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Review Article

Thermotropic liquid crystalline drugs

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Abstract

Crystalline solids are characterized by long-range positional and orientational order in three dimensions, whereas amorphous liquids lack long-range order in any dimension. Liquid crystals (mesophases) show structural, mechanical and optical properties intermediate to those of crystalline solids and the amorphous, liquid state of matter. There are two principle types of liquid crystals: thermotropic liquid crystals (TLCs) and lyotropic liquid crystals (LLCs). TLCs can be formed by heating a crystalline solid or by cooling an isotropic melt of a TLC-forming molecule (mesogen). In the first part of this review the types of liquid crystals are defined and classified and the structural properties of mesogens are explained. In the second part, ten case studies of thermotropic mesomorphous drugs and pharmaceutically relevant molecules (arsphenamine, nafoxidine hydrochloride, L-660711, palmitoyl propranolol hydrochloride, penbutolol sulfate, itraconazole hydrochloride, fenoprofen sodium, fenoprofen calcium, ciclosporin and cholesteryl esters) are presented and their thermotropic mesomorphism is described. The review closes with a brief discussion of the unusual properties of drug mesophases and a potential use of drugs and excipients in this fourth state of matter.

The liquid crystalline state of matter

Crystalline solids are characterized by long-range positional and orientational order in three dimensions, whereas amorphous liquids lack long-range order in any dimension (Fisch & Kumar 2001; Müller-Goymann 2002). Liquid crystals (mesophases) show structural, mechanical and optical properties intermediate to those of crystalline solids and the amorphous, liquid state of matter (Figure 1). Liquid crystals, however, are not merely a mixture of solids and liquids, but indeed a separate state of matter. The transition from an isotropic, liquid melt to an anisotropic, liquid crystalline, nematic phase (for explanation of phases, see below under Thermotropic liquid crystals) always exhibits a first-order transition (Fisch & Kumar 2001) according to the Ehrenfest classification (Atkins 1994), which manifests itself for example as a peak in a differential scanning calorimetry (DSC) thermogram (Neubert 2001a). Also, transitions from one liquid crystalline phase to another (indicating the existence of mesomorphous polymorphism (Neubert 2001a)) usually are first-order transitions (Fisch & Kumar 2001). The transition from a nematic to a smectic A mesophase, however, can also be second order according to the Ehrenfest classification, which manifests itself for example as a step in the baseline in the DSC thermogram (i.e. a change in the heat capacity upon transition), thus being more comparable with the glass transition in amorphous solids (Neubert 2001a).

There are several excellent books and reviews about liquid crystals available (Demus & Richter 1978; Demus et al 1998; Fisch & Kumar 2001; Müller-Goymann 2002), and it is therefore neither necessary in this review to report the classification systems of liquid crystals nor the structural differences between the various types of liquid crystals in great detail. However, a basic introduction to the various types and structures of liquid crystals will be given below to gain a better understanding of the nature of liquid crystals.

Liquid crystals are a condensed state of matter formed by anisotropic organic molecules. While not all anisotropic molecules can form liquid crystals, all liquid crystals are formed by anisotropic molecules (Fisch & Kumar 2001). These molecules are termed mesogens and are either of rod-like (prolate) or, less commonly, disc-like (oblate) shape (Fisch & Kumar 2001).

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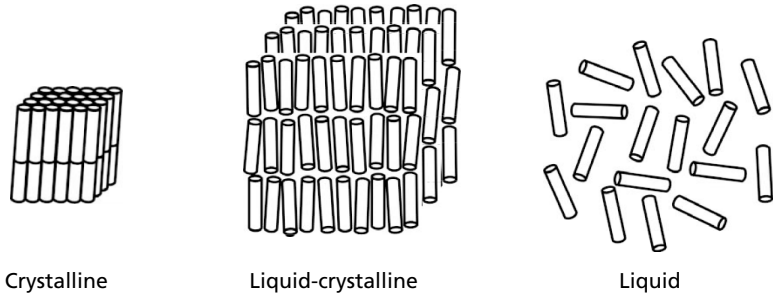

Figure 1 Schematic of typical crystalline, liquid crystalline and amorphous systems.

There are two principle types of liquid crystals: thermotropic liquid crystals (TLCs) and lyotropic liquid crystals (LLCs). TLCs can be formed by heating a crystalline solid or by cooling an isotropic melt of a mesogen. LLCs can be formed by the addition of a liquid, in most cases water or an organic polar solvent, to a solid or liquid mesogen. The principle difference between the two types of liquid crystals, therefore, is that the minimal number of components necessary to form the mesophase is one in the case of TLCs and two in the case of LLCs. Applying Gibbs phase rule (Hillert 1998) it therefore follows that the degrees of freedom that need to be specified (at constant pressure) to describe a one-phase liquid crystalline system is one in the case of TLCs (usually temperature) and two in the case of LLCs (usually concentration of the mesogen and temperature). Temperature plays an important role in both TLCs and LLCs, as mesogens can only form thermotropic and lyotropic mesophases in certain temperature ranges. Mesomorphous polymorphism is a function of temperature in TLCs but may also be a function of temperature in LLCs (other important factors for lyotropic mesomorphous polymorphism include solvent type and mesogen concentration) (Müller-Goymann 2002).

Lyotropic liquid crystals

LLCs are used in pharmacy and cosmetics in surfactant gels, ointments and creams, liposomal (multilamellar liposomes can be regarded as dispersions of lamellar liquid crystals in a polar continuous phase) and other colloidal dispersions, transdermal patches and for sustained drug release. The literature investigating the use of LLCs in drug delivery has recently been reviewed by Müller-Goymann (2002). Although the current review deals with thermotropic liquid crystalline drugs, the different types of LLCs are discussed briefly, as most of the thermotropic liquid crystalline drugs described below also show lyotropic mesomorphism, additionally to their thermotropic mesomorphism.

