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Fabrication of organogels achieved by prodrug-based organogelators of ketoprofen

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The treatment strategy of curing diseases using prodrugs of an anti-inflammatory drug is widespread. In the present study, we report on the synthesis of prodrugs of ketoprofen, consisting of a derivatization of ketoprofen and long hydrocarbon chain of fatty acids with diacylhydrazine linkage. The presence of an acidic moiety in ketoprofen may lead to ulceration in the gastrointestinal tract that reduces the efficacy of the drug with an increased adverse effect. The synthesis of prodrugs of ketoprofen involves the use of fatty acids as a carrier and hydrazine as a spacer. Synthesized prodrugs were characterized by infrared, ^{1}H-NMR and mass spectroscopy. The resulting prodrugs were found to be insoluble in water and precipitated out in acetonitrile, hexane, benzene, and so on. The synthesized prodrugs are slightly soluble in chloroform, methanol and ethanol and only form a gel like structure in carbon tetrachloride. The resulting gels are referred as organogels and prodrugs are referred to as organogelators. The surface morphology of the prepared organogels were studied by field emission-scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM), and other spectral characteristics were also investigated. FE-SEM and TEM images revealed that there were continuous elongated fiber-like structures present that were in the nanometer size range. Gel–sol temperature profiles of the prepared organogels were also studied using differential scanning calorimetry. The results from all these techniques are presented and discussed from point of view of the use of the derivatives as prodrug formulations. It is demonstrated that the prodrug containing the diacylhydrazine moiety has the ability to form a gel.

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Introduction

Development of drugs with minimum side effects is a never ending saga for pharmaceutical chemists. The prodrug concept is emerging as a new strategy in the field of drug delivery. Prodrugs provide sustainable release of a drug with efficient site availability, and improved bioavailability with minimum side effects.[1] The prodrug concept is that there is an inert derivative of the drug which is used to enhance the bioavailability and solubility during administration of parent drug. A prodrug is the bioreversible derivative of the parent drug, which changes the physiochemical properties and the pharmacological profiles of a drug molecule.[2] A synthetic strategy to design prodrugs includes minimization of side effects with easy administration of the parent drug. Non-steroidal anti-inflammatory drugs (NSAIDs) have limitations that are attributed to their ulcerogenic side effects in the gastrointestinal tract and poor water solubility.[3] Ketoprofen is the NSAID widely used in the treatment of tissue injuries and which diminishes inflammation associated various diseases by arresting prostaglandin synthesis.[4] An earlier pharmacological study of ketoprofen shows some adverse effects because of the presence of an acidic moiety in ketoprofen which causes ulceration in the stomach with prolonged use and also reduces its bioavailability.[5,6] It is well documented that prodrugs increase solubility and bioavailability of the parent drug.[7] The ketoprofen–saccharide conjugates and a polymeric prodrug can be synthesized successfully by using enzymatic or chemo-enzymatic reactions with biocompatibility and low toxicity.[5,6]

Many pharmaceutical chemists address drug delivery problems through use of prodrugs, gels, emulsion formulation, and so on. The gels are intermediate states of matter containing both solid and liquid components. Formation of gels requires a continuous network which may be achieved from fiber enlargement or trapping the mobile phase. Gelation takes place in organic media as a result of the binding forces between the gelator, intermolecular hydrogen binding or metal coordination bonds.[8] The formation of gel is because of non-linear polymerization of the molecule and the degree of polymerization depends upon the solute–solvent interactions. The structural details of the gel includes the conformation of molecule, nature and the extent of side-chains as well the solvent characteristic and inter-molecular forces.[9] Weak physical forces of attraction such as van der Waals interaction, hydrogen bonding, and π–π stacking are assumed to be responsible for the flexibilities of

