Chapter 4

Aminopyridinyl Tricosanamide Based Pseudoplastic and Thermoreversible Oleogels for pH-Dependant *in vitro* Release of Metronidazole

4.1 Introduction

In recent years, oleogels have received the attention of researchers community because of having their immense prospective towards developing formulations for cosmetics, nutraceuticals, pharmaceuticals and foods.[1] They can be used either as jellies, creams, lotions, ointments, and gels.[2] The scheme of formation of these types of products is very complicated and tedious. Alongwith this, there is a big concern allied with the durable stability of the majority of these products which reduces their shelf-life. Oleogels may be referred as non-crystalline, viscoelastic, semi-solid systems, in which edible oils get immobilized within the feasible spaces of the three dimensional self-assembled network created via physical interactions among the self-assembled network of oleogelators.[3] In oleogelation, oil is taken as a liquid component and here solid component is termed as oleogelator.[4] The concentration and the behaviour of gelator decide the architecture of the network. This type of gels possesses an advantage over ordinary gels because they do not require any additives as preservatives or stabilisers and also exhibit resistivity towards moisture.[5] Oleogels are superior for topical and transdermal applications because of their uniform spreadabilty over skin due to their homogenous microstructure arrangement.[6,7] The uniform microstructural arrangement develops a permanent macrostructure which retain its structural reliability during the experimentation.[1] The preparation method of oleogelation is similar as that of organogelation. The more complex behavior of the edible oils (mixture of monoglycerides, diglycerides, triglycerides and several fat-soluble compounds) make the oleogels more attractive. Some of the familiar examples of oleogelators are sorbitan esters, ceramides, fatty acids, phospholipids, fatty alcohols, phytosterols, animal and plant waxes.[8] Oleogels are receiving more attention in developing formulations for topical and transdermal

drug delivery applications due to their ability to enhance the diffusion of lipophilic drug by altering the barrier properties associated with chemical, physical, immunological and bio-chemical factors, across the skin (stratum corneum) through interactions.[9,1,2] The oleogels formulation permit the drug delivery into the systemic circulation just by spreading over the skin surface.[10] The oleogel network offers a good delivery system for the researcher to develop controlled release system for the lipophilic drugs and can aid natural fluidization, better hydration of the skin and concurrent transport across the skin. The good extradurability, fine spreadability, satisfactory thermal stability, suitable organoleptic properties and nice controlled drug release makes the oleogels ideal for topical drug delivery.[5] Mustard oil and olive oil are good for topical drug delivery because they give nourishment to the surface of skin.[4] Oleogels can be formed by using edible oil as a solvent and 22DAP gelator, which is off white solid at room temperature and is an amide of 2,6-diaminopyridine and behenoyl (docosanoyl) chloride. 22DAP forms a clear solution in mustard oil (MO) and olive oil at temperature >60 ^{o}C. Extraction of the mustard oil was done from the mustard seeds (Brassica juncea L.).[11] Mustard oil particularly contains 21% of polyunsaturates, 60% of fatty acids with monounsaturation (MUFA), 12% of saturated fats and 1% allyl isothiocyanate.[12,13] MO has good cholesterol reducing and antioxidant properties due to low saturated fat content and also stimulate digestion, assist to avoid heart diseases, detoxify the human body, blocks microbial growth and reduce the inflammation.[14,15] Alongwith, MO also contains several essential vitamins like vitamin E and B vitamins.[13] Mustard oil is generally used as cooking oil and also used in several clinical treatments due to its rubefacient, anti-cancer and anti-microbial activities.[16-18] But currently, the clinical use of mustard oil is decreasing because of facing the

problem in its handling, ordinarily, spillage, and it can be control by immobilizing the oil in three dimensional arrangement of suitable oleogelator.[19] Refined mustard oil is usually considered as safe compound, permitted by the US Food and Drug Administration (FDA) for food and topical applications.[20] Mustard paste has been considerably used topically to relieve the pain.[20,21] Olive oil (OO) contains oleic acid as a major component which enhance the permeability of drugs through the skin during topical and transdermal applications.[22,23] In addition of the above, OO has broad antifungal, antimicrobial and antiplasmal activity which are related to the phenolic glycoside, unsaturated aldehydes and various phenol compounds.[24] Olive oil based oleogels add extra benefits during topical application on skin due to its anti-microbial and antioxidant behaviour. In addition, the pH of olive oil is attuned with skin pH and no reverse effects have been reported until now.[25,26] Keeping the above facts in mind, development of MO and OO derived oleogels for topical and transdermal applications may be defended. Alongwith pharmaceutical applications, oleogels have various applications in cosmetic and food industries.[27,28] The oleogelation helps to avoid the migration of the oils in complex food, cosmetics and pharmaceutical products and hence intensify the life-span of the products.[28,29]

The objective of current study is, to synthesize oleogels of MO and OO, to obtain rheological and thermal behaviour of gels at their critical gelation concentration (CGC), controlled and pH dependent drug release profile for topical and transdermal applications.

4.2 Characterization of oleogels

Oleogels can be characterized by the following different methods.

4.2.1 Test for gelation/determination of critical or minimum gelation concentration

Typically, the gelation is confirmed by inversion of sample vial or inverted test tube method. In this technique, a fixed amount of oleogelator is taken into a particular amount of oil or solvent in a sample vial and heated the mixture upto complete dissolution of the gelator and after that the mixture was allowed to cool at room temperature for sufficient time. After that the gelation is confirmed by inversion of sample vial, no flow of solvent on inversion of the sample vial confirms the gelation.[30,31]

4.2.2 Gel-Sol transition temperature

Gel-to-sol transition temperature (T_g) is the temperature above which a gel loses its structural reliability and the solvent started to flow along gravity on inversion of the sample vial, while, below this temperature (T_g), gel retains its structural integrity and does not shows any flow of solvent along gravity. The transition temperature can be determined by either simple tube inversion method,[31,32] glass ball drop method,[33] and or by bubble motion.[34] It is typically dependent on chemical and physical properties of oleogelator and solvent, in addition to their interactions (either chemical or physical). The T_g of oleogels increases with increase in the gelator concentration.[35] Thermal stability of oleogels can be obtained by plotting T_g against concentration of the gelator. Permanent gels can be formed by the chemical interaction among the large polymeric molecules and do not show gel to sol transition.

4.2.3 Analytical methods

Analytical methods like X-Ray diffraction, FT-IR and NMR Spectroscopy[36] analysis have been used for the characterization of oleogels. These methods afford precious

information about molecular interaction in aggregation of oleogelator molecules. The FT-IR analysis provides the important information about hydrogen bonding. The hydrogen bonding (intermolecular) can be confirmed with the help of NMR spectroscopy.[37,38] The shape of fibrous assembly of oleogelators can be considered by small-angle neutron scattering (SANS) method.[39] Information regarding molecular organization of organogels, morphology, internal mobility of the constituents and specific interactions can be procured by NMR measurement (magic angle spinning (MAS) in the solid-state NMR, multiple-quantum (MQ) spectroscopy, spin relaxation times, the pulse field gradient (PFG) method, and magnetic resonance imaging (MRI).[40]

4.3 Applications of oleogels

4.3.1 Pharmaceutical

Skin acts as an effectual obstacle for majority of the drugs except clonidine, nitroglycerine scopolamine, estradiol, nicotine, lidocaine, fentanyl, oxybutinin and testosterone. Hence, there is always a need of topical formulation with enhanced permeability of drug molecules through the skin.[41] Many organogelators like lipids perform as a penetration enhancer and hence provide an extra benefits to the organogel formulations. Organogels have been explored as dermal and topical pharmaceuticals.[42] Transdermal and topical drug delivery can avoid severe gastric irritation caused by oral uptake of aceclofenac.[43] It can be a choice of drug for osteoarthritis, ankylosing spondylitis, and rheumatoid arthritis.

4.3.2 Oleogels in cosmetics

Emulsion-based skin care products which contain oil and water are in trend today. Alongwith, oleogels are very popular for making creams, lotions and ointments. They

are mainly suggested for skin problems and used in the formulations for cutaneous cosmetics. Oleogels have semi-solid uniformity identical to cream emulsions. Oleogels are mainly recommended for the lips caring, for cold creams, hand care products, sun protection products and also for the care of perianal skin disorders and diabetic skin. Cracked and dry foot skin becomes smooth and soft by use of the oleogels because they also provide nourishment to the skin. A major advantage of oleogels is that they show resistancy against perspiration and water. Oleogels can be applied for massage creams because of having semisolid consistency. They are also recommended for the formulations of decorative cosmetics like eye shadows, makeup and mascara. Oleogels meet the foremost preconditions for corneotherapy which was invented in the late nineties by Albert M. Kligman. The "outside-in-therapy" needs that the formulation of the product is physiologically modified to the skin and so that fruitless effects by non-physiological supplments can be avoided. Both the necessities pertain for oleogels.[44]

4.3.3 Nutraceutical applications

Researchers modified the physiological properties of oils by providing specific texture and rheology to these oil based materials. In this approach, numerous food goods demanding a specific rheology and texture can be formulated with these novel oleogels without causing major changes in the quality of final product. Mostly these oleogels can be formed by incorporation of specific molecules (polymers, waxes and amphiphiles) into the edible oils to form oleogels. Some of the oleogels are chemically more stable than grease, like, Ethylcellulose based oleogels, and have been used for topical application.[45] Castor oil based oleogels are preferred as environmentally pleasant lubricating greases having good mechanical and thermal stabilities.[46] Glyceryl monostearates and sorbitan based oleogels have been potentially used as biodegradable

substitutes to conventional lubricating greases, and show good viscoelastic functions because of use of low viscous oils.[47]

4.4 Experimental

4.4.1 Materials

2,6-Diaminopyridine, triethylamine, behenic acid and oxalyl chloride were procured from CDH (Central Drug House). Metronidazole and analytical grade solvents were bought from Sigma Aldrich. All the solvents were distilled before purification techniques. Olive oil and mustard oil were acquired from local market. Throughout the experimental studies double distilled water was used.

4.4.2 Preparation of oleogels

Accurately weighed **22DAP** was taken in particular amount of the olive oil and mustard oil in glass sample vials and heated the solution up to complete dissolution of the gelator. Then the mixture was cooled at room temperature to acquire gelation. Gelation was confirmed by tube inversion method.[5] The critical gelation concentration was obtained by changing the concentration of the **22DAP** gelator. CGC is the minimum concentration of the gelator at which gelation takes place and there will be no flow of the liquid component on inversion of the sample vial.[5] In the similar way metronidazole loaded oleogels were prepared. The drug concentration was taken as 1% (w/w) within the oleogels. For the preparation of drug loaded oleogels, firstly the oleogels were prepared as mentioned above after that the gels were heated at 70 ^{o}C and then accurately measured one equivalent of metronidazole was added to the mixture continuing the heating up to 15 minutes. Then the solutions were cooled to room temperature to acquire gelation. All the oleogels were kept at room temperature for further experimental studies.

