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Synthesis and characterisation of palymitoyl propanolol hydrochloride auto-lymphotrophs for oral administration

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Abstract

Self-associating supramolecular organisation of molecules above critical micellar concentrations (c.m.c.) offers enormous potentials in figuring distinguished behaviour both within formulations as well as in the bioenvironment. The present study deals with the enhancement of the oral bioavailability of propranolol HCl by synthesising the amphiphathic prodrug, which tends to aggregate in supramolecular orientations. The palmitoyl derivative of propranolol HCl was prepared by esterification of the secondary OH group. The prepared palmitoyl propranolol HCl (PPH) was characterised for its structure by IR and NMR spectroscopy as well as its physicochemical properties, hydrolysis profile and interfacial behaviour. The degree of hydrolysis as well as the aminolysis of aggregated and molecular PPH was monitored as a function of varying pH. The aminolysis could be effectively ablated in the case of the aggregated form of PPH. The in vivo bioavailability was determined by calculating the area under the curve in the blood plasma profile after oral administration of PPH in the form of a liquid crystalline dispersion and molecular dispersion. The possible mechanism operating for the enhancement of oral bioavailability was established as lymphatic transportation.

Keywords: Autolymphotrophs; Propranolol palmitate; Supramolecule; Liquid crystals; Lymphatic transport; Oral delivery

1. Introduction

Propranolol HCl is one of the most commonly used β-blocker available in the market. When it is orally administered it is metabolised and partially inactivated due to extensive hepatic first pass metabolism (Dvornik et al., 1983). According to the reports in literature some of the serious drawbacks of the drugs could be effectively circumvented by covalent linkage of the drug to fatty acids (Mantellp et al., 1985). The resulting lipidised prodrug could be used for effective lymphatic transportation for systemic delivery after oral administration. These lipidic prodrugs, if provided with some surface-active property, tend

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to form supramolecular assemblages (liquid crystalline to micellar) in aqueous media. Supramolecules are defined as the self-assemblages, whose functions and properties are determined by the molecular orientation of the assembling units (Wolf and Wolf, 1948). Vizoglu and Speiser, (1992) have previously reported pindolol glycerolmonostearate and buperinol hydrochloride glycerol dipalmitate esters with self-dispersion properties.

Various vesicular systems including liposomes (Gregoriadis and Florence, 1993) and niosomes (Baillie et al., 1985), have demonstrated their potential for application in effective drug delivery. Pharmacosomes have been introduced as a relatively recent version of a novel drug delivery system with well identified advantages. Drug covalently bound to lipids may exist as a colloidal dispersion or ultrafine-vesicular micellar or hexagonal aggregates, which are referred to as pharmacosomes or prodrug mesophases. Thus the pharmacosomes combine the properties of the active drug principle (pharmacon) and the carrier (soma). Contrary to various classical vesicular drug delivery systems, problems of drug incorporation and leakage from the carrier are effectively alleviated in the case of pharmacosomes (Viazoglu and Speiser, 1986; Goymann and Hamann, 1991).

Any drug possessing a free carboxyl group can be esterified with the hydroxyl group of a lipid molecules (glyceride, phosphatide, etc.); similarly, the drugs with an active hydrogen atom, i.e. -OH or -NH, etc., can be esterified. Synthesis of such compounds results in strongly amphiphilic molecules that facilitate transmembrane transfer. These amphipathic prodrug mesogens may serve as building blocks by participating in supramolecular assemblages and thus acquire a colloidal state. The latter may possibly offer a modified newer in vivo fate for the derivatised drug(s).

The present work was undertaken with the intention of enhancing the oral absorption of propranolol HCl as it is extensively metabolised during the first pass hepatic metabolism: nearly 60–80% of the administered dose suffers first hepatic elimination (Dvornik et al., 1983)and, therefore, a major portion of drug remains pharmacodynamically unavailable. Propranolol HCl has been derivatised so as to obtain an amphiphathic prodrug with a long hydrophobic chain, and hydrophilic head group. The prodrug could orient to form supramolecular nanoconstructs. These may be expected to be absorbed well via lymphatic transportation or at least display some modified absorption and distribution characteristics.

2. Materials and methods

2.1. Materials

Propranolol HCl was a gift sample from Cipla India, Mumbai. Phosphatidyl choline and olive oil were purchased from Sigma, USA. Dimethyl aminopyridine was from Merck India, Mumbai, and palmitoyl chloride was from Fluka, Switzerland. All other chemicals and solvents were obtained from BDH, a division of E. Merck India (Mumbai) unless otherwise specified.

2.2. Methods

2.2.1. Prodrug

2.2.1.1. Synthesis of Prodrug. Palmitoyl propranolol hydrochloride (PPH) was synthesised by the esterification of propranolol hydrochloride with palmitoyl chloride, employing the procedure specified of Pech et al. (1996), replacing timolol maleate with propranolol HCl in the same stoichiometric ratios. The compound obtained was purified using column chromatography (Keislgur) with a mobile phase of diethyl ether–acetic acid (95:5) and characterised using NMR and IR spectroscopy.

2.2.1.2. Identification of the prodrug. IR and NMR spectroscopic analysis were conducted to confirm the synthesis of the prodrug using Shimadzu (Japan) IR and Perkin-Elmer NMR (360 MHz) systems.

