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RESEARCH PAPER

Spontaneous Gelation of a Novel Histamine H4 Receptor Antagonist in Aqueous Solution

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ABSTRACT

Purpose Low molecular weight hydrogelators typically require a stimulus such as heat, antisolvent, or pH adjustment to produce a gel. This study examines gelation of a novel histamine H4 receptor antagonist that forms hydrogels spontaneously at room temperature.

Methods To elucidate the mechanism and structural moieties responsible for this unusual gelation, hydrogels were characterized by rheology, optical microscopy, and XRD. SEM was performed on xerogels; NMR measurements were conducted in gelator solutions in the presence of a gel-breaker. The influence of temperature, concentration, pH, and ionic strength on elastic and viscous moduli of the hydrogels was evaluated; gel points were established via thorough rheological criteria.

Results The observed are "true" gels with a fibrillar texture and lamellar microstructure. On a molecular level, the gels are composed of aggregates of partially ionized species stabilized by hydrophobic interactions of aromatic moieties. The gel-to-sol transition occurs at physiologically relevant temperatures and is concentration-, pH-, and ionic strength-dependent.

Conclusions We hypothesize that this spontaneous gelation is due to the so-called "spring" effect, a high energy salt form that transiently increases aqueous solubility above its equilibrium limit. Upon equilibration, this supersaturated system undergoes aggregation that avoids crystallization and produces a hydrogel.

KEY WORDS gelation · low molecular weight hydrogels · rheology · supramolecular assembly · xerogels

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INTRODUCTION

Gels represent a class of soft matter often described as consisting of three-dimensional networks that immobilize a liquid component in a continuous structure of macroscopic dimensions that is permanent on the time scale of the experiment (1). Owing to this structure, gels can exhibit mechanical properties of a solid even when comprised predominantly of the liquid component. This unique behavior is the underlying reason why gelled systems have been attracting immense scientific and practical interest. Hydrogels, i.e. aqueous gels containing no less than 90% of liquid mass, are of particular significance because of their important role in nature and extensive commercial use, including pharmaceutical (2,3) and biomedical applications (4,5).

Traditional gelators of water are high molecular weight compounds such as synthetic polymers, polysaccharides, and proteins. Hydrogels formed by non-covalently bound small molecules (referred to as supramolecular or, simply, molecular hydrogels) have also been described. However, with the exception of classic amphiphiles such as surfactants and lipids, low molecular weight (LMW) hydrogelators received little attention until the last decade, when it was realized that applications for these systems can be as diverse

as those for their polymeric counterparts (6–8). LMW hydrogels represent a special type of self-assembly manifested by formation of highly anisotropic (typically, fibrillar) aggregates that, at very low concentrations, are capable of propagating through the entire volume of solvent, yielding a physically branched or entangled network (6,7). Although seemingly related to their more well-developed counterparts, molecular organogels (9–11), LMW hydrogels are fundamentally different due to the nature of forces that drive gelation in these two cases. Highly directional hydrogen bonding, the key driving force for aggregation in organogels (6,7,10), is deteriorated in aqueous environments. Instead, hydrophobic interactions become the main driving force for supramolecular assembly in water (6,7). However, hydrophobic interactions lack precise directing ability, which presents a peculiar challenge for anisotropic self-assembly and makes LMW hydrogelation a rather rare phenomenon.

Examples of LMW hydrogelators do not appear common in the pharmaceutical industry and warrant special attention, as intermolecular aggregation may have profound effects on drug behavior and formulation development. Moreover, formation of hydrogels by pharmaceutically active molecules may open new delivery routes for such molecules. Thus, Xu *et al.* reported on hydrogelation of the well-known antibiotic vancomycin modified with a pyrene group (12,13). This derivative exhibited a manifold higher antibiotic activity than the parent, and the activity enhancement was reported to be due to self-assembly of vancomycin-pyrene on the bacterial cell surface. The same group described formation of a supramolecular hydrogel in a mixture of Fmoc-L-leucine and Fmoc-(N^{ε})-L-lysine (14). These amino acids belong to a novel class of anti-inflammatory agents, offering the potential to use the gel as a wound dressing. A handful of other examples of molecular hydrogels of therapeutic agents can be found in recent reviews (6,15).

Virtually all LMW hydrogelators reported thus far required some sort of external stimulus to form a gel (6,16–18). Most commonly it is through heating to an isotropic supersaturated solution followed by a drop in temperature below the gelator's solubility limit (19–23). Other "stimuli" include introduction of an anti-solvent (24) or another compound (25), pH adjustment (14,26), and sonication (27). In fact, the only example of an LMW hydrogel obtainable simply by addition of water found in our extensive literature search was reported by Bieser as "exceptional" and relied on re-hydration of a xerogel initially produced through heating (28). Here, we report formation of an LMW hydrogel that occurrs spontaneously without any external stimuli. Compound 1, structure shown in Fig. 1, is a novel human histamine H4 receptor antagonist for the treatment of inflammatory disease. It is

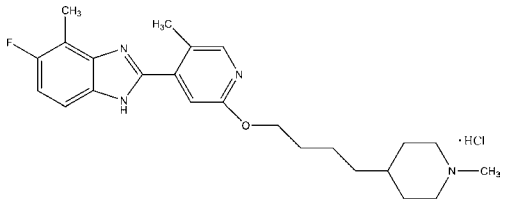

Fig. 1 Chemical structure of compound 1.

a hydrochloride salt of a small organic molecule with molecular weight 447 g/mol and apparent aqueous solubility of >100 mg/ml. During early pharmaceutical development, it was serendipitously discovered that compound 1 was able to gel aqueous media upon equilibration at room temperature. This behavior was unexpected not only because of its spontaneous nature but also since compound 1 did not structurally fall into the conventional categories of LMW hydrogelators, such as classic or gemini surfactants, bolaamphiphiles, sugar-based molecules, and cholesterol derivatives. Inasmuch as this, to our knowledge, was the first account of spontaneous gelation of a small pharmaceutical molecule in water, elucidation of the gelation mechanism of compound 1 and its structural moieties responsible for hydrogelation was intriguing from the academic standpoint. It was also necessary for practical reasons, since compound 1 headed a pipeline of human histamine H4 receptor antagonists and, especially, since liquid formulations of highly soluble compounds are typically considered for pre-clinical and Phase 1 development. Several techniques were used to gain insight into the supramolecular behavior of compound 1 in water, including rheology, NMR spectroscopy, microscopy, and X-ray diffraction.