4.4.3 Microscopic studies

A ZEISS Stemi 508 Greenough Stereo Microscope (with Axiocam ERC 5S camera) was used for determining the microstructure of the gels. The oleogelation mechanisms was analysed by changing the concentration of the oleogelator in oils and drug loading mechanism was studied by varying the concentration of drug into the oleogels. Microscopic images were taken from the microscope. The sample was placed on the glass slide and examined into the microscope with capturing the images with microscope camera Axiocam ERC 5S followed by the software (Zeiss).

4.4.4 Fourier transform infrared spectroscopy studies

The infrared spectra of the gels were obtained by using FTIR spectrophotometer in diminished total reflectance ATR model (Alpha-E, Bruker, Germany). The scan range for the samples was 4000 to 667 cm^{-1} to determine the interactions among the oleogel components.

4.4.5 Organoleptic properties

The colour, texture and appearance of the gels were noted as their organoleptic properties.

4.4.6 Rheological studies

The rheological analysis of the oleogels was done by using Anton Paar rheometer (Rheolab QC Rotational Rheometer. All the readings were taken out at 25 ^{o}C using the freshly prepared oleogel samples.

4.4.7 Spreadability tests

The spreadability of oleogels was checked by the reported method.[48] A fixed amount of the gel (0.25 g) was taken between two glass slides of like weight and surface area. The

preliminary spreading diameter of the gel was noted, before putting the weight. After that, different weights of 10, 15, 25, 50 and 100 g were taken over the upper glass plate. The ending diameter was obtained after 60 s. The spreadibility % was calculated by the following **equation (1)**.

$$\% \text{ Spreadibility} = \frac{D_2 - D_1}{D_1} \times 100 \quad (1)$$

Where D_1 is the initial diameter of gel spreading and D_2 is the final diameter of gel spreading.

4.4.8 pH determination

The pH of the oleogels was obtained by Bench pH meter (EUTECH Instruments pH tutor). The pH of oleogels was reported by dipping the glass electrode of digital pH meter in the gel samples.[23]

4.4.9 Thermal stability

The thermal analysis of the gels was done by tube inversion method, by determining the gel to sol phase transition temperature (T_g). The gel was prepared in a glass sample vial and then, the vial was placed in an oil bath at 20 ^oC. After that, the temperature of the oil bath was increased with a rate of 1^oC/min. The temperature at which the oleogel start to flow on inversion of the sample vial was noted as T_g.[49]

4.4.10 Drug loading

A fixed amount of gelator (**22DAP**) was added to 1 mL of solvent (MO or OO) and heated to form a clear solution. Then 1 equiv. of metronidazole was added to the clear gelator solution at 70 ^oC. The solution was mixed thoroughly and allowed to cool at temperature 20 ^oC for 1 h. The amount of metronidazole was increased up to 10 equiv. and stable gel formation achieved.

4.4.11 Drug release study

pH-Responsive *in vitro* drug release study of the metronidazole loaded oleogels was carried out in phosphate buffers at pH 5.8 and 7.4 respectively. The drug release study was also done in double distilled water (ddw) for a comparative release rate. Appropriately, metronidazole encapsulated oleogel was prepared and after that, ddw was added to the oleogel for drug release study. The degradation pattern of oleogel was obtained in ddw by noting down the absorbance at 319.5 nm. In the similar way degradation pattern of the drug loaded oleogel was also determined in buffer solutions at pH 5.8 and 7.4 respectively at 37 ^{o}C.

4.5 Results and discussion

4.5.1 Preparation of oleogels

We have synthesized amide derivatives of fatty acids (**12DAP**, **16DAP**, **18DAP, 18UDAP**, **20DAP** and **22DAP**)[50] for the oleogelation.

Table 4.1: Oleogelation study of DAP derived fatty acid amides

entry	Gelater	Solvent *(v/v; 1 mL)*	Gel	Gelation time	CGC (mg/mL)	T_{gel} (K)[a]
1	**12DAP**	MO	No	-	-	-
2	**16DAP**	MO	No	-	-	-
3	**18DAP**	MO	No	-	-	-
4	**20DAP**	MO	No	-	-	-
5	**22DAP**	MO	Yes	<30 min	18	313
6	**18UDAP**	MO	No	-	-	-
7	**12DAP**	OO	No	-	-	-
8	**16DAP**	OO	No	-	-	-
9	**18DAP**	OO	No	-	-	-
10	**20DAP**	OO	No	-	-	-
11	**22DAP**	OO	Yes	<30 min	20	321
12	**18UDAP**	OO	No	-	-	-

[a]Tube Inversion Method; CGC = Critical gelation concentration

Gelation of these synthesized fatty acid amide gelators were carried out in OO and MO by heating-cooling technique and their gelation study is briefly discussed in **Table 4.1**.

It was found that only **22DAP** was able to prepare oleogel in both the oils (MO and OO). **22DAP** was dissolved in OO and MO at 75 ^{o}C, for complete dissolution of the oleogelator. Then the mixture was allowed to cool at room temperature, the gelator molecules self-assembled to form oleogel by physical interactions among each other in 3D network organization at CGC. And the gelation was confirmed by inversion of the sample vial (no flow of oil from the gel was observed against gravity). But if the concentration of the gelator was lower than the CGC, gel was not formed, only cloudy suspension was obtained, while if the concentration of the gelator was taken more than CGC, in that case opaque gel was formed.[51,52]

In case of MO, the CGC was noted to be 1.8% (w/v), while in case of OO, it was found to be 2.0% (w/v). But both the gels were prepared with the same concentration of the gelator for comparative drug loading and release study. Both the oleogels were stable even after two months at room temperature.

4.5.2 Organoleptic evaluation

The MO and OO oleogels were yellow and light yellow in colour, respectively. With increase in the concentration of gelator, stability of gels was enhanced. All the gel samples were smooth in texture and oily in touch.

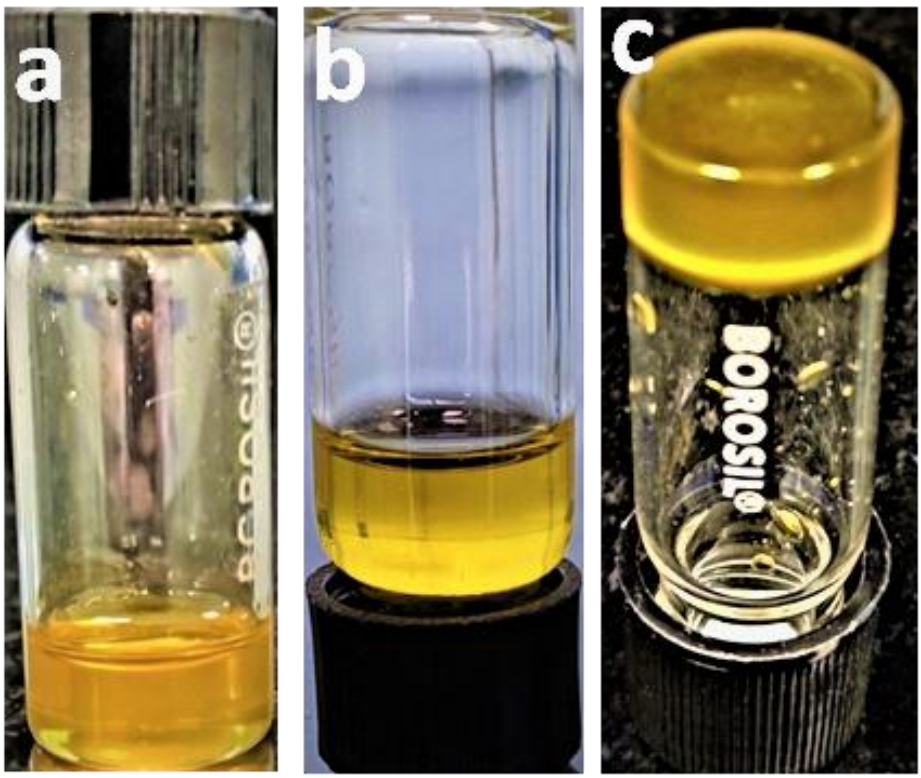

Figure 4.1. 22DAP/MO mixture (a) below CGC, (b) on melting gel and (c) the gel above CGC.

4.5.3 Thermal analysis

The thermal stability of the oleogels was checked by tube inversion method and their gel-to-sol transition temperature (T_g) was esteemed (**Table 4.2**). The T_g of MO gel (1.8% w/v) was found to be 40 ^oC, but after incorporation of the drug (metronidazole), the T_g of the oleogel was reduced by 2 °C and became 38 °C. In case of OO gel (2% w/v), the T_g was noted as 48 °C which was reduced by 1 °C and became 47 °C after drug incorporation. Thermal analysis of the gels before and after incorporation of drug, resulted out that the addition of drug molecules deceased the fibre-fibre interaction among the gel molecules in both the oleogels (OO and MO). It was also observed that the thermal stability of OO oleogel was higher as compare to MO.

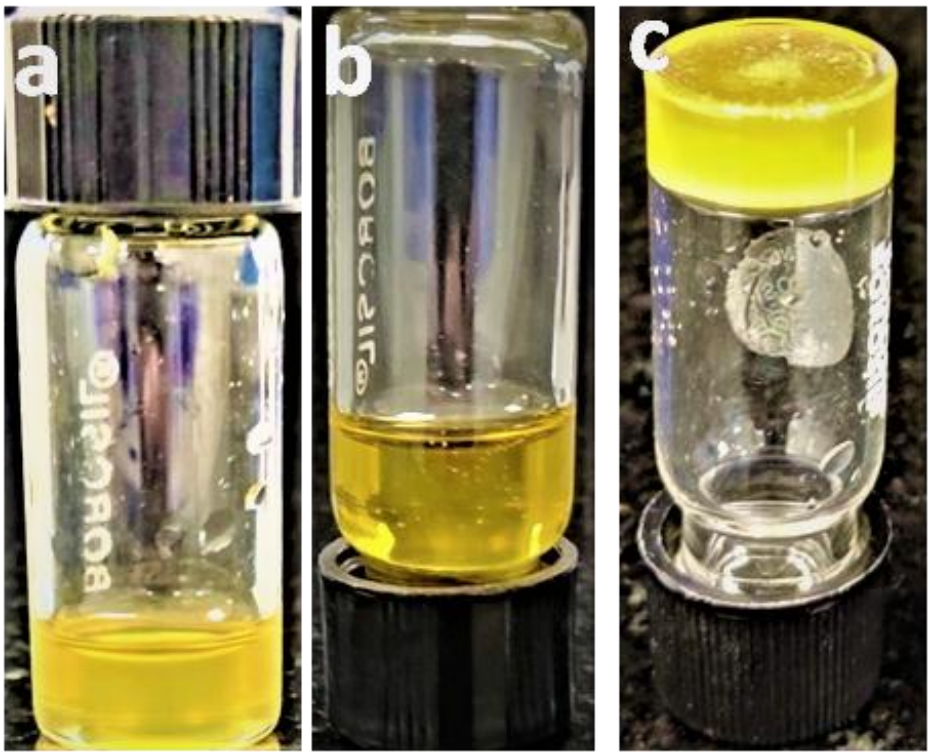

Figure 4.2. 22DAP/OO mixture (a) below CGC, (b) on melting gel and (c) the gel above CGC.