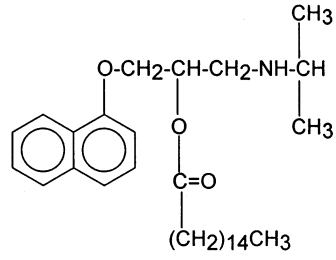
IR: 2910 (C–H stretching vibration CH_3), 2830 (C–H stretching vibration CH_2), 1730 (C═O stretching vibration), 1612 (NH bending vibration CH_3), 1575 (band due to phenyl ring), 1385 (C–H

bending vibration CH_3), 1225 (C–O–C stretching vibration), 1155 (C–O stretching vibration), 924 (C–H out of play vibration in aromatic region)

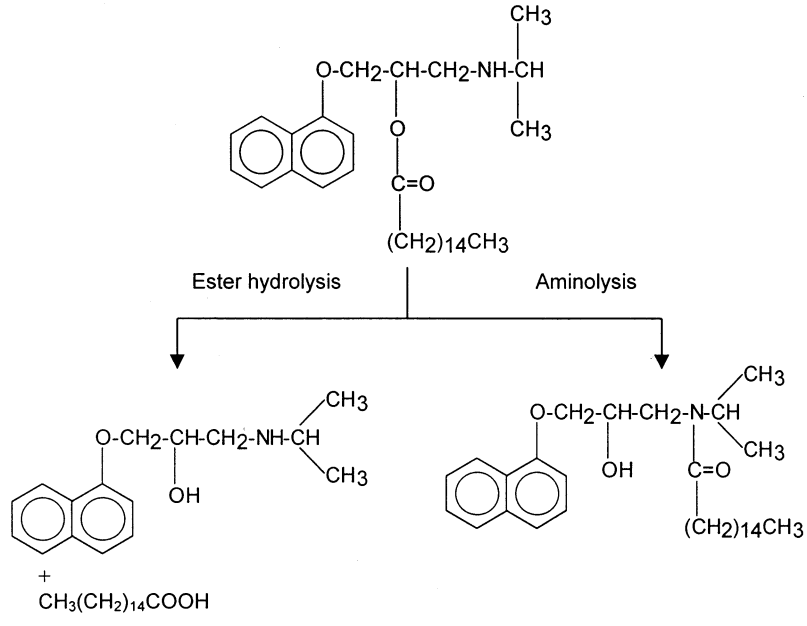
NMR: ($CDCl_3$) δ in ppm values 0.82 (3H, H_3-16), 0.9 (7 H, s, $CH(CH_3)_2$), 1.2 (24 H, s H_2-15), 1.57 (m, H_2-3), 2.33 (m, H_2-2), 3.12 (1H, m, H′-γ), 3.48 (1H, d, H″-γ) 4.2 (2H, m, $OCOCH_2$), 5.9 (1H, w, H-α), 6.8–7.8 (five medium peaks due to aromatic ring)Scheme 1a.

2.2.1.3. Physicochemical characterisation of the prodrug. The physicochemical parameters, i.e. melting point, partition coefficient and solubility of the prodrug were determined and the data were compared with propranolol.

2.2.1.4. Propranolol content. Accurately weighed prodrug was hydrolysed using 0.1 N HCl by heating at 90°C for 2 h. The solution was filtered and following appropriate dilution with phosphate saline, estimated spectrophotometrically ($\lambda_{max} = 290$ nm) using a Systronics 119 UV/VIS spectrophotometer for propranolol content (Clark, 1969).



Structure of PPH



Scheme 1. (b) Possible mode of degradation of PPH Scheme 1.

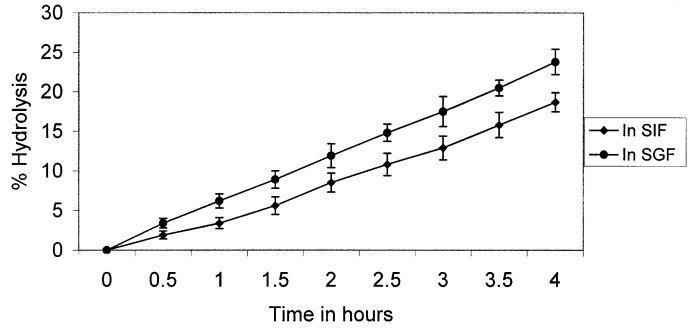


Fig. 1. In vitro hydrolysis of PPH.

2.2.1.5. Hydrolysis profile. The hydrolysis profile of the prodrug was followed in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). The standard dialysis method was applied using Sigma dialysis tubes. The samples were withdrawn periodically and analysed for propranolol content spectrophotometrically as described earlier (Systronics 119 UV/VIS spectrophotometer at $\lambda_{max} =$ 290 nm) (Fig. 1).

2.2.1.6. Tensiometric studies. The tensiometric study was conducted using a Lauda Mgw tensiometer (Konigshofen, Germany). The samples of different concentrations of PPH were prepared in a concentration range 0.1–0.001 w/w in phosphate saline buffer (pH 7.4). The surface tension was recorded at ambient temperature over different time intervals up to 30 min (Pech et al., 1997). Different concentrations of palmitoyl propranolol hydrochloride (PPH) were prepared (0.5, 0.1, 0.05, 0.01, 0.005, 0.001 and 0.0005% w/w) for the determination of critical aggregation concentration and the measurements were recorded until equilibrium was attained. The results are presented in Fig. 2a and b.

2.2.1.7. Microscopic examination. The liquid crystalline phases were observed microscopically using a plain polarised light microscope. The textures and morphology of liquid crystals was identified as described by Rosevear (1954). Dispersion of prodrug in water was studied between 90° and 0.05% w/w at ambient temperatures using a Nikkon (Japan) microscope equipped with crossed polariseres. The samples were briefly heated to 50°C to facilitate the dispersability of poorly water-soluble prodrug. The anhydrous samples of PPH were, however, observed microscopically using a thermostated stage maintained at a temperature ranging between 20–120°C (Fig. 3a, b and c).