LLCs can be thought of as assemblies of micelles, which in turn are assemblies of amphiphilic molecules (anisotropic, prolate mesogens), such as surfactants. In terms of increasing order (from micellar solution to crystalline dispersion or solvate) one can differentiate the following types of LLC:

- the *lyotropic nematic phase*, which is composed of rod-like micelles, and which shows a long-range orientational order (LOO) with respect to the symmetry axis of the micelle, but no long-range positional order (LPO);
- the *lamellar phase* (layered packing of indefinitely extended disc-like micelles), which usually has a bilayered structure as repetition unit, and which shows LPO in one dimension (normal to the layers) and LOO within the layer;
- the *hexagonal phase* (hexagonal packing of rod-like micelles), which shows LPO in two dimensions (normal to the symmetry axis of the rods);
- the *cubic phase* (cubic packing of spherical micelles), which shows LPO in three dimensions (but the mesogens show rotational-diffusional motion on the level of the micelles forming the cubic arrangement).

The hexagonal phase can exist in a normal form, with the polar head groups of the mesogens pointing outwards (with respect to the symmetry axis of the rod) if the solvent component is polar, and in a reverse form with the polar head groups of the mesogens pointing inwards if the solvent component is lipophilic. Micellar cubic phases can also exist in a normal and in a reverse form. Moreover, the cubic phase can exist in various bicontinuous forms, which cannot simply be understood as assemblies of spherical micelles, but are classified as cubic mesophases, because, like the micellar cubic phases, and unlike the other LLC, they are isotropic (as is the cubic crystalline lattice) (Atkins 1994).

Both the lamellar and the hexagonal phase can be identified using polarising light microscopy (PLM) as they exhibit a range of textures that are typical for the respective LLC. For the lamellar phase, these are based on certain defect structures in the liquid crystalline order (Rades & Müller-Goymann 1997), whereas the undisturbed lamellar phase often shows pseudo-isotropic behaviour (Horányi et al 2004) if the bilayers arrange themselves parallel to the plane of the object slide and cover glass. The mesogens are then oriented parallel to the incoming beam of light and no birefringence can occur. Excellent polarising light micrographs are published for lamellar and hexagonal phases, showing the typical oily streaks, Maltese crosses and pseudo-isotropic textures of the lamellar phase as well as the fan-shaped texture of the hexagonal phase (Müller-Goymann 2002; Horányi et al 2004). The transmission electron microscope,

especially using the freeze-fracture technique for sample preparation (Müller-Goymann 2002), can also be used to identify the various types of TLC, and striking examples of the microstructure of lamellar, hexagonal and cubic phases have been published (Müller-Goymann 1984; Schütze & Müller-Goymann 1993). Other techniques to investigate liquid crystals (e.g., X-ray diffraction (XRD)) are mentioned below, in context with the case studies. For overviews on experimental techniques to study liquid crystals (both LLCs and TLCs) see for example Müller-Goymann (2002) and Fisch & Kumar (2001).

Thermotropic liquid crystals

In the following text, the discussion will be restricted to TLCs formed by rod-like molecules and will neglect the disk-shaped, banana-shaped and polymeric TLCs (Fisch & Kumar 2001) as, to the knowledge of the authors, liquid crystalline pharmaceutical drugs are from the prolate group only. Rod-like organic mesogens usually exhibit a length-to-diameter ratio of 3–8 (Fisch & Kumar 2001). Structurally, "*a common feature of all the molecules of this type is that they all comprise a central rigid core connected to a flexible alkyl chain at one or both ends*" (Fisch & Kumar 2001). Neubert (2001b) has recently reviewed the chemical structure–property relationships in thermotropic mesogens (and included in her review other monographs, book chapters, books and databases on the structures of thermotropic mesogens). The general structure of a typical rod-like mesogen is shown below:

$$T_1 - R_1 - C - R_2 - T_2$$
$$\qquad\quad | \qquad\quad |$$
$$\qquad LS_1 \qquad LS_2$$

The rigid rod is formed by two ring systems (R_1 and R_2). These are connected by a central (or connecting) group (C), and linked to two terminal groups (T_1 and T_2), at least one of which is a flexible alkyl chain. It should be noted, however, that while these are typical structural elements of a TLC mesogen, not all these elements necessarily have to feature in every mesogen (see below). Furthermore, there might be lateral substituents present on the mesogen. These are usually connected to the R_1 and R_2 position but off the molecular axis (LS_1 and LS_2) (Neubert 2001a).

The central groups (C) are usually polar, such as azo-groups, azoxy-groups, imines, esters, thioesters and amides, but also alkynes, alkenes or even alkanes. Generally, if the central group adds to the rigidity of the molecule, this will give better mesogenic properties. Neubert points out that to be mesogenic the molecule has to be rod-like, and therefore, as molecules containing a central group that can exist in *cis*- or *trans*-conformation (such as stilbenes or cinnamates), only the molecules with *trans*-conformation will be mesogenic (Neubert 2001b). As early as 1962, Athenstaedt found that, basically for the same reason, a single CH_2 group as the central group does not produce mesogens if the connecting group is in the *para*-position to the terminal groups T_1 and T_2 (Athenstaedt 1962). The same holds true inter-alia for carbonyl, amine, ether and thiolether groups as connecting groups between two phenyl rests (Neubert 2001b). On the other hand, the fact that many biphenyls are good mesogens shows that sometimes a central group is not necessary at all to form mesogens (Demus & Zaschke 1984).

