JPP Journal of Pharmacy and Pharmacology

JPP 2010, 62: 430–439

Received August 05, 2009
Accepted Jan 07, 2010
DOI 10.1211/jpp/62.04.0004
ISSN 0022-3573

Research Paper

Surfactant systems for nasal zidovudine delivery: structural, rheological and mucoadhesive properties

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Abstract

Objectives Zidovudine is the antiretroviral drug most frequently used for the treatment of AIDS. Although its effectiveness is recognized, it undergoes extensive first-pass metabolism and exhibits poor oral bioavailability. The nasal route is an option for enhanced therapeutic efficacy and to reduce the extent of the first-pass effect. There are some mechanisms that limit intranasal absorption, such as mucociliary clearance, which rapidly removes the formulation from the nasal cavity. To improve the nasal residence time of zidovudine on the nasal mucosa, we aimed to develop a mucoadhesive surfactant system for zidovudine nasal administration.

Methods Systems composed of PPG-5-CETETH-20 as surfactant, oleic acid and water were characterized by polarized light microscopy, small-angle X-ray scattering and rheological measurements. Mucoadhesion was investigated by phase behaviour studies, rheological synergism and mucoadhesive strength determination.

Key findings Results indicate that the original formulations were microemulsions that displayed phase transition to a lamellar phase when brought into contact with aqueous nasal simulated mucus. The phase transition was accompanied by an increase in system elasticity and, irrespective of phase behaviour, all the systems showed a good mucoadhesive force. Thus, a viscous and mucoadhesive liquid crystalline matrix could be formed when the formulations were in contact with simulated mucus, which may prolong the residence time of zidovudine in the nasal cavity.

Conclusions These findings indicate a potentially useful system for nasal administration of zidovudine.

Keywords liquid crystal; microemulsion; mucoadhesion; oscillatory rheology; phase behaviour

Introduction

The human immunodeficiency virus (HIV) pandemic remains the most serious infectious disease challenge to public health. The estimated number of people living with HIV worldwide in 2007 was 33.2 million.[1] Although antiretroviral drug therapy has contributed significantly to improvement of acquired immunodeficiency syndrome (AIDS) disease management, its current use is associated with several disadvantages and inconveniences to the patient. Zidovudine (AZT), the first anti-HIV compound approved for clinical use, is widely used for the treatment of AIDS, either alone or in combination with other antiviral agents.[2] However, the main limitations to the therapeutic effectiveness of zidovudine are its dose-dependent haematological toxicity, low therapeutic index, short biological half-life and poor bioavailability.[3] Owing to its short half-life, patients have to take frequent doses to maintain constant therapeutic drug levels.[2] The severe side effects associated with the complicated dosing schedules of antiretroviral therapy may be a cause of low adherence to the treatment.[2] Strategies currently being investigated to overcome these limitations include the design and development of novel drug delivery systems that can improve the efficacy of both existing and new antiretroviral drugs.[4]

Novel drug delivery systems can be designed to administer drugs by alternative routes when the oral route is ineffective. Nasal drug delivery has generated widespread interest as an alternative route for drugs such as zidovudine that are susceptible to first-pass hepatic

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metabolism. Various factors that synergistically enhance the permeation of nasally administered drugs are: the relatively large surface area due to the presence of a large number of microvilli in the nasal passages, a porous endothelial membrane and a highly vascularized epithelium.[5,6] Despite the established potential of the nasal route, there are some factors limiting the intranasal absorption of some types of drugs, such as the physical removal of the drug from the site of deposition in the nasal cavity by the mucociliary clearance mechanisms.[6] Nasal mucociliary clearance largely determines the absorption profile of nasal drug delivery, since the residence time of drugs delivered to the nasal cavity is limited by this mechanism.[6,7] To optimize nasal uptake, bioadhesive drug delivery systems have been studied.[8]

Bioadhesion can be defined as the binding of a natural or synthetic polymer to a biological substrate.[9,10] When this substrate is a mucous layer, the term mucoadhesion is often used. Mucoadhesive drug delivery systems provide a means of enhancing retention time at defined sites and, if systemic uptake occurs, the use of mucoadhesive polymers will promote a wider distribution of the drug. As a result of adhesion and intimate contact, the formulation stays longer at the delivery site, improving drug bioavailability, while lower drug concentrations can be used for disease treatment.[10–12] The reduction of doses and the avoidance of first-pass metabolism may then reduce dose-related side effects of some drugs such as zidovudine.

