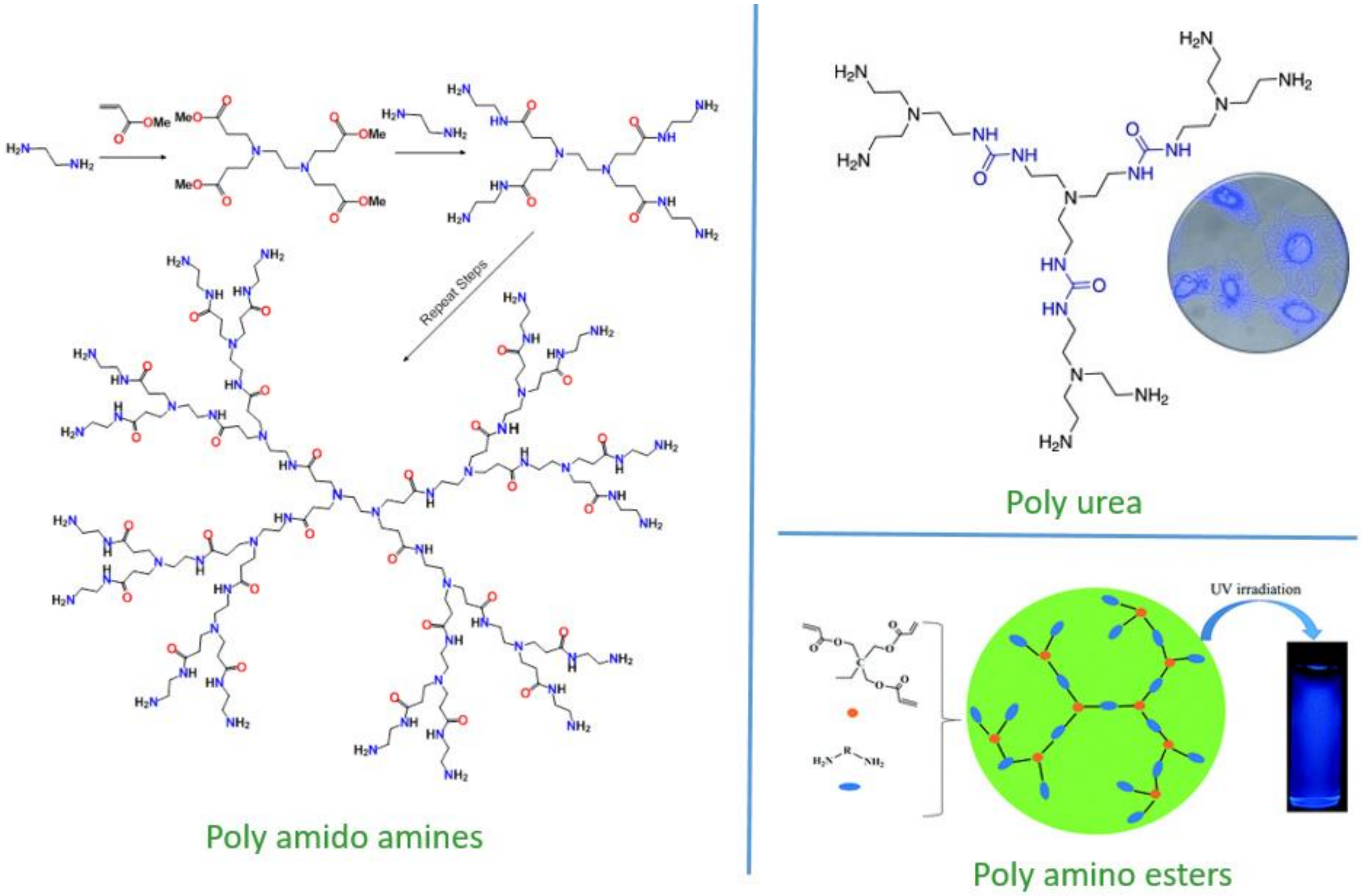
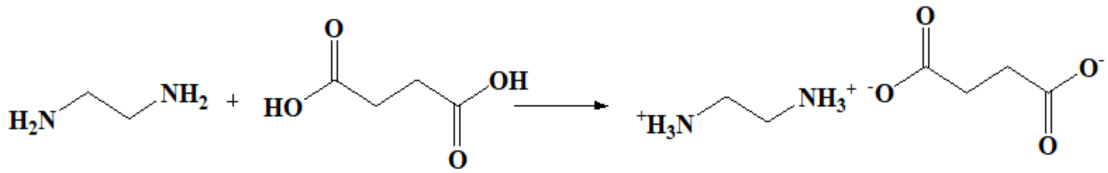


Fig.1. Amine containing non-conjugated polymers which showing fluorescence.[2, 4]

Maleimide fluorophores with remarkable fluorescent properties such as tunable emissions among the visible colour range (blue to yellow), high fluorescence quantum yields (up to 64%) and solvafluorochromism. They investigated the effect on optical properties when altering the halogen group (Cl, Br and I) and the donor groups (amines and alcohols). This, in turn, will aid the design of future maleimide fluorophores with small size, multi-functionalization and high fluorescence efficiency for biological and chemical sensing applications.[5] The development of novel organic fluorophores is of great interest in the areas of biological labels and probes, medical diagnostics and photoactive materials. The rational design of organic fluorophores has provided a straightforward method for generating various fluorescent functional materials. Currently, many bulky planar fluorophores suffer from complex syntheses, large size and poor solubility, limiting their applications in fluorescence labelling and functionalization. Among reported organic dyes, maleimide-based fluorophores, one of the smallest fluorophores, exhibit promising properties, such as high emissivity, large stokes shifts, and ease of modification. Unsubstituted maleimides have been extensively reported as effective fluorescence quenchers through direct conjugation to fluorophores. The low-lying n–π* transition of the maleimide ring can provide a non-radiative pathway for excited state decay and significantly reduce the emission of fluorophores.[5] The breakthrough done by Garg, Szostak and Zou groups in the metal-activation of amidic bond, several methodologies have been developed involving amides as electrophilic coupling partners in cross-coupling reactions. Diverse amides have been studied and among them, N-Boc-amide has shown good activities towards various cross coupling reactions and a high synthetic usefulness. However, some drawbacks exist concerning these N-Boc amides. First of all, the N–Boc bond is a weak bond and can be cleaved in some harsh reaction conditions especially at high temperature. This would lead to a complete deactivation of the amidic bond and limit the possible reaction with them. Second, concerning their synthesis, classical methods to introduce Boc group were completely ineffective notably for hindered substrates and therefore they have to be prepared from acyl chloride. Consequently, future challenges would be to find new activating groups which could be introduced easily and possess strong bond in order to avoid potential scission of the N–activating group bond. It would be also interesting to find new catalytic systems allowing the decarbonylative process using these N-Boc amides in mild conditions. This would enhance the use of N-Boc amides as arylating or even alkylating coupling partners.[6] Despite the continuing popularity of radical reactions in organic synthesis, the field is far from being exhausted and much remains to be explored. Nitrogen-centred radicals, for instance, have not received the attention they deserve and their considerable synthetic potential has remained largely unappreciated.[1] Progress in this area has been slow, hampered hitherto by a dearth of convenient routes for generating these reactive species and a lack of awareness concerning their reactivity. There are broadly two approaches for generating nitrogen radicals. The more general route involves the scission of a weak N–X bond, where X is a halogen (excepting fluorine), a nitrogen, an oxygen, or a sulphur group. Cleavage of the strong N–C bond is possible in special cases, such as in aziridinylmethyl radicals or in cyclohexadienyl systems, where aromatisation provides the necessary driving force. It can be included in the category of direct oxidation reactions, where an N–H bond is ultimately broken. The second strategy is indirect; it relies on a radical addition to an unsaturated nitrogen group such as a nitrile, an imine, an oxime, a hydrazone, or an azide. Only cases where the nitrogen radical is captured to produce a new C–N bond, and not merely reduced by hydrogen atom transfer.[7]

Zhen et al recently reported, room-temperature phosphorescence (RTP) in the non-aromatic organic small molecule cyanoacetic acid (CAA), with a fluorescence lifetime (τ) value as long as 862 ms. The strong intermolecular interactions through hydrogen bonds are the main driving force for its phosphorescence emission. The obtained experimental results confirmed that without the presence of classic aromatic groups, RTP is observed in nonaromatic pure organic compounds, opening a new angle for the consideration of organic luminogens. Also, RTP presents for the CAA crystal but not in solution, which demonstrates the importance of molecular packing and presence of possible molecular interactions. This phenomenon could be termed as a Molecular Uniting Set Identified Characteristic (MUSIC). Perhaps, the traditional thoughts on the light emitting materials should be further expanded from the aromatic to nonaromatic ones, with much deeper understanding of the inherent mechanism.[9]

Quingui Wang published that, the aliphatic amide salt, like ethylene succinimide salt, precipitated out by blending ethylene diamine with succinic acid in methanol. Similar amide salts were prepared with diamines and diacids of different carbon number, and they were all fluorescent with a quantum yield of about 8%.



Scheme 1. Reported synthesis of amide salt (ethylene succinimide salt).

They focused on the amide salt, revealed that this amide salt was fluorescent in their solid powder and in their aqueous solution, a novel type of non-conventional luminogen. The emission intensity was in constant increase distinctly with the increase in concentration due to cluster formation. Among fourteen metal ions tested, only Cu^{2+} was shown to quench the amide salt emission with a good linearity between its concentration and the emission intensity. Cytotoxicity evaluation of the amide salt against HeLa cells revealed the amide salt was not toxic, of good biocompatibility and was effectively used for cellular imaging and Cu^{2+} monitoring. Compared with all reported luminogens up to date, this amide salt is facile to prepare with low costing, of high photostability, excellent water solubility, low cytotoxicity, and easy to scale up production. All these features endow the amide salt with great potential as a sensitive fluorescent probe for cell imaging and sensitive detection of Cu^{2+} under physiological conditions (Scheme 1).[8]

As per above literature, amine containing and non-conjugating fluorescence is rarely studied. It was proven that tertiary amine dendrimers can show the blue fluorescence. After that it was also proven that small molecules, oligomers containing even primary and secondary amine also show fluorescence by heat treatment or forming the amide salt. Fluorescence intensity can be enhanced by aggregation induced effect (AIE) due to H-bonding.

Conventional drug formulations such as tablets, solution, suspension and emulsions have limitations of high dose, low availability, instability and nonlinear plasma concentration. Novel drug carriers are needed to meet the requirement of model drug delivering agents. The nanoparticles based drug delivering agents have sizes ranging from 10-100 nm. Due to small size, they have the advantage of increased solubility of the drug and therefore its bioavailability. Nano-carriers have benefits of increased half-life in the body for clearance, increased specificity for its receptors, and targeted delivery for low drug concentration and reduced non-target tissue toxicity. It helps to cross organ or tissue barriers due to small size.[10] The nano-carriers for drug delivery are made from natural or synthetic polymers. Natural polymers include chitosan, alginate, xanthan gum, lipid, protein and carbohydrate. Synthetic polymers include linear polymers like PLGA, PGA, PEG and hyper branched globular polymers called dendrimers like PAMAM. Inorganic metal nano-carriers also have been synthesized and evaluated for drug delivery including gold, iron, manganese, silver etc.[11] metal nanoparticles are more toxic than polymeric nanoparticles due to their smaller size, varying shape and morphology, surface charge and therefore its catalytic activity.[12] Polymeric nanoparticles are inexpensive, biodegradable, biocompatible, non-immunogenic, and nontoxic.[13]

In this study, we synthesized a low molecular weight gelator (LMWG) by using oleylamine (mono amine) and maleic anhydride (Fig. 2). Enhancing the fluorescence intensity by incorporating reactive anhydride for π bonds in the system. Free radical forming agent used for aggregation induced effect (AIE). Prepared compounds were applied for fluorescence material for drug carrier property. The prepared compounds were also explored for specific and selective metal sensors.

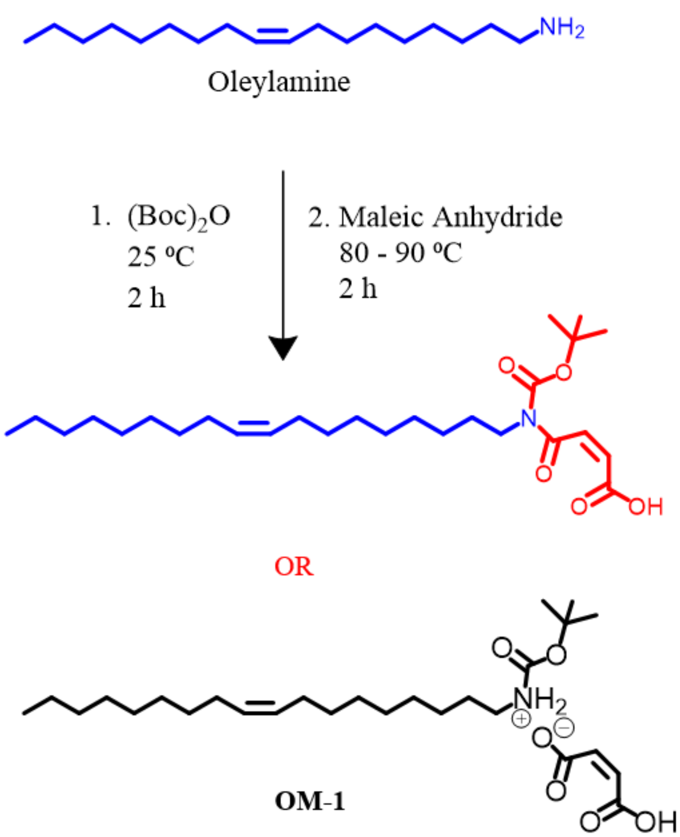
5.2 Experimental

Materials

Oleylamine, dodecylamine, maleic anhydride, succinic anhydride and maleic acid from S. D. Fine Chem. Ltd. Di-tert-butyl dicarbonate (Boc anhydride) from Spectrochem Private Ltd. and 2,2′-azobis(2-methylpropionitrile) (AIBN) from Avara Synthesis Private Ltd. Benzene, ethyl acetate, acetone and other solvents from S. D. Fine Chem. Pvt. Ltd.