The ring systems (R_1 and R_2) can be aromatic, alicyclic, aromatic heterocycles, saturated heterocycles or condensed ring systems. The number of ring systems in a mesogen is not restricted to two, but more than two can be linked together by more than one connecting group (Demus & Zaschke 1984; Neubert 2001b). With the exception of benzoic acid derivatives and cinnamic acid derivatives individual benzene rings, however, do not allow the formation of thermotropic mesophases. Benzoic acid and cinnamic acid derivatives form linear dimers through hydrogen bonds between two carboxyl groups. These two carboxyl groups form the central group of the mesogen, and the benzoic or cinnamic acid groups of two molecules thus form the R_1 and R_2 ring systems (Neubert 2001b).

The vast majority of thermotropic rod-like mesogens contain two terminal groups (T_1 and T_2). It is, however, not surprising that there are exceptions to this rule (Demus & Zaschke 1984; Neubert 2001b), which only contain a single terminal group. Chemically, the terminal groups can be straight or branched alkyl or alkoxy chains (which may additionally contain functional groups such as alkenes, alkynes, ethers, ketones, esters, etc.) or substitutes such as halides, nitro- or cyano-groups (Demus & Zaschke 1984; Neubert 2001b). Rod-like amphiphilic mesogens that contain exclusively aromatic building blocks and no flexible alkyl chains have also recently been synthesized (Kolbel et al 2000).

Furthermore, a large variety of lateral substituents (LS_1 and LS_2) of different sizes are described in the literature and influence the properties of the mesogen according to their type, size, number and position (Demus & Zaschke 1984; Neubert 2001b).

Besides rod-like mesogens of the above-described classical type (Figure 1), Demus & Zaschke (1984) list derivatives of amino acids, steroids, alkali salts of aliphatic acids and hydrochlorides of aniline and pyrimidinone derivatives as rod-like mesogens.

A great range of structurally different TLCs have been described, with over 20 different smectic phases alone (Fisch & Kumar 2001). It is thus not surprising that several ways to classify TLCs exist.

According to Fish & Kumar (2001), TLCs can be differentiated according to the position of the symmetry axis of the mesogen (director) with respect to the layers formed in the liquid crystal into: non-tilted TLC phases (including the nematic, smectic A, and hexatic B phase); tilted TLC phases (including the smectic C, and hexatic F phase); chiral tilted TLC phases (including the chiral nematic phase, previously called cholesteric phase, and the chiral smectic phases).

Neubert (2001a), on the other hand, differentiates: nematic phases; chiral nematic phases (cholesteric

phase); fluid smectic phases (including smectic A, smectic C, chiral smectic C); hexatic smectic phases (including hexatic B, hexatic F); crystalline mesophases with molecular rotational-diffusional motion; crystalline mesophases with molecular jump-diffusional motion. This classification reflects the increasing order from isotropic melt to nematic and chiral nematic mesophase to fluid smectic phase to hexatic smectic phase to higher-ordered TLC, called crystalline mesophases and finally to crystals.

Unfortunately, as we will see below, within the group of pharmaceutical drugs, for the examples that have been studied so far, only rarely has the type of TLC been unequivocally identified. There is therefore no need here to discuss the subtleties of the different TLCs in detail, especially as this has been done for organic molecules in general (e.g. see Demus & Richter 1978; Fisch & Kumar 2001). Figure 2 may thus be sufficient to illustrate the differences between some of the different forms of TLCs mentioned above.

Like LLCs, TLCs can be identified by their characteristic textures using PLM. Several texts are available that show differences in optical appearance of the various TLCs in the polarising light microscope (Demus & Richter 1978; Gray & Goodby 1982). Two important and helpful rules for the identification of TLCs are that identical mesophases will be miscible in all proportions, and, if the mesogen exhibits mesomorphous polymorphism, that certain sequences of types of liquid crystals exist corresponding to decreasing order upon heating (see above).

Case studies

Arsphenamine

As early as 1923, Freundlich and co-workers reported on colloid-chemical observations on arsphenamine (salvarsan, Figure 3A) and the structurally closely related neosalvarsan (Figure 3B) (Freundlich et al 1923). They found that these drugs in concentrated aqueous solution form a mesophase that exhibits birefringence in the polarising light microscope. Ten years later, Zocher described the mesophase formed as nematic (Zocher 1933). It has to be clearly stated that this mesophase is an LLC, as water is present as a second component. It is not known (at least to the authors of this review) whether the drug itself forms a TLC upon heating. The chemical structure of arsphenamine, however, indicates that this might well be the case, as several of the structural requirements for a typical mesogen are present. Athenstaedt (1962) points out that the -As = As- group is structurally related to the azo group or the -CH = CH- group, well-known central groups in many mesogens. The -As = As- group is, however, not mentioned in the lists of either Neubert (2001b) or Demus & Zaschke (1984).

Nafoxidine hydrochloride

In 1978, Mlodozeniec published a study investigating the thermodynamics and physical properties of the anti-oestrogen compound nafoxidine hydrochloride (Mlodozeniec 1978). This cationic drug (Figure 3C) has amphiphilic properties, with a cationic pyrrolidine head group and a somewhat unusual lipophilic tail group (diphenyl dihydronaphthalene). As a function of increasing drug concentra-