2.2.1.8. Viscosity. The rheological property, viscosity, in particular for the prodrug PPH in aqueous medium was determined using an Ostwald capillary viscometer. The viscosities of the prodrug aqueous dispersions were measured at 30°C as well as 45°C (Fig. 4).

2.2.2. Formulation

2.2.2.1. Preparation of prodrug formulations

Liquid crystalline dispersion. A 10% w/v liquid crystalline dispersion of PPH (PLCD) was prepared using the method described by Jaitely and Vyas (1999). The dispersion was heated to 60°C so as to achieve the isotropic phase and slowly cooled to ambient temperature under continual stirring in order to achieve the anisotropic phase. The self-assembled liquid crystalline phase of PPH was surface stabilised by incubating at 40°C with phosphatidyl choline (PC) (10% w/w with respect to the weight of PPH). The preparation was used as such without any further processing.

Lipid solution. A lipid solution of PPH (PLS) was prepared in olive oil. PPH equivalent to 2 mg of propranolol content in 1ml of oil was introduced into olive oil and sonicated briefly to obtain a clear solution.

2.2.2.2. In vitro release profile. The in vitro release profiles of the PLCD and PLS was determined using the dialysis method. Accurately weighed dispersions (PLCD or PLS) were placed in dialysis tubes (Sigma, USA) and the tubes were placed in the dialysing medium (SGF pH 1.2 or SIF pH 7.5) under constant stirring maintained at 37 ± 2°C. The samples were withdrawn periodically and were estimated spectrophotometrically for drug content (Systronics 119 UV–Vis spectrophotometer at $\lambda_{max} = 290$ nm). Each time the withdrawn sample was replaced with an equal volume of respective dialysing medium (Fig. 5).

2.2.2.3. pH stability. The esters of propranolol HCl have been previously reported to follow some degradation not only by ester hydrolysis but to some extent by an aminolysis hydrolysis process as well (Burr et al., 1988). The degradation of palmitoyl propranolol HCl was determined for its aggregated as well as aqueous molecular monomeric forms. The extent of degradation was determined as a function of pH by measuring liberated parent drug by an HPLC method as described by Burr et al. (1988) using an LKB Pharmacia system. The relative height of the peaks in reference to those of standards under similar conditions was taken as the parameter for estimation. In the HPLC chromatogram the decrease in the height of the peak corresponding to *O*-palmitoyl propranolol and a simultaneous increase in the height of the peak corresponding to *N*-palmitoyl propranolol (formed due to aminolysis (Scheme Ib) was utilised for quantitative estimation of aminolysis (amide formation). The degradation profile was monitored in the case of (a) palmitoyl propranolol HCl dispersion below CMC, (b) palmitoyl propranolol HCl dispersion above CMC, (c) liquid crystalline dispersion (PLCD), and (d) lipid solution (PLS).

2.2.2.4. In vivo performance evaluation. Albino rats of either sex were weighed (250–300gms) and divided into five groups of six animals each. All

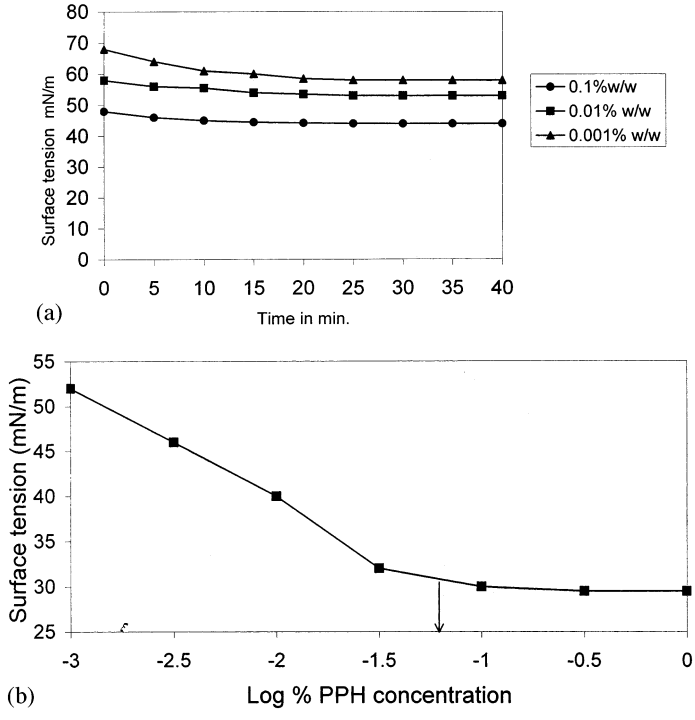
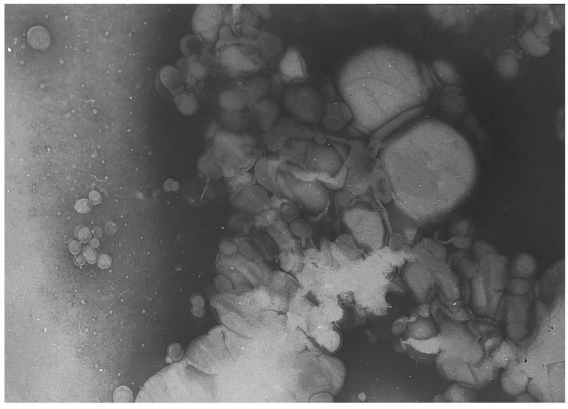
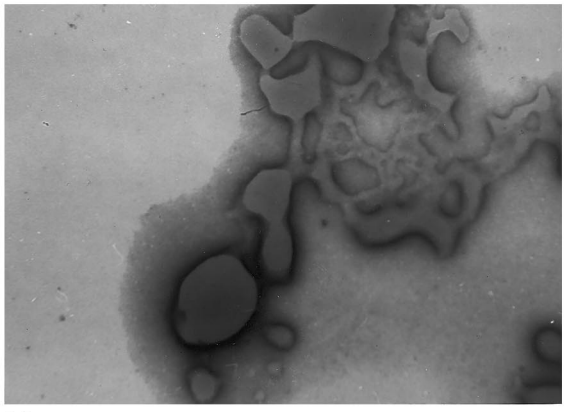