Synthesis of OM-1 and its gel form

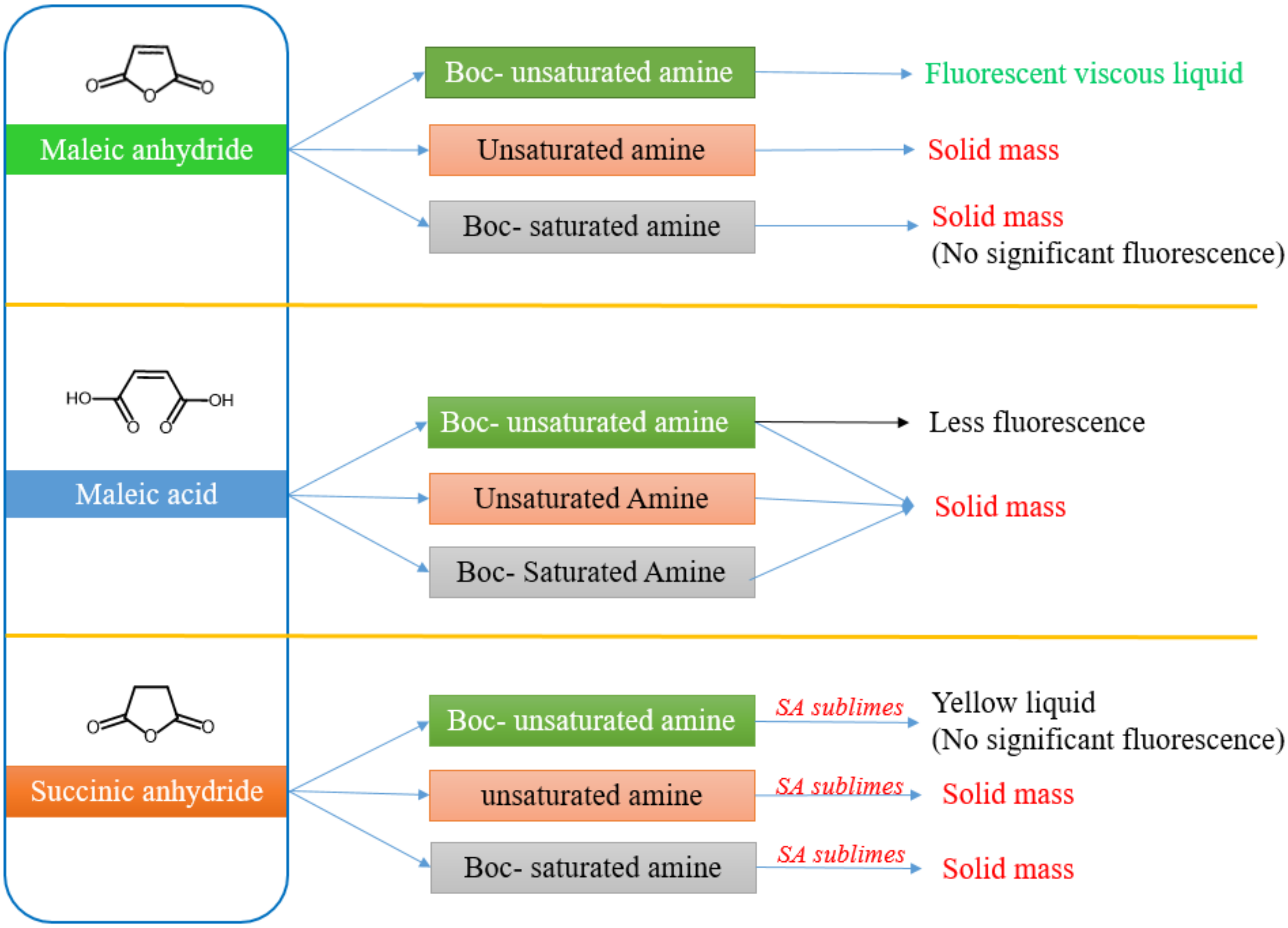
Maleic anhydride (2.0 g, 0.02 mole) and Boc protected oleylamine (7.5 g, 0.02 mole) were charged in a RBF having 100 mL capacity. The RBF was kept in cool condition using ice-salt to freeze the reactants and apply vacuum to remove trapped air and refilled with nitrogen gas, the process was repeated for 5-6 times (freeze-thaw method). The flask was placed in oil bath under nitrogen atmosphere and applied temperature to 80-90 ^{o}C for 24 h. Dark yellow reaction mass viscous liquid obtained. The reaction mass was washed with petroleum ether (5 mL*3 times) to remove unreacted Boc-oleylamine and AIBN followed by water washes (10 mL*2 times) to remove unreacted maleic anhydride. The viscous mass was dried at 80-90 ^{o}C under vacuum. Fluorescent viscous liquid (**OM-1)** was used for characterization and application purpose. For the formation of gel, during the process, radical generating reagent AIBN (0.33 g, 0.002 mole) was added (Scheme 2).



Scheme 2. Synthesis of fluorescence compound (**OM-1**) from Boc-oleylamine and maleic anhydride.

Visible light UV light (365 nm) Gel formation with AIBN

Fig. 2. Images of **OM-1** in visible and UV lights with its gel form.



Scheme 3. Design of experiments (DoE) to understand the emergence of fluorescence.

5.3 Results and Discussion

To understand the cause of fluorescence of **OM-1**, we designed experiments to understand the role of functionality. We synthesized the same material by systematic changing the functionality present in the molecular framework. We did these by either removing Boc protection or altering anhydride functionality in the molecular framework. For the elimination of Boc protection from the amine, we just allow to react maleic anhydride with olelyl amine under the same reaction conditions. We observed the semi-solid product. However, the intensity of fluorescence of the semi-solid product was significantly low. This observation suggests that the Boc protecting group of the amine has a significant role in fluorescence as it is responsible to generate tertiary amine in the molecular framework. To eliminate unsaturation from the anhydride moiety, maleic anhydride was replaced by succinic anhydride keeping the other experimental conditions same. It resulted the semi-solid product and showed negligible fluorescence (Scheme 3).

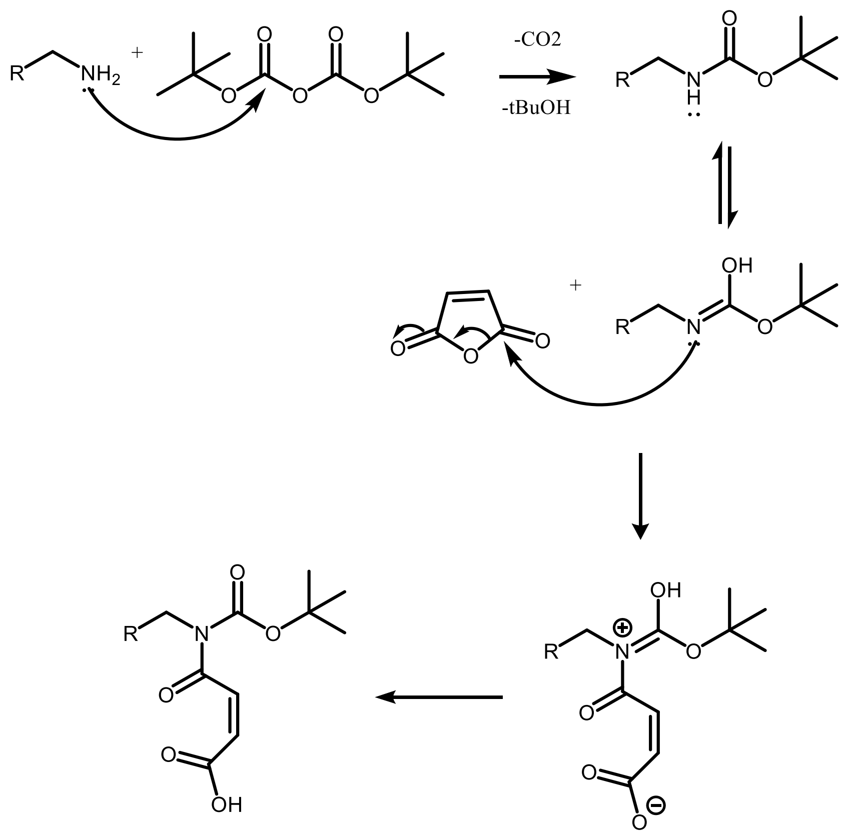
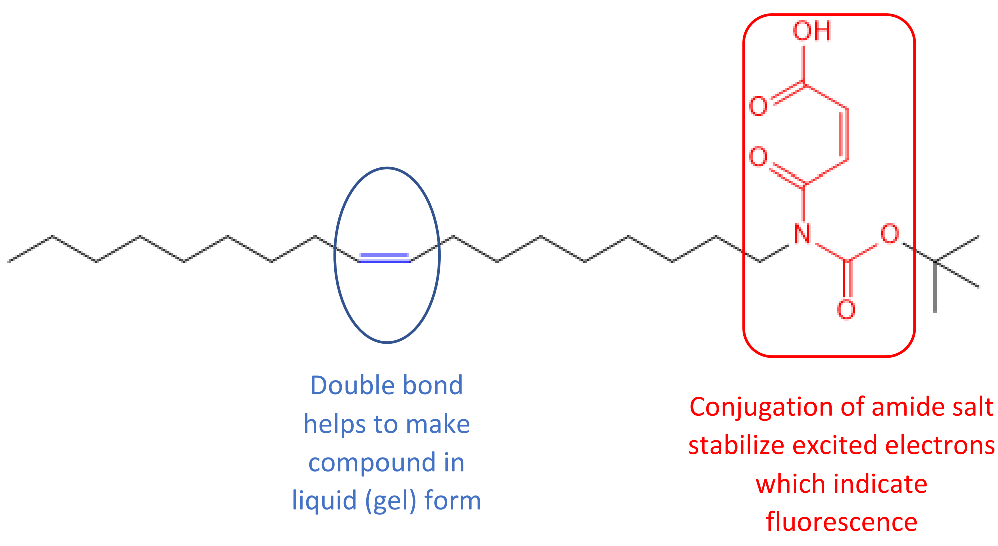


Fig. 4. Proposed mechanism for the formation of **OM-1.**

To understand the role of anhydride moiety in fluorescence, experiments were carried out on reacting maleic acid with Boc-oleyl amine, oleyl amine and dodecyl amine. There was no fluorescence observed at 90 ºC as reaction mixture was not in homogeneous phase due to melting point of maleic acid is higher than 90 ºC. So, on increasing temperature to 120 ºC, it forms a dark brown coloured gel mass. CO_2 effervescence also observed in case of Boc oleylamine. Oleylamine was found better instead of dodecyl amine. There is a quite possibility to form amide salts with maleic acid. On addition of radical initiator (AIBN) during the reaction between maleic anhydride and olelyl amine under solvent free conditions, gel product was formed rather than viscous liquid. Surprisingly, the product showed intense fluorescence than the liquid form. It indicates that the radicals have an important role to form aggregate by bonding the molecules through radical reactions which form the gel. It infers that entanglement of molecules plays an important role to alter the intensity of fluorescence (Fig. 2). Hence, all the functionalities of the as-synthesized molecule (**OM-1**) contribute to enhance the fluorescence or reduce the effect of quenching interactions. Maleic anhydride lowers the melting point of corresponding acid, Boc group forms carbamate which increases the hydrogen bonding sites, alkene bond of maleic anhydride play a role for intermolecular interactions among the molecules which forms a stable fluorescence gel of amide based compound **OM-1**.

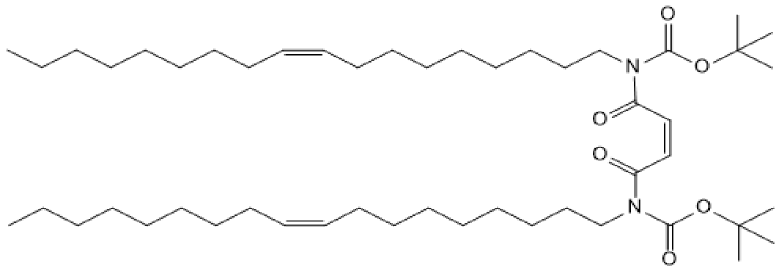
The physical properties of **OM-1** were evaluated by solubility behaviour, its thermal and rheological stability, oligomers content and fluorescence estimation. It was found soluble in very specific solvents having dielectric constant 5-20, such as ethyl acetate, acetone, diethyl ether, chloroform and tetrahydrofuran due to the hydrophilic and lipophilic contents as well as ionic characteristic. It was not soluble even in acidic as well as in alkaline water (Table S1). The gel permeation chromatography (GPC) analysis revealed the presence of 78% of monomer, 12% of dimer and 10% of tetramer in **OM-1** (Fig. S9).