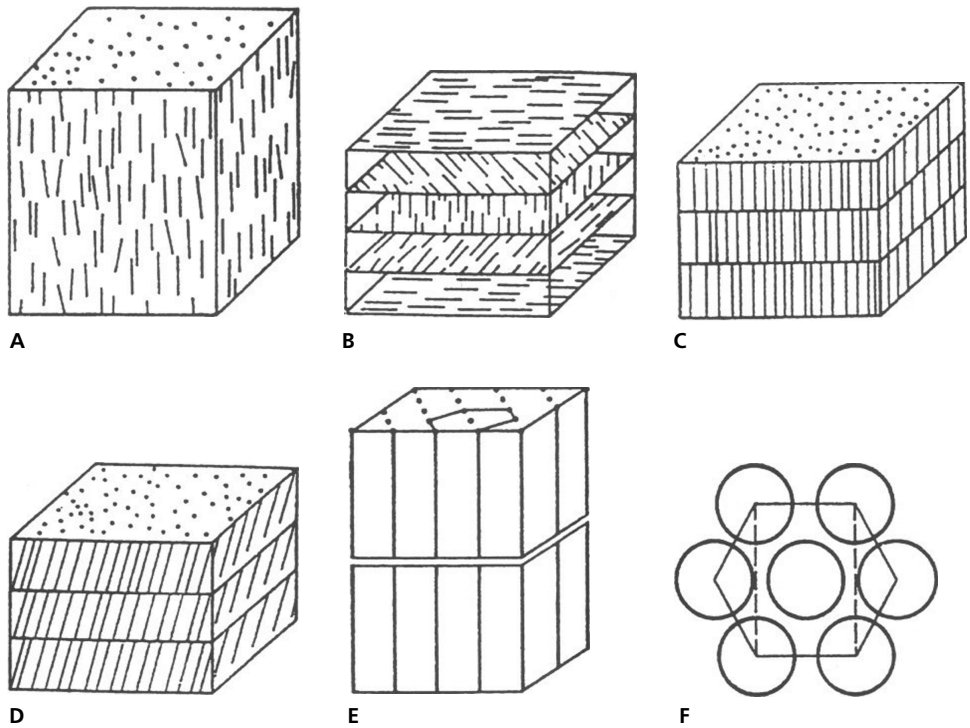

Figure 2 Schematic of different thermotropic liquid crystalline phases: nematic phase (A), chiral nematic phase (B), smectic A phase (C), smectic C phase (D), hexatic B phase (E), hexatic B phase (xz-plane) (F) (from Demus & Richter 1978).

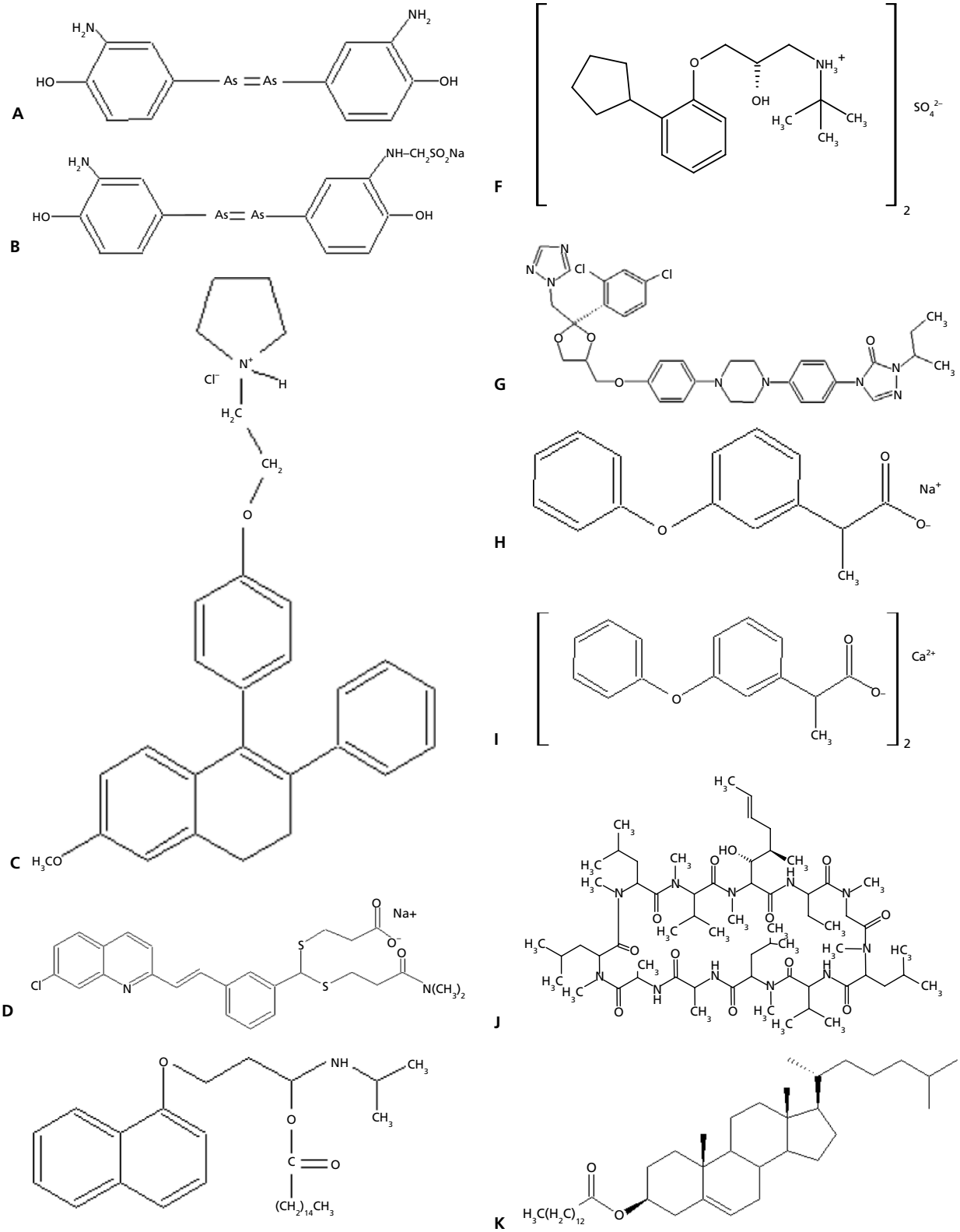

Figure 3 Structural formulas of thermotropic liquid crystalline drugs: salvarsan (A), neosalvarsan (B), nafoxidine hydrochloride (C), L-660711 (D), palmitolyl propranolol hydrochloride (E), penbutolol sulfate (F), itraconazole hydrochloride (G), fenoprofen sodium (H), fenoprofen calcium (I), ciclosporin (J), cholesteryl myristate (K).