Table 4.2: Oleoogelation study of 22DAP in MO and OO

Gelater	Solvent *(1 mL)*	Drug (equiv.)	Gelation time	CGC (mg/mL)	T_{gel} (K)[a]
22DAP	MO	-	60 min	18	313
"	MO	1	60 min	18	311
"	OO	-	60 min	20	321
"	OO	1	60 min	20	320

[a]Tube Inversion Method; CGC = minimum gelation concentration; T_{gel} = Gel-to-sol phase transition temperature

4.5.4 Microscopic study

The microstructures of the oleogels were determined from light microscope (**Figure 4.3** and **4.4**). The micrographs indicated that on increasing the concentration of the oleogelator, the **22DAP** molecules self-assembled to form fibrous organisation with rough surface. It was pointed out that at CGC, gelator fibres organised a three dimensional networked assembly which resists the flow of liquid component (MO and

OO) against gravity.[53,54] The interactions amongst the gelator (long acyl chain of gelator) and oil molecules may be weak van der Wall forces. The stability of oleogels enhanced due to hydrophobic interactions among the gelator-oil components.[55] In case of concentration, higher than CGC, the stability of oleogels was enhanced due to increase in hydrophobic forces.[56]

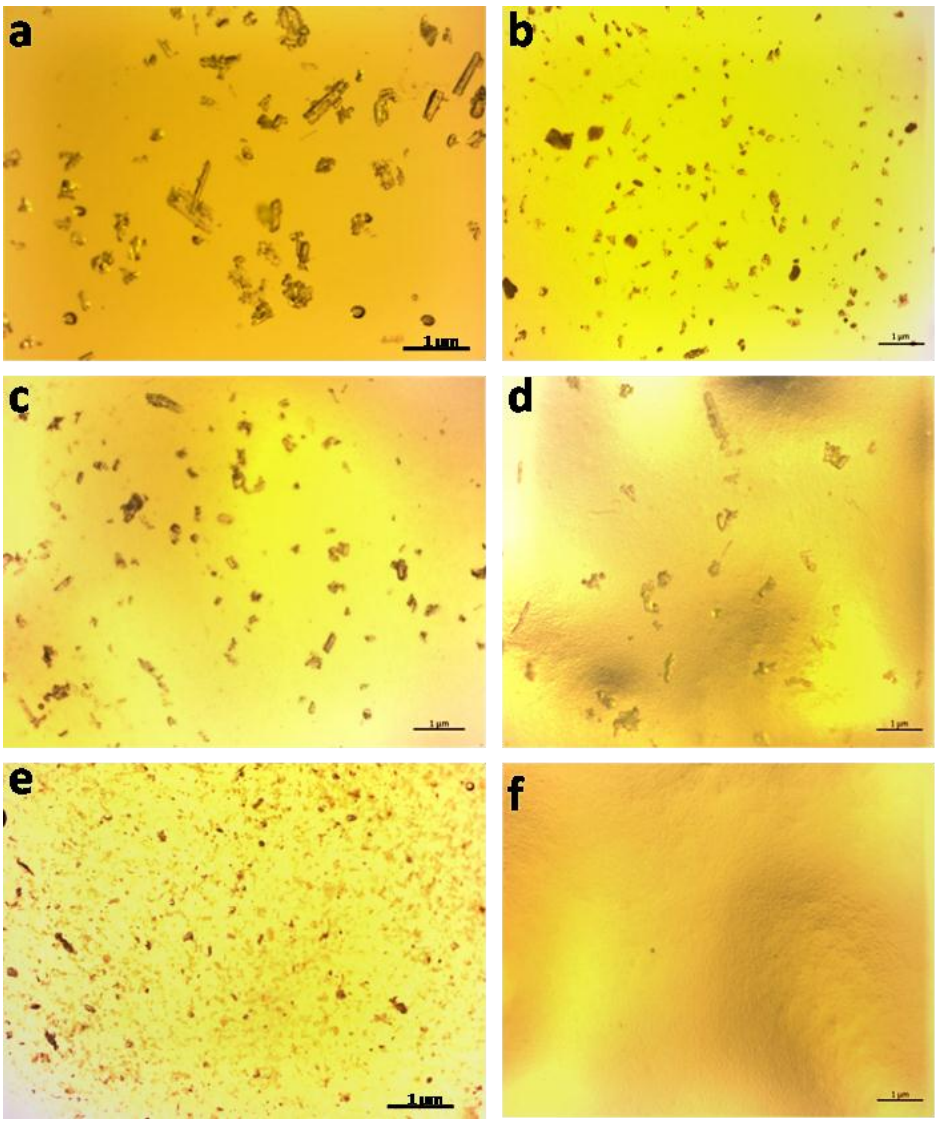

Figure 4.3. Micrographs of **22DAP**/MO mixture with drug incorporation, at varying concentration of **22DAP** and Drug: (**a**) 0.5% 22DAP/MO (w/v) with 2 equiv. metronidazole, (**b**) 1.0% 22DAP/MO (w/v) with 1 equiv. metronidazole, (**c**) 1.5% 22DAP/MO (w/v) with 1 equiv. metronidazole, (**d**) 2.0% 22DAP/MO (w/v) with 1 equiv. metronidazole, (**e**) 2.0% 22DAP/MO (w/v) with 10 equiv. metronidazole and (**f**) 2.0% 22DAP/MO (w/v) without drug incorporation.

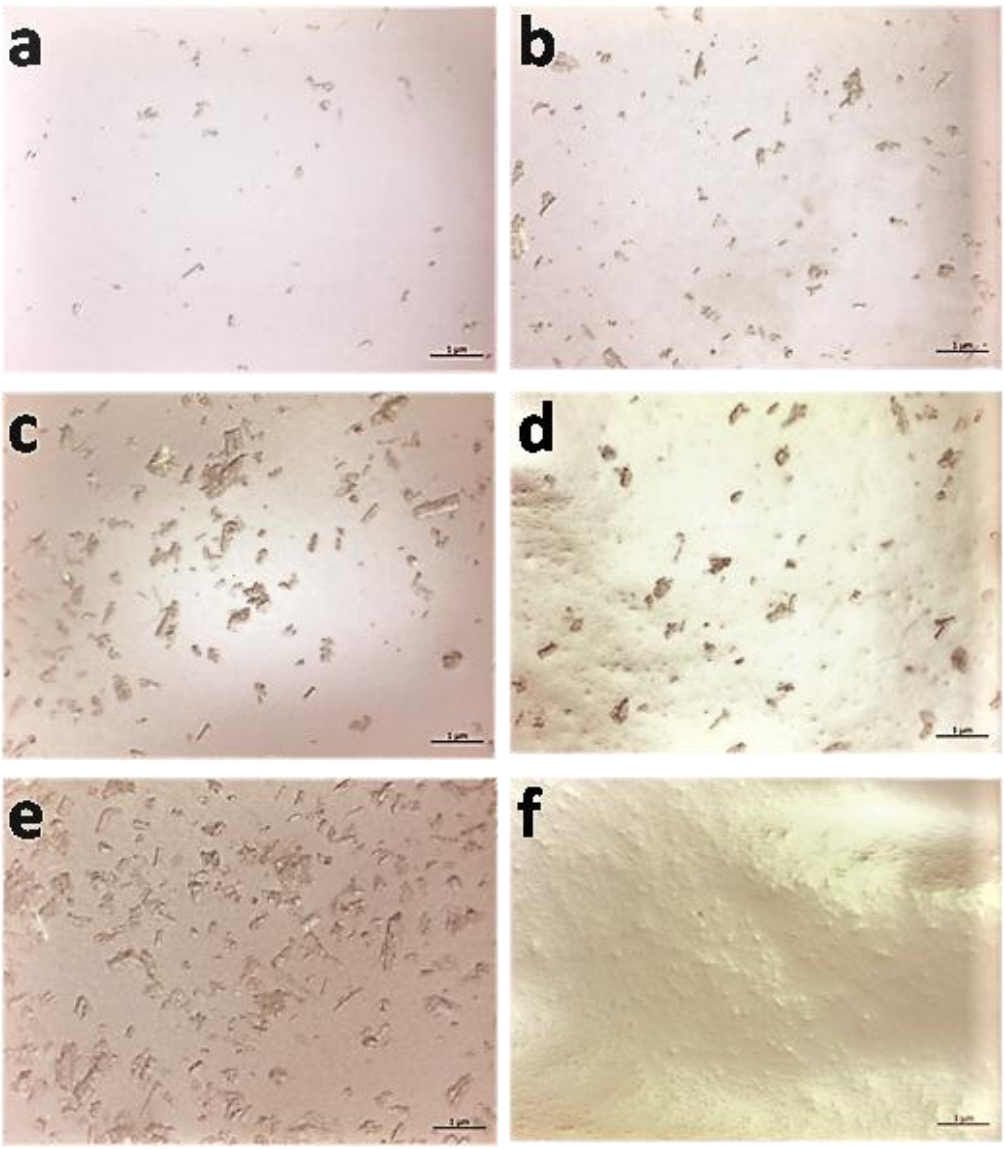

Figure 4.4. Micrographs of **22DAP**/OO mixture with drug incorporation, at varying concentration of **22DAP** and Drug: (**a**) 0.5% 22DAP/OO (w/v) with 2 equiv. metronidazole, (**b**) 1.0% 22DAP/OO (w/v) with 1 equiv. metronidazole, (**c**) 1.5% 22DAP/OO (w/v) with 1 equiv. metronidazole, (**d**) 2.0% 22DAP/OO (w/v) with 1 equiv. metronidazole, (**e**) 2.0% 22DAP/OO (w/v) with 10 equiv. metronidazole and (**f**) 2.0% 22DAP/OO (w/v) without drug incorporation.