MATERIALS AND METHODS

Materials

Compound 1 was synthesized at Johnson & Johnson Pharmaceutical Research & Development, L.L.C. in greater than 96% chemical purity. The compound is a crystalline, anhydrous, and non-hygroscopic hydrochloride salt. Aqueous solubility of compound 1 measured prior to gelling exceeds 100 mg/ml. Equilibrium solubility of compound 1 in pure water was estimated to be below 20 mg/ml based on observed gelation. The exact aqueous solubility at equilibrium was not quantified due to difficulties in separating dissolved and undissolved species in the gelled state. pK_a values of 9.79 (protonated form of the piperidine moiety) and 3.61 (unresolved between protonated forms of the pyridine moiety and benzimidazole moiety) were measured for compound 1 using multiplex

capillary electrophoresis (performed at Analiza Inc.). The second pK_a of the benzimidazole moiety was not observed up to pH = 11, the maximum pH tested. These values are in good agreement with pK_a values calculated using ACD Labs software: 3.83 (protonated form of the pyridine moiety), 9.25 (protonated form of the piperidine moiety), and 12.52 (second dissociation of the benzimidazole moiety).

Preparation of Hydrogels and Xerogels

Required quantity of compound 1 was mixed with 10–20 ml of DI water in a scintillation vial. The resulting mixture typically formed a non-viscous clear homogeneous solution immediately after preparation and was allowed to equilibrate at room temperature to produce the hydrogel. Hydrogels containing added ionic strength were prepared in a similar fashion by dissolving compound 1 in 50 mg/ml and 100 mg/ml aqueous NaCl solutions. Hydrogels with adjusted pH were prepared by adding up to 0.5% vol. of 1 N HCl or NaOH to freshly prepared non-gelled solutions of 50 mg/ml compound 1 in DI water (additional ionic strength introduced this way was very minor to negligible). All samples were allowed to equilibrate for at least 48 h prior to final visual testing and rheological characterization. Compound 1 was shown to be chemically stable for at least 1 week under the above experimental conditions.

To obtain xerogels, hydrogels were quickly frozen with liquid nitrogen and lyophilized.

NMR

^{1}H NMR measurements were conducted using a Varian 400 MHz spectrometer, with averaging of 64 scans. Samples were prepared as 1 mg/ml solutions of compound 1 in D_2O. Samples with cyclodextrin contained additionally 5 mg/ml of sulfobutyl-ether-cyclodextrin (SBE-β-CD).

Optical and Scanning Electron Microscopy

Optical microscopy was performed using a Zeiss Axioskop 40 microscope equipped with a hot-stage and an AxioCam MRc digital camera. A thin smear of the gel was placed on a microscope slide, covered with a cover slip, and immediately imaged in order to avoid sample drying.

Scanning Electron Microscopy was performed using a Fenom FEI SEM. Samples were sputter-coated with gold prior to imaging.

X-Ray Diffraction

X-ray diffraction (XRD) was carried out on a Rigaku D/Max Rapid wide angle X-ray diffractometer in transmission mode using a copper source with a 0.3 mm collimator and power settings of 46 kV/40 mA. Samples were loaded into 0.3 mm quartz capillary sample holders. Exposure time was 5 and 10–15 min for powders and gels, respectively.

Rheology

Rheological characterizations were carried out in controlled-stress mode on a TA Instruments AR 2000 rheometer equipped with a 60 mm 2° cone and plate geometry. Measurements were performed in the dynamic oscillatory mode at the stress amplitude of 0.12 Pa and the frequency of 1 Hz (stress/frequency sweeps were done to define the linear viscoelastic region and select an appropriate stress amplitude/frequency). Temperature sweeps were performed at 2°C increments with an equilibration time of 5 min at each step. A solvent trap was used to reduce water evaporation during measurements.

RESULTS AND DISCUSSION

Visual Observation of Gelation

Unlike typical LMW gelators that require a stimulus (heat, anti-solvent, pH-adjustment, etc.) to form a gel (6,14,16–28), compound 1 was observed to gel in pure water at concentrations as low as 20 mg/ml and higher simply upon equilibration at room temperature. Figure 2 illustrates the evolution of a gel in a 50 mg/ml solution. Initially, compound 1 appears to dissolve completely, resulting in a clear non-viscous liquid. Within approximately 1 h of equilibration at room temperature, long fibrous structures that can be seen by the naked eye occur in the solution. The formation of such fibers indicates the ability of the compound to self-assemble into highly anisotropic aggregates, which is generally considered a prerequisite for a LMW gelator. As time progresses, the fibrous structures formed by compound 1 do not precipitate, which is another crucial requirement for a gelator, but instead propagate rather homogeneously throughout the medium. The solution becomes translucent, and its viscosity, as assessed by

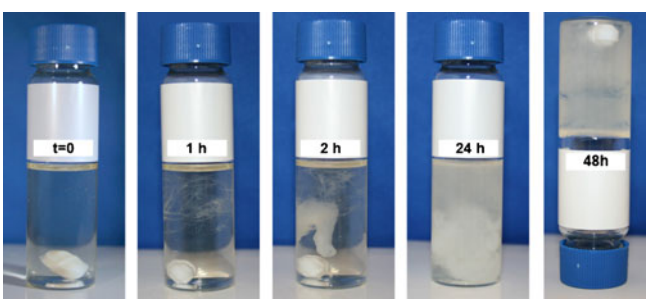

Fig. 2 Evolution of a gel in 50 mg/ml solution of compound 1 in water.

the appearance and feel, increases. Eventually, the entire volume becomes trapped into an intertwined network of non-covalently assembled molecules of compound 1. At the 48-h time-point, a 50 mg/ml gel is strong enough mechanically to withstand the tube inversion test as well as the falling sphere test—the two most common visual tests of gelation.

Remarkably, the free base form of compound 1 did not gel in water and remained completely segregated from the aqueous phase at concentrations above 0.0036 mg/ml, the solubility limit. Moreover, closely related analogs (also HCl salts) of compound 1 showed no signs of gelation in water under similar conditions (internal communication; chemical structures of the analogs could not be disclosed at this time). These results indicate a very delicate balance between molecular structure and salt form, on one hand, and intermolecular interactions and gelation, on the other.