tion in water at room temperature or at 37°C it initially forms a molecular solution, followed by a micellar solution (critical micelle-forming concentration (CMC) approx. 0.7–1.0 mg mL^{-1}), a hexagonal mesophase, a cubic phase (microstructure unknown, probably bicontinuous) and a lamellar mesophase. Lyotropic liquid crystalline structures are present from about 5–95% of drug.

This drug, however, also forms a thermotropic mesophase, which has been identified as smectic using PLM. Textures including the focal conic texture, fan-like texture and oily streak texture were found. The type of smectic thermotropic mesophase has not been further identified and cannot unequivocally be inferred from the micrograph presented in the paper (Mlodozeniec 1978), but could be a smectic A phase. The smectic phase has been found between 175 and 186°C, but interestingly only formed when the β-polymorph of the drug was heated. For the α-polymorph a direct transition from crystal to melt has been observed by differential thermal analysis. This indicated that different conformations of the drug in the different crystalline forms play a role in formation or lack of formation of the mesophase upon heating. Looking at the structure of the drug in terms of the typical structures of thermotropic mesogens, it should be noted that Demus & Zaschke (1984) list derivatives of 2-phenyl naphthalene as a potential source for mesogens. This molecule thus might be a thermotropic mesogen because it has a dihydronaphthalene-phenyl ring system (no connecting group, comparable with the biphenyls structure, mentioned above), one methoxy group as terminal group and no second terminal group. The second phenyl group, together with the pyrrolidine rest has to be regarded as a lateral substituent. With such a structure, a rather large lateral substituent, it would be very interesting to determine the layer thickness of the smectic phase (e.g. by using small angle XRD).

L-660711

Solid-state phase transitions of the leukotriene D4 receptor agonist L-660711, 3-(((3-(2-(7-chloro-2-quinolinyl) ethenyl) phenyl) ((3-(dimethylamino)-3-oxo-propyl) thio) methyl) thio) propanoic acid (Figure 3D), have been investigated by Vadas et al (1991). The authors report that the drug is able to form lyotropic and thermotropic mesophases if heated above 80°C. In the anhydrous crystalline form, the drug undergoes a first-order transition (as detected by DSC) from the crystalline state to a liquid crystalline state (identified as mesophase due to the "*shimmering colours*" (Vadas et al 1991) by PLM). Upon cooling, this mesomorphic state remained metastable and did not convert back to the crystalline form. Instead the authors found a lower-energy endothermic transition between 35 and 55°C upon reheating, which may represent a liquid crystalline polymorphic transition. Exposure of the crystalline state to moisture could also lead to a transition to a mesophase, indicating that this drug might again be able to form TLCs and LLCs. The nature of the TLC and of the potential liquid crystalline polymorphism has not been identified and the polarising light micrographs also do not allow determination the type of mesophase. The authors present wide-angle X-ray diffractograms (5–35° 2θ) of the drug exposed to various relative humidities for various times. While the peaks in the diffractograms decrease in intensity due to exposure to humidity (decreased crystallinity), there are still many peaks in the wide-angle range, indicating presence of the crystalline form of the drug. No small-angle X-ray diffractometric measurements were performed, and the presence of a smectic mesophase therefore cannot be confirmed by the presented X-ray data. The finding that the mesophase could be maintained upon cooling might be useful to manipulate solubility or even bioavailability of this drug, although the stability of this mesophase over time has not been determined and this substance is highly water soluble anyway.

Palmitolyl propranolol hydrochloride

Vyas et al (1999) produced an amphiphilic derivative of the beta-blocker propranolol hydrochloride, by esterification of the secondary hydroxyl group of the drug with palmitoyl chloride (palmitoyl propranolol hydrochloride, Figure 3E). The focus of their work was on aqueous dispersions of this amphiphilic drug, which formed lyotropic liquid crystalline dispersions (so-called pharmacosomes) (Vaizoglu & Speiser 1986; Müller-Goymann & Hamann 1991). The amphiphilic nature of the drug was determined by measuring the CMC using a Wilhelmy plate tensiometric method (CMC was 0.06% w/w). The authors however, also investigated the thermal properties of the anhydrous sample. At room temperature the drug is described as exhibiting "*an amorphous form with typical birefringent microcrystals*" (Vyas et al 1999). This somewhat ambiguous description nevertheless indicated the drug to be in a crystalline state. The melting point of palmitoyl propranolol hydrochloride was stated as 49 ± 0.5°C or as in the temperature range of 45–55°C (for comparison, the melting point of propranolol hydrochloride is 163–164°C (Windholtz 1983)). Initially, the drug melted to an isotropic melt but upon cooling, a thermotropic liquid crystalline phase appeared, with a "*typical radiant pattern*" (Vyas et al 1999). This TLC phase was present upon cooling between 48 and 35°C. Below 35°C the drug crystallised again. The type of TLC has not been determined and PLM micrographs were also not shown. The LLC phase was of a "*smectic type*" (Vyas et al 1999) lamellar nature, resembling the textures of aqueous phospholipid dispersions, including the formation of myelin bodies, but at this stage it can only be speculated whether the TLC phase was also smectic.