Figure 4.5 and **4.6**, the difference between micrographs of drug composite oleogels before release (**4.5a** and **4.6a**) and after release (**4.5b** and **4.6b**) clearly indicated that, after drug release from both the oleogels (MO and OO), no drug molecules are present in the gels (only receptor medium droplets are there in **4.5b** and **4.6b**).

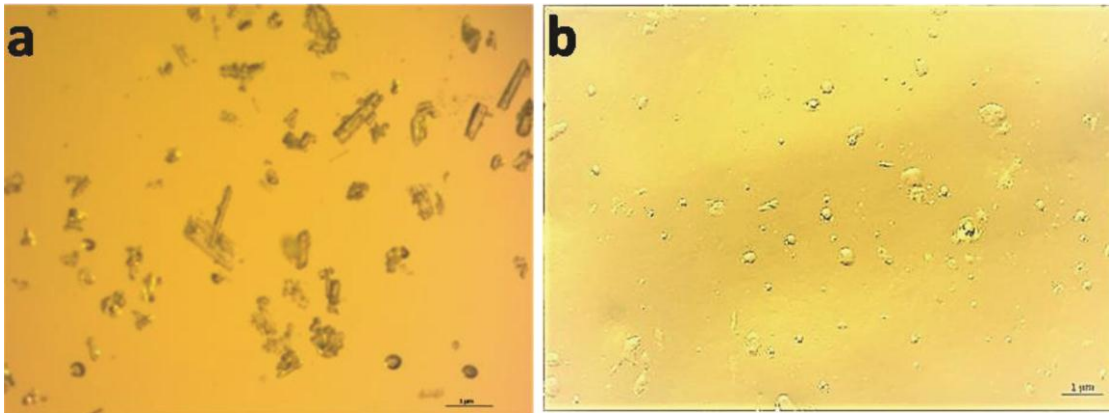

Figure 4.5. Micrographs of drug composite **22DAP**/MO (MOG+D) **(a)** before drug release and, **(b)** after complete drug release.

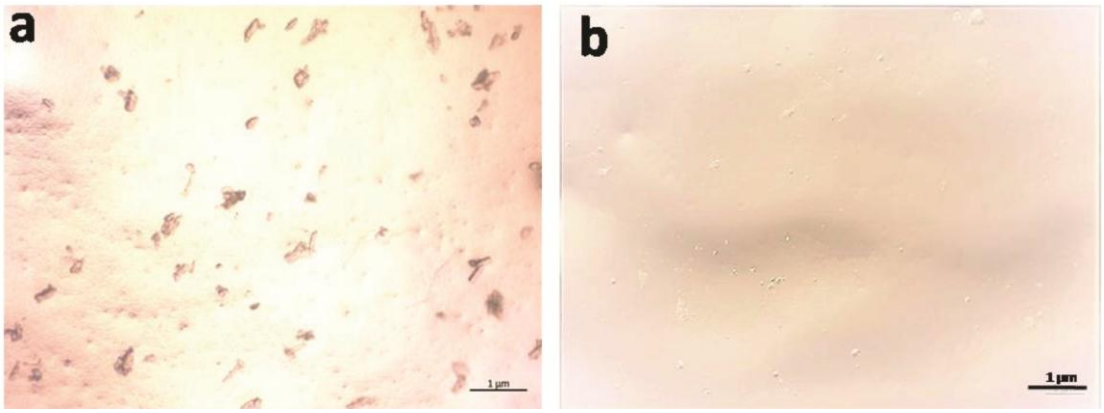

Figure 4.6. Micrographs of drug composite **22DAP**/OO (OOG+D) **(a)** before drug release and, **(b)** after complete drug release.

4.5.5 Rheological studies

The rheological studies of the oleogels are shown in the **Figure 4.7** and **4.8**. The rheological profile showed that the apparent viscosity of the OO gel is higher as compare to MO gel showing better mechanical strength of OO gel as compare to MO. From the graph, it was obtained that the shear viscosity of the oleogels was decreased on increasing the shear rate from 12 to 95 s^{-1}.

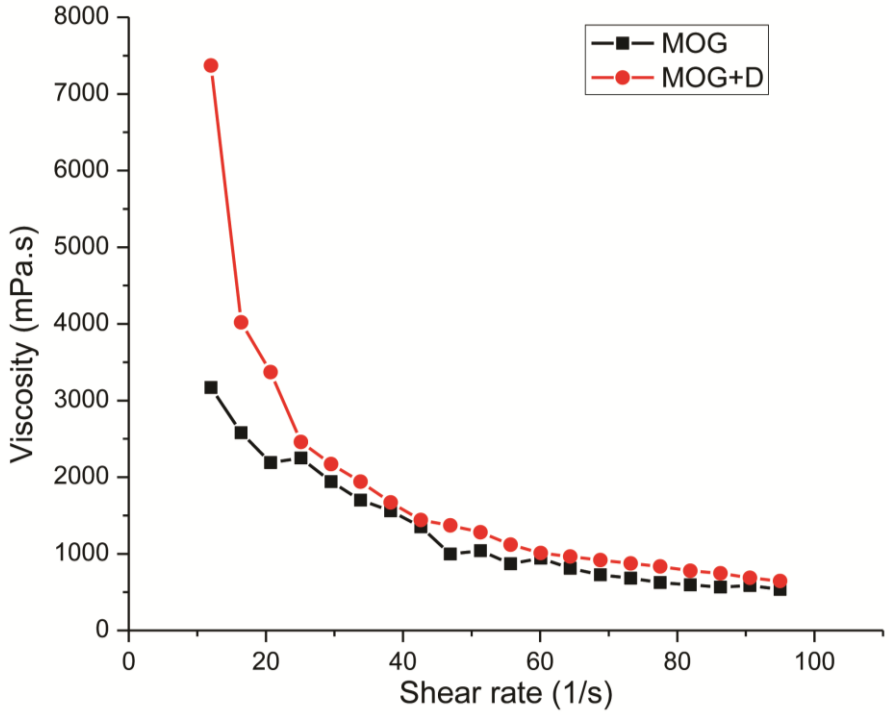

Figure 4.7. Viscosity profile of the MO oleogel (MOG) and metronidazole loaded MO gel (MOG+D) with respect to shear rate.

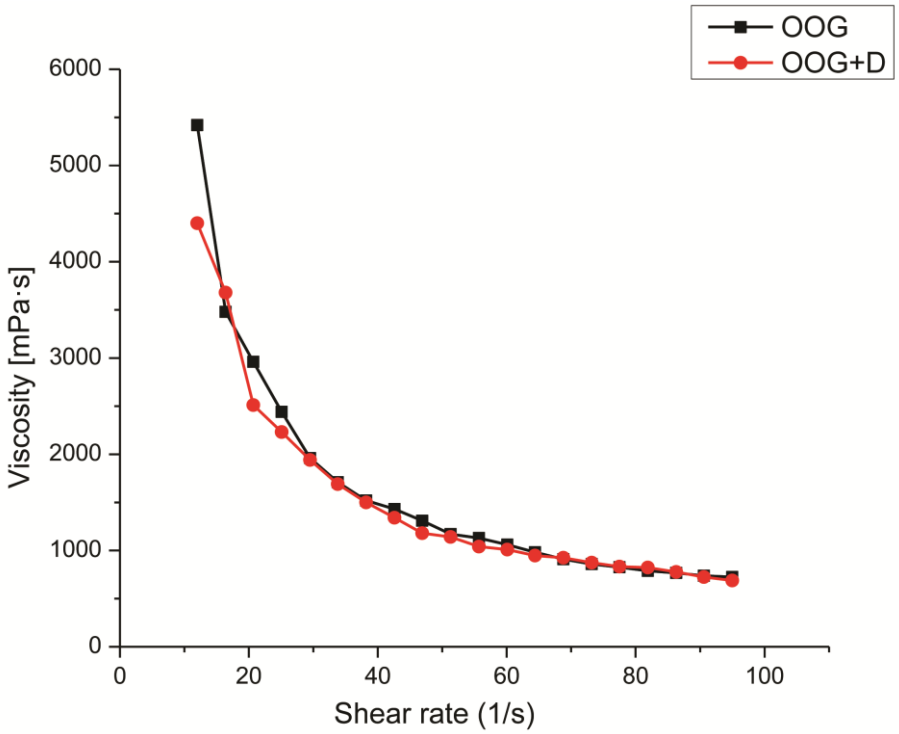

Figure 4.8. Viscosity profile of the OO oleogel (OOG) and metronidazole loaded OO gel (OOG+D) with respect to shear rate.

The decrease in the shear viscosity with increase in shear rate showed the shear thinning behaviour of the oleogels.[57] Both the metronidazole loaded oleogels and blank oleogels indicated the shear thinning behaviour which is common in ointments, creams and gels.[58] The flowing nature of the oleogels was determined by the modified power law (**equation 2**).[59]

$$\eta = K \gamma^{n-1} \quad (2)$$

Where η is the apparent viscosity (Pa.s), K is the flow consistency index, γ is shear rate (s−1) and n value determines the flowing 186ehaviour of the oleogels. From **equation 2**, it was calculated that the n values of the gels were < 1 which presented that the oleogels follows the non-Newtonian pseudoplastic fluid behaviour. Pseudoplasticity is known to be important property for topical formulations, because on increasing the shear stress, the gels will penetrate good through the skin.[60,61] The semi-solid nature of MO and OO oleogels provide them long term stability during storage.[60,62]

4.5.6 Comparative rheological study of MOG and OOG oleogels

The rheological studies of the MO and OO oleogels, and drug composite MO (MOG+D) and OO (OOG+D) oleogels are shown in the **Figure 4.9** and **4.10** respectively. The rheological profile showed that the apparent viscosity of the OO gel is higher as compare to MO gel showing better mechanical strength of OO gel as compare to MO. From the graph, it was obtained that the shear viscosity of the oleogels was decreased on increasing the shear rate from 12 to 95 s^{-1}.