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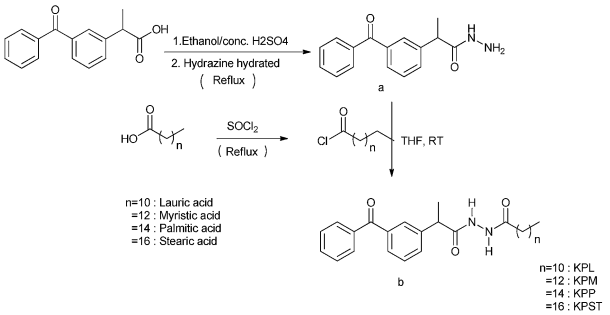
gels and their stability.[10,11] These gels play an important role in the polymer sciences, drug delivery, pharmaceuticals, food industry, cosmetics, making athletic shoes, and so on.[12] The gels can be differentiated into hydrogels and organogels on the basis of the solvent used for their preparation. Hydrogels are prepared in water whereas organogels exist in organic solvents of a low-medium and a low polarity. The microscopic framework of the organogel is a branched network of interlocking fibers that improve transdermal drug delivery.[13] Nowadays these gel-like structures and the study of them are claimed to form a sixth state of matter. Therefore many scientists are now engaged in the study of the mechanisms involved in the nucleation and growth of the gel network in a suitable solvent system.[14] Yuk *et al.*[15] have reported a pH-sensitive drug delivery system using an oil/water emulsion and also a hydrogen bonded complex gel in water for the temperature sensitive drug delivery system.[16]

It is always a challenging task to design organogelators because so many factors are known to govern their formation such as steric effects, ability to gel, polarity, thermal stability, and so on. Examples of the various organic building blocks that can be utilized are: saccharides, nucleic acid, cholesterol derivative, ureas, esters, amides, peptides, and so on.[17] Biodegradable and biocompatible materials are used to prepare gelators in order to avoid side effects for sustained release.[18,19] Generally, fatty acids have been used to prepare organogels of NSAIDs.[20] We report on studies related to the synthesis of prodrug-based organogelators containing long carbon chains and hydrazine as a spacer. The use of hydrazine as an active center containing carbon and nitrogen is responsible for the physical and chemical properties of the hydrazines and they are used for the synthesis of prodrug-based organogelators.



Scheme 1 Synthesis of prodrug-based organogelators. KPL: *N*′-(2-(3-benzoylphenyl)propanoyl)tetradecanehydrazide, KPM: *N*′-(2-(3-benzoylphenyl)propanoyl)tetradecanehydrazide, KPP: *N*′-(2-(3-benzoylphenyl)propanoyl)palmitohydrazide, KPST: *N*′-(2-(3-benzoylphenyl)propanoyl)stearohydrazide.

Table 1 Gelation study of organogelators in various solvents[a]

Solvents	KPST	KPP	KPL	KPM
Water	I	I	I	I
Chloroform	S	S	S	S
Methanol	S	S	S	S
Ethanol	S	S	S	S
Carbon tetrachloride	G	G	G	G
Acetonitrile	P	P	P	P
Hexane	P	P	P	P
Benzene	P	P	P	P
o-Xylene	P	P	P	P
m-Xylene	P	P	P	P
p-Xylene	P	P	P	P
n-Heptane	P	P	P	P
n-Octane	P	P	P	P

[a] I: insoluble, P: precipitate, G: gel, S: soluble.

Result and discussion

Designing prodrug-based organogelators of ketoprofen

The therapeutic efficacy of ketoprofen can be increased by preparing its prodrug, which is of great importance for contemporary drug delivery. Recently researchers have synthesized a prodrug of ketoprofen, but there were problems in the drug administration. We aimed to synthesize prodrugs of ketoprofen that contained a long hydrocarbon chain followed by incorporating diacylhydrazine linkage (Scheme 1) which may cleaved to release the parent drug. The gel formation of the synthesized prodrug was investigated. However, the gelation occurs in organic solvents, thus the prepared prodrugs are referred to as organogelators.