Penbutolol sulfate

Also for the beta-blocker penbutolol sulfate (Figure 3F) the formation of a thermotropic mesophase has been described that was, however, not investigated in much detail (Kuhnert-Brandstätter & Völlenklee 1987). In a study focussing on the crystalline polymorphism of this drug, the formation of thermotropic liquid crystals was observed upon slow cooling of the melted drug. The liquid crystalline phase displayed tiny spherical structures with Maltese crosses in the polarising light micrograph, which fused to larger structures upon further cooling and finally

crystallised. The clear point of the thermotropic mesophase was determined as 191°C.

Itraconazole hydrochloride

In 2001, the thermal behaviour of the antifungal drug itraconazole hydrochloride (Figure 3G) has been investigated by Six et al (2001). The authors found that when glassy itraconazole hydrochloride (prepared by cooling an itraconazole melt to 40°C at the moderate cooling rate of 20°C min^{-1} or by preparing a dichloromethane solution of the drug and rapid evaporation of the solvent) was heated, besides the glass transition temperature at 59°C, two endothermic events were detected at 74 and 90°C using modulated temperature DSC. The authors concluded that the transitions required the presence of a liquid (melt or solution) state before glass formation. The endothermic events at 74 and 90°C were reversible so that exothermic transitions were found upon cooling at approximately the same temperatures. By using HPLC, the authors could exclude degradation or impurities as being responsible for the observed transitions, and using hot-stage PLM they found formation of a birefringent texture at 90°C when cooling the isotropic melt, and a change in texture (and increase in viscosity) at 74°C upon further cooling. As with the DSC traces, these events observed by PLM were reversible. Using variable-temperature XRD, it could be shown that upon cooling after formation of the mesophases, the drug did not crystallise, but the mesomorphous state was "*frozen into a glassy state*" (Six et al 2001). The authors state that the chemical structure of itraconazole is not typical for a mesogenic molecule, although the piperazine group can be regarded as a central group. This group is indeed not mentioned in the lists of Neubert (2001b) or in Demus & Zaschke (1984). There is, however, a paper from Lattermann et al (1992), describing mesogenic amide and ammonium derivatives of piperazine and triazacyclononane. The drug molecule is anisotropic and recently it has been shown that thermotropic mesogens do not necessarily need to contain a linear aliphatic side chain (Kolbel et al 2000), although indeed many do. The presence of chiral centres in the mesogen also allows for the formation of a chiral nematic phase. Six et al (2001) conclude that the microscopic texture present between 74 and 90°C is compatible with that of a chiral nematic phase (Figure 2). The transition at 74°C is thought to be due to a restriction in rotational motion of the molecules, which also would explain the increase in viscosity. Unfortunately, small-angle X-ray diffraction of the liquid crystalline phases of this drug or mixing experiments with known chiral nematic mesogens were not performed, so that the identification of the TLC phases of itraconazole hydrochloride is not completely certain. It is very interesting that the drug retains its liquid crystalline order upon cooling and is "*frozen into a glassy state*" (Six et al 2001). This is in contrast to the general assumption that TLCs do not show a marked super-cooling effect (Neubert 2001a), but is in line with the findings of Rades & Müller-Goymann (1994) on fenoprofen calcium, as well as recent observations on ciclosporin (Lechuga-Ballesteros et al 2003), whose thermotropic mesophases can also be retained at room temperature (see below).

Fenoprofen sodium

The potential thermotropic mesomorphism of the non-steroidal anti-inflammatory drug fenoprofen sodium dihydrate (Figure 3H) was initially investigated by hot-stage PLM (Rades & Müller-Goymann 1994). The drug melted between 58 and 80°C to an isotropic melt. Upon further heating, however, at 105°C birefringent structures (a so-called fan-like texture was visible in the polarising light micrograph) began to appear. This thermotropic mesophase remained present until 160–180°C before it turned into a second isotropic melt. Cooling of the substance from the thermotropic liquid crystalline state to room temperature led to a preservation of the texture in the polarising light micrograph (i.e. as in the case of itraconazole hydrochloride the mesophase could be super-cooled). However, after a few hours of storage at 20°C and 60% relative humidity, the super-cooled mesophase transformed back into the crystalline dihydrate state.

To identify the type of TLC formed by fenoprofen sodium, PLM, transmission electron microscopy (TEM) and XRD investigations were carried out. The polarised light microscopic texture pointed either at a smectic or a cholesteric (chiral nematic) mesophase (Rades & Müller-Goymann 1994). The reason for the similarities in the appearance of the smectic and the cholesteric mesophase is that both types of liquid crystals have a layered structure. In the former, the molecules are arranged in layers perpendicular to the layer plane and in the latter, parallel to the layer plane. A layer thickness in the order of about 2 nm could be detected by TEM. Using XRD it was found that the mesophase had a smectic structure (the ratio of the Bragg spacings in the small angle range of the diffractogram was $1:\frac{1}{2}:\frac{1}{3}$ compatible with a smectic structure) with a layer thickness between 2.2 and 2.3 nm, which is about twice the length of one fenoprofen sodium molecule.

The authors concluded that the thermotropic mesophase of fenoprofen sodium is of the smectic type, arranged in double layers (smectic A_2) (Fisch & Kumar 2001). This is comparable with the crystalline dihydrate form of fenoprofen sodium that also has a layered arrangement, as well as to an LLC that can be formed by this drug in aqueous dispersions, which is lamellar in nature. At higher temperatures, a layer thickness of only 1.4–1.5 nm was detected by small-angle XRD, possibly due to a higher mobility of the molecules that allows for a partial overlap of the terminal phenyl groups of two opposite fenoprofen sodium molecules, resulting in what is known as a partially interdigitated smectic A_d phase (Fisch & Kumar 2001).