The structures of **OM-1** monomer and dimer are predicted on the basis of the spectroscopic analysis (^{1}H, ^{13}C, DEPT 135 NMR and FTIR) (Section 5.6.2) and literature references.[8]

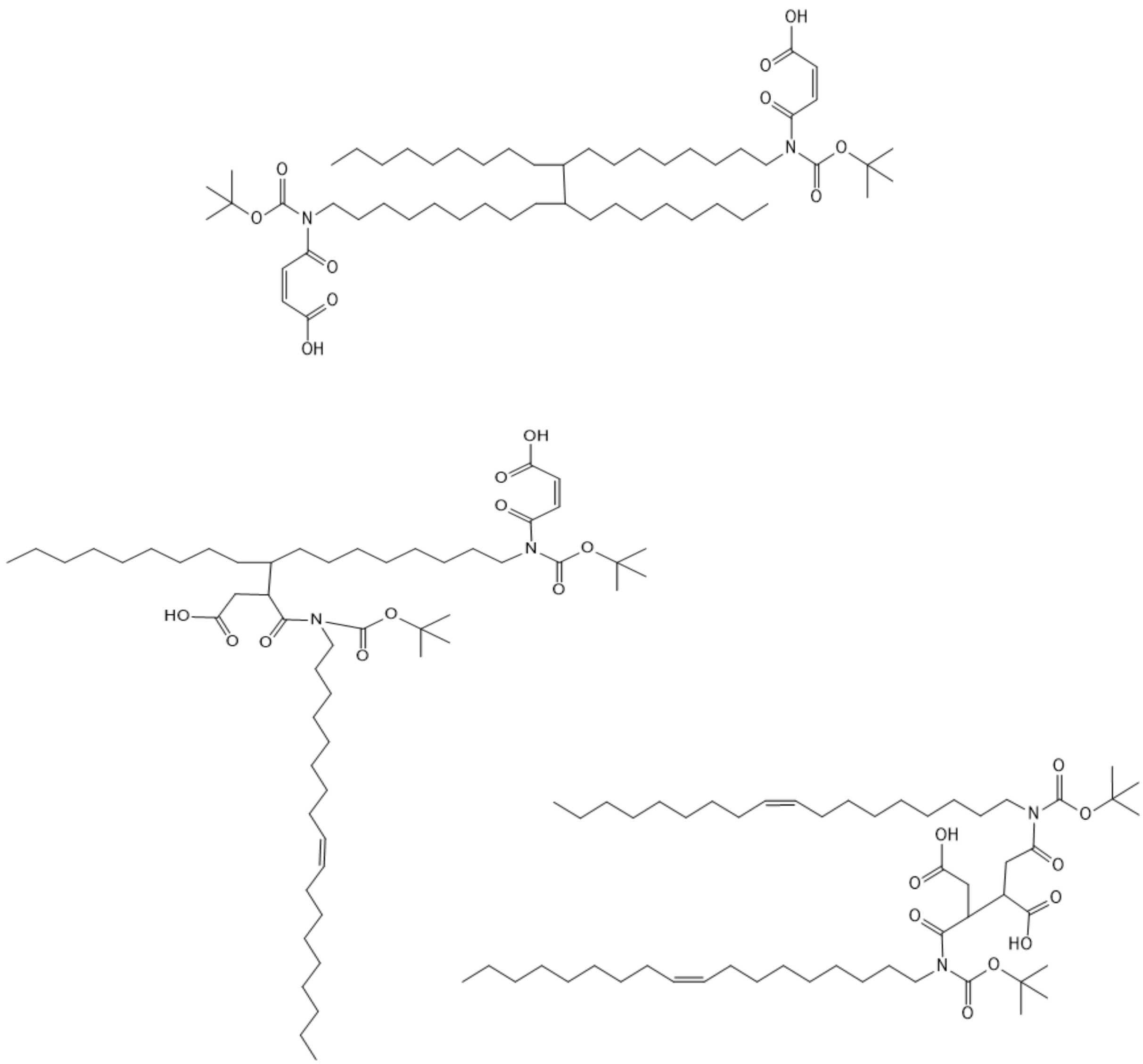


Molecular weight: 483.68 g/mole

Fig. 5. OM-1 functional group importance for material properties.



Molecular weight: 851.29 g/mole



Predicted dimer, Molecular weight: 969.38 g/mole

Fig. 6. Predicted structures of **OM-1** monomer and dimer.

Molecular weights from GPC analysis seems quite matching with its theoretical mass calculation. The thermal behaviour of **OM-1** gel was studied by DSC. The phase transitions at 20 ºC (Tg) and 88 ºC (Tm) was observed (Fig. S10). It was thermally stable up to 170 ºC measured by TGA (Fig. S11). The rheological studies indicated that the fluid behaviour was matching with Newtonian fluids and viscosity gradually decreased with increasing the shear rates (Fig. S12).

Fluorescence properties of **OM-1** gel was estimated by quantum yield and excitation-emission matrix (EEM) analysis (Fig. 7). The 5.3% quantum yield was measured which was matched with the reported fluorescent amide compound.[8] Optimum condition for the fluorescence was 460 nm emission wavelength at 355 nm excitation wavelength, estimated from EEM analysis. Fluorescence intensity was observed increasing gradually with dilution. The fluorescence of **OM-1** was affected by acidic as well as alkaline pH which indicated the presence of ionic structure. While experimenting with 20 metal salts, an interesting observation captured for the selectivity and sensitivity for a specific metal ions. The fluorescence of **OM-1** quenched in presence of Cu^{+2}, Fe^{+2} and Fe^{+3} metal ions in aqueous solution and unaffected by Mn, Co, Ni, Cu^{+}, Zn, Pd, Cd, Ce, Nd, Li, Na, K, Pb, Mg, Ca, Ag and Hg salts in aqueous solution (Fig. 12).

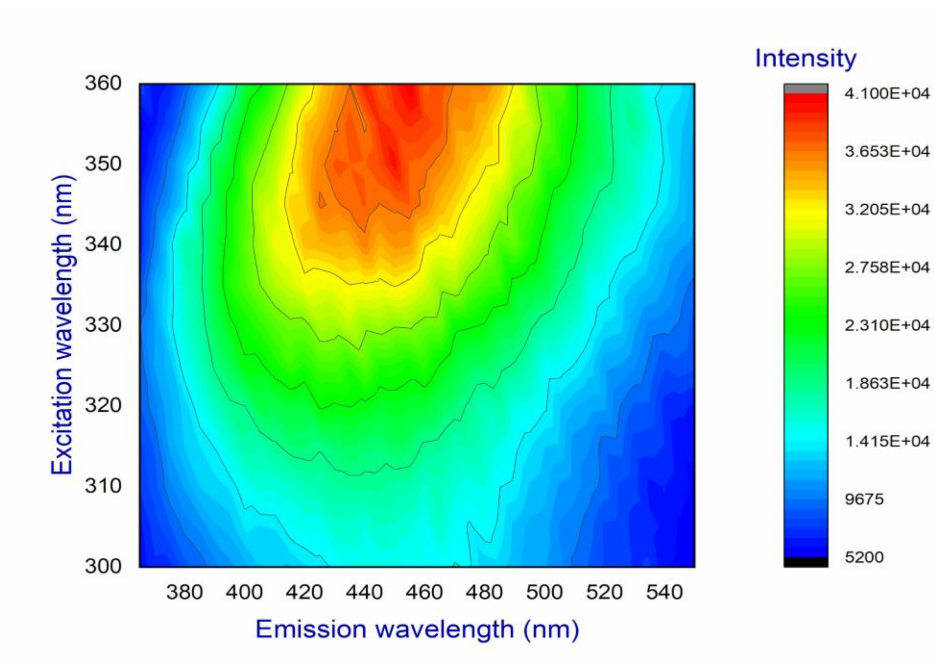


Fig. 7. Contour plot of **OM-1** EEM analysis.

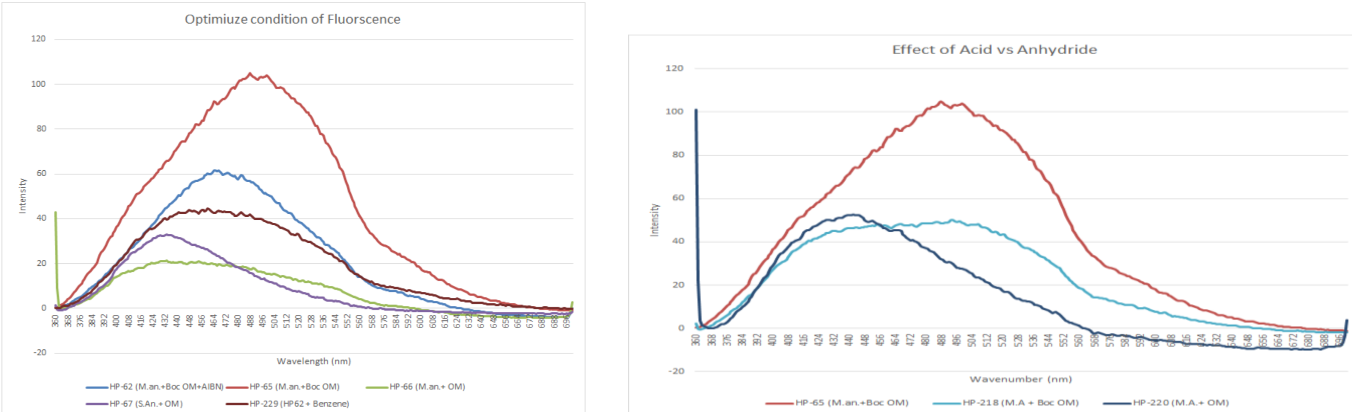


Fig. 8. (a) Optimization of fluorescence condition (b) effect of maleic acid instead of maleic anhydride.

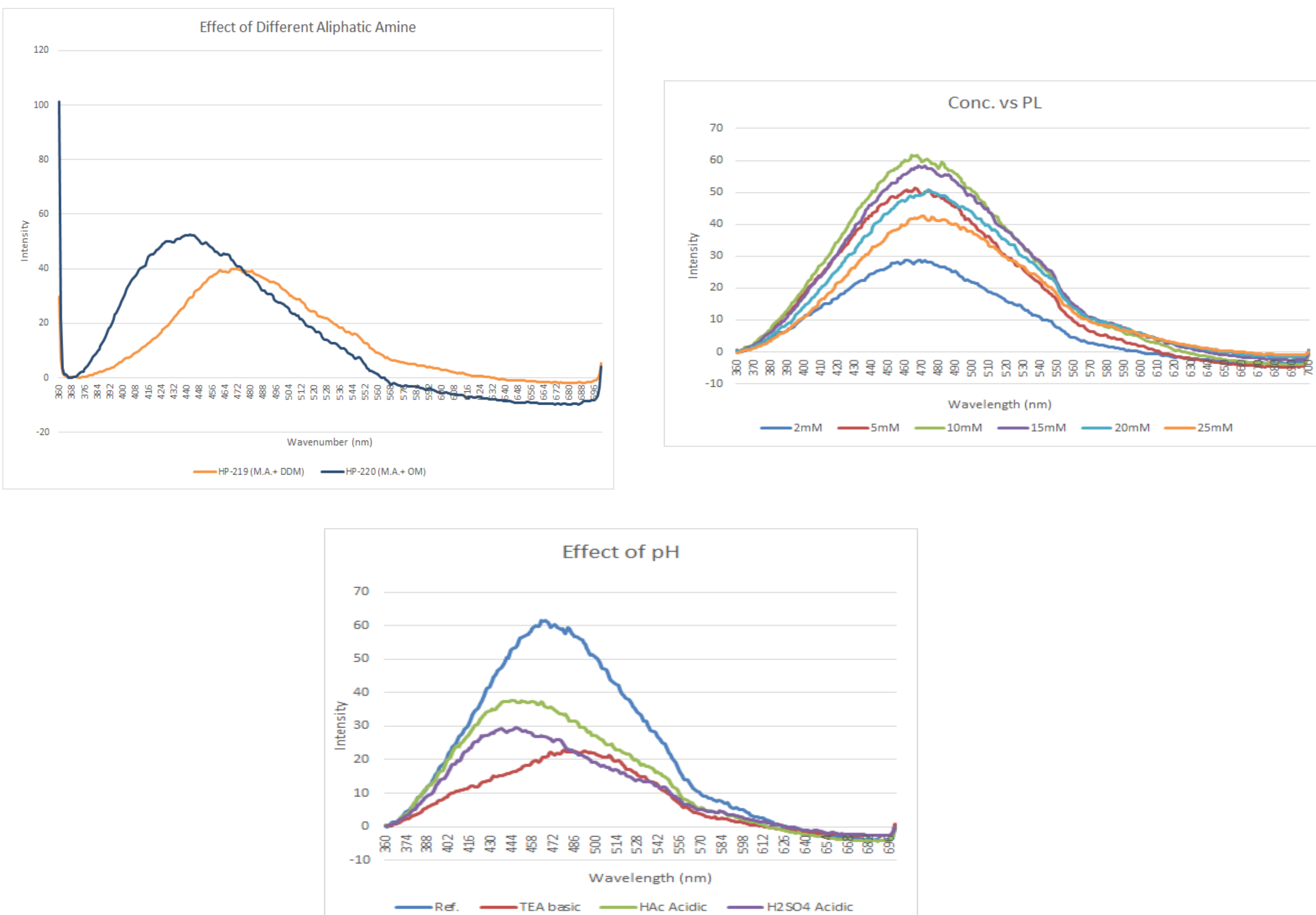


Fig. 9. (a) Effect of dodecyl amine instead of oleylamine (b) optimum concentration for high fluorescence (c) effect of pH on fluorescence.