Gelation studies of prodrug-based organogelators

Synthesized prodrug-based organogelators were introduced to a wide range of solvents (Table 1). An appropriate amount of organogelators were poured into different glass vials containing a particular amount of solvents (1–10% w/v). The solutions obtained were heated slowly to cause dissolution of the organogelators, because as the heating period increases, the dissolution of the organogelators increases. The resultant solutions were cooled at room temperature to visualize gel formation. This gel formation is because of the intermolecular force of attraction, which causes aggregation of molecules that promote formation of gels having a well defined pattern of fibers. Gel formation was confirmed by inverting the glass vial and if no gravitational flow was observed, then a gel had been formed.

Prepared organogelators do not aggregate in water, they remain insoluble even after prolonged heating. In chloroform, methanol and ethanol organogelators were soluble but unable to form gels, whereas in acetonitrile, hexane and the other solvents tested (Table 1), organogelators were precipitated out. It was found that in carbon tetrachloride, molecules of prodrug-based organogelators were aggregated and formed a gel of excellent quality.

Gel–sol transition temperature (T_{gel})

The gel–sol transition temperature (T_{gel}) is the temperature at which the gel starts to melt and becomes a solution, this was investigated by a previously reported method.[21] In organogels prepared at 1–10% w/v, the T_{gel} occurs between 40–60 °C and after 5% w/v the gels are nearly at the same T_{gel} (Fig. 1). The concentration of organogelators were increased from 1% to 5%

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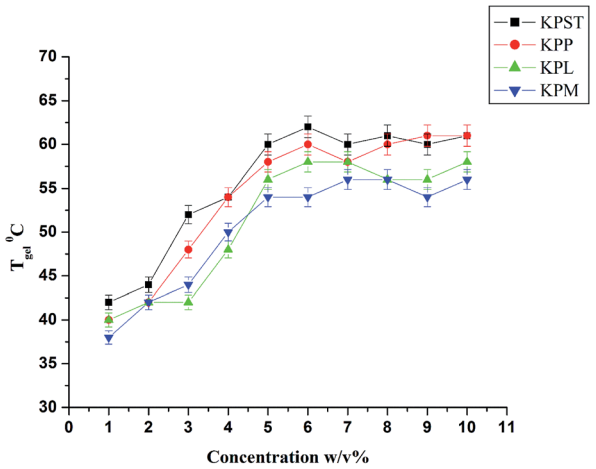

Fig. 1 Gel–sol transition temperature of organogels prepared from KPST, KPP, PKM, and KPL.

w/v for the gels tested, and it was observed that up to 5% w/v, T_{gel} also rises and it remains approximately the same from then onwards. This may be because of the length of the carrier which affects the T_{gel} to a certain extent, because as the number of carbons increases, the strength of the gel also increases. Thus, it can be concluded that the long chain of the carbon is responsible for the stability of the gels.

In differential scanning calorimetry (DSC) measurements, when the gel was heated slowly, the temperature rises and at a certain temperature, the gel melts suddenly exhibiting an endothermic peak at 52.94 °C. The enthalpy of transition from gel to solution is at $\Delta H = 126$ J g^{-1} calculated from the DSC thermogram (Fig. 2). When the gel was cooled it shows an exothermic peak at 47.26 °C having $\Delta H = -94.62$ J g^{-1}. Therefore, it can be concluded that organogels have a well-defined thermoreversible sol–gel transition.

Morphology of organogels

Surface morphology of all the organogels were studied by field-emission-scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM). The images at a 5% w/v concentration of KPST, KPP, KPM and KPL organogels show a well defined pattern of long thinner fibers having widths between 1.03 μm and 145 nm (Fig. 3). In contrast FE-SEM images obtained at a 1% w/v concentration exhibited no such network of thin fibers (Fig. 4). Therefore, it is clear that gels obtained at a 5% w/v concentration are well aggregated and form a better quality of gels than those obtained using 1% w/v concentration. TEM images of organogels obtained at a 5% w/v concentration are shown in Fig. 5, and the images reveal that the prepared organogels have fiber thickness of approximately 212 nm. However, fiber thickness also varies up to 0.26 μm. FE-SEM and TEM images of KPST, KPP, KPM and KPL organogels showing long, continuous, uniformly elongated fibers. The characterization suggested that organogels are fabricated in a well defined network pattern. Thus, prodrug-based organogelators assemble in a fibrillar network with a well defined pattern. An increased length of organogelators facilitates the formation of aggregates and also promotes gelation.