Following the visual observation of gelation in pure water, similar tests were performed in the presence of various salts and acids to assess their effect on gelation of compound 1. Two caveats accompany this experiment. First, instantaneous solubility of compound 1 above pH 5 did not exceed the μg/ml level (as compared to solubility exceeding 50 mg/ml below pH 5). Likely as a result of the low solubility, completely segregated liquid and solid phases were obtained at equilibrium above pH 5. Thus, gelation experiments at neutral and basic condition would be irrelevant. Second, attempts to use a large excess of added salt (>200 mM) or high ionic strength buffers (the typical approaches to decoupling the effects of pH and ionic strength) resulted in precipitation of compound 1 even below pH 5. Furthermore, due to nearly instantaneous formation of extremely thick gels at some of the conditions tested, consistent determination of final pH values across the experimental grid was not feasible. Therefore, a possibility of interplay between changes in pH and ionic strength must be kept in mind.

Visual gelation tests in the presence of various salts suggested that added ionic strength facilitated gelation of compound 1 with little sensitivity to the salt identity besides its effect on the resulting ionic strength (Table I). Thus, the minimum gelation concentration of compound 1 dropped from >10 mg/in going from pure water and 17 mM NaCl to 17 0 mM NaCl. Identical trends were revealed with NaBr and NH_4Cl, hinting that it is primarily an ionic strength effect. Supporting this speculation, in higher ionic strength $CaCl_2$ solutions, the minimum gelation concentration drops below 10 mg/ml at 17 mM $CaCl_2$ and further to ≤1 mg/mL at 170 mM $CaCl_2$. Indeed, higher ionic strength is expected to facilitate supramolecular hydrogelation by decreasing electrostatic repulsion and increasing hydrophobic and van der Waals interactions between LMW gelator molecules.

Table I Visual Observation of Compound 1 Gelation in the Presence of Salts

Solvent	Concentration of compound 1 (mg/l)			
	1	10	50	100
Deionized water	−	−	+	+
NaCl, 17 mM	−	−	+	+
NaCl, 170 mM	+	+	+	+
NaBr, 17 mM	−	−	+	+
NaBr, 170 mM	+	+	+	+
NH_4Cl, 200 mM	+	+	+	+
$CaCl_2$, 17 mM	−	+	+	+
$CaCl_2$, 170 mM	+	+	+	+

+ Gelling; − No gelling

Visual gelation tests in the presence of hydrochloric, phosphoric, and sulfuric acids indicated that added acidity disrupts the gelation. As shown in Table II, phosphoric and sulfuric acid solutions with initial pH values of 2 and 1, respectively, completely inhibit gelation of compound 1 in the entire range of gelator concentrations tested. However, in their less acidic solutions, gelling was observed at the gelator concentration ≥50 mg/ml, which is approximately the minimum gelation concentration in pure water. The "acidic inhibition" trend was even more clearly manifested in HCl solutions, where the minimum gelation concentration was found to increase from 1 to 10 to 50 mg/ml with the initial pH values decreasing from 3 to 2 to 1, respectively. From the mechanistic standpoint, such pH-sensitivity indicates that the aromatic moiety (pK_a 3.61) plays an essential role in supramolecular assembly of compound 1. From the practical standpoint, the above observations indicate that compound 1 may be more prone to gelling in simulated gastric fluid (HCl, pH 1–2) than in water. If not taken into account during formulation development (especially, since highly soluble drugs are often dosed orally as aqueous solutions in pre-clinical and Phase 1 studies), this behavior may compromise dosage performance *in vivo*. However, for a discussion regarding the gelling potential under gastric conditions, an understanding of the gel behavior under physiological temperatures is necessary. Also, one may notice that, at a given pH, the minimum gelation concentrations in the presence of HCl were generally lower than those observed in phosphoric or sulfuric acid solutions. While this may be a hint at the importance of chloride as the common counterion, another explanation may pertain to a possible synergistic interplay between pH and ionic strength. Thus, further investigation is needed to adequately interpret the effect of pH on gelation of compound 1. Due to potentially very subtle changes in gel behavior in response to minimal alterations

Table II Visual Observation of Compound 1 Gelation in the Presence of Acids

Solvent (Ionic strength)	Concentration of compound 1 (mg/ml)			
	1	10	50	100
Deionized water	−	− (pH 4.8)	+ (pH 3.7)	+ (pH 2.9)
HCl, pH 1 (100 mM)	−	−	+	+
HCl, pH 2 (10 mM)	−	+	+	+
HCl, pH 3 (1 mM)	+	+	+	+
H_2SO_4, pH 1 (~150 mM)	−	−	−	−
H_2SO_4, pH 2 (~15 mM)	−	−	+	+
H_2SO_4, pH 3 (~1.5 mM)	−	−	+	+
H_3PO_4, pH 1 (~140 mM)	−	−	−	−
H_3PO_4, pH 2 (~11 mM)	−	−	−	−
H_3PO_4, pH 3 (~1 mM)	−	−	+	+

+ Gelling; − No gelling

of ionic strength or pH, such an investigation must rely on a technique capable of characterizing gelation more accurately than the visual tests. In addition, this technique must also offer a convenient method to monitor sol-gel transition as a function of temperature, allowing construction of a phase diagram showing the sol-gel boundaries within the physiologically relevant range.

Rheological Behavior and Gelation Conditions

Due to the unusual spontaneous manner of gelation, it was imperative to confirm gelation of compound 1 with a robust characterization tool. Methods that rely on visual observation and feel, such as the aforementioned tube inversion or falling sphere tests, may be misleading in interpreting gelation: A viscous liquid can be mistaken for a gel or, vice versa, a gel with a small yield stress can be mistaken for a liquid. Rheology is a technique that quantitatively characterizes viscoelastic properties of materials and, thus, can differentiate a gel from a sol (liquid) in an accurate and reproducible manner. Rheologically, materials can be categorized in terms of their dynamic elastic modulus G′, which represents the ability of the material to store energy under stress, and dynamic viscous modulus G″, which represents the tendency of a material to dissipate energy and flow under stress. Generally, a material is considered a solid when G′>G″. Conversely, a material is considered a liquid when G′<G″. At the crossover point, there is a balance between solid and liquid-like structure. Thus, the gel point is often estimated as the crossover of the G′ and G″ curves. A more comprehensive criterion introduced by Winter and Chambon defines the gel point as the instance at which the G″/G′ ratio is independent of frequency (29). All gels tested within the scope of this publication obeyed the Winter-Chambon criterion. Moreover, gel points determined per Winter-Chambon and per G′-G″ crossover were practically indistinguishable, as illustrated in Fig. 3. For the ease of reading, only the latter criterion will be used hereafter, as it is less graphically complicated.