Fig. 2. (a) Change in surface tension with time for different concentrations of PPH. (b) Plot used for determination of critical micellar concentration of PPH.



(a)



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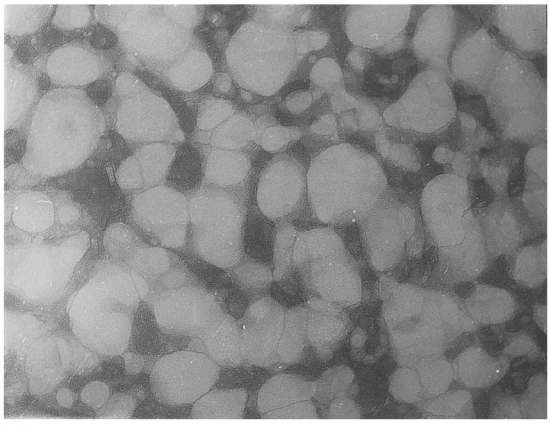

(c)

Fig. 3. Photomicrographs of PPH dispersion in water under plain polarised light. (a) Lamellar phase of PPH showing typical myelinic system. (b) Myelinic tubes with characteristic narrowing and (c) cellular texture characteristics showing liquid crystalline phase.

the animals were kept for overnight fasting but given free access to water. All the animals of group 1 were given an oral dose of PLCD; group 2 was given orally a dose of the lipid dispersion; the animals of group 3 were given orally an aqueous dispersion of prodrug (PPH) and group 4 were given an aqueous solution of propranolol HCl, in equivalent doses, whereas the 5th group served as control (doses for all the formulations were calculated to be equivalent for propranolol).

All the animals were anaesthetised by urethane injection (1.2 g/kg body weight). The animals were dissected and the thoracic duct was cannulated as described by Warshaw (1972). Simultaneously, the jugular vein was also cannulated. All the animals were kept in the supine position and infused with normal saline solution at the rate of 4 ml/h/kg. Lymph and blood samples were collected periodically and analysed for the propranolol content using the fluorometric method for determination of propranolol as reported by Trivedi et al. (1986) (Figs. 6 and 7).

3. Result and discussion

Supramolecular assemblages are described as an ordered orientation that molecules adopt to attain a thermodynamically stable state (Vyas et al., 1997). The formation of the assemblages is attributed to the architectural behaviour of the molecules ascribed to the intrinsic properties of the molecule in a particular solvent, like amphiphilic compounds (e.g. sodium lauryl sulphate, cetyl trimethyl ammonium bromide), which have been known to form ordered assemblages. The formation of these ordered assemblages for drug molecules leads to modifications in the pharmacokinetic and pharmacodynamic behaviours of drug molecules in a biological milieu. The assemblages offer protection against bioenvironmental challenges through supramolecular orientation (Kataoka et al., 1993).

Propranolol HCl is a water soluble β-blocker which on oral administration metabolised extensively during first hepatic pass as well as being partially inactivated during intestinal absorption in the gastrointestinal tract (Dvornik et al., 1983).

Nearly 75% of the administered dose is inactivated (metabolised) by the liver during the first hepatic passage, leading to a low bioavailability of propranolol on oral administration. The present work was undertaken with the intention of increasing the overall oral bioavailability of propranolol HCl utilising intestinal lymphatics transportation.

The prodrug of propranolol HCl was prepared by esterification at the secondary alcoholic group with palmitoyl chloride by the method described by Pech et al. (1996). The product obtained was purified using column chromatography.

The isolated prodrug was studied for its chemical structure with the help of IR and NMR spectroscopy. The IR spectra for the synthesised prodrug *O*-palmitoyl propranolol hydrochloride (PPH) when compared against that of propranolol HCl, clearly revealed a peak for OH bending vibration at 3332 cm^{-1} while a prominent peak at 100 cm^{-1} disappeared. In addition, an additional peak at 1740 cm^{-1} appeared. The later peak corresponds to C=O vibration of the ester group formed depicting the esterification of propranolol HCl with palmitoyl chloride. The presence of the ester linkage was further confirmed by NMR spectroscopy (peaks previously indicated).

The synthesised prodrug (PPH) was studied for its physicochemical parameters. The melting point of the prodrug was found to be 49 ± 0.5°C. The amphiphilic nature of the prodrug was shown by the solubility determination. It was found that PPH was differentially soluble in polar as well as in non-polar solvents, however, it demonstrated higher solubility in the organic solvents. The partition coefficient in the octanol–water system was 1.857 ± 0.045 indicating the amphiphilic nature of PPH, however, the hydrolysis of PPH was affected in SGF and SIF. Fig. 1 clearly shows that the propranolol ester was quite stable in acidic as well as in basic environments. The hydrolysis, at the gastric pH was found to be relatively faster as compared to the rate of hydrolysis at the intestinal pH. At the intestinal pH the formation of *N*-palmitoyl propranolol as a derivatised base could be one mechanism of degradation, which avoids ester hydrolysis.