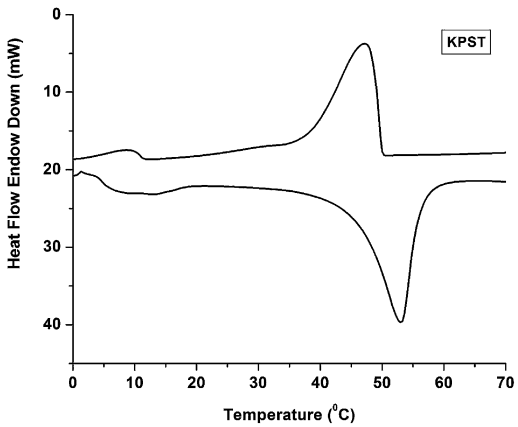

Fig. 2 DSC thermogram of KPST organogel.

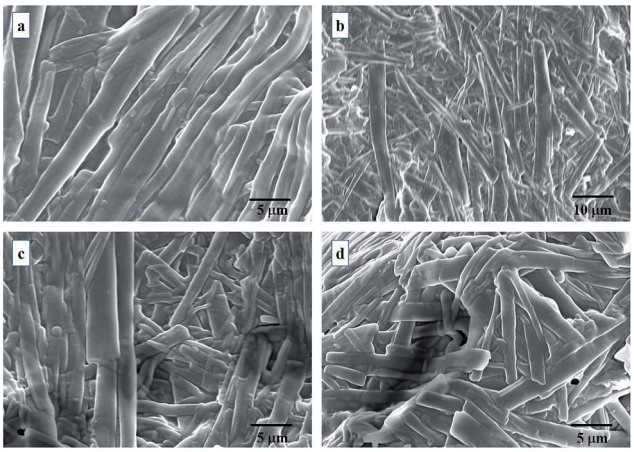

Fig. 3 FE-SEM images of organogels obtained at 5% w/v. (a) KPST organogel; (b) KPP organogel; (c) KPM organogel; (d) KPL organogel.

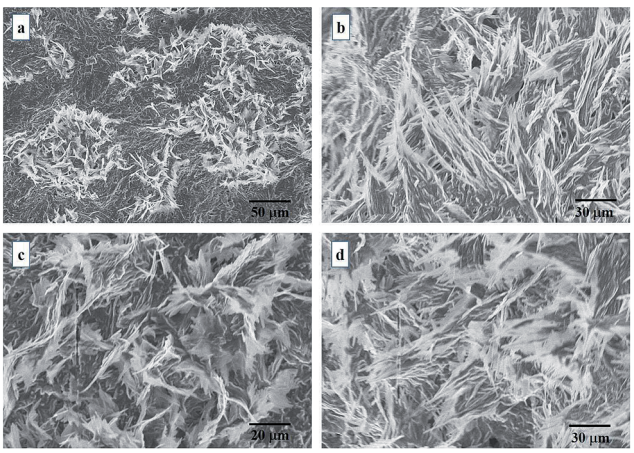

Fig. 4 FE-SEM images of organogels obtained at 1% w/v. (a) KPST organogel; (b) KPP organogel; (c) KPM organogel; (d) KPL organogel.