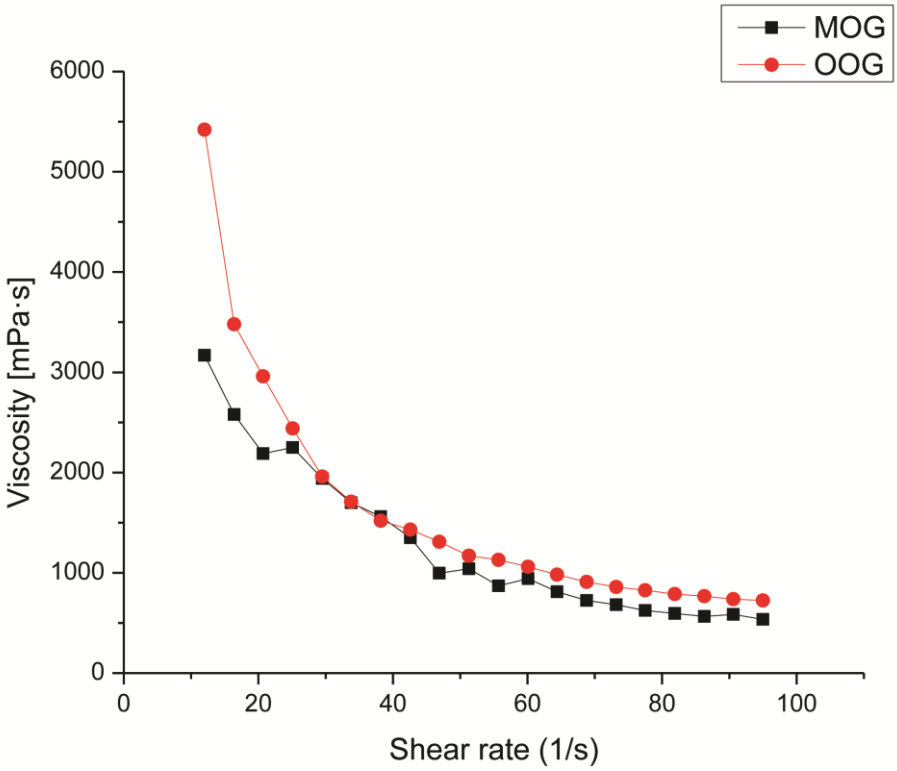

Figure 4.9. Viscosity profile of the MO (MOG) and OO (OOG) oleogels with respect to shear rate.

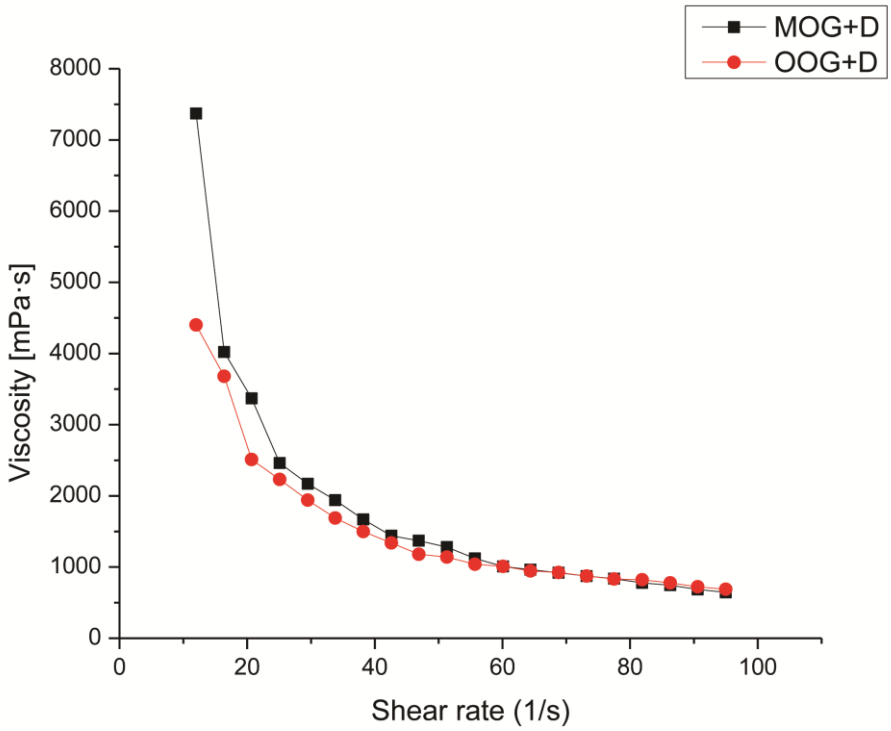

Figure 4.10. Viscosity profile of the drug composite MO (MOG+D) and OO (OOG+D) oleogels with respect to shear rate.

4.5.7 Spreadability studies

The % spreadability of the oleogels with respect to different weights has been shown in **Figure 4.11**. On increasing the applied load, the % spreadibility was significantly increased as shown in **Figure 4.11**. The gels were uniformly spread without loss of structural integerity after applying the load on to the oleogels, which advised good mechanical strength of gels. Good % spreadability suggested that the gels may act as a medium in transdermal and topical drug delivery applications.[63,64,65]

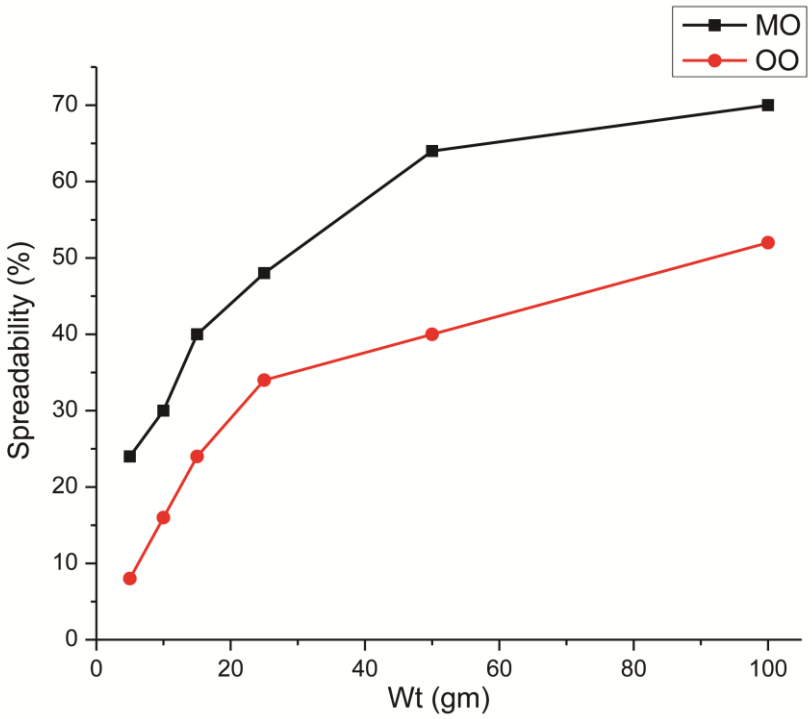

Figure 4.11. The graph indicating the % spreadability of oleogels with respect to applied weight.

It was obtained that MO oleogel had higher % spreadability (25-70%) than the OO gel (10-52%) on applying the load of 5, 10, 15, 25, 50 and 100 g respectively, and also the gel-to-sol transition temperature of MO oleogel was lower as compare to OO gel which resulted out that the OO gels had higher mechanical strength due to stronger interactions among the gel components.[66,67]

4.5.8 *In vitro* drug release studies

The release behaviour of drug molecules from the drug loaded oleogels depends on the dissolution and partition factor of the drug in the oil. For drug delivery application we have chosen metronidazole as a model drug. Metronidazole is a good antiseptic and antimicrobial agent and this is commonly used to treat adult periodontal diseases.[68-72] For drug delivery studies, 1% (w/w) metronidazole was loaded in both the oleogels (MO and OO). The release study of drug was obtained at 37 ^{0}C at a stirrer with 100 rpm.

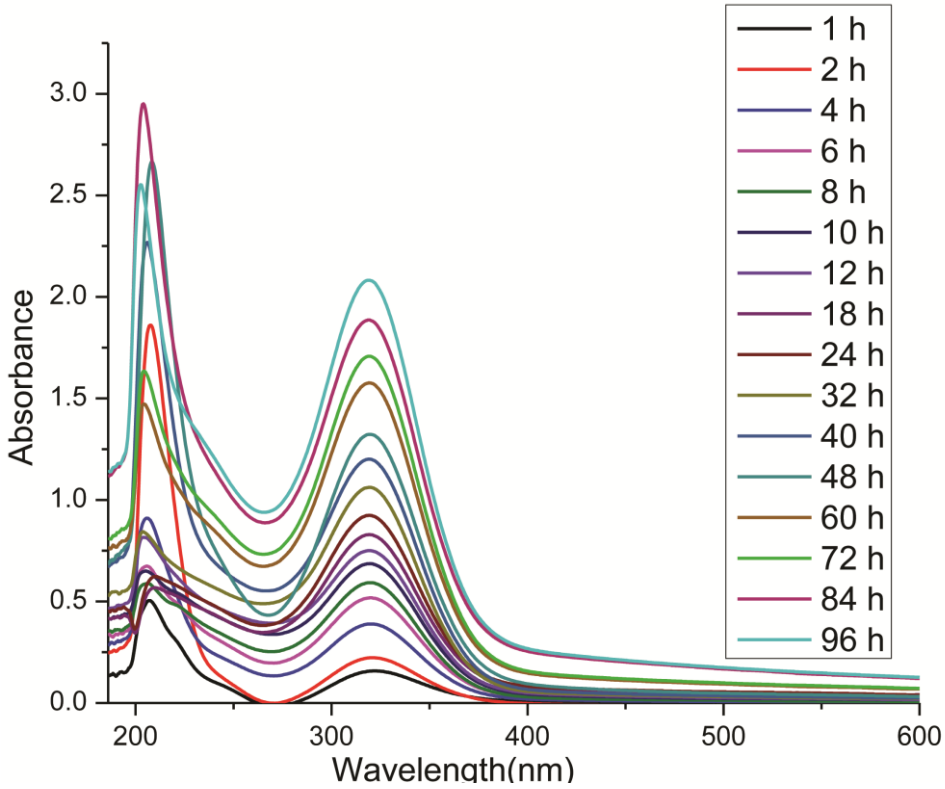

Figure 4.12. UV spectra of kinetic degradation of drug composite OO oleogel at pH 7.4.

The kinetic degradation of the drug composite OO oleogel in phosphate buffer receptor medium of pH 7.4 is as shown in **Figure 4.12.** The released amount of the drug metronidazole was calculated by the intensity of the characteristic peak of the metronidazole at λ_{max} 319.5 as shown in **Figure 4.12**. And it was observed that in case

of drug composite OO oleogel, initially only 5.96% of the drug was released within 1 h. After that the release was esteemed at a regular interval of time and it was found that the drug release was slow and nice controlled i.e. 35.09% in 24 h, 50.24% in 48 h, 59.85% in 60 h, 64.83% in 72 h, 71.62% in 84 h and 79.07% in 96 h respectively and after that the gel degraded completely and no more release was observed.