The self-assembling nature of PPH was established with the help of tensiometric studies. The amphiphilicity of PPH can be appreciated by measuring the surface tension of dispersion against time using the Wilhelmy plate method (Davis and Ridear, 1963) as illustrated in Fig. 2a and b at pH 7.4. It was found that the surface tension decreased with increasing concentration of PPH (equilibrium surface tension of 0.1, 0.01 and 0.001% w/w solution were 44, 53 and 58 mN/m). The concentration also significantly affected the kinetic profile, for lower concentrations the initial surface tension values decreased slowly till equilibrium surface tensions were attained (Fig. 2a).

The aggregation concentration was determined from a plot prepared between the surface tension and concentration (log scale) (Fig. 2b). It was found that after an initial linear decrease, the surface tension was almost constant. The point of

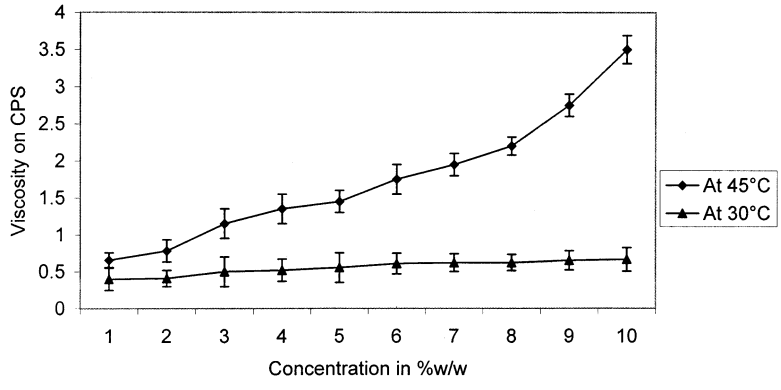

Fig. 4. Change in viscosity with concentration at different temperatures.

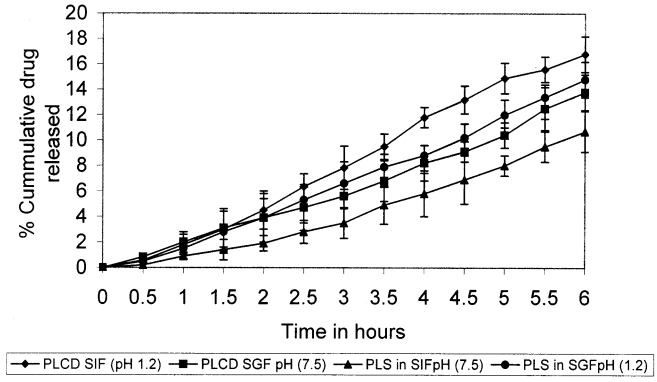

Fig. 5. In vitro release rate profile.

deviation from linearity and the corresponding concentration was considered as the critical aggregation concentration, which was found to be 0.06% w/w (corresponding log value -1.22). This observation further substantiates the amphipathic nature of the PPH. This could be attributed to the presence of a hydrophobic palmitoyl chain in association with an intrinsic hydrophilic group of propranolol (quaternary ammonium salt). The amphiphilicity and the presence of a long hydrophobic chain of the prodrug may account for the assemblage of PPH molecules with a particular orientation leading to the formation of a supramolecular system.

The anhydrous state of the prodrug (PPH) at room temperature exhibited an amorphous form with typical birefringent microcrystals scattered in all directions appearing spherulitic in shape with irregular maltese crosses. In the temperature range 45–55°C complete isotropic melting was observed. However, the liquid crystalline phase reappeared on cooling with a typical radiant pattern. On reheating, melting occurred regularly at 48°C. The crystalline phase of PPH existed below 35 ± 1°C. Above 50 ± 2°C the isotropic melting of PPH was observed.

Smectic type liquid crystalline phases which are referred to as 'lamellar phases' were observed in the preparation. These 'lamellar phases' were identified by the presence of myelinic systems with characteristic labyrith like patterns. These myelin systems are clearly visualised in Fig. 3a that resemble closely to that of phospholipids. The cylindrical structures of myelinic systems showed various alterations and narrowings as seen in Fig. 3b. These shapes are the result of subsequent deformations in the myelinic structures and lamellar textures. The characteristic cellular textures of the lyotropic crystalline phase are clearly seen in Fig. 3c.

At 30°C and up to 35°C the PPH dispersion was observed microscopically to be heterogeneous distinctively marked by the existence of solid crystals and a liquid crystalline phase, whereas below 30°C mainly a solid crystal rich phase was seen. Similarly, in the microscopic observation made at 45°C and above, an explicit phase transition from a solid crystalline state to an absolutely liquid crystalline phase was recorded. Simultaneously, the viscoelastic studies were conducted at 30° and 45°C. The system prepared displayed typical temperature dependent viscoelastic behaviour as suggested by Kirwet and Muller-Goymann (1993). The viscosity was enhanced at a lower temperature, i.e. 30°C, presumably due to the presence of the solid crystalline phase. It was observed to be independent of the concentration of PPH. At a higher temperature, i.e. 45°C, the system, however, demonstrated a linear increase in viscosity with increasing concentration of PPH. The intrinsic viscosity measurement was found to be $\eta = 2.5\phi$, corresponding to spherical to cylindrical assemblages (Kirwet and Muller-Goymann, 1993). Moreover, the conversion of the isotropic phase to the anisotropic phases with a concomitant change in temperature from 45°C and above

was witnessed by varying viscoelastic behaviour. It also suggests the formation of lamellar liquid crystalline phases of PPH as it was also optically observed under the stated temperature conditions with the help of a polarising microscope. The presence of liquid crystals supports viscoelastic behaviour and the findings are in accordance with those suggested by Chang and Bodmier (1997) for monoglycerides.