5.4 Potential Applications of as-synthesized OM-1

5.4.1. OM-1 as an azithromycin/erythromycin drug carrier

Synthesis of drug loaded nanoparticles

5 mg of **OM-1** and 2.5 mg of antibiotic (Azithromycin (Az) or Erythromycin (Er)) were dissolved in 1 mL of acetone. The solution was added drop wise to 12 mL of water in a round bottom flask under high speed agitation at 800 rpm to form drug loaded nanoparticles AzNP and ErNP. The nanoparticles stirred overnight to evaporate acetone. Blank nanoparticles (BNP) were prepared without using any antibiotics and following the same protocol as above. The formed nanoparticles were dialyzed against water for 30 min using 14 kDa molecular weight cut off membrane to remove unloaded drug. The nanoparticles of **OM-1** characterized by particle size analysis and lyophilized for FTIR analysis. Drug loading on **OM-1** was confirmed by UV analysis (Fig. S13-S16).

Antimicrobial activity of drug loaded nanoparticles

20 mL of sterile Luria agar poured in a sterile petri dish and allowed to solidify for 30 min. 5 mL of 0.8% soft agar was allowed to cool down at 40 ºC. Then, 0.5 mL of bacterial culture was added and mixed well. The mixture poured onto the Luria agar plate and allowed to solidify for 30 min. The wells punctured using sterile cup borer with 6 mm diameter. 50 µL of nanoparticles, dilutions 1:2 and 1:10 were added to the agar plate well. For positive control 1 mg/mL solution of antibiotic was used. The plates kept in a refrigerator for 10 min. Then plates transferred to incubator at 37 ºC for 18 h. The zone of inhibition measured in mm. All the work was done in sterile condition. The overnight grown pathogenic bacterial culture was used for the antimicrobial study (Fig. 11).

Microscopic analysis of nanoparticles

The **OM-1** nanoparticles mounted on a glass slide and viewed under fluorescence microscope with excitation wavelength 265 nm. The particles have spherical shapes with different sizes. The smallest size of nanoparticles observed constantly moving under microscope with 100X magnification, hence not able to capture image for size measurement. The particles smaller than that could not be visible under the microscope due to its resolution limit. From microscopic analysis, all particles have a globular form and few particles have a big size in the range of 1.5µ to 12µ (Fig. 10). The compound **OM-1** was fluorescent hence, could be used as a tracker molecule for various applications.

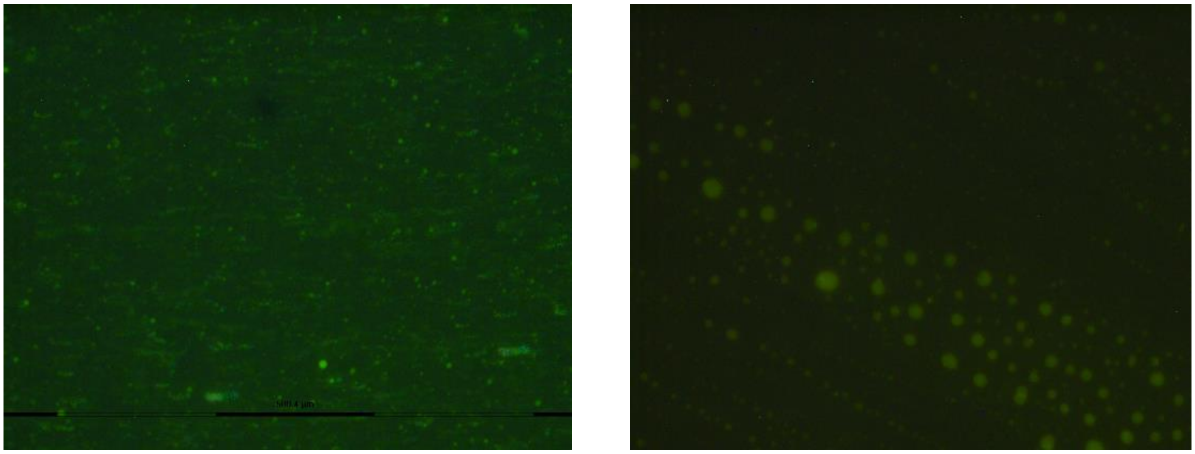
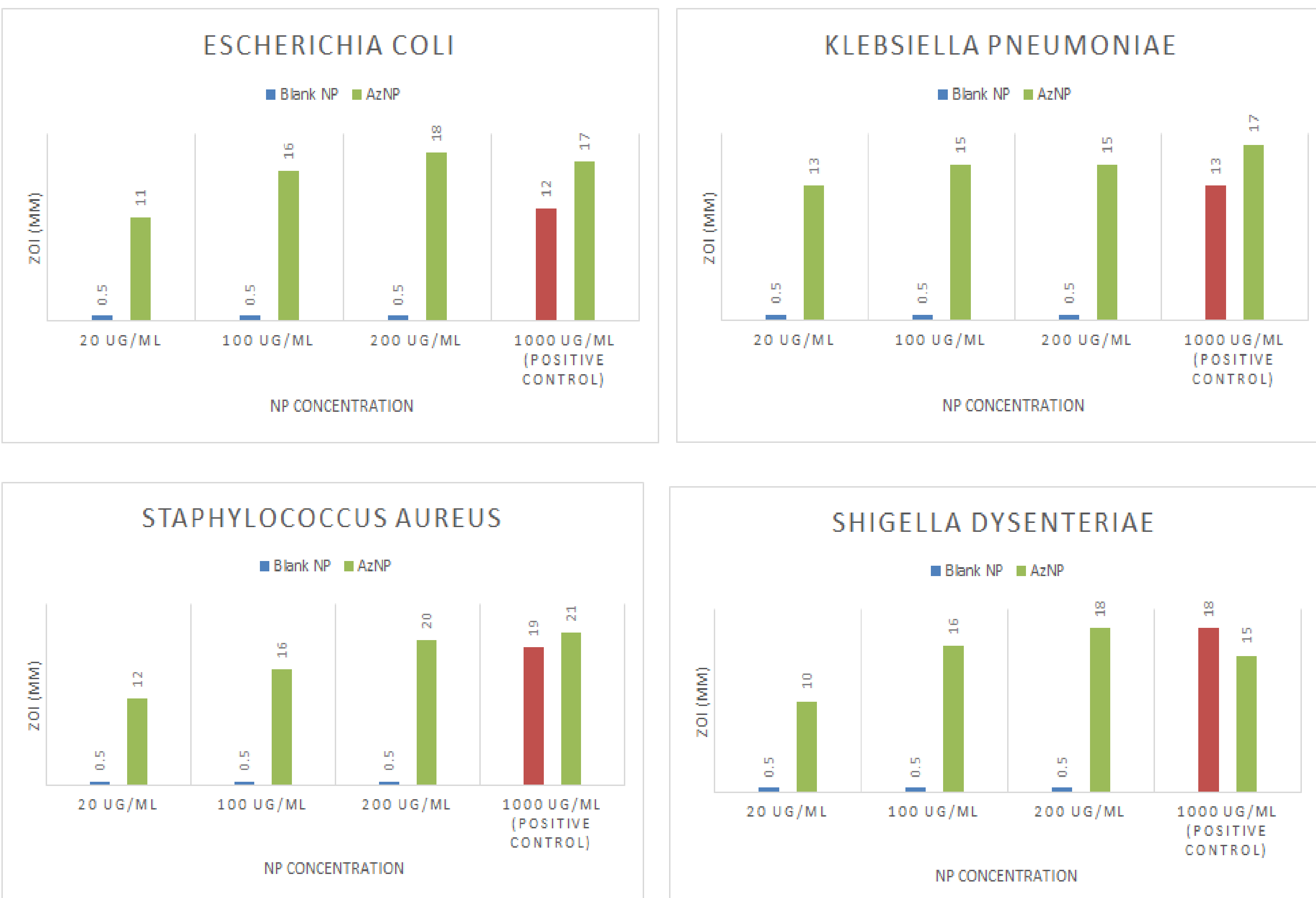


Fig. 10. Fluorescence microscopic images of the drug loaded nanoparticles (AzNP).

**5 mg AzNP in 1 ml acetone.*



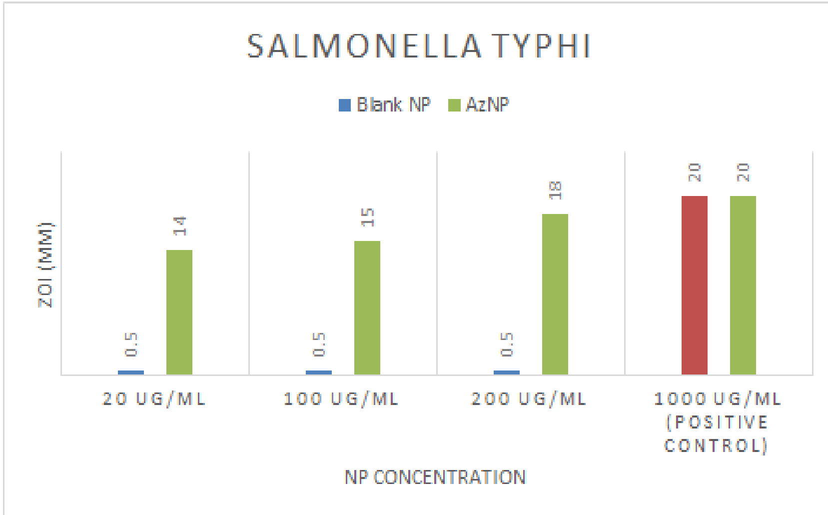


Fig. 11. Antibacterial activity with five different bacterial culture using AzNP with positive control of azithromycin.

The blank nanoparticles (BNP) did not show inhibition of bacterial growth. This confirmed that **OM-1** was non-toxic to pathogenic bacteria. The drug loaded nanoparticles showed a zone of inhibition (ZOI) with increasing diameter from diluted to undiluted nanoparticles. The positive control contains 50 µg of pure antibiotic, whereas undiluted, 1:2 and 1:10 nanoparticles contain 10.4, 5.2 and 1.04 µg of antibiotic respectively. The size of ZOI of positive control was near to undiluted nanoparticles with 5 times less drug concentration. The drug loaded nanoparticles observed more effective which contains less drug concentration as compared to without nano-particles. The **OM-1** could use as a drug delivering agent due to its biocompatible nature.

The antibiotic Azithromycin (Az) is less soluble in water. The nano-formulation AzNP has more mobility/penetration power into the agar matrix due to its small size. The ZOI of 50 µg of azithromycine (Az) showed the highest mm of ZOI for each bacterial strain. The AzNP of undiluted, 1:2 and 1:10 contained 10.5 µg, 5.2 µg and 10.4 µg drug respectively. The ZOI using AzNP observed similar to azithromycin drug but the concentration was 50 times less which indicated that **OM-1** increased efficacy of the azithromycin.

The results can be justified by reported study of dendrimer PAMAM loaded antibiotics. The dendrimer PAMAM is a branched polymer. The number of functional groups increases logarithmic with generation numbers. Antibiotic erythromycin is less soluble in water. PAMAM loaded with erythromycin has increased the solubility and therefore its antibacterial efficacy.[14] Vancomycin is sensitive to gram positive bacteria and insensitive to negative bacteria due to cell membrane permeability. The PAMAM loaded with vancomycin when used to treat gram negative bacteria, showed significant antibacterial activity.[15]