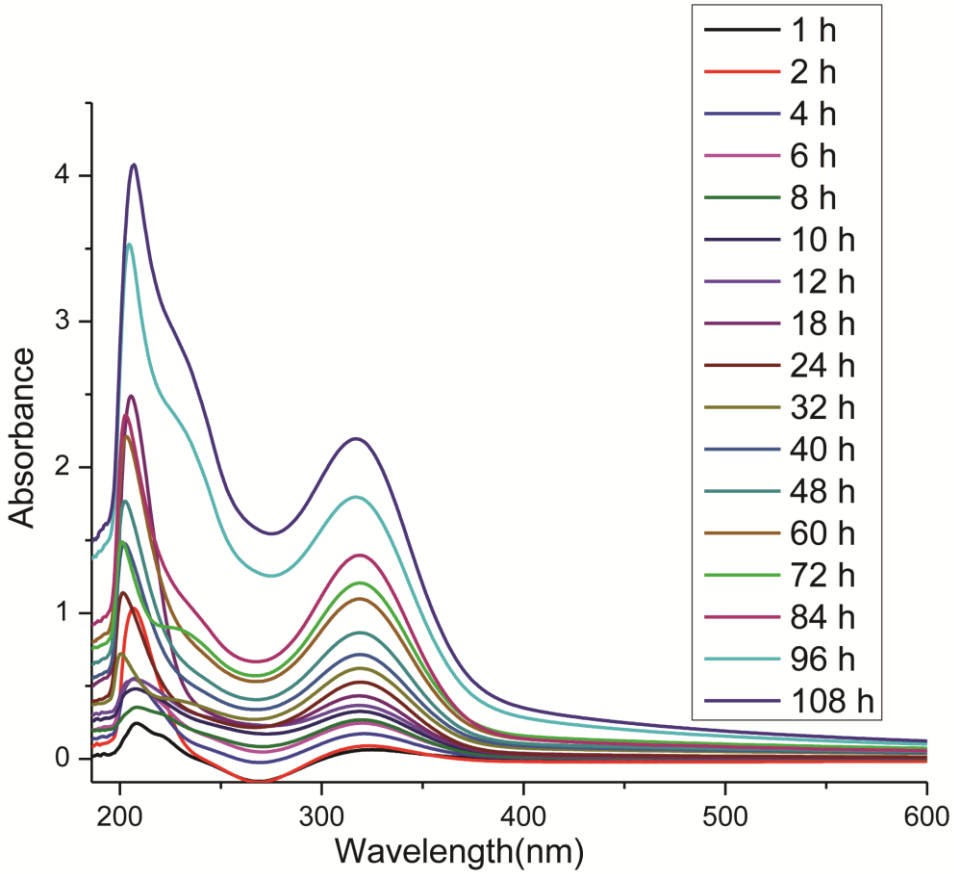

Figure 4.13. UV spectra of kinetic degradation of drug composite MO oleogels at pH 7.4.

The kinetic degradation of the drug composite MO oleogel in phosphate buffer receptor medium of pH 7.4 is as shown in **Figure 4.13**. And it was observed that in case of drug composite MO oleogel, initially only 2.31% of the drug was released within 1 h. After that the release was esteemed at a regular interval of time and it was found that the drug release was slow and nice controlled i.e. 19.98% in 24 h, 32.85% in 48 h, 41.66% in

60 h, 45.80% in 72 h, 53.01% in 84 h, 67.94% in 96 h and 83.06% in 108 h respectively and after that the gel degraded completely and no more release was observed.

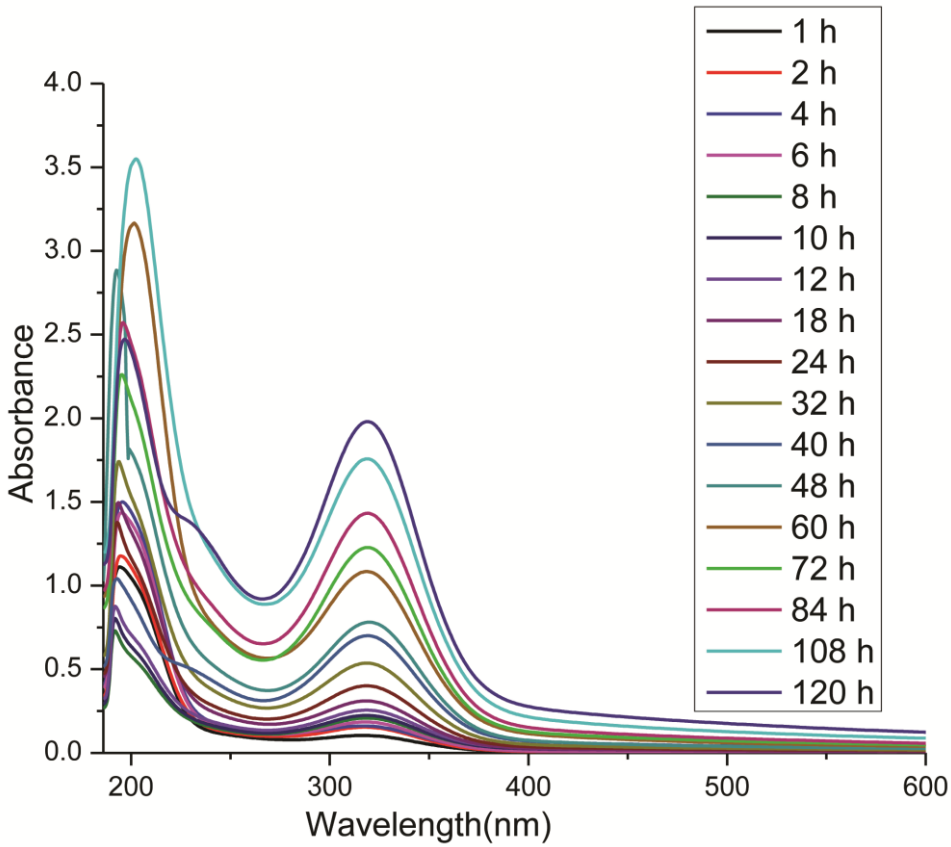

Figure 4.14. UV spectra of kinetic degradation of drug composite OO oleogel at pH 5.5.

The kinetic degradation of the drug composite OO oleogel in phosphate buffer receptor medium of pH 5.5 is as shown in **Figure 4.14**. The released amount of the drug metronidazole was calculated by the intensity of the characteristic peak of the metronidazole at λ_{max} 320.5 as shown in **Figure 4.14**. And it was observed that in case of drug composite OO oleogel, initially only 3.95% of the drug was released within 1 h. After that the release was esteemed at a regular interval of time and it was found that the drug release was slow and nice controlled i.e. 15.23% in 24 h, 29.66% in 48 h, 41.13% in 60 h, 46.60% in 72 h, 54.38% in 84 h, 66.73% in 96 h and 75.19% in 120 h

respectively and after that the gel degraded completely and no more release was observed.

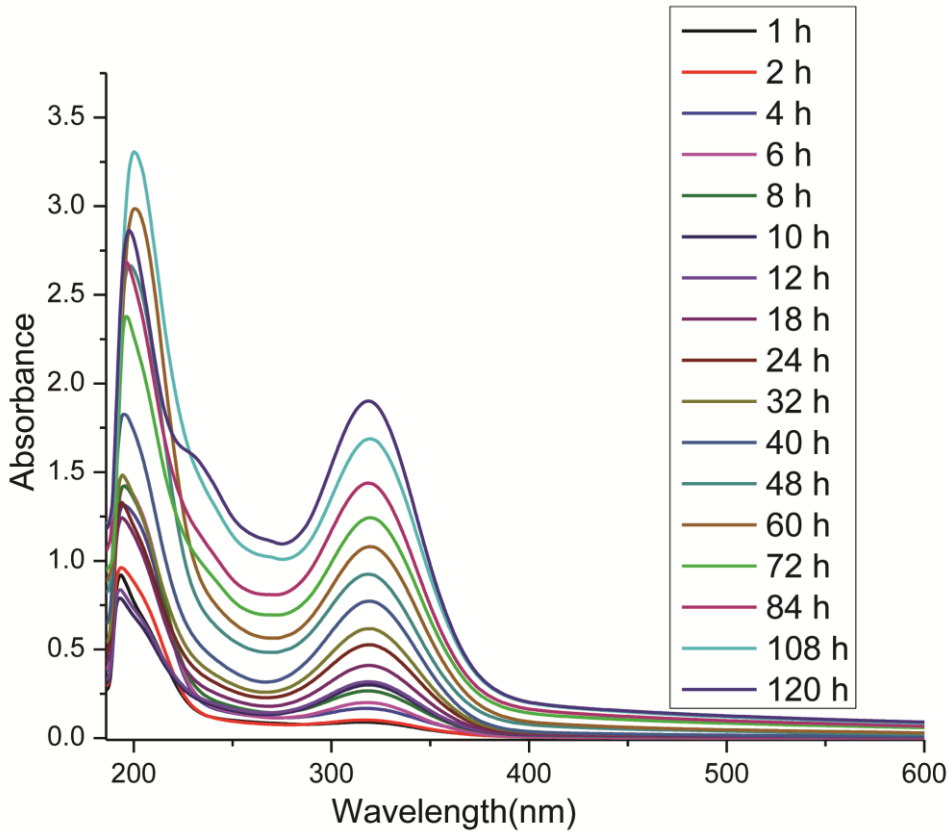

Figure 4.15. UV spectra of kinetic degradation of drug composite MO oleogels at pH 5.5.

The kinetic degradation of the drug composite MO oleogel in phosphate buffer receptor medium of pH 5.5 is as shown in **Figure 4.15**. The released amount of the drug metronidazole was calculated by the intensity of the characteristic peak of the metronidazole at λ_{max} 320.5 as shown in **Figure 4.15**. And it was observed that in case of drug composite MO oleogel, initially only 3.41% of the drug was released within 1 h. Afterthat the release was esteemed at a regular interval of time and it was found that the drug release was slow and nice controlled i.e. 19.98% in 24 h, 35.09% in 48 h, 41.01% in 60 h, 47.20% in 72 h, 54.58% in 84 h, 64.11% in 96 h and 72.16% in 120 h

respectively and after that the gel degraded completely and no more release was observed.