The overall oral bioavailability of the prodrug (PPH) was determined following its oral administration in various formulations, i.e. in assembled (dispersion) as well as solution forms. PPH was found to convert to its anisotropic forms due to specific amphiphilic properties and microscopic phase behaviour under varying temperature conditions. PPH was restricted to its liquid crystalline aggregated supramolecular phase using phosphatidyl choline as the surface stabiliser as well as protecting it from G.I. environmental challenges as suggested by Satpadar et al. (1993) for the preparation of nanocrystals (PLCD). The lipid solution of PPH was prepared in olive oil in order to study the effect of lipid vehicle and lipophilicity of drug on its lymphatic transportation.

The in vitro release profile obtained (Fig. 5) in SGF and SIF indicates that all the formulations of PPH are fairly stable throughout the gastric environment. Release of the free drug was $13.8 \pm 0.25\%$ in the SGF in 6 h whereas $16.83 \pm 0.354\%$ of the drug was estimated in SIF in the case of PLCD. However, only 10.2 ± 0.25 and $12.83 \pm 0.54\%$ of the drug was released in SGF and SIF, respectively, in the case of PLS. It indicates the high shielding effect offered by the molecular geometry of aggregated system as well as the surface phospholipid layer highlighting the suitability of its use as a carrier for intestinal delivery of drugs.

The hydrolysis of PPH leading to the liberation of free drug was studied in lieu of the fact that the esters of propranolol (acetyl, propionyl, butyryl, and pivitoyl) may form amides at alkaline pH as reported by Burr et al., 1988. The study was conducted as per the method utilised by Burr et al., 1988. PPH dispersions (below aggregation concentration as well as above aggregation concentration) and in the prepared PLCD were studied using HPLC chromatograms and by monitoring the peaks height together with retention time using standards. The ester of propranolol HCl can undergo degradation by two consecutive pathways, one by ester hydrolysis and another by aminolysis (Scheme Ib).

Fig. 5 shows pH versus percent amide formation (percent amide = 100 − percent propranolol formed) and clearly illustrated that the amide formation could effectively be arrested as the PPH concentration attains an aggregated state. The results are in accordance with the findings of Burr et al. (1988), which suggested that as the bulkiness of the substituted group, increases the degree of aminolysis decreases. Seemingly, palmitoyl being a highly bulky group (15.5% of amide formation

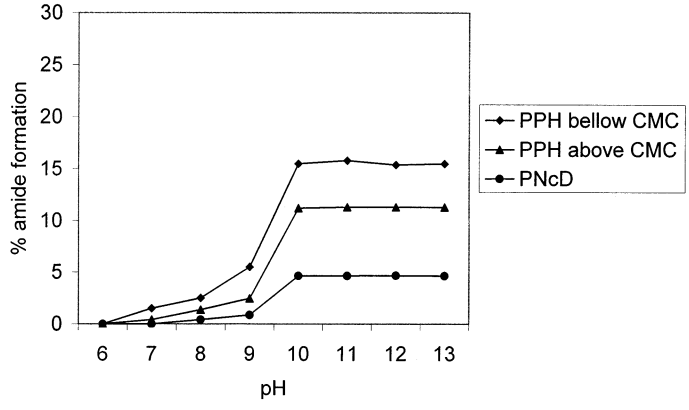

Fig. 6. Degree of aminolysis in PPH.

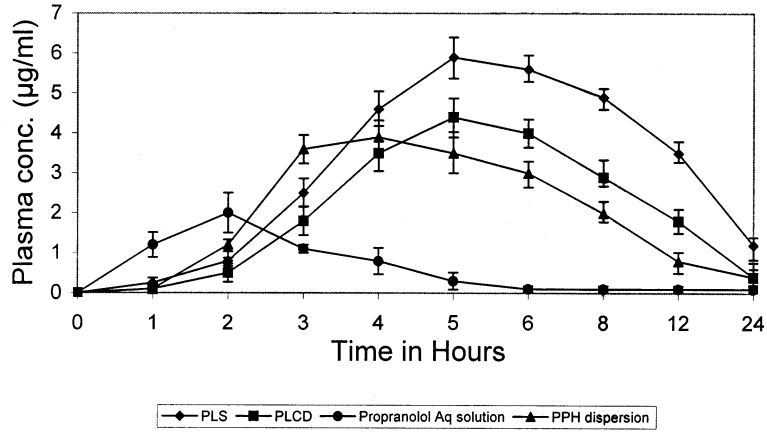

Fig. 7. Plasma profile of PPH and its supramolecular aggregates.

in the normal dispersion) could prevent aminolysis.

The prodrug PPH when taken below the aggregation concentration degrades via ester hydrolysis as well as to some extent by aminolysis where the latter accounts for 15.5% of the degradation at a pH above 9.0. However, above CMC in the aggregated state the degree of aminolysis was found to decline to 11.2%. Negligible to almost no aminolysis was found in the case of PPH liquid crystalline dispersion (PLCD) as well as in lipid solution (PLS). This could be accounted to the shielding effect and the molecular orientation in the bioenvironment leading to higher stability. Similar reports have been made for methyl prednisolone 21-hemiester micelles by Anderson et al. (1983). Hence it could be inferred that the aggregated state protects the palmitoyl propranolol hydrochloride from aminolysis. However, ester hydrolysis remains an effective pathway of prodrug hydrolysis resulting in the liberation of free propranolol.