5.4.2. OM-1 as selective metal sensor for Cu^{+2}, Fe^{+2} and Fe^{+3}

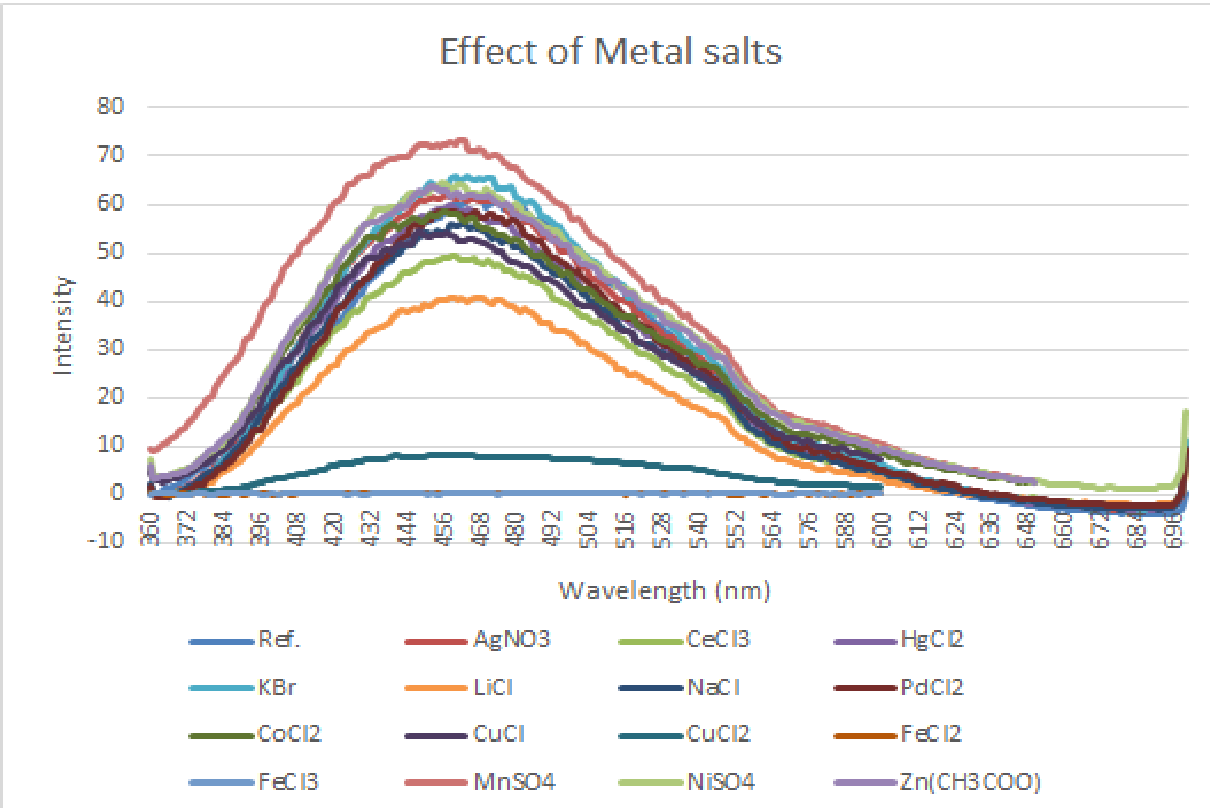
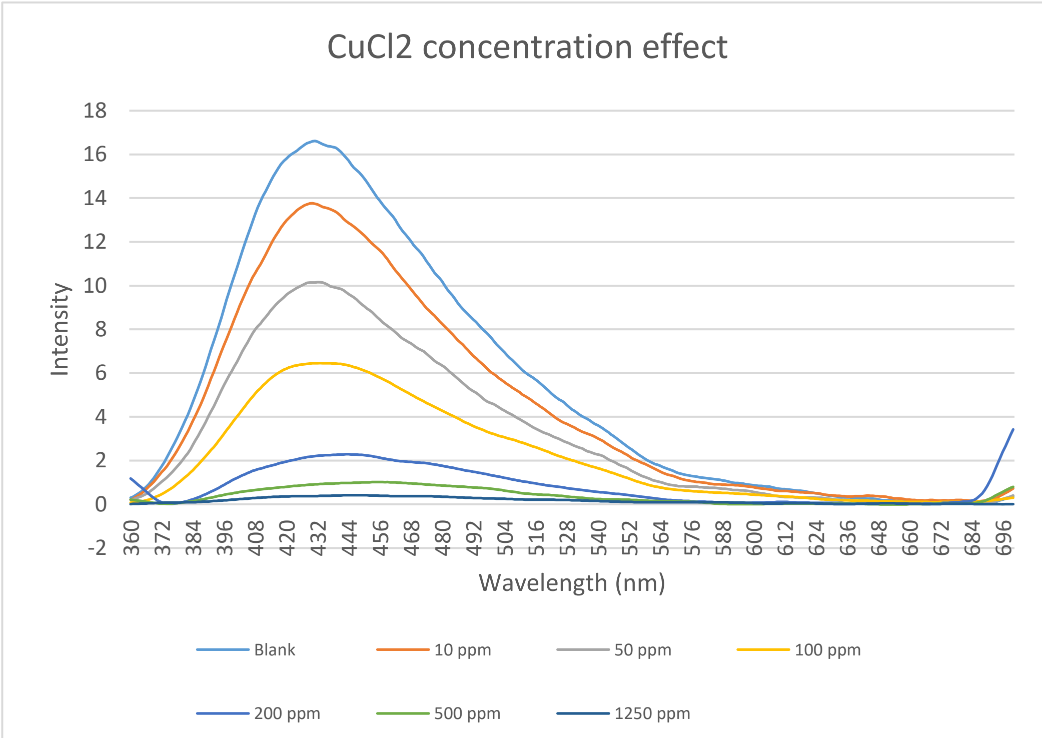


Fig. 12. Effect of various metal salts on **OM-1** fluorescence.



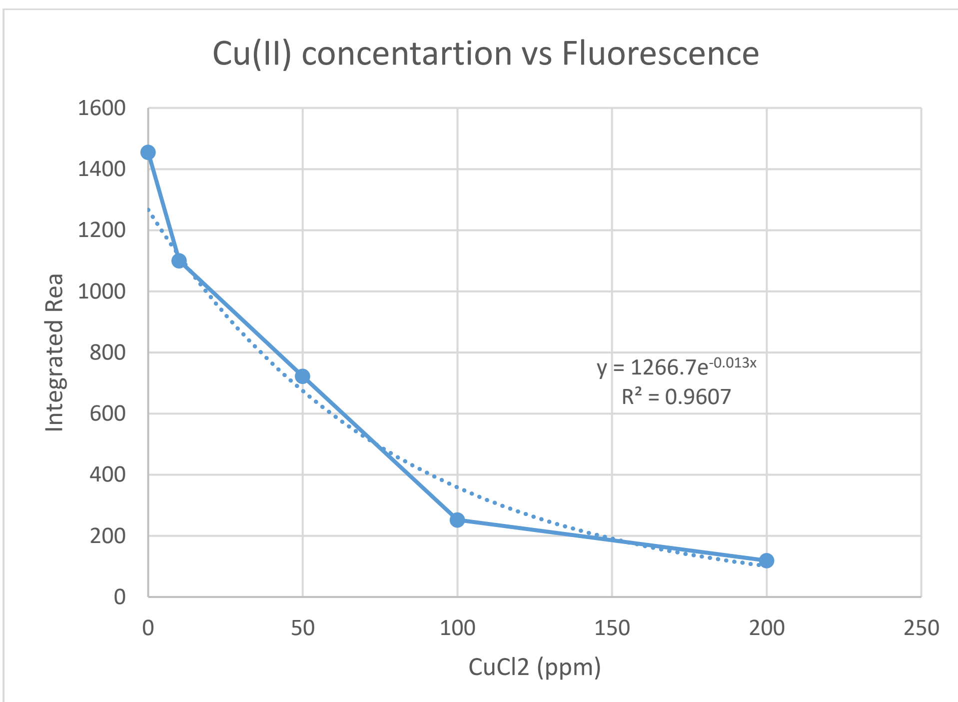


Fig. 24. Correlation plot of Cu^{+2} concentration vs **OM-1** fluorescence intensity.

5.5 Conclusion

OM-1 in various forms like the aliphatic amide salt, fluorescence viscous liquid or gel, was synthesized from naturally occurring and non-toxic compounds like oleylamine and maleic anhydride. It is soluble in specific solvents having dielectric constant 5-20. **OM-1** fluoresces at 465 nm on excitation at 355 nm, having quantum yield 5.3%. It was characterized by spectroscopic, thermo gravimetric, rheological and chromatographic analysis. Non-toxic behaviour of **OM-1** is an attraction for biological applications such as a drug carrier for azithromycin/erythromycin like antibiotics and it helped to increase the efficacy of these antibiotics on lower dosages. It can be also explored for the other costly drugs. **OM-1** fluorescence found sensitive for Cu^{+2} and Fe^{2+}/Fe^{3+} metal ions. Hence, it has a potential to explore for the metal sensing applications.

5.6 Characterization

The Functional groups of **OM-1** characterized by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and fourier-transform infrared spectroscopy (FTIR). Excitation and emission spectra recorded by ultraviolet analysis (UV) and photoluminescence spectroscopy (PL). Excitation emission matrix (EEM) and quantum yield (QY) analysis was done from Sophisticated Analytical Instrument Facility (SAIF)-IIT Madras. Oligomer content and molecular weights characterized by GPC analysis. Thermal and rheological properties studied by dynamic scanning calorimetry (DSC), thermal gravimetric analysis (TGA) and rheology analysis.

^{1}H NMR ($CDCl_3$): (ppm) 0.9 (3H, s), 1.28-1.52 (27H, m), 2.02 (6H, s), 3.1-3.52(4H, m), 5.38 (2H, m), 6.3-8.05 (2H, m). ^{13}C NMR: ($CDCl_3$ ppm): 14.2 (CH_3), 22.7, 26.9-32.7 (CH_2, CH_3), 40.7 (qC), 128.5-130.2 (C=C), 132.5-135.6 (C=C), 166.6-171.0 (C=O). DEPT-135: indicated that there were two types of primary carbons (oleyl CH_3 and Boc CH_3) and tertiary carbon (-HC=CH-) on positive side, carbonyl carbon (>C=O) was not observed and secondary carbons ($-CH_2-$) were observed on negative side.

<u>Supporting Information (SI)</u>

5.6.1. Solubility of OM-1 in various solvents

Table S1. solubility of **OM-1** was screened from high to low dielectric constant and polarity, coded with insoluble (-1), partial soluble (0) and completely soluble (1).

Solvent	Solubility	Solubility Code	Polarity Index	Dipole moment	Dielectric Constant
methanol	●	-1	5.1	2.87	32.7
DMF	●	-1	6.4	3.86	36.71
DMSO	●	-1	7.2	4.1	46.68
Water/Acidic/Basic	●	-1	10.2	1.87	80.1
Hexane	●	0	0.1	0.08	1.88
Toluene	●	0	2.4	0.31	2.38
2-Propanol	●	0	3.9	1.66	19.92
Pet Ether/DEE	●	1	2.8	1.15	4.33
Chloroform	●	1	4.1	1.15	4.81
Ethyl acetate	●	1	4.4	1.88	6.02
THF	●	1	4	1.75	7.58
Acetone	●	1	5.1	2.69	20.7

5.6.2. Spectroscopic analysis of OM-1

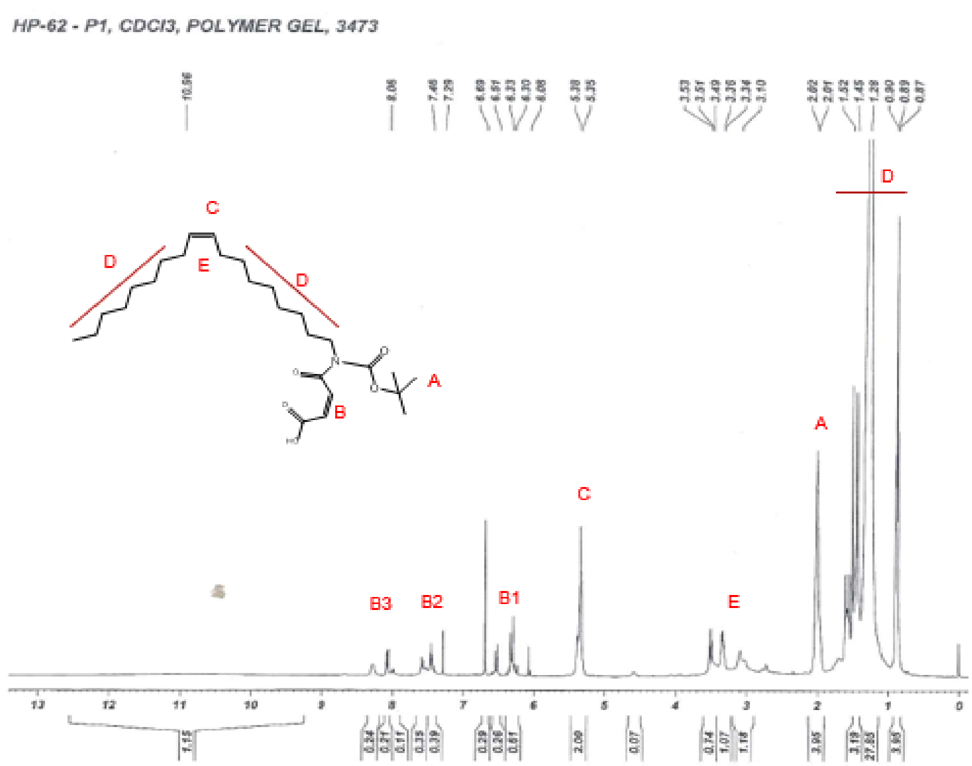


Fig. S1. [1]H NMR of **OM-1** gel.