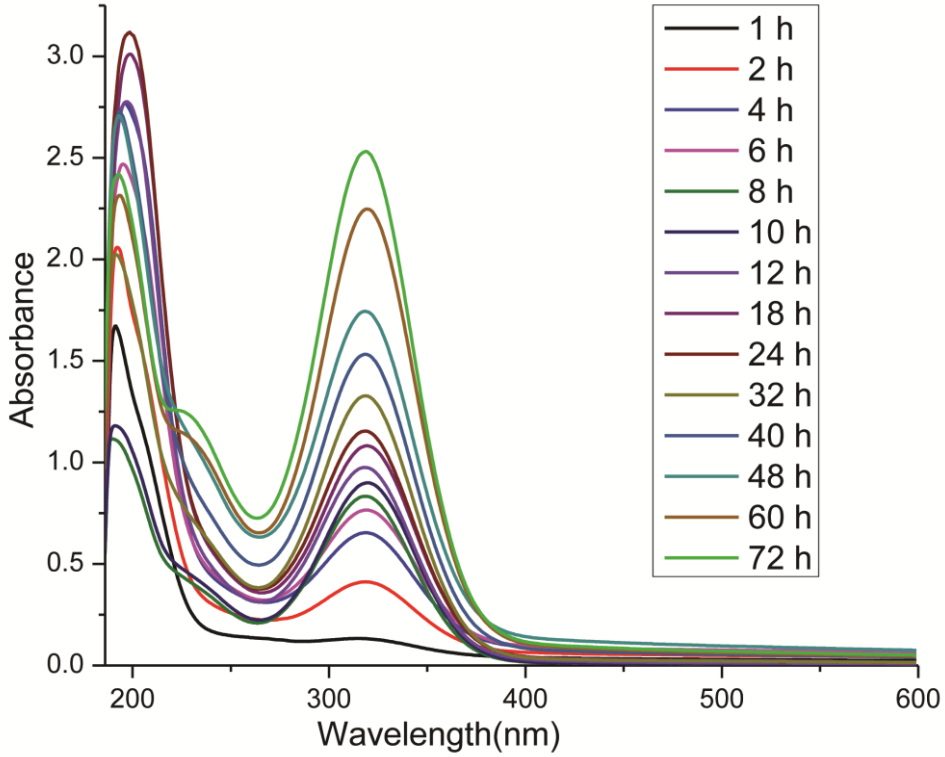

Figure 4.16. UV spectra of kinetic degradation of drug composite OO oleogels in ddw.

The kinetic degradation of the drug composite OO oleogel in ddw (double distilled water) is as shown in **Figure 4.16**. The released amount of the drug metronidazole was calculated by the intensity of the characteristic peak of the metronidazole at λ_{max} 320.0 as shown in **Figure 4.16**. And it was observed that in case of drug composite OO oleogel, initially only 4.97% of the drug was released within 1 h. Afterthat the release was esteemed at a regular interval of time and it was found that the drug release was slow and nice controlled i.e. 43.79% in 24 h, 66.16% in 48 h, 85.34% in 60 h and 96.09% in 72 h respectively and after that the gel degraded completely and no more release was observed.

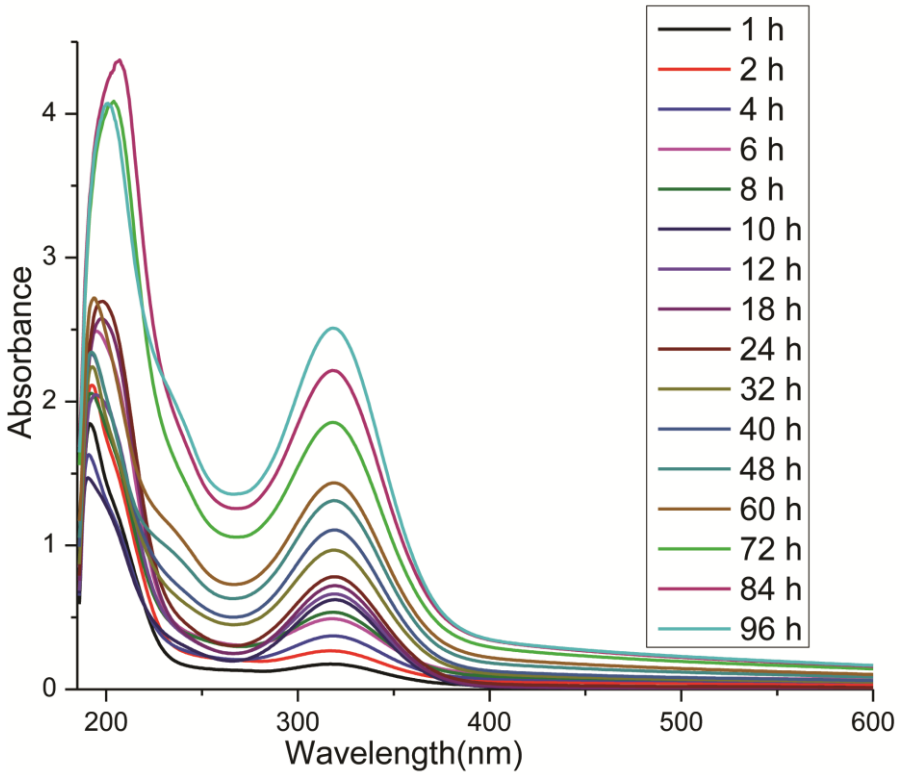

Figure 4.17. UV spectra of kinetic degradation of drug composite MO oleogels in ddw.

The kinetic degradation of the drug composite MO oleogel in ddw (double distilled water) is as shown in **Figure 4.17**. The released amount of the drug metronidazole was calculated by the intensity of the characteristic peak of the metronidazole at λ_{max} 320.0 as shown in **Figure 4.17**. And it was observed that in case of drug composite OO oleogel, initially only 6.65% of the drug was released within 1 h. After that the release was esteemed at a regular interval of time and it was found that the drug release was slow and nice controlled i.e. 29.69% in 24 h, 49.75% in 48 h, 85.34% in 60 h, 96.09% in 72 h, 84.04% in 84 h and 95.25% in 96 h respectively and after that the gel degraded completely and no more release was observed.

Figure 4.18, 4.19 and **4.20** clearly showed that the release rate of drug molecules was higher in case of OO gel as compare to MO gel in buffer receptor mediums at pH 7.4, 5.5 and in ddw respectively.

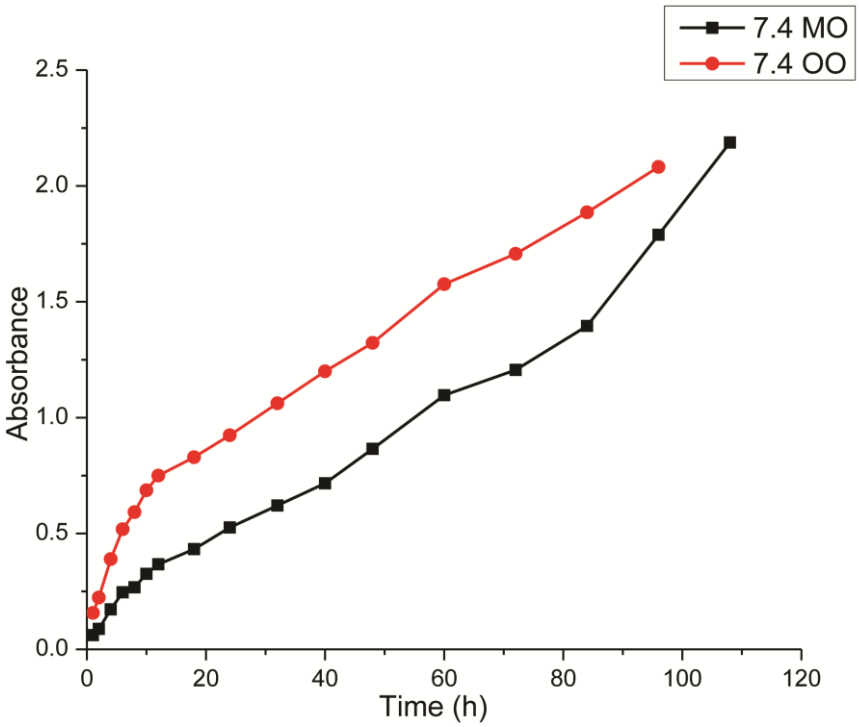

Figure 4.18. pH-Responsive cumulative release of metronidazole from the metronidazole composite oleogels (MO and OO) in phosphate buffer at pH 7.4.

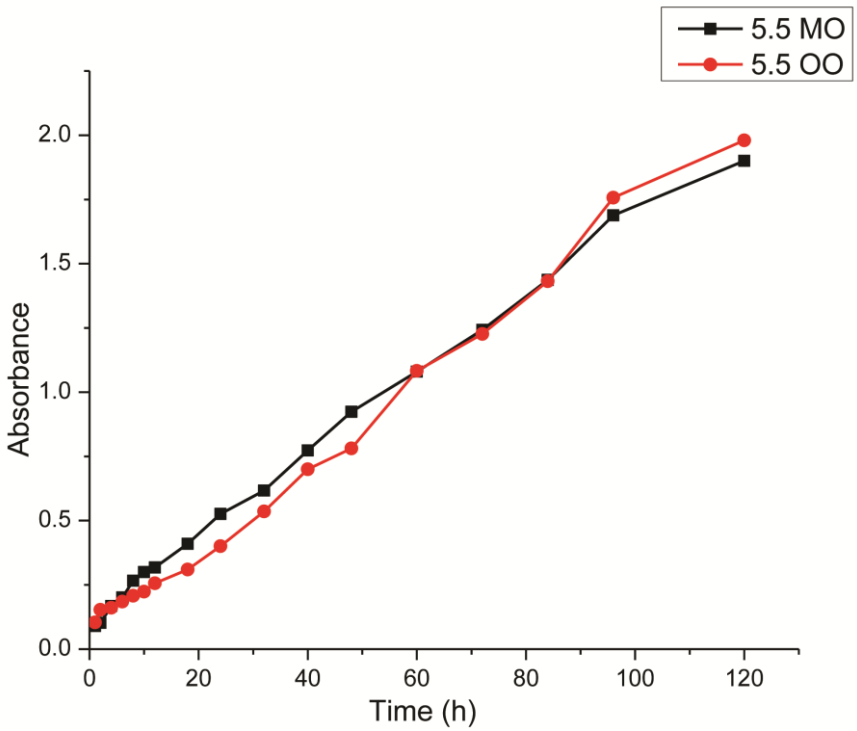

Figure 4.19. pH-Responsive cumulative release of metronidazole from the metronidazole composite oleogels (MO and OO) in phosphate buffer at pH 5.5.