The in vivo performance evaluation of the prodrug solution and in the form of its supramolecular aggregates or dispersion was conducted on albino rats. The rats were given an initial oral dose of soybean oil in order to swell the lymphatic duct to make it accessible for cannulation. A double cannulated anaesthetised rat model was used to investigate the potential lymphatic transportation of PPH in its supramolecular assemblages. Periodically collected plasma and lymph were estimated for propranolol content. Fig. 7 shows the plasma profile. The data obtained were subjected to appropriate statistical treatment. The bioavailability of propranolol following various treatments was determined by computing the area under the curve of the drug plasma profile. The area under the curve (AUC) was determined employing a trapezoidal rule. A significantly higher bioavailability of propranolol HCl following the administration of PPH was recorded as compared to the drug plasma profile and AUC recorded the plain drug administration. A significant statistical difference was recorded ($P < 0.05$) (Table 1). Similarly the bioavailability estimated for PLCD was much higher than the others, whereas it was maximum amongst the various formulations in the case

Table 1
Comparative bioavailability of various formulations determined from plasma data

Sample No.	Formulation	AUC ($n = 6$) (µg/ml/h)
1.	Propranolol HCl aqueous solution	218.46 ± 1.09
2.	PPH dispersion in water	276.77 ± 1.24
3.	Liquid crystalline dispersion (PLCD)	292.83 ± 0.25
4.	Lipid solution of PPH (PLS)	326.34 ± 0.78

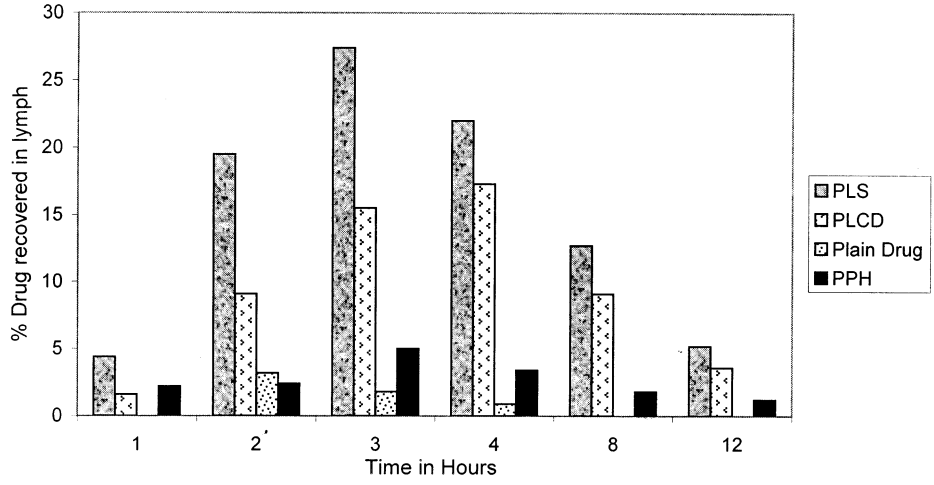

Fig. 8. In vivo lymphatic uptake of various formulations.

of PLS. The AUC recorded were 292.83 ± 0.25 and 326.34 ± 0.78 μg/ml/h ($n = 6$) for PLCD and PLS, respectively, which were significantly higher ($P < 0.05$) than the AUCs recorded for plain drug and prodrug. Thus on the basis of multiple statistical competitive evaluation the preparation based on prodrug of propranolol in the form of self aggregated supramolecular forms demonstrated the best results by producing significantly higher propranolol blood plasma levels and overall AUC reflecting better bioavailability too.

In the case of supramolecular PLCD as well as PLS an initial lag phase was observed. This is suggestive of the mechanism, which is operative in uptake and trafficking of the drug to the systemic circulation via lymphatics as well as by normal systemic absorption. The lymph was simultaneously collected and a periodic lymphatic drug concentration profile was generated (Fig. 8) to support the absorption mechanism.

The lymphatic drug concentration profile indicates that lymphatic uptake and trafficking of the PPH are apparently responsible for the increased oral bioavailability. The effects, one contributed by the lipophilicity and the other by the supramolecular assemblage of PPH, are seemingly responsible for an increased lymphatic uptake which would have been further enhanced by the presence of lipidic vehicle. The increased bioavailability in the case of oral administration of PPH contained in a lipid vehicle could be attributed to the effect of the vehicle, which reportedly enhances the enterocyte production, and in turn chylomicron expression. The latter is a well-documented process of lipid uptake (Palin and Wilson, 1984). Obviously the lipid vehicle digestion may largely be attributed to better uptake of PPH, which is more lipophilic as compared to its drug molecule. The relatively better systemic absorption via lymph in the case of supramolecular assemblages of PPH tensiogen may be accounted for by its chylomicron mimicking architecture; surface stabilised with phospholipid is critical in negotiating lymphatic transportation. However, a well-designed study protocol should be followed in order to establish the exact mechanism that operates for better availability.

The intestinal lymphatics are characterised by a centrally located vessel; a lacteals within the intestinal villi which join a plexus of lymphatic capillaries in the mucosa and submucosa and drain via the mesenteric lymph vessels into the cisterna chyli. The lymph from the cisterna chyli is drained by the thoracic lymph duct, which empties directly into the general circulation at the junction of the left internal jugular and left subclavian veins, thus avoiding hepatic first pass metabolism (Granger et al., 1988). Therefore, lymphatic transportation could be largely responsible for the higher bioavailability of PPH after oral adminis-

tration. The avoidance of hepatic first pass extraction is seemingly a pivotal factor that could account for improved bioavailability.