Figure 4 shows G′ and G″ for a series of concentrations of compound 1 in water as a function of temperature. At temperatures below 25°C, all samples exhibit characteristics of a soft solid with G′>G″, i.e. behave as a gel. On an absolute basis, G′ is rather small and, for any of the concentrations tested, is less than an order of magnitude higher than G″. This indicates a relatively weak elastic structure of the gels and that a significant dissipation of energy occurs through viscous mechanisms. As the temperature increases, the elastic component becomes less and less dominant. Eventually, the gel structure "melts" resulting in the G′<G″ behavior characteristic of a liquid, and, above

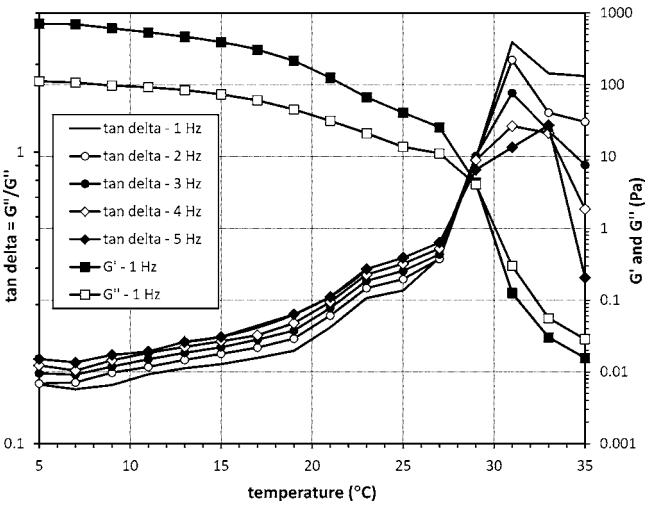

Fig. 3 Rheological determination of the gel point for 50 mg/ml hydrogel of compound 1. The gel point can be estimated as the crossover of the elastic modulus (G′) and viscous modulus (G″) or, following the more rigorous Winter-Chambon criterion, as the point at which the G″/G′ ratio is independent of frequency. As illustrated in this example, gel points determined by the two approaches were practically indistinguishable.

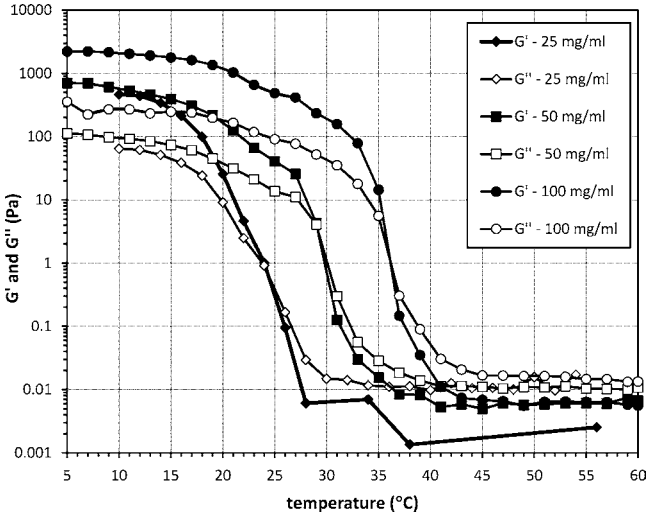

Fig. 4 Elastic (G′) and viscous (G″) moduli of compound 1 solutions in water as a function of temperature.

45°C, all the tested samples behave as continuous low-viscosity solutions with no measurable change in G′ and G″. The temperature of the G′-G″ crossover (i.e. the gel point) is concentration-dependent and increases from 25°C to 36°C as the concentration increases from 25 mg/ml to 100 mg/ml. The gel-sol phase diagram (Fig. 5) illustrates this concentration-temperature relationship more explicitly. The increase in the transition temperature as well as in G′ and G″ with increasing gelator concentration is consistent with typical behavior of LMW gels and reflects formation of the more cohesive 3D network that immobilizes the liquid component to a greater degree: Increasing the gelator concentration increases the number of gelator-built fibrillar aggregates as well as the number of crosslinks between aggregates such that more energy is required for the gel-to-sol transition. It should be noted that gel-sol transition of compound 1 in water is reversible and, when cooled back below the gel point, the sols restore their elastic structure (the data are not presented). Like most LMW hydrogels that do not display lower or upper critical solution temperature behaviors observed in polymeric systems (6,7), hydrogels of compound 1 did not swell or shrink upon temperature cycling but instead melted and re-formed.

To gain a better understanding of the gelation mechanism and conditions, the influence of ionic strength and pH on rheological behavior of aqueous solutions of compound 1 was examined. As previously noted, attempts to use fixed ionic strength buffers or a large excess of added salt (the typical approaches to decoupling the effects of pH and ionic strength) resulted in precipitation of compound 1 and segregation of the gels. Therefore, the influences of ionic strength and pH were examined by titrating the gels with NaCl and HCl/NaOH, respectively, and cross-evaluating the obtained trends to address possible interactions between these variables. Both solution pH and ionic strength were found to have a dramatic effect on gel behavior. As shown in Figs. 6 and 7, addition of NaCl makes the gels "stronger" (evident from the higher G′ and G″ values) and more temperature-stable (evident from the increase of the gel point temperature). These data quantitatively confirm the qualitative results of visual tests presented in Table I, further suggesting that added ionic strength facilitates gelation of compound 1.