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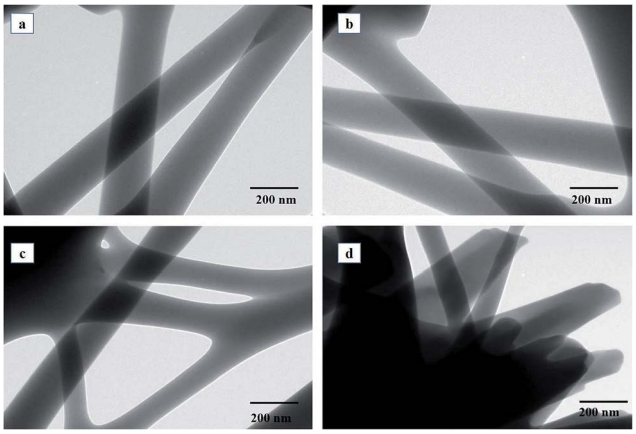

Fig. 5 TEM images of organogels obtained at 5% w/v. (a) KPST organogel; (b) KPP organogel; (c) KPM organogel; (d) KPL organogel.

^{1}H nuclear magnetic resonance (^{1}H-NMR) and Fourier transforms infrared spectrophotometry (FT-IR) study

The ^{1}H-NMR spectra of organogels exhibits quite similar features to that of its respective organogelators, even the aromatic region does not indicate any shifting of signals (Fig. 6). Therefore, it may be concluded that there is no π–π stacking involved in the fabrication of the network. An FT-IR study revealed that the amide bond of KPST had NH and CO stretching at 3261 and 1739, 1705, 1668 cm^{-1} and this was attributed to presence of a non-hydrogen bonded amide group. The FT-IR spectra of the KPST gel showed that the amide proton is merged in the alkyl region and does not show sharp bands because of the strong intermolecular hydrogen bonding, whereas the amide carbonyl exhibits shifts at 1696, 1654 and 1584 cm^{-1}. This clearly indicates that the two amide bonds are participating in stronger intermolecular hydrogen bonding, which results in aggregation of molecules and promotes gelation. Thus, the organogels encompassing the parallel sheet pattern of a fibrillar network, because of the hydrogen bonding between the carbonyl group of the amide group of one molecule with the amide group of another molecule (Fig. 7).

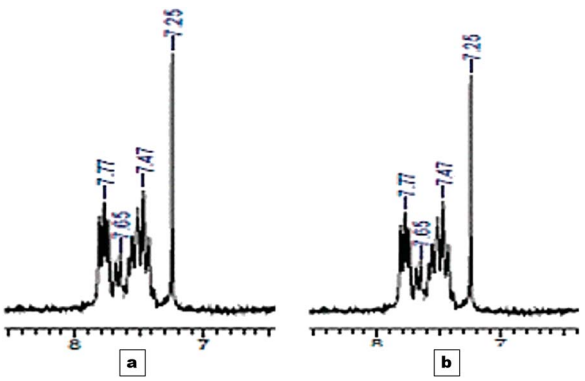

Fig. 6 ^{1}H-NMR spectra for the aromatic region of (a) KPST gelator; (b) KPST organogel.

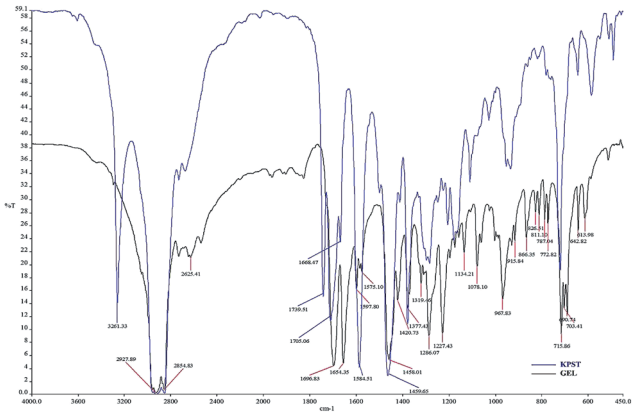

Fig. 7 Comparison of FT-IR spectra for KPST and the organogel of KPST.

Experimental

Materials

The Ketoprofen used in this study was a gift from Enaltech Laboratories, Mumbai (M.S.), India. All the chemicals and solvents used were of synthetic grade (SD Fine-Chem, Mumbai, India). All solvents and chemical were used as obtained.