4. Conclusion

It could be concluded from the study that the palmitoyl prodrug of propranolol having an amphiphilic nature is a tensiogeneic mesogen that assembles to form supramolecules convertible into liquid crystalline states. The nanoconstructs restricted to this particular orientation by surface stabilisation are transported to the systemic pool via lymphatics and thus bypass hepatic metabolism and as a result enhance the systemic availability of propranolol. Furthermore, the major constraint related to propranolol ester, i.e. aminolysis, has been effectively addressed especially through supramolecular assemblages. Hence the supramolecular aggregates could serve as stable lymphotrophs exploitable for effective drug delivery for better bioavailability of the drugs which undergo extensive first hepatic pass metabolism.

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References

Anderson, B.D., Conradi, R.A., Johnson, K., 1983. Influence of permilcellar and micellar association on reactivity of methyl-prednisolone 21-hemsiters in aqueous solution. J. Pharm. Sci. 72 (4), 448–453.

Baillie, A.J., Florence, A.T., Hume, L., Muirhead, G.T., Rogerson, A., 1985. The preparation and properties of niosomes-non-ionic surfactant vesicles. J. Pharm. Pharmcol. 37, 863–868.

Burr, A., Bundgaard, H., Lee, V.H.L., 1988. Prodrugs of propranolol: hydrolysis and intramolecular aminolysis of various propranolol esters and an oxazolidine-2-one derivative. Int. J. Pharm. 42, 51–60.

Chang, C.M., Bodmier, R., 1997. Swelling and drug release from monoglyceride based drug delivery systems. J. Pharm. Sci. 86, 747–752.

Clark, E.G.C., 1969. Isolation and Identification of Drugs, vol. 1. Pharmaceutical Press, London, p. 521.

Davis, J.T., Ridear, E.K., 1963. Surface Chemistry: The Physics of Surface. Academic Press, New York.

Dvornik, D., Kraml, M., Duvue, J., et al., 1983. Relationship between plasma propranolol conventional and dose of long acting propranolol (Inderal ® LA). Curr. Ther. Res. 39, 595–605.

Goymann, C.M., Hamann, H.J., 1991. Pharmacosomes-multilammelar vesicels consisting of a pure drug. Eur. J. Pharm. Biopharm. 37, 113–117.

Granger, D.N., Korthius, R.J., Kvietys, P.R., Tso, P., 1988. Intestinal microvascular exchange during lipid absorption. Am. J. Physiol. 255, 690.

Gregoriadis, G., Florence, A.T., 1993. Liposomes in drug delivery. Drugs 45, 15–28.

Jaitely, V., Vyas, S.P., 1999. Development and characterisation of supramolecular autovectoring system for selective drug delivery. J. Drug Target. 6 (5), 315–322.

Kataoka, K., Kwon, G.S., Yokoyama, M., Okano, T., Sakuri, Y., 1993. Block co-polymer micelles as vehicles for drug delivery. J. Control. Release 24, 119–132.

Kirwet, K., Muller-Goymann, C.C., 1993. Binary diclofenac diethylammonium water systems: micelles, vesicles and lyotropic liquid crystals. Eur. J. Pharm. Biopharm. 39, 234–238.

Mantellp, S., Speiser, P., Hauser, H., 1985. Phase behaviour of diglyceride prodrug, spontaneous formation of unilamellar vesicles. Chem. Phys. of Lipids 37, 329–343.

Palin, K.J., Wilson, C.G., 1984. The effect of different oil on absorption of probucol in rats. J. Pharm. Pharmacol. 36, 641.

Pech, B., Duval, O., Richomme, P., Benoit, J.P., 1996. A timolol prodrug for improved ocular delivery: synthesis conformational study and hydrolysis of palmitoyl timolol malonate. Int. J. Pharm. 128, 179–188.

Pech, B., Proust, J.E., Bouloguand, Y., Benoit, J.P., 1997. Tensioactivity and supramolecular organisation of the palmitoyl prodrug of timolol. Pharm. Res. 14 (1), 37–41.

Rosevear, F.B., 1954. The microscopy of the liquid crystalline neat and middle phases of soap and synthetic detergents. J. Am. Oil Chem. Soc. 31, 628–639.

Satpadar, P., Bruno, J., Liversidge G. et al., 1993. Nanocrystals Pilosulfan the prototype or newer intravenous delivery from for highly insoluble antitumor agents. Gorden Conf. on Chemotherapy of Experimental and Clinical Cancer, 3–8 October , 1993, Isee, Germany.

Trivedi, B.M., Gohel, M., Chawa, H., 1986. Flurorometric determination of propranolol. Ind. J. Pharm. Sci. 48, 142–143.

Viazoglu, O., Speiser, P., 1986. Pharmacosomes—a novel drug delivery system. Acta Pharm. Suec. 23, 163–172.

Vizoglu, O., Speiser, P., 1992. The pharmacosomes drug delivery approach. Eur. J. Pharm. Biopharm. 38, 1–6.

Vyas, S.P., Jaitely, V., Kanaujia, P., 1997. Self-assembling supramolecular biovectors a newer dimension in novel drug delivery system. Die Pharmazie 52 (4), 259–267.

Warshaw, A.Z., 1972. A simplified method for cannulating the intestinal lymphatics of rat. Gut 13, 66.

Wolf, K.L., Wolf, R., 1948. Supramolecules. Angew. Chem. 61, 191.