Rheological examination of the effect of pH indicates that the gels are the strongest in a very narrow range of pH. As illustrated in Figs. 8 and 9 for a 50 mg/ml gel, a half-unit deviation from pH 3.7 noticeably weakens the gel (observed both visually and by rheology) and drops the gel point temperature, roughly by the same value regardless

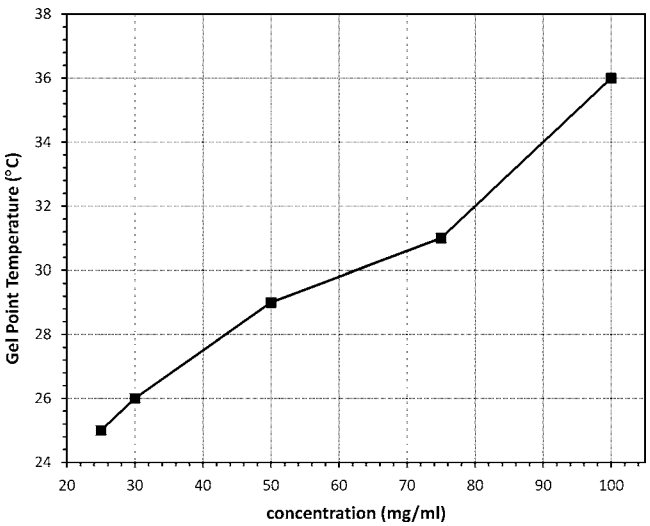

Fig. 5 Sol-gel phase boundaries for compound 1 in water determined by rheology.

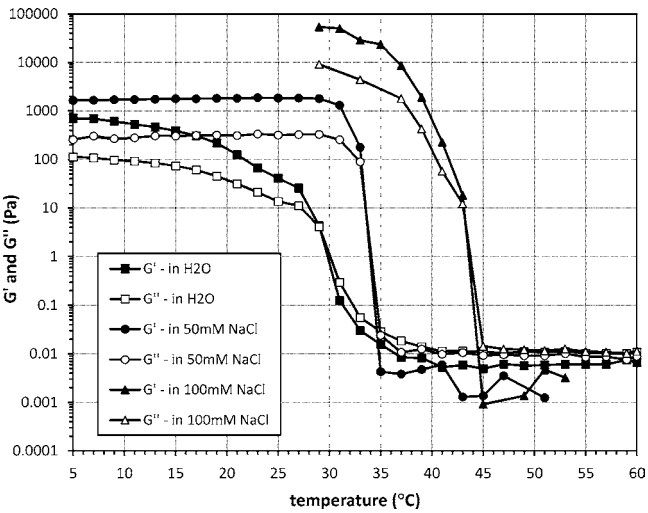

Fig. 6 Effect of added ionic strength on rheological behavior of 50 mg/ml solution of compound 1.

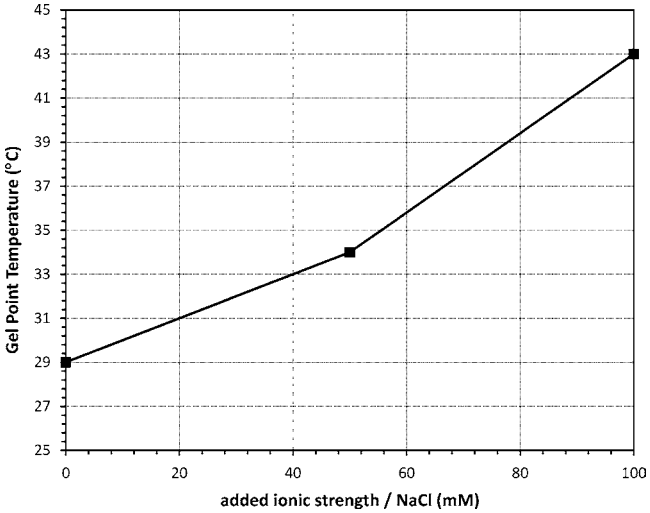

Fig. 7 Effect of added ionic strength on the gel point of 50 mg/ml solution of compound 1.

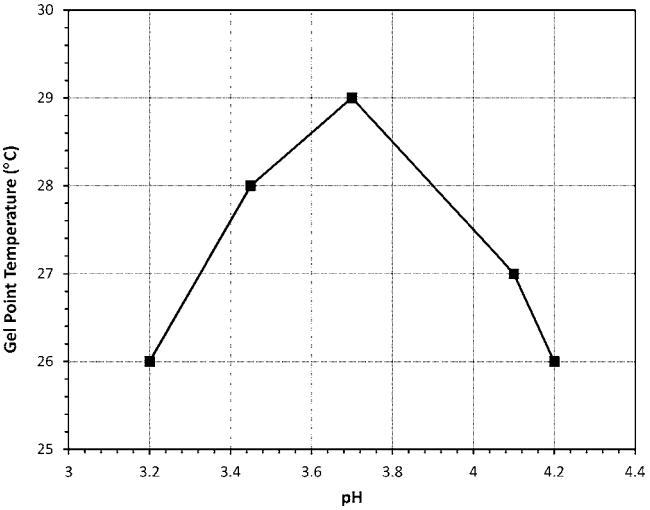

Fig. 9 Effect of pH on the gel point of 50 mg/ml solution of compound 1.

the direction of pH adjustment. Although the amount of HCl/NaOH introduced to adjust the pH was very minor to negligible compared to concentration of compound 1, the ionic strength was not kept constant, strictly speaking. Nevertheless, the observed gel weakening is clearly pH-inflicted and is not a result of a slight increase of the ionic strength: as explained in the previous paragraph, increasing the ionic strength facilitates (not weakens) gelation. It is important to note that increasing NaCl concentration from zero to 100 mg/ml was accompanied by a slight but measurable change of gel pH (from 3.7 to 3.3). However, in this case, the gel strengthening effect of ionic strength appears to be predominant.

Two conclusions regarding the effects of pH and ionic strength can be made: 1) Higher ionic strength facilitates gelation of compound 1, likely due to decreased electrostatic repulsion and increased hydrophobic and van der Waals interactions between the molecules of compound 1 in solution; 2) the sharp changes in rheological properties near pH 3.7 suggest that the charge of the aromatic moiety of the molecule (pK_a 3.61) plays an essential role in its supramolecular assembly. At the molecular level, facilitation of gelation in the vicinity of pK_a suggests that the gels are composed of aggregates of the ionized and unionized species which further assemble into higher-order supramolecular entities (18). Additionally, the critical involvement of the aromatic moiety in self-assembly of compound 1 is supported by NMR studies. Sulfobutyl-ether-cyclodextrin (SBE-β-CD) was found to disrupt gelation of compound 1 in our preliminary studies. ^{1}H NMR spectra of dilute D_2O solutions of compound 1 with and without 5 mg/ml of the SBE-β-CD were characterized by notable differences (Fig. 10): in the presence of SBE-β-CD, downfield shifts of the aromatic proton peaks (6.6–8.2 ppm region) as well as two of the three methyl peaks (2.2–2.6 ppm region) were observed, which suggests that the aromatic moiety (but not the N-methyl piperidinyl moiety) is involved in an interaction with the cyclodextrin. Since hydrophobic interactions almost always constitute a major driving force in cyclodextrin complex formation (32,33), the NMR data are consistent with the aromatic moiety being involved with intermolecular hydrophobic interactions during supramolecular assembly of compound 1.