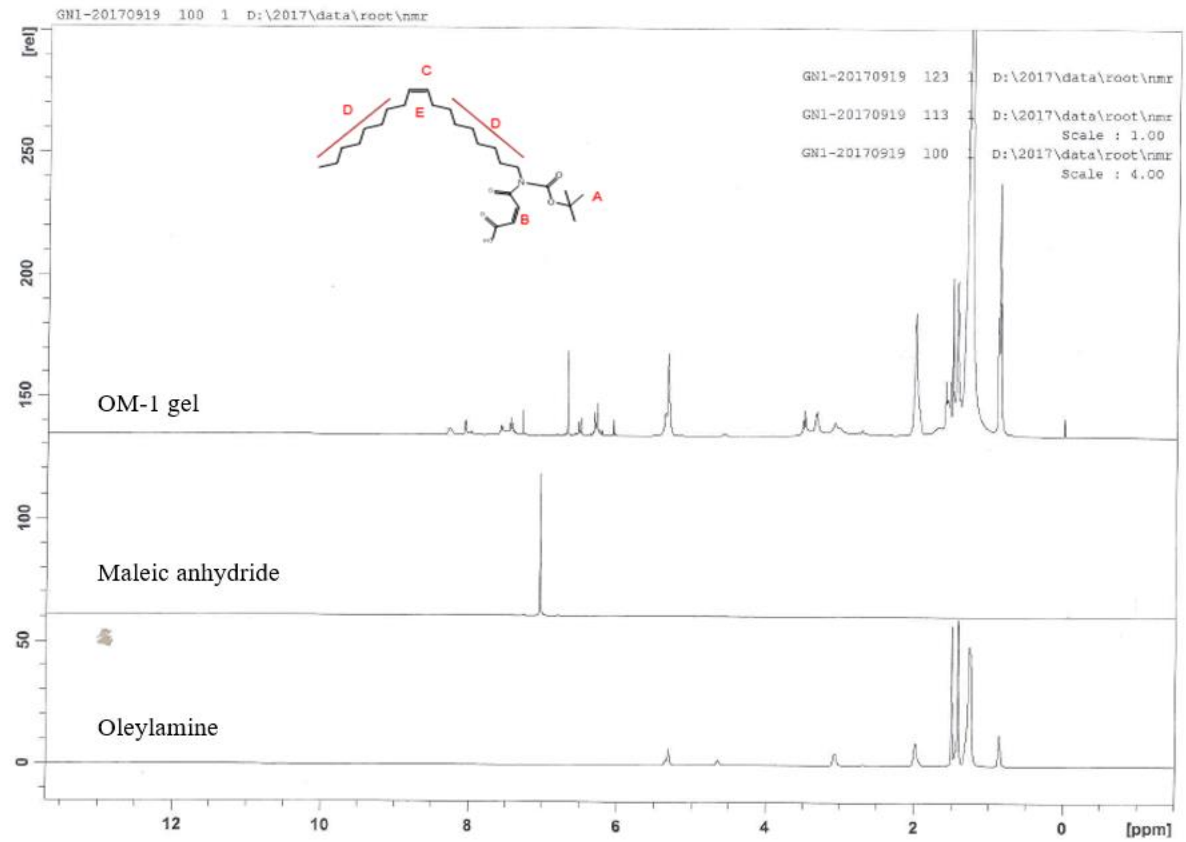


Fig. S2. [1]H NMR comparison of product (a) **OM-1** with (b) maleic anhydride and (c) Boc oleylamine.

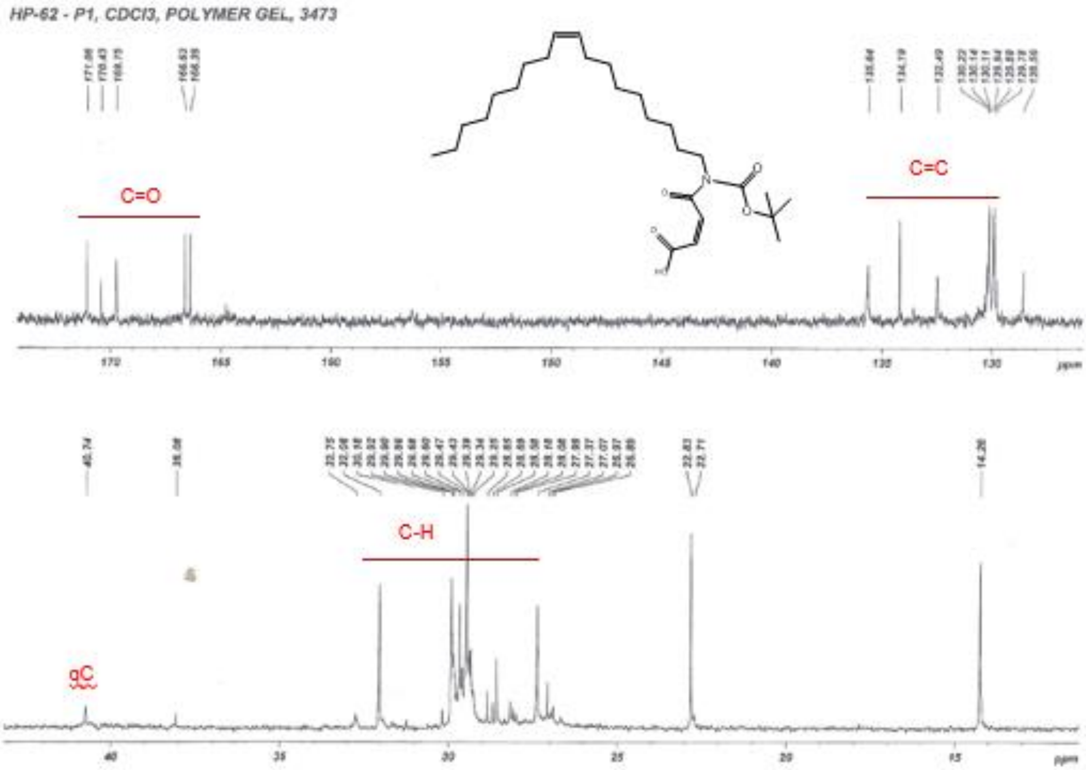


Fig. S3. ^{13}C NMR of **OM-1** gel.

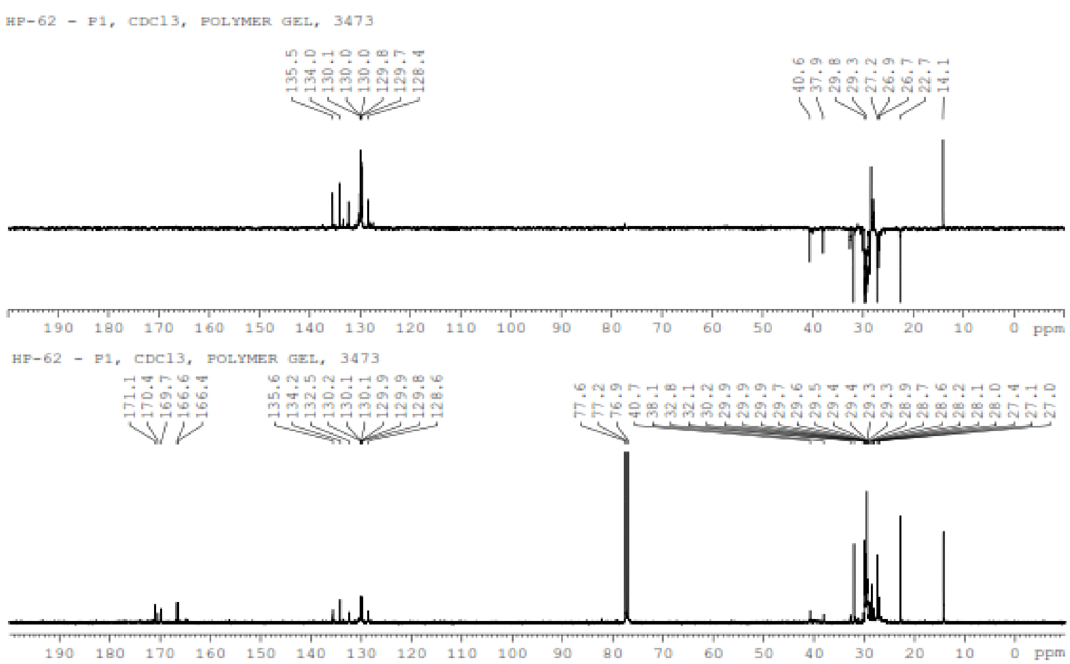


Fig. S4. ^{13}C DEPT 135 analysis of **OM-1** gel.

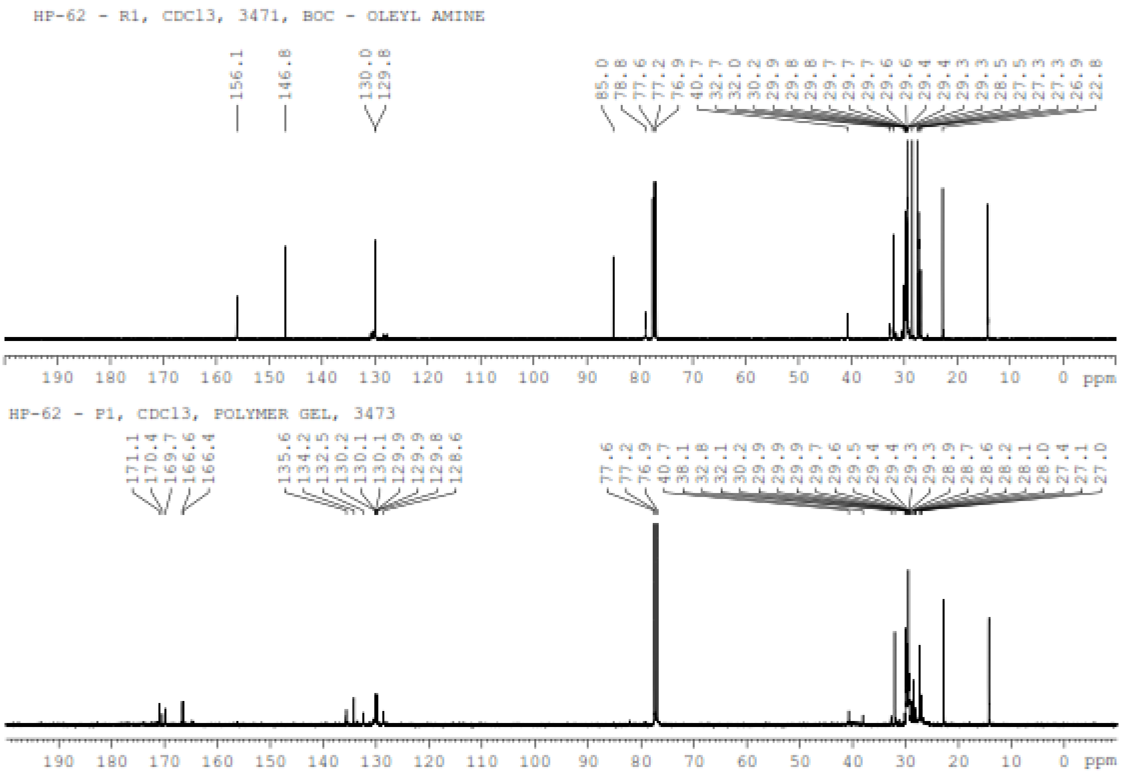


Fig. S5. Comparison of ^{13}C NMR of **OM-1** gel with Boc oleylamine.

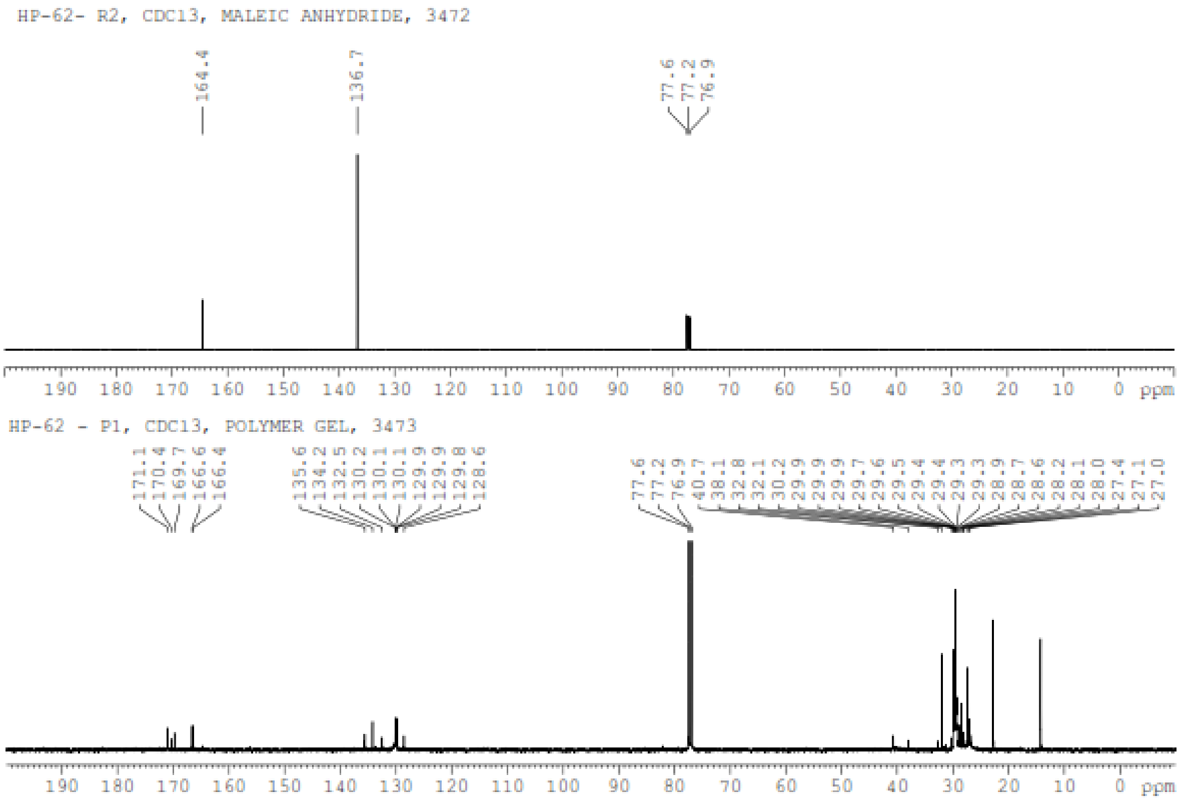


Fig. S6. Comparison of ^{13}C NMR of **OM-1** gel with Maleic anhydride.