Synthesis of organogelators

The overall reaction scheme was adapted to synthesize a fatty acid derivative of ketoprofen which is a prodrug-based organogelators and is shown in Scheme 1.

Synthesis of 2-(3-benzoylphenyl)propane hydrazide (a). To the mixture of ketoprofen (0.0039 mole) and absolute ethanol (10 ml), 1–2 ml of conc. H_2SO_4 was added dropwise with constant stirring and the reaction mixture was refluxed. The progress of the reaction was monitored using thin-layer chromatography (TLC). After completion of the reaction, the hydrazine hydride (99.9%; 0.0058 mole) was added dropwise with continuous stirring. Then the reaction mixture was refluxed for few hours. The completion of the reaction was checked using TLC. After completion of the reaction, the reaction mixture was poured in to a crushed ice-water mixture. The synthesized product was collected from the organic layer using water as a phase transfer medium. The product in organic layer was washed with brine solution, yielding a yellowish colored solid product and the presence water and vaporized impurities were removed by applying a vacuum at 60 °C. The synthesized 2-(3-benzoylphenyl)propanehydrazide (**a**) was purified by column chromatography (hexane–ethyl acetate (2 : 8)).

Synthesis of fatty acid derivatives of 2-(3-benzoylphenyl)-propanehydrazide as organogelator. An equimolar mixture of 2-(3-benzoylphenyl)propanehydrazide (**a**) was dissolved in tetrahydrofuran (10 ml), and to this solution acyl chlorides of the corresponding fatty acids were added. Then the reaction mixture was stirred at room temperature. The progress of the reaction was monitored using TLC. The separated solids were collected by filtration and washed with cold water and purified by column chromatography. The organogelators prepared were

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characterized using infrared, NMR, mass spectral studies, and the detailed are given next.

N′-(2-(3-Benzoylphenyl)propanoyl)stearohydrazide (KPST). Yield: 86%, FT-IR (ν_{max}/cm^{-1}): 3261 (NH); 2927 (alkyl CH); 1739 (CO of ketone); 1708, 1668 (CO of amide). ^{1}H-NMR (δ ppm): 7.77 (3H, m); 7.65 (3H, m); 7.47 (3H, m); 5.75 (2H, s); 3.56 (1H, q); 2.33 (2H, m); 1.59 (3H, d); 1.50 (2H, m); 1.21–1.24 (28H, m); 0.87 (3H, m). Mass *m/z*: 535 (M + 1) ($C_{34}H_{50}N_2O_3$).

N′-(2-(3-Benzoylphenyl)propanoyl)palmitohydrazide (KPP). Yield: 82%, FT-IR (ν_{max}/cm^{-1}): 3226 (NH); 2920 (alkyl CH); 1735 (CO of ketone); 1701, 1660 (CO of amide). ^{1}H-NMR (δ ppm): 7.77 (3H, m); 7.51 (3H, m); 7.45 (3H, m); 5.75 (2H, s); 3.76 (1H, q); 2.34 (2H, m); 1.62 (3H, d); 1.54 (2H, m); 1.21–1.24 (28H, m); 0.87 (3H, m). Mass *m/z*: 507 (M + 1) ($C_{32}H_{46}N_2O_3$).

N′-(2-(3-Benzoylphenyl)propanoyl)tetradecanehydrazide (KPM). Yield: 70%, FT-IR (ν_{max}/cm^{-1}): 3226 (NH); 2922 (alkyl CH); 1737 (CO of ketone); 1704, 1662 (CO of amide). ^{1}H-NMR (δ ppm): 7.79 (3H, m); 7.61 (3H, m); 7.48 (3H, m); 5.79 (2H, s); 3.74 (1H, q); 2.34 (2H, m); 1.58 (3H, d); 1.51 (2H, m); 1.21–1.25 (28H, m); 0.87 (3H, m). Mass *m/z*: 479 (M + 1) ($C_{30}H_{42}N_2O_3$).