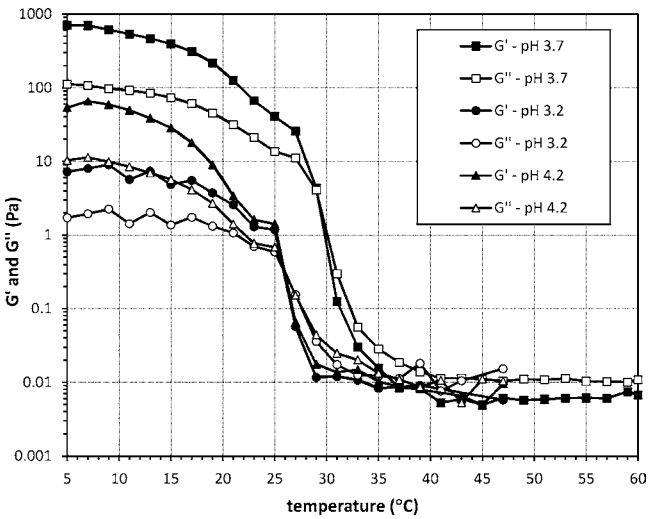

Fig. 8 Effect of pH of rheological behavior of 50 mg/ml solution of compound 1.

In summary, rheology has demonstrated that gelation temperature of compound 1 can be effectively influenced by gelator concentration, ionic strength, and pH. For example, for 50 mg/ml gel, it can be as low as 26°C (at pH 4.2) and as high as 43°C (in 100 mM NaCl). Occurrence of the gel-to-sol transition in the physiologically relevant range of temperature and pH is noteworthy due to its potential impact on formulation development and

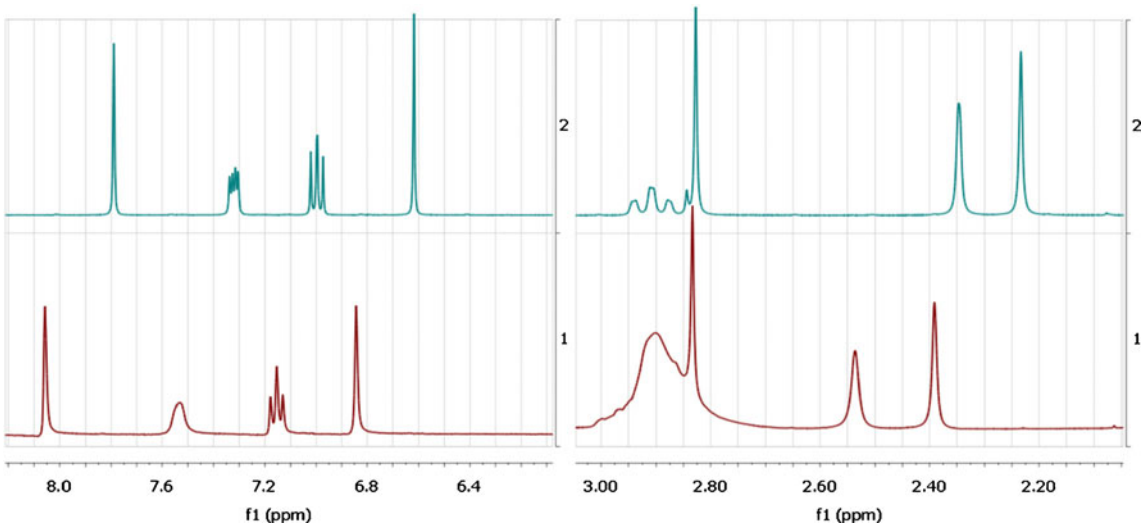

Fig. 10 ^{1}H NMR spectra of D_2O solutions of compound 1 without (*top*) and with (*bottom*) SBE-β-CD present.

formulation performance *in vivo*. In light of the continued interest to gels as innovative materials for oral drug delivery (30), one can think of formulating compound 1 as an excipient-free softgel-like dosage form that will "melt" upon ingestion. Potentially by simply adjusting its drug load and, thereby, gel-sol transition temperature, such soft gel can be formulated to act like an immediate release dosage or like a prolonged release dosage. On the other hand, softening of the gels at temperatures near the skin surface temperature (32°C) could allow topical application (31), a delivery route desirable in many instances for anti-inflammatory drugs such as this novel histamine H4 antagonist. Normal human skin has a surface pH of 4–6, which at the lower end may be compatible with gelling of compound 1, providing appropriately chosen gelator and salt concentrations.

Microstructure Elucidation

To elucidate the structure of the gels, optical microscopy (OM) and polarized optical microscopy (POM) were performed. Optical micrographs of 50 mg/ml and 100 mg/ml gels obtained at room temperature display a distinct fibrous texture (Fig. 11a and c). These fibers are hundreds of micrometers in length and can also be