Mucoadhesive polymers are generally hydrophilic networks that contain numerous polar side-groups. The attachment and bonding of mucoadhesive polymers to biological substrates occurs mainly through interpenetration of polymer chains followed by secondary non-covalent bonding between polymer and substrate, mainly due to hydrogen-bond formation, since the polymers possess hydrophilic groups such as those in poly(ethylene oxide) (PEO).[11]

PEO-based polymers have found use in mucoadhesive drug delivery.[13] Poloxamers (block copolymers of PEO and poly(propylene oxide) have a phase transition from liquid to mucoadhesive gel at body temperature and will therefore allow in-situ gelation at the site of interest. These copolymers have also been chemically combined with poly(acrylic acid)s to produce systems with enhanced adhesion and retention in the periodontal pocket[14] and nasal cavity.[9] In-situ gelling and mucoadhesive polymer-based vehicles composed of poloxamer, polycarbophil and PEO showed much higher absorption and prolonged nasal retention of plasmid DNA.[13]

PEO polymers can also associate to form a range of different liquid crystalline structures.[15] It has been recognized for several decades that lyotropic liquid crystal phases formed from aqueous surfactant systems can provide matrices for the sustained release of drugs with different physicochemical properties.[16] When a lyotropic surfactant is added to water, it undergoes various phase transformations, forming lamellar, hexagonal and cubic phases, depending on the water content in the matrix. Liquid crystal phases have been found to be mucoadhesive with a range of mucosal surfaces, although the mucoadhesion mechanism differs somewhat from that described in other mucoadhesive theories that explain the mucoadhesive process (e.g. wetting, adsorption, diffusion and mechanical theories).[9,10] In the case of liquid crystal phases, the mechanism of mucoadhesion probably involves the rheological properties of the system, which are similar to those of in-situ gelling vehicles. The liquid crystal system can be arranged in a very strong and viscous matrix that favours mucosal retention, impeding the immediate removal of the formulation by mucociliary clearance. Cubic phases have been suggested as a mucoadhesive matrix, due to their high viscosity,[17] but this characteristic can hinder nasal administration. To circumvent these handling problems, precursor formulations of liquid crystalline mesophases are proposed.[17] The lamellar phase has been used as a precursor of the cubic phase which, upon water uptake, forms the cubic phase.[17–19] Since mucus is an aqueous fluid, the liquid crystal precursor system can be designed to change spontaneously to a more viscous phase by absorbing physiological aqueous fluid from the mucosa. This property can be used to improve the retention of the formulation for a prolonged period of time in the nasal cavity.

The main aim of this study was to develop a zidovudine delivery system composed of a PEO surfactant able to form liquid crystal phases when in contact with the mucus layer in the nasal cavity and increase the viscosity of the system, to avoid its rapid elimination by mucociliary clearance. In previous studies the nonionic PEO surfactant polyoxypropylene (5) polyoxyethylene (20) cetyl alcohol (PPG-5-CETETH-20) combined with oleic acid was shown to stabilize various phases, such as microemulsions, liquid crystals and emulsions, in the presence of water.[20,21] The structural, rheological and mucoadhesive properties of these systems were the object of investigation in this study. The formulations were characterized by polarized light microscopy (PLM), small-angle X-ray scattering (SAXS) and oscillatory rheological measurements. Mucoadhesive properties were assessed by phase behaviour studies using PLM and SAXS analyses, followed by in-vitro measurement of rheological properties and mucoadhesive strength.