N′-(2-(3-Benzoylphenyl)propanoyl)tetradecanehydrazide (KPL). Yield: 65%, FT-IR (ν_{max}/cm^{-1}): 3210 (NH); 2924 (alkyl CH); 1737 (CO of ketone); 1712, 1666 (CO of amide). ^{1}H-NMR (δ ppm): 7.77 (3H, m); 7.58 (3H, m); 7.41 (3H, m); 5.70 (2H, s); 3.71 (1H, q); 2.33 (2H, m); 1.54 (3H, d); 1.50 (2H, m); 1.21–1.24 (28H, m); 0.86 (3H, m). Mass *m/z*: 551 (M + 1) ($C_{28}H_{38}N_2O_3$).

Fourier transform infrared spectrophotometry, ^{1}H-nuclear magnetic resonance and mass spectroscopy measurements

The synthesized organogelators were analyzed on a PerkinElmer Spectrum One spectrophotometer using a Nujol mull sampling method. ^{1}H-NMR spectra were recorded on a Varian Mercury 300 MHz NMR, and scanned at 300 MHz deuterated chloroform as solvent. Chemical shifts were reported in ppm and compared to tetramethylsilane as an internal standard. The mass spectra were recorded on a Shimazdu LCMS-QP 8000 LC-MS spectrometer.

Differential scanning calorimetry

DSC analysis of the prepared organogels was performed using a PerkinElmer DSC-400 instrument. The weighed samples (10 mg) were placed in an aluminium pan and the samples were scanned from 0 °C to 200 °C at a heating rate of 10 °C min^{-1}.

Field emission-scanning electron microscope

The prepared organogels were scanned using a Hitachi High Technologies S-4800-II SEM with an accelerating voltage of 5000 V and the emission current was 10 100 nA.

Transmission electron microscopy

TEM of the samples was performed using an FEI-Tecnai-F20 electron microscope operating at 10 000 kV. A small amount of gel sample was placed on the copper grid, it was dried at room temperature and directly observed under the TEM.

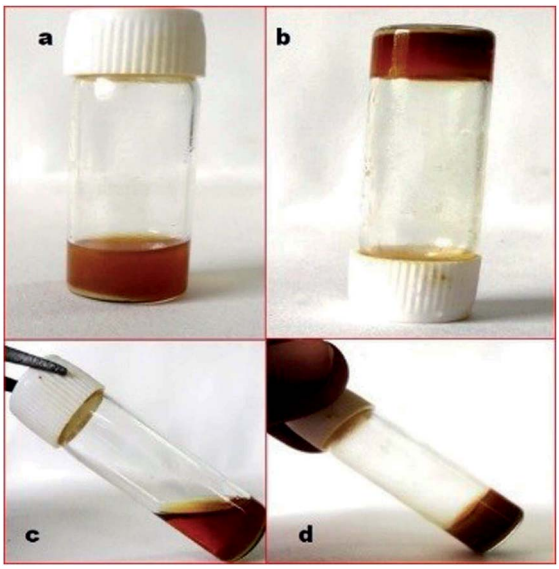

Fig. 8 Organogelators dissolve in the carbon tetrachloride: (a) before gelation; (b) after gelation; (c) organogel at 60 °C; (d) organogel at 25 °C.

Preparation of organogels

Organogels were prepared by the prodrug-based organogelators by taking appropriate amounts of solvents and gelators (110% w/v) in scintillation glass vials which were sealed with a screw cap. These vials were heated up to 60–80 °C until the gelators were completely dissolved in the solvents. Then the vials were cooled slowly to room temperature, and after approximately 70–80 min, a viscous gel was obtained for each particular solvent. The formation of the organogel (Fig. 8b) was confirmed by inverting the tube, and if no gravitational flow was observed on inverting the tube, then a gel had been formed.

Gel–sol transition temperature (T_{gel})

The gel to solution transition of the prepared organogels was determined by using the 'Inversion tube method'.[21] In this method, gels of 1–5% w/v gelators were placed in oven in such a way that the upper side of vial faces downwards and the vial and its contents were heated slowly. At a certain temperature, the gel melts and flows downwards in the inverted vial and this point is referred to as the gel–sol transition temperature.