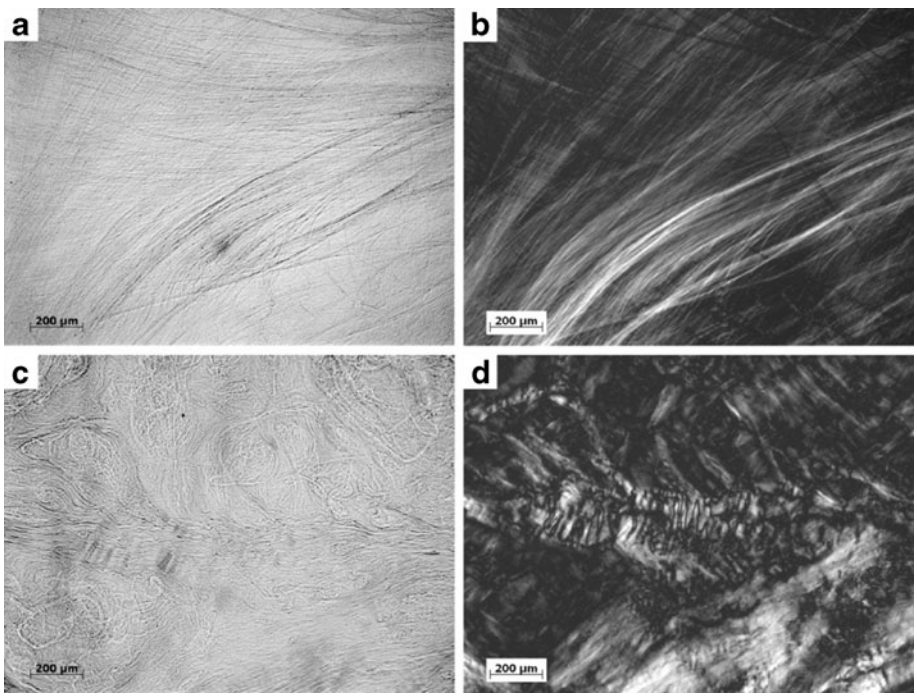

Fig. 11 Optical (OM) and polarized optical (POM) micrographs of aqueous gels of compound 1 (scale bar—200 μm): (**a**) OM, 50 mg/ml gel; (**b**) POM, 50 mg/ml gel (same area as in **a**); (**c**) OM, 100 mg/ml gel; (**d**) POM, Schlieren textures in 100 mg/ml gel (same area as in **c**).

observed through crossed polars (Fig. 11b and d). As tested by hot stage microscopy (Fig. 12), the fibrous structures "melt" between 35 and 40°C, which is consistent with the rheologically determined gel points. Another structural feature of these gels is the presence of birefringent domains. The so-called Schlieren textures, as seen in Fig. 11d, are often associated with liquid crystalline mesophases (28,34). Indeed, with its two aromatic moieties and the flexible aliphatic spacer, compound 1 possesses the prerequisites of a mesogenic molecule.

The liquid crystalline structure of the hydrogels is also supported by powder XRD. Both 50 and 100 mg/ml gels show a broad but distinct reflection in the wide angle region at 2θ of ~24°, corresponding to short spacings or intermolecular distances of ~0.37 nm (Fig. 13a and b). In the small angle region, 100 mg/ml gel reveals a series of

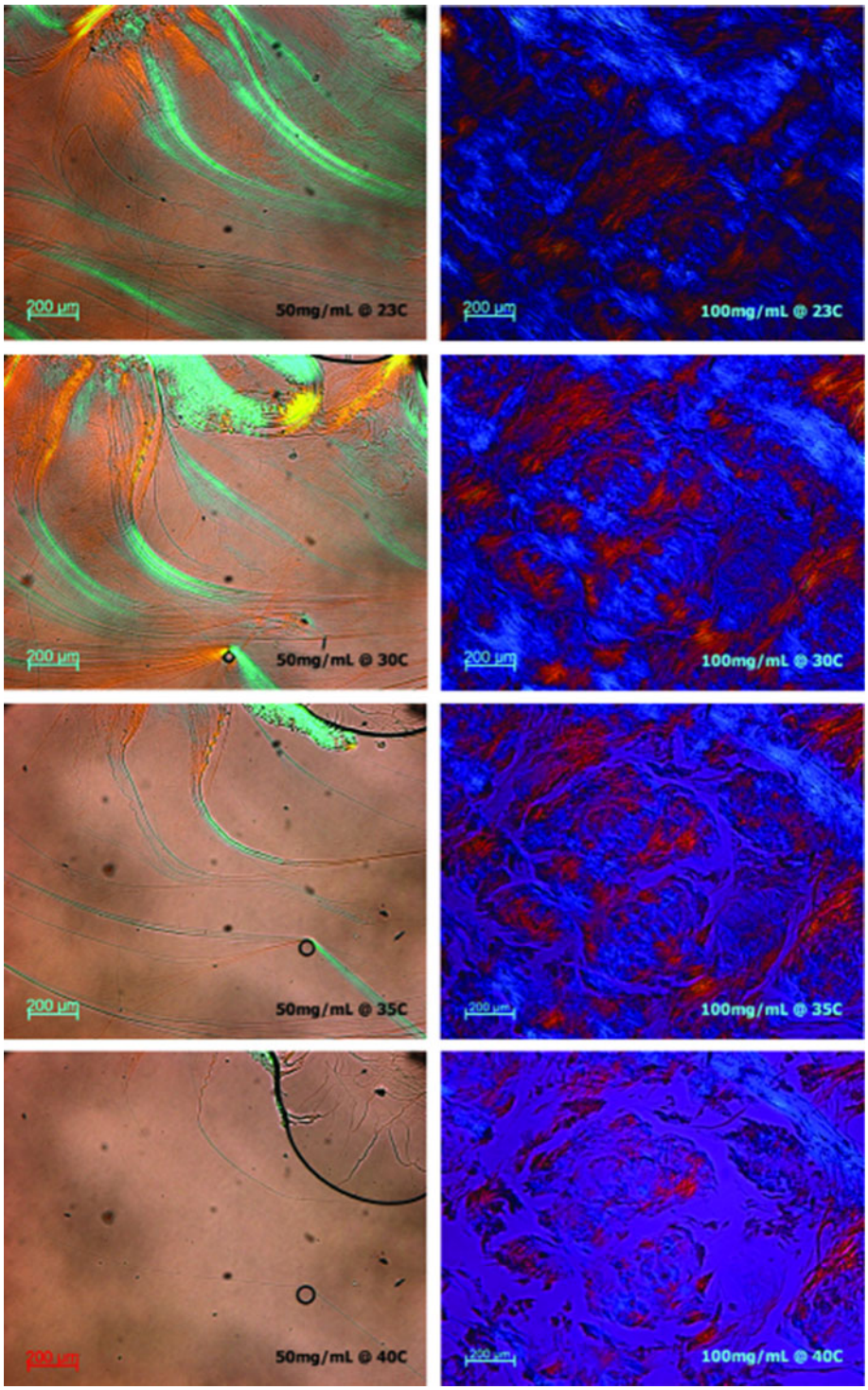

Fig. 12 Hot stage optical micrographs of 50 mg/ml and 100 mg/mg aqueous gels of compound 1 (scale bar—200 μm).