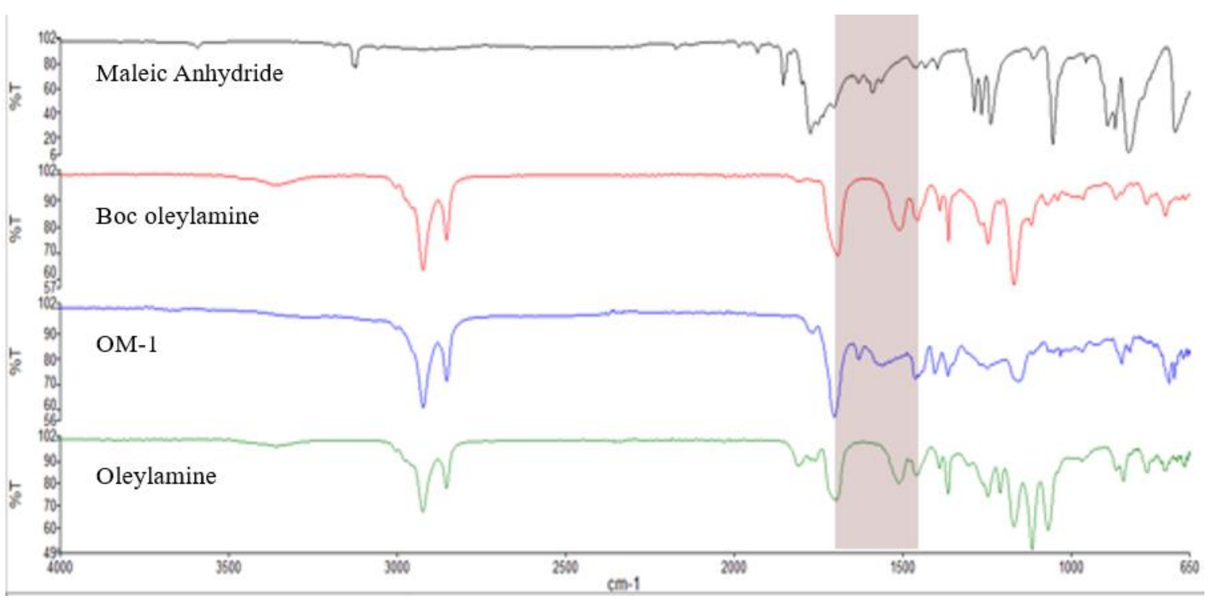


Fig. S7. Comparison of FTIR of (a) maleic anhydride (b) Boc oleylamine (c) **OM-1** (d) oleylamine.

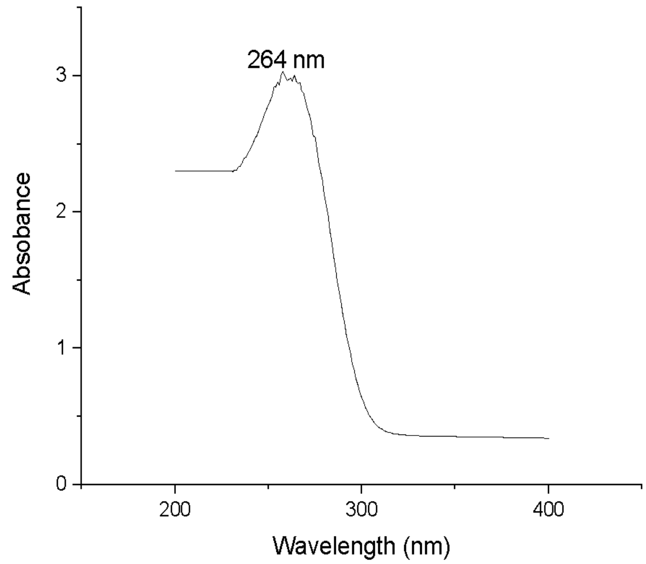
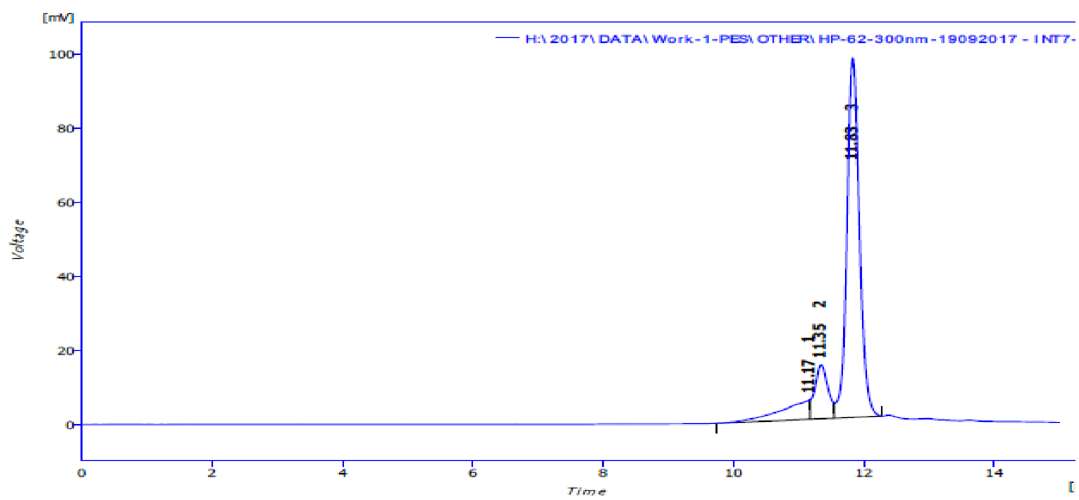


Fig. S8. UV spectrogram of **OM-1** in acetone (1 mg/mL).

5.6.3. Chromatography analysis



Result Table (PS-18092017 - Narrow Flow Rate Corr. Simplified Calculations -
H:\2017\DATA\Work-1-PES\OTHER\HP-62-300nm-19092017 - INT7-1)
Equation: Y = - 0.59773* X + 9.76026
Correlation Factor: 0.9996664

	Max. RT	Mn	Mw	Mz	Mz1	PD	Area [%]
1	11.17	1857	2154	2608	3217	1.1598	9.65
2	11.35	934	948	962	976	1.0149	12.33
3	11.83	476	485	494	503	1.0182	78.02

Fig. S9. Gel permeation chromatogram (GPC) of **OM-1** gel.

**GPC system was calibrated with polystyrene calibration with dichloromethane as mobile phase.*

5.6.4. Thermal analysis

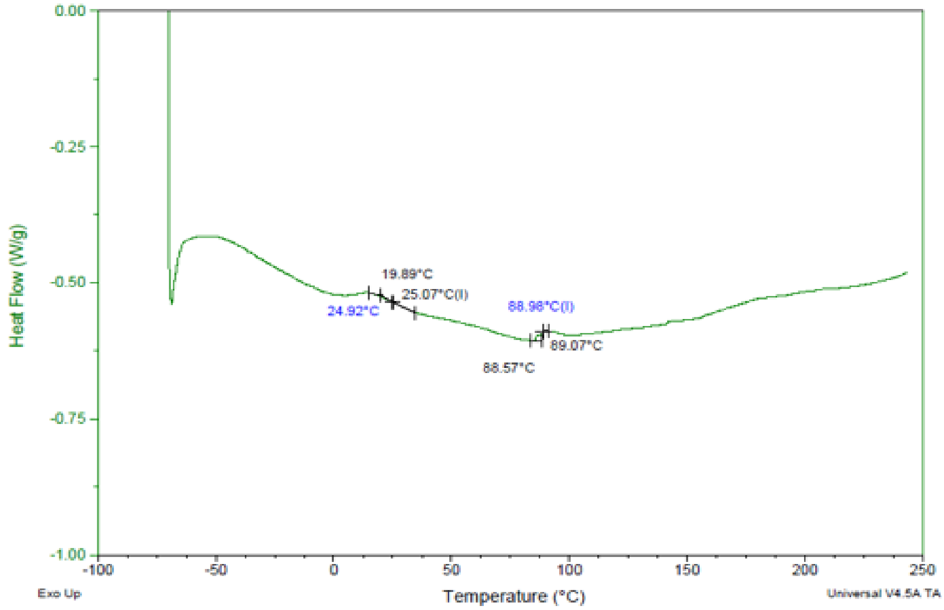


Fig. S10. Differential scanning calorimetry (DSC) of **OM-1** gel.

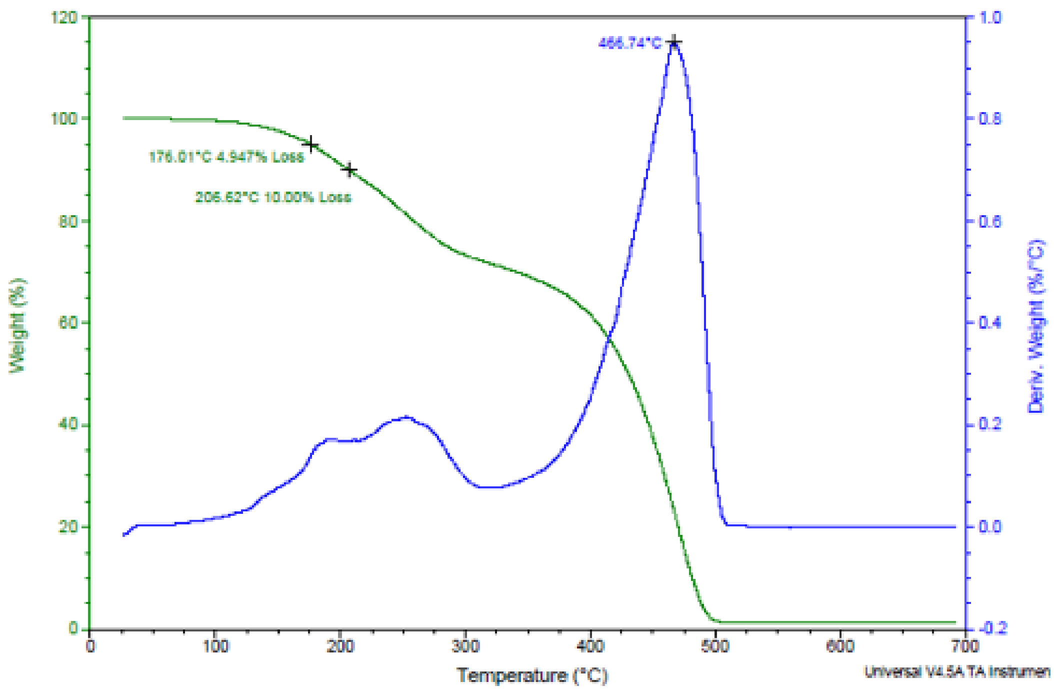
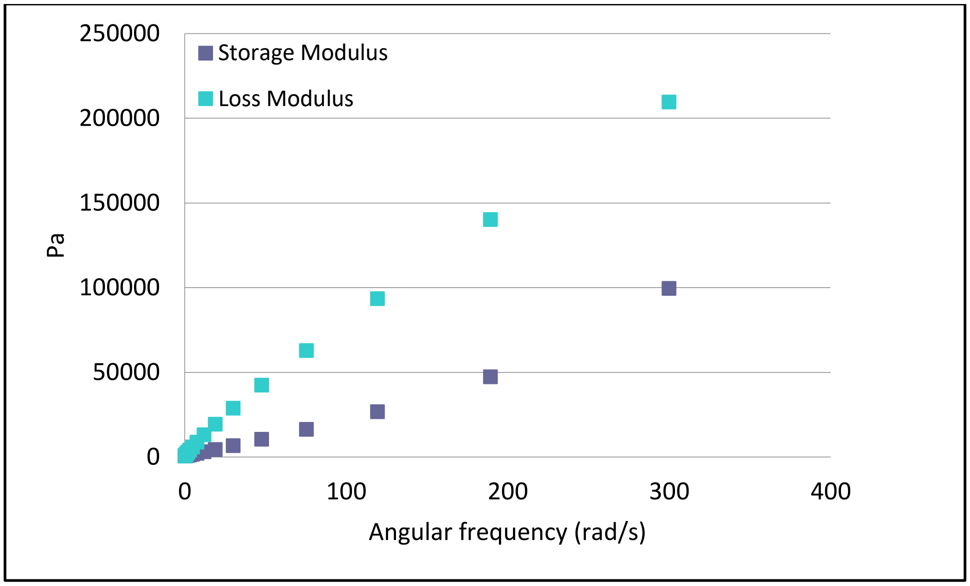


Fig. S11. Thermal gravimetric analysis (TGA) of **OM-1** gel.



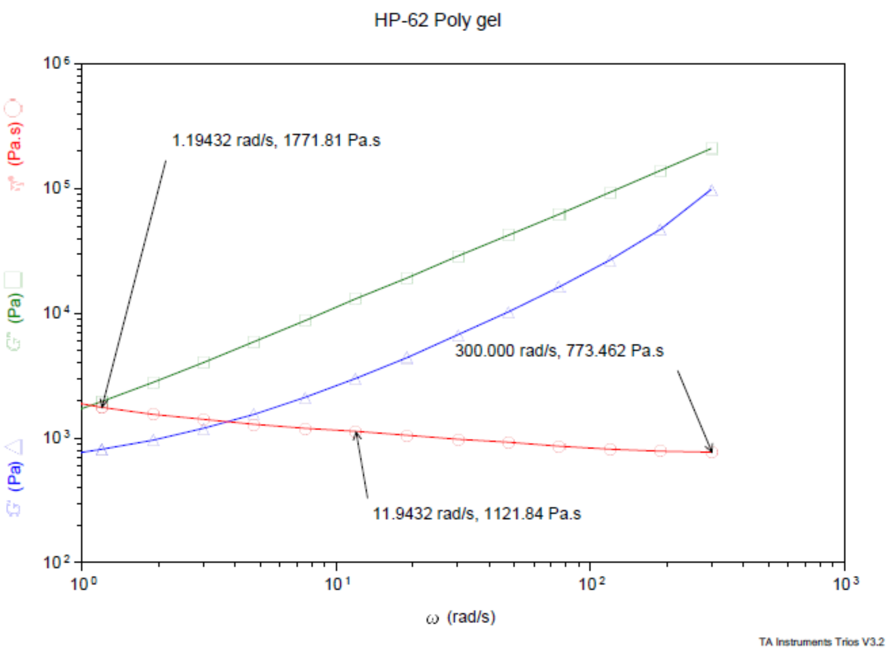


Fig. S12. Rheological studies of **OM-1** gel.