Conclusion

We have reported the synthesis of prodrugs of ketoprofen. In many cases prodrugs are not supposed to reached the site of the drug's action, however, they are required as a carrier. In the present work we introduced a long carbon chain *via* a diacylhydrazine linkage to the ketoprofen. The overall purpose of this study is to design prodrugs of ketoprofen. The prepared prodrugs must be able to form gels, thus the gelation ability of the synthesized prodrug was also studied. An excellent gel quality, with the formation of a fibrillar network was observed using carbon tetrachloride. Thus, the prodrugs prepared are

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referred to as prodrug-based organogelators. The resulting gels have a good themoreversible sol–gel transition.

Acknowledgements

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Notes and references

1 C. Bao, M. Jin, B. Li, Y. Xu, J. Jin and L. Zhu, *Org. Biomol. Chem.*, 2012, **10**, 5238.
2 J. S. Sohn, J. I. Jin, M. Hess and B. W. Jo, *Polym. Chem.*, 2010, **1**, 778.
3 M. Babazadeh, *Int. J. Pharm.*, 2008, **356**, 167.
4 H. Y. Wang, C. Li, N. Wang, K. Li, X. W. Feng, T. He and X. Q. Yu, *Bioorg. Med. Chem.*, 2009, **17**, 1905.
5 X. Cai, N. Wang and X. Lin, *Polymer*, 2006, **47**, 6491.
6 V. K. Tammara, M. M. Narurkar, A. M. Crider and A. M. Khan, *Pharm. Res.*, 1993, **10**, 1191.
7 M. Sugimoto, T. Okagaki, S. Narisawa, Y. Koida and K. Nakajima, *Int. J. Pharm.*, 1998, **11**, 160.
8 K. J. Skilling, F. Citossi, T. D. Bradshaw, M. Ashford, B. Kellam and M. Marlow, *Soft Matter*, 2014, **10**, 237.
9 F. Zhao, M. L. Ma and B. Xu, *Chem. Soc. Rev.*, 2009, **38**, 883.
10 A. Vintiloiu and J. C. Leroux, *J. Controlled Release*, 2008, **125**, 179.
11 S. Murdan, *Expert Opin. Drug Delivery*, 2005, **2**, 489.
12 D. J. Abdallah and R. G. Weiss, *Langmuir*, 2000, **16**, 352.
13 P. F. C. Lim, X. Y. Liu, L. Kang, P. C. L. Ho, Y. W. Chan and S. Y. Chan, *Int. J. Pharm.*, 2006, **311**, 157.
14 A. R. Hirst, I. A. Coates, T. R. Boucheteau, J. F. Miravet, B. Escuder, V. Castelletto, I. W. Hamley and D. K. Smith, *J. Am. Chem. Soc.*, 2008, **130**, 9113.
15 S. H. Yuk, S. H. Cho and H. B. Lee, *J. Controlled Release*, 1995, **37**, 69.
16 K. S. Oh, S. K. Han, Y. W. Choi, J. H. Lee, J. Y. Lee and S. H. Yuka, *Biomaterials*, 2004, **25**, 2393.
17 I. A. Coates, A. R. Hirst and D. K. Smith, *J. Org. Chem.*, 2007, **72**, 3937.
18 A. Motulskya, M. Lafleurb, A. C. Couffin-Hoaraua, D. Hoarauc, F. Bouryd, J. P. Benoitd and J. C. Lerouxa, *Biomaterials*, 2005, **26**, 6242.
19 Y. A. Shchipunov, *Russ. Chem. Rev.*, 1997, **66**, 301.
20 T. Penzes, G. Blazso, Z. Aigner, G. Falkay and I. Eros, *Int. J. Pharm.*, 2005, **298**, 47.
21 P. K. Vemula, G. A. Cruikshank, J. M. Karp and G. John, *Biomaterials*, 2009, **30**, 383.

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