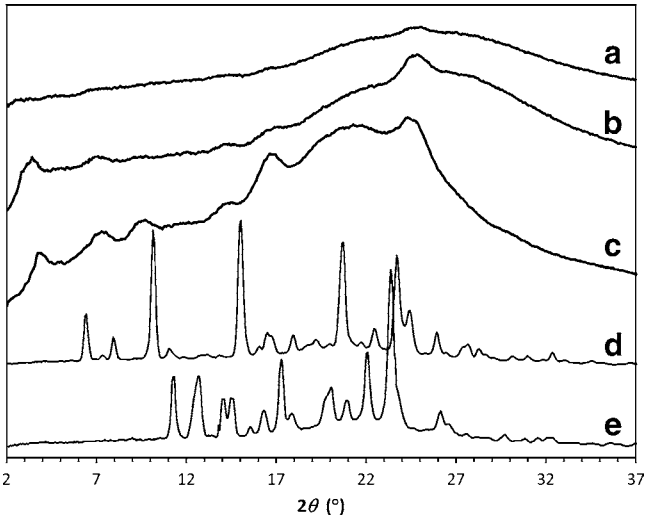

Fig. 13 XRD patterns of (**a**) 50 mg/ml and (**b**) 100 mg/ml aqueous gels of compound 1, (**c**) xerogel obtained from 50 mg/ml hydrogel, (**d**) dry powder HCl salt, and (**e**) dry powder free form. Intensities scaled for a better representation. The broad amorphous "halo" signal is due to scattering from quartz capillary sample holder.

reflection peaks 3.3, 6.6, 9.3, 13.9 and 16.5°. The relative of peak positions nearly perfectly follow 1:2:3:4:5, indicating a lamellar ordering. Although hints of small angle reflections can be seen in the XRD pattern of the 50 mg/ml gel, the low signal-to-noise ratio precludes a reliable interpretation.. In an effort to gain better insight into the gel structure, xerogels obtained by freeze-drying of 50 mg/ml hydrogels were examined XRD and SEM. Although caution must be taken when ascribing gel morphology based on xerogel state due to drying effects, it has been established in many studies that the self-assembled hydrogel structure can be reflected by its xerogel in some aspects (27,28). Indeed, as illustrated in Fig. 10c, XRD of a 50 mg/ml xerogel reveals the already noted peak in the wide angle region as well as distinct peaks as 3.7, 7.1, 9.4, 14.0, and 16.5° consistent with relative position of 1:2:3:4:5. We can therefore assume that at both 50 and 100 mg/ml concentrations, compound 1 assembles into an ordered lamellar structure. The first-order reflection at 3.3–3.7° corresponds to the lamellar spacing of 2.67–2.39 nm. This spacing is slightly greater than the molecular length of compound 1 (2.16 nm for an idealized extended conformation calculated with Chem3D software) but significantly smaller than twice the extended molecular length. This cautiously implies a monolayer molecular packing within the lamella. However, a bilayer with some interdigitation, perhaps combined with molecular tilting with respect to the lamellar planes, cannot be ruled out.

The improved definition of pattern C also allowed comparison against powder diffraction patterns D and E, which indicated that the ordered structure of the hydrogels/xerogels is not a result of re-crystallization of compound 1 into the HCl salt (the starting material) or the free form (a possible disproportionation product). Moreover, SEM of xerogels reveals a 3D structure comprised of continuous "wrinkled" sheets and intertwining fibers (Fig. 14). This morphology is inconsistent crystallization and once again confirms that the ordering observed in diffraction patterns of the xero- and hydrogels is not due to conventional crystallization. More likely, this ordering represents a hydrophobically stabilized intermediate state that gives rise to the gel and is also known as the secondary structure of a self-assembled physical gel (7).

CONCLUSION

For the first time, spontaneous formation of hydrogels by a small pharmaceutical molecule, a novel human histamine H4 receptor antagonist, was observed. This gelation was rigorously tested and confirmed by rheology. Experiments toward elucidation of solution conditions inducing gelation and structural moieties of the drug responsible for the supramolecular assembly suggest that the gels are composed of aggregates of partially ionized species, which are stabilized by hydrophobic interactions of the aromatic groups. Experiments toward elucidation of the hydrogel microstructure revealed a fibrillar texture with a lamellar microstucture and confirmed that the observed ordering is not due to re-crystallization into the HCl salt (the starting material) or its free form. We hypothesize that spontaneous gelling behavior of compound 1 is related to the so-called "spring" effect of the HCl salt (35,36). Following this hypothesis, high energy of the HCl salt provides the driving

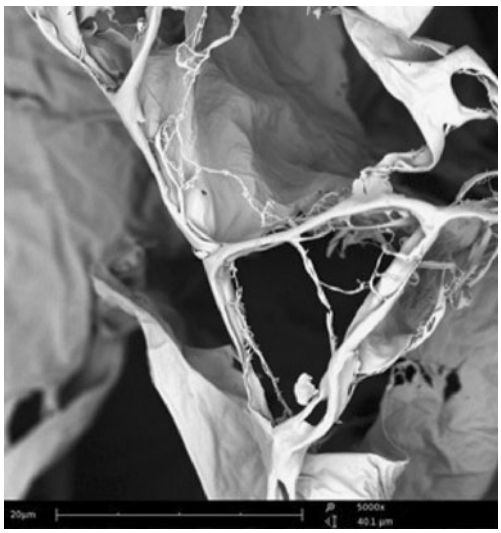

Fig. 14 SEM of a xerogel obtained from 50 mg/ml hydrogel of compound 1 (horizontal scale bar—20 μm).

force to solubilize the compound at a concentration greater than its equilibrium solubility, resulting in a meta-stable supersaturated solution. Upon equilibration, the molecules start to aggregate to satisfy the thermodynamic solubility. However, rather than crystallizing out of the solution, the aggregates get trapped in an intermediate hydrophobically stabilized liquid crystalline state, giving rise to a gel. Utilizing high-energy salt forms or, for that matter, polymorphs as a route to produce low molecular weight hydrogels at room temperature has not been previously reported and may have practical implications for pharmaceutical formulation development.

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