5.6.5. Quantum yield measurement

$$\mathbf{Q = Q_{ref}\ \eta^2/\eta^2_{ref}\ I/A\ A_{ref}/I_{ref}}$$

Relative Standard: **QUININE SULFATE** (Abs: at 320 nm)

Where,

η = Refractive Index of the solvent used.

η_{SAMPLE} = 1.372 for ETHYL ACETATE

η_{ref} = 1.33 for 0.1M H_2SO_4 SOLUTION.

Q_{ref} = 0.54 (Quantum yield of QUININE SULFATE)

I = Integrated Fluorescence intensity of sample

I_{ref} = Integrated Fluorescence intensity of reference

A = Absorbance at the excitation wavelength of sample

A_{ref} = Absorbance at the excitation wavelength of reference

Absorbance (QS)	**Integrated Fluorescence Intensity of Reference (QS)**	**Absorbance (OM-1)**	**Integrated Fluorescence Intensity of sample (OM-1)**	**Quantum Yield (QY)**
0.08644	1.20209×10^9	0.08184	1.26552×10^8	0.06389
0.07138	1.06014×10^9	0.07095	1.08448×10^8	0.05913
0.03079	7.12668×10^8	0.03182	6.67887×10^7	0.05211
0.02745	6.39933×10^8	0.02006	3.20126×10^7	0.03932
			Average	**0.05361**

Quantum Yield of OM-1 = 5.36 %

5.6.6. Particle size analysis

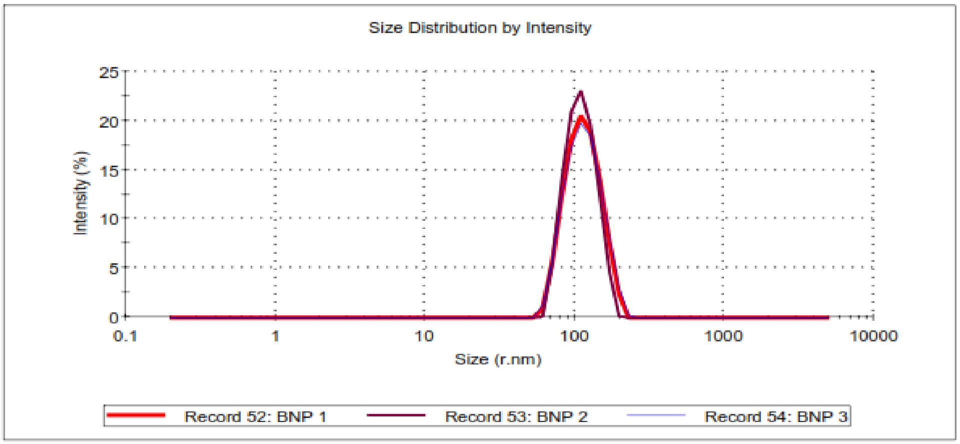


Fig. S13. Dynamic light scattering (DLS) of **OM-1** (BNP: blank nanoparticles).

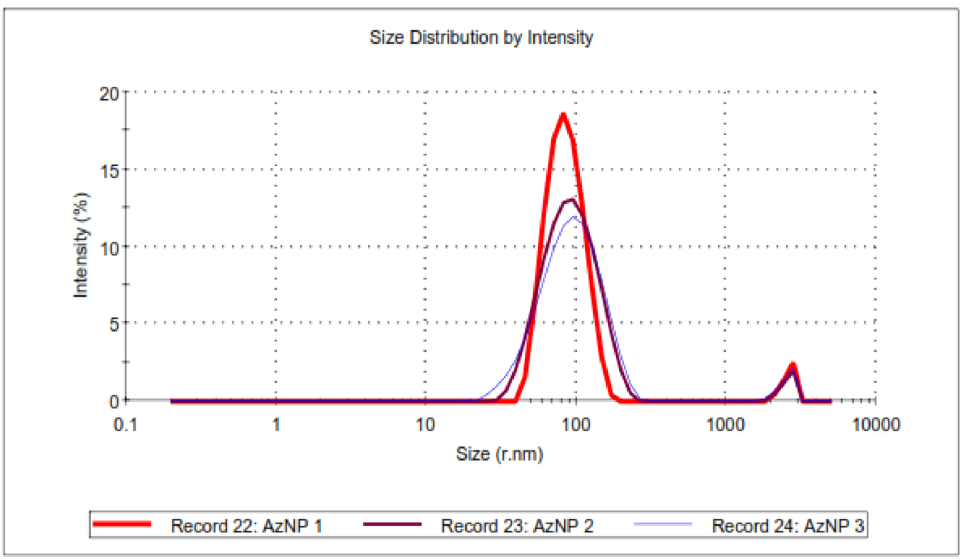


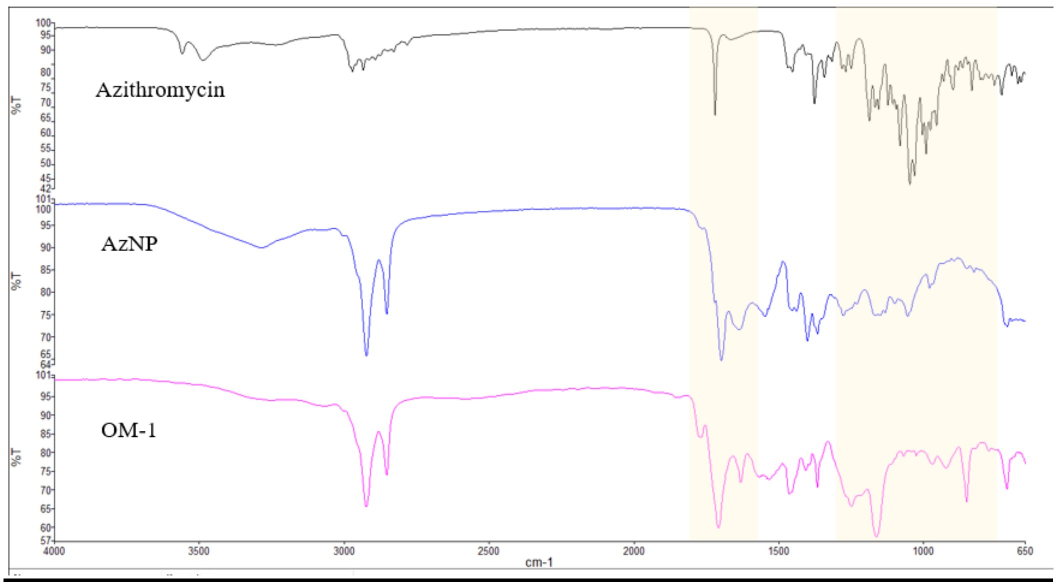
Fig. S14. Dynamic light scattering (DLS) of AzNP (azithromycin loaded nanoparticles).

	Particle Size (nm)	**% Intensity**	**PDI**
BNP	109.1	100	0.085
AzNP	92.75	95.6	0.294
AzNP*	91.71	95.5	0.3

**analysis of same sample after 8 days*

The particles size of **OM-1** nanoparticles in range of 40-100 nm with narrow polydispersity index. The nanoparticles observed stable at room temperature for 8 days without any additional stabilizer.

5.6.7. Spectroscopic analysis of drug loaded OM-1 nanoparticles



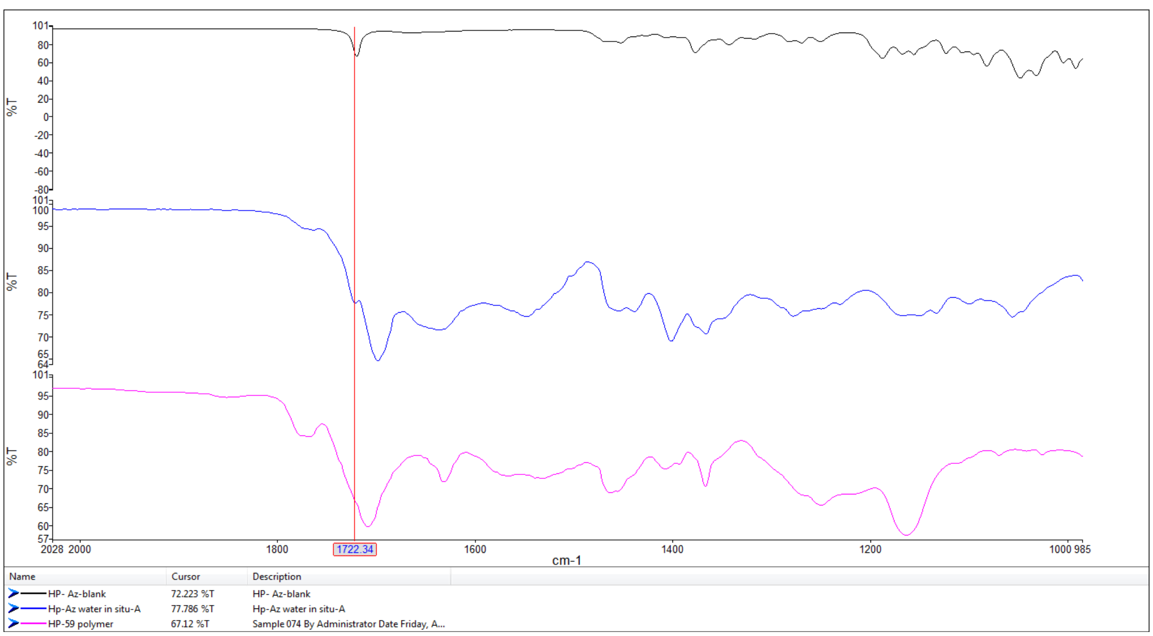


Fig. S15. FTIR analysis: comparison of OM-1 nanoparticles (BNP), azithromycin (Az) and drug loaded nanoparticles (AzNP).

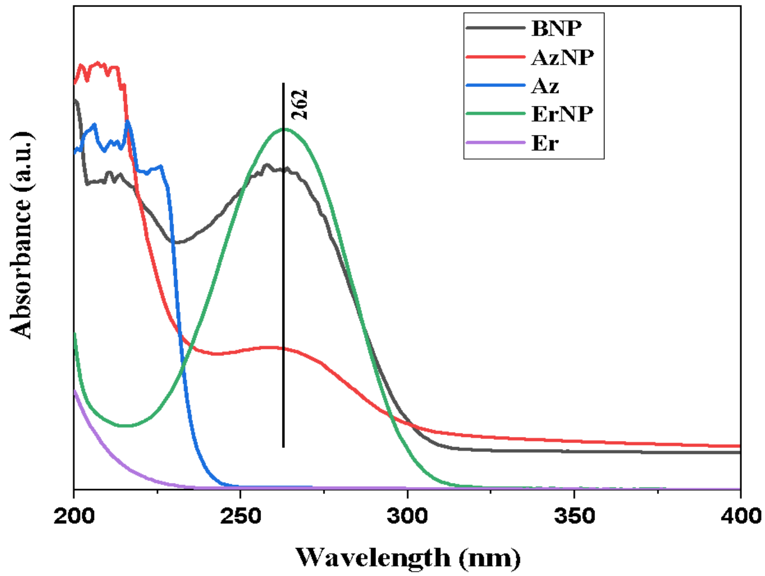


Fig. S16. Comparison of UV analysis of **OM-1** nanoparticles (BNP) and AzNP.

5.6.8. Economic importance of OM-1 fluorescent material

Average price of Azithromycin in bulk: 115 USD/kg (8390 INR/kg) as of June 2021 *(ref. www.pharmacompass.com)*

Estimated cost for **OM-1** preparation in bulk:

Raw materials	Qty. (g)	Cost amount (Rs.)*
Oleylamine	185	464
Boc Anhydride	218	272
Maleic anhydride	98	7
AIBN	50	17.5
Total	***483***	***760***
Total cost for kg	***1000***	***1573***

*Cost (INR) from Sigma Aldrich in June 2021.

Estimated cost for drug formulation:

Formulation Reagent	Qty. (g)	Cost (Rs.)
Azithromycin drug	50	420
Fluorescent gel (OM-1)	50	78
water base	2.5 L	25
Other solvent	100 mL	10
Total cost for	***50 g Drug***	***533***
Total cost for	***1 kg of drug***	***10660***

As per the mentioned experimental data, 5 times (optimum) less drug required for the equivalent effect of 500 mg of azithromycin. Increasing efficacy of the drug also leads to the reduction in side effects in the human body. As per above calculations, 100 mg tablet cost will be 1.06 Rs. The current cost for a 500 mg tablet will be 4.2 Rs (~20 Rs including all tax and profit). Main drug content required less so, other related expenses also reduced. As this process is eco-friendly, we can also reduce carbon footprint indirectly.

Disclaimer: Here, the mentioned, considered and calculated amounts or numbers estimated are in general terms just to relate the benefits of the synthesised material. Accurate and in-depth (detailed) calculations might be required.

5.7 References

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