It is somewhat surprising that the fenoprofen sodium molecule is able to form a TLC, as the biphenyl-ether structure usually does not form thermotropic mesophases (Neubert 2001a) due to the lack of rigidity of the central group. It is thus possible that the polar head groups of two opposite fenoprofen sodium

molecules form the central group, in analogy to cinnamic and benzoic acids. In that case, the first ring of each of the two fenoprofen sodium molecules belongs to the ring systems and the phenoxy groups of each of the two molecules are terminal groups.

Fenoprofen calcium

The second fenoprofen salt of which thermotropic mesomorphism has been studied is fenoprofen calcium (Figure 3I). In the crystalline state, like the sodium salt of the drug, it forms a dihydrate (i.e. a monohydrate, with respect to the fenoprofen anion). However, in contrast to fenoprofen sodium, fenoprofen calcium does not have a smectic structure, as was shown by SEM and XRD-investigations (Rades & Müller-Goymann 1994; Rades et al 1996).

Heating the substance in an open and in a closed system leads to a very different melting behaviour of the calcium salt. Using a sealed object holder, the sample melts at 120°C to an isotropic liquid (PLM, DSC). No indication of the formation of thermotropic mesophases could be found. Cooling the substance to room temperature, the liquid melt transforms into an amorphous glass, which after some days crystallised. Quite different, however, was the thermal behaviour of the substance heated in an open system. The substance changed its appearance slightly at temperatures higher than 80°C. Using thermogravimetry, the authors could show, that (as in the case of fenoprofen sodium) the crystalline-bound water had left the system before a TLC could develop (Rades & Müller-Goymann 1994; Rades et al 1996).

Cooling the TLCs to room temperature led to the formation of a super-cooled thermotropic mesophase. Within a period of one month no crystallisation of the super-cooled TLC was found (PLM, XRD). This behaviour is different to that of fenoprofen sodium and was explained by a different molecular arrangement of both mesophases (see below). Temperature-dependent XRD confirmed the liquid crystalline nature of the substance at temperatures higher than 120°C and in the super-cooled state at room temperature: X-ray interferences were only found in the small-angle range, while in the wide-angle range of the diffractogram there was only a halo, indicating no true crystalline order. The ratio of the Bragg reflections was $1:1/\sqrt{3}:1/\sqrt{4}$, compatible with the interference pattern of a hexagonal or a reverse hexagonal phase. Because of sterical reasons and the absence of water in the system, it seems to be likely that a reverse hexagonal phase has developed.

The distance of two neighbouring tubes was calculated to be 1.8 nm. As in the case of the fenoprofen sodium mesophase at high temperatures, it was assumed that there was partial overlap of the lipophilic parts of the fenoprofen calcium molecules (Rades & Müller-Goymann 1994).

Although the reversed hexagonal phase is unusual for TLCs and more common in LLCs, in the early sixties the structure of TLC phases of anhydrous sodium and calcium salts of fatty acids was investigated (Scoulios & Luzatti 1959; Spegt & Scoulios 1960). These authors found a lamellar phase as the high temperature form of sodium soaps and a reverse hexagonal phase as the high temperature phase of the calcium soaps, underlining the importance of the counter ion on the type of mesophase formed.

In a later study, Patterson et al (2002) further investigated the physical stability and solubility of the super-cooled liquid crystalline form of fenoprofen calcium alone and in a proprietary tablet formulation (Nalfon). No interference of tablet excipients with the thermal behaviour of the drug in the tablet formulation was observed. The super-cooled TLCs alone and in preheated and ground tablets were physically stable when stored in a dry environment or at 33% relative humidity at both 20 and 40°C for two months. At 40°C and 75% relative humidity, the super-cooled TLCs however, converted to the crystalline dihydrate within a week. Confirming results from an earlier study (Rades et al 1996), the solubility of the crystalline dihydrate alone and from the tablet formulation (2.8 mg mL^{-1} and 3.0 mg mL^{-1}, respectively) could be increased to 5.0 mg mL^{-1} and 6.9 mg mL^{-1} for the super-cooled TLCs alone and in the presence of excipients, respectively.

Ciclosporin

A special solid form, assigned as a "*super-cooled thermotropic liquid crystal*", has been reported for the immunosuppressive peptide drug ciclosporin (Figure 3J), prepared by spray-drying from ethanolic solution (Lechuga-Ballesteros et al 2003). In contrast to other solid forms of ciclosporin, this material does not display crystalline order as obvious from the lack of sharp wide-angle X-ray reflections. In the polarising light micrograph it is, however, birefringent and remains so even during a sharp solid-to-liquid phase transition observed around 120–125°C. Birefringence disappears only upon heating above 170°C. The birefringent material displays a small-angle reflection corresponding to a long-range repeating unit of about 22.1 nm at both 10°C and 150°C (but displays no higher order reflections). These characteristics were attributed to the presence of a mesophase or super-cooled mesophase (at room temperature) that is thermotropic in nature as the material contained only very little (around 1%) residual solvent. The exact structure of the liquid crystalline phase could not be unequivocally assigned. Taking the crystallographic structure of the peptide molecule into consideration, the authors suggested that ciclosporin might behave as a rod-like molecule and form a calamitic smectic structure. The thermal behaviour of the liquid crystalline material was quite remarkable. Even though a sharp, melt-like solid-to-liquid phase transition was observed, macroscopic liquefying of the material was reflected as a C_p change accompanied by an enthalpic relaxation pointing to a second-order T_g-like transition in DSC. Also dielectric relaxation spectroscopy pointed to a transition characteristic of glassy or amorphous material. On the other hand, exposure of the super-cooled liquid crystalline material to moisture did not result in crystallisation as would usually be expected for amorphous substances (and was for example also observed for super-cooled liquid crystalline fenoprofen sodium). In addition to the solid–liquid transition, a broad DSC

endotherm was detected between 10°C and 80°C for the native material. This endotherm included evaporation of solvent, as well as an enthalpic relaxation not associated with a phase transition. The enthalpic process at the beginning of the endotherm was tentatively attributed to structural rearrangements, probably due to conformational changes, which were also reflected in a change of textures observed in PLM below 60°C.