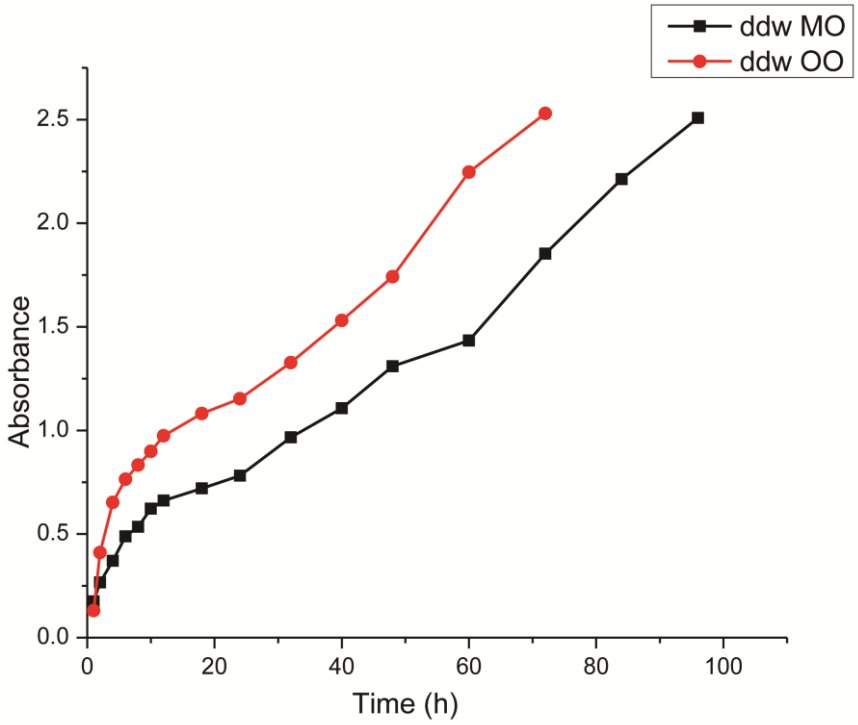

Figure 4.20. pH-Responsive cumulative release of metronidazole from the metronidazole composite oleogels (MO and OO) in ddw.

This may be due to the more compact arrangement amongst the gel component in OO oleogel, which produce greater hinderance for drug molecules to interact with the gel components which resulted out the faster release of drug molecules.

The cumulative drug release percentage in double distilled water and buffers at different pH is shown in **Figure 4.21**. **Figure 4.21** clearly indicates the effect of pH of receptor medium on the release of metronidazole from the oleogels prepared by the **22DAP**. It was noticed that the release rate of drug molecules was higher in case of OO gel as compare to MO gel in buffer receptor mediums at different pH and also in ddw respectively. This may be due to the more compact arrangement amongst the gel component in OO oil gel, which produce greater hindrance for drug molecules to interact with the gel components which resulted out the faster release of drug

molecules. The release rate and quantity was maximum in ddw receptor medium of both the oleogels. It was also observed that the drug release rate was slower and more controlled in case of acidic buffer (pH 5.8) from both the oleogels (MO and OO) as compare to slightly basic buffer (pH 7.4) because in acidic buffer metronidazole shows less solubility due to the formation of zwittorionic compound. It was also noticed that due to low solubility of drug in acidic receptor medium, the release rate has not much difference in both the oleogels (MO and OO). The drug release % in case of MO was 95.2% in 96 h (ddw), 83.1% in 108 h (pH 7.4) and 72.16% in 120 h (pH 5.5), while in case of OO the release % was 96.1% in 72 h (ddw), 79.1% in 96 h (pH 7.4) and 75.19% in 120 h (pH 5.5), respectively.

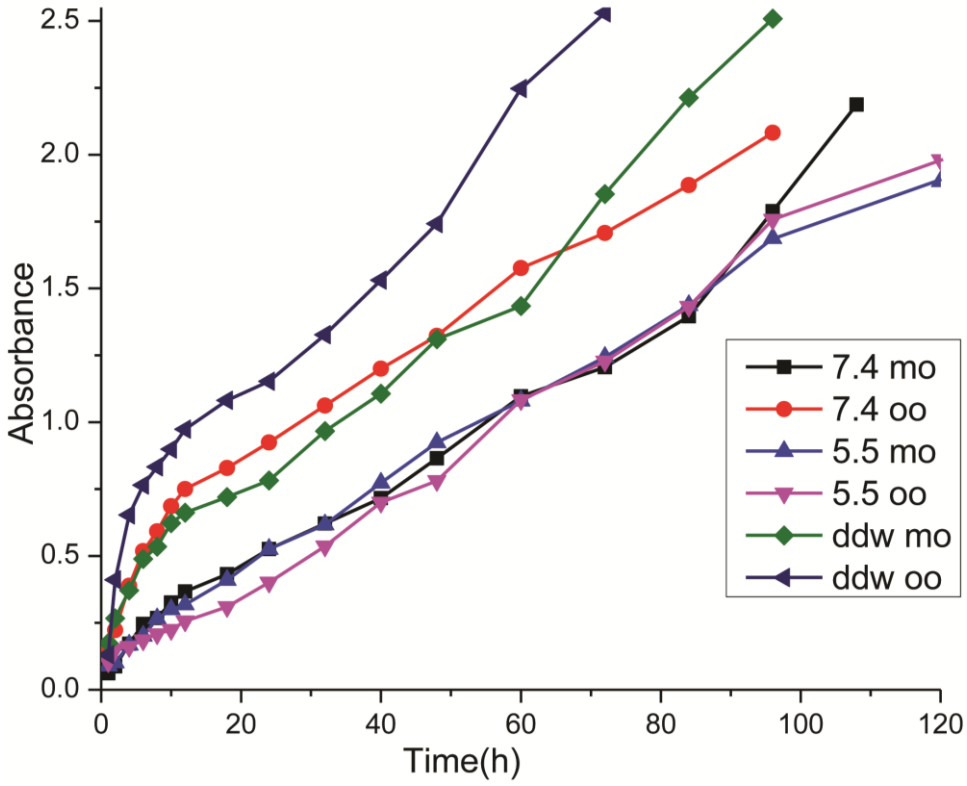

Figure 4.21. pH-Responsive cumulative release of metronidazole from the metronidazole composite oleogel at pH 7.4, 5.5 and ddw.

In summary, the release rate of both the oleogels was slow and nice controlled release due to longer fibre length of **22DAP** oleogelator which might results into higher area of overlapping and cross-linking providing good gel strength.[73]

4.5.9 FTIR analysis

Figure 4.22 shows the FTIR spectra of MO, OO, oleogels and met (metronidazole) composite oleogels. By FTIR spectral analysis, information regarding type of interactions amongst the drug and gel components can be determined. The FTIR spectra of gelator **22DAP** and oils was compared with the FTIR spectra of respective oleogels and then the FTIR spectra of the oleogels were compared with their respective drug (metronidazole) composite oleogels. The FTIR spectrum of the Metronidazole showed a broad peak at 3230 cm^{-1} which represents the presence of O–H stretching vibrations. While in case of spectrum of metronidazole composite MOG this peak at 3230 cm^{-1} is disappeared indicating the intermolecular H-bonding of metronidazole with MOG components.

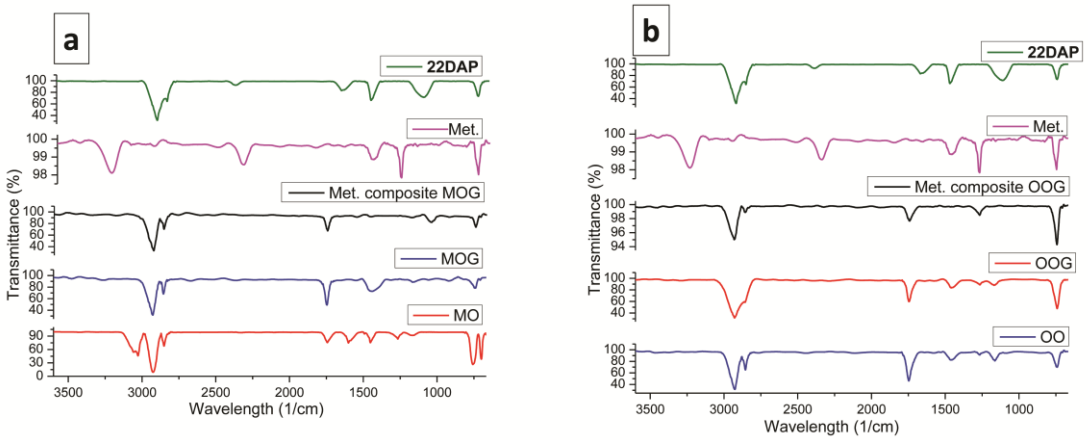

Figure 4.22. a) FTIR spectra of **22DAP**, drug Metronidazole, Met. composite MOG, MOG and MO, **b)** FTIR spectra of **22DAP**, drug Metronidazole, Met. composite OOG, OOG and OO.

Also the N-H stretching peak at 3480 cm^{-1} in case of MOG get shifted to 3449 cm^{-1} in Metronidazole composite MOG indicating the involvement of intermolecular H-bonding of the **22DAP** gelator components in MOG with drug molecules. While the C=N peak at 1460 cm^{-1} in metronidazole shifted to 1450 cm^{-1} in case of drug composite MOG. Similar variations were observed in case of OOG and drug composite OOG. Along with this, rest of spectral pattern of OO, OOG, Met. composite OOG, **22DAP**, MO, MOG and Met. composite MOG has no significant differences showing that the chemical functionality of the oleogel components was not changed even after the incorporation of the drug.

4.5.10 pH Measurement

The pH values of oleogels were noted to be in the range of 4.82 to 5.28 (**Table 4.3**) which was found to be in the range of skin pH. The pH range indicated that the synthesized oleogels may not irritate the skin and therefore they may be good vehicles for topical and transdermal applications without any side effects.[67,74,75]

Table 4.3: pH of Oleogels at 30.6°C

Gel	Drug (equiv.)	pH
22DAP/MO	-	4.82 ±0.30
22DAP/MO	1	5.09 ±0.30
22DAP/MO	-	5.01 ±0.30
22DAP/MO	1	5.28 ±0.30

4.6 Conclusion

In this study, MO- and OO-based oleoogels were successfully prepared. 22DAP was taken as an oleogelator. The oleogels were found to be pseudoplastic, shear thinning, semi-solid, opaque and thermo-reversible in behaviour. The thermal analysis showed

that the OO gels were thermally more stable as compare to MO. The rheological analysis proposed that OO gels were more viscous and having high mechanical strength. A comparative pH dependent drug release study of oleogels revealed that the release is faster in case of OO gel as compare to MO gel due to more compact nature of OO gel (the drug molecules may be weakly interact with OO gel). The drug release studies of the oleogels indicated a good and nice controlled release of the drug from the oleogels which suggested that long term antimicrobial activity may be assisted with these drug loaded oleogels and may have great potential in topical and transdermal drug delivery applications because of their good shear thinning and pseudoplastic behaviour.

4.7 References

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