As the molecular structure of the cyclic peptide ciclosporin deviates markedly from those of the other substances covered by this review, a somewhat unusual behaviour is not so surprising. With regard to the increasing importance of peptidic drugs for therapeutic purposes, deeper investigations into the properties of this 'frozen thermotropic mesophase' would be highly desirable, particularly as many interesting questions concerning its structure and behaviour still remain unresolved.

Cholesteryl esters

The first description of observations relating to the thermotropic liquid crystalline state of matter dates back to Reinitzer who investigated the melting behaviour of cholesteryl esters in 1888 (Reinitzer 1888). Thermotropic mesomorphism in the form of cholesteric (chiral nematic) or nematic liquid crystalline phases is typical for this physiologically important group of lipids. The phase behaviour of cholesteryl esters has been studied intensively inter-alia by PLM and electron microscopy, XRD and calorimetry with regard to the different types of phases, their thermal relations and structure (Ginsburg et al 1984). If a given ester can exist in both the smectic and the cholesteric mesophase, the smectic phase is always the one with the lower melting point. The mesophases can be stable (i.e. forming upon melting of the crystalline material and being indefinitely stable at the given temperature) or metastable (i.e. forming from an isotropic liquid upon super-cooling; metastable mesophases will transform into the thermodynamically stable state with time). Also for the smectic mesophase (e.g., of cholesteryl myristate, Figure 3K), considerable super-cooling can be observed before crystallisation (Snow & Phillips 1990). This super-cooling tendency, which is even higher in the dispersed state, has recently been utilised to develop colloidal dispersions of cholesterol esters in the highly supercooled smectic state as a novel type of drug carrier system (Kuntsche et al 2004). Cholesteryl myristate, which always exists as a crystalline solid at room temperature in the bulk, was dispersed into nanoparticles of 100–200 nm mean particle size by high-pressure homogenisation of a hot premix containing phospholipid and bile salt or polyvinyl alcohol as stabilisers. In contrast to the situation in the bulk material, the smectic state of the matrix lipid was preserved over one year of storage at room temperature under optimised stabilisation conditions in the resulting dispersions. The phase behaviour of the cholesteryl ester was, in principle, retained in the nanoparticles with somewhat broadened and shifted phase transitions upon heating and a strongly decreased smectic–crystalline phase transition temperature upon cooling. The shape of the smectic nanoparticles as derived from electron microscopic investigations is often non-spherical (although indications for the presence of layered, spherical particles were also found in some dispersions), which probably reflects the smectic order of the molecular layers of the cholesteryl ester. The highly viscous, but still fluid, structure of the smectic particle matrix may offer interesting potential for drug incorporation and sustained release. Loading of the particles with 10% (related to the cholesteryl ester) ibuprofen, miconazole and etomidate did not alter the liquid crystalline state of the nanoparticles and did not lead to precipitation of these lipophilic model drugs within the dispersion.

Conclusion

The few examples of thermotropic liquid crystalline pharmaceutical drugs found in the literature and presented here indicate that the presence of thermotropic mesomorphism in drugs is rather small. However, it has been estimated that approx. 5% of all organic molecules are able to form this so-called fourth state of matter. One can thus assume that drugs other than the ones described in this review are also able to form TLCs. Moreover, it is conceivable, as in the case of palmitoyl propranolol hydrochloride, that many drugs can be chemically modified to gain thermotropic liquid crystalline properties.

In some of the examples shown in this review, unusual properties of the drug mesophases compared with traditional TLC molecules were found (such as the presence of a biphenyl ether structure of fenoprofen that still allows for TLC formation), the fact that the liquid crystalline order could be super-cooled (e.g. in the case of itraconazole hydrochloride, fenoprofen calcium, ciclosporin and cholesteryl esters) and the general tendency of pharmaceutical mesogens to form both TLCs and LLCs (e.g. in the case of nafoxidine hydrochloride, L660711, palmitolyl propranolol hydrochloride and fenoprofen sodium). These properties make it worthwhile, from a fundamental physico-chemical viewpoint, to study these molecules in more depth.

Thermotropic liquid crystalline properties could be exploited, as shown in the example of fenoprofen calcium, to increase the solubility of poorly soluble crystalline drugs (i.e. those belonging to type II of the biopharmaceutical classification system) (Amidon et al 1995) if it is possible to stabilise their thermotropic liquid crystalline state at room temperature. As stated above, usually, the tendency of thermotropic mesophases to super-cool is regarded as low. However, if the crystalline form is a hydrate, the stability of the thermotropic liquid crystalline non-hydrate may be high, provided water addition to the drug upon storage is restricted.

A new area of formulation research that, to the knowledge of the authors, has not been attempted yet, would be the mixing of thermotropic mesomorphous drugs with small molecules or polymers that have thermotropic liquid crystalline properties at room temperature. These small or polymeric molecules could form the basis of a new class of excipients to create TLC mixtures as a new type of drug delivery system. The liquid crystalline cholesteryl ester

nanoparticles described above are interesting candidates for this type of processing, particularly for the formulation of poorly water-soluble drugs.

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