Materials and Methods

Materials

PPG-5-CETETH-20, which is available commercially as Procetyl AWS, was purchased from Croda (Campinas, Brazil) and oleic acid from Synth (Diadema, Brazil). Mucin from porcine stomach was from Sigma Aldrich (Steinheim, Germany). Zidovudine was a gift from FURP (Fundação para o Remédio Popular, Guarulhos, Brazil). High-purity water was prepared with a Millipore Milli-Q plus purification system.

Preparation of the formulations

Formulations were prepared from PPG-5-CETETH-20 as surfactant, oleic acid as oil phase and water as aqueous phase. Zidovudine was incorporated by dissolving the drug powder directly in the systems. The compositions of the formulations are shown in Table 1.

Table 1 Composition of zidovudine-loaded surfactant system formulations, structural parameters obtained from SAXS measurements, elastic (G') and viscous (G'') modulus at representative frequency of 1.5 Hz and mucoadhesion force

	Composition (%)			SAXS parameters				Viscoelastics modulus		Adhesion measurements
	O	S	W	d (Å)	d_1/d_2	d_1/d_3	d_1/d_4	G′	G″	Force (N)
1A	35	55	10	52.3				0.07	1.27	0.306 ± 0.038
1A30				76.1	2	3		0.16	2.51	–
1A50				86.5	2	3		15.57	16.15	0.262 ± 0.038
1A100				110.0	2	3		452.4	44.28	–
2A	30	55	15	57.1				1.50	3.41	0.279 ± 0.035
2A30				75.6	2	3		2.94	10.74	–
2A50				84.0	2	3		526.2	103.8	0.304 ± 0.040
2A100				111.0	2	3		867.3	57.36	–
3A	25	55	20	62.8				0.04	2.5	0.247 ± 0.026
3A30				80.4	2	3		356.0	58.65	–
3A50				92.1	2	3		910.5	131.7	0.283 ± 0.043
3A100				118.0	2	3	4	734.7	55.55	–

Formulations were loaded with 40 mg/g of zidovudine. O, oil (oleic acid); S, surfactant (PPG-5-CETETH-20), W, water. G′ (elastic modulus) and G″ (viscous modulus) were measured at a representative oscillatory frequency of 1.5 Hz. Force (measured at 32°C) is presented as means ± SD, $n = 7$

Characterization of the formulations

Polarized light microscopy

An Optical Leica Microscope was used to analyse various fields of each sample at room temperature. The isotropic or anisotropic behavior of the samples was observed. Pictures were taken at a magnification of 20 000×.

Small-angle X-ray scattering

Data was collected at the Synchrotron SAXS beam line of the National Laboratory of Synchrotron Light (LNLS, Campinas, Brazil), equipped with an asymmetrically cut and bent Si (1 1 1) monochromator (λ = 1.608 Å) that yields a horizontally focused beam. A vertical position-sensitive X-ray detector and a multichannel analyser were used to record the SAXS intensity, I(q), as a function of the modulus of the scattering vector q, $q = (4\pi/\lambda)\sin(\varepsilon/2)$, ε being the scattering angle. The samples were placed in a cell at 25°C. The parasitic scattering produced by slits was subtracted from the total scattering intensity.

Oscillatory rheological measurements

The rheological analysis of samples was performed at 25°C with a controlled-stress Carrimed CSL 100 rheometer (TA Instruments, Sao Paulo, Brazil), in oscillatory mode, using a stainless-steel parallel plate geometry (2 cm diameter), separated by a gap of 200 μm. Samples were carefully applied to the lower plate, ensuring that formulation shearing was minimized, and allowed to equilibrate for at least 3 min before analysis. Oscillatory analysis of each formulation under examination was performed after determination of its linear viscoelastic region at 25°C, where stress was directly proportional to strain and the storage modulus remained constant. Frequency sweep analysis was performed over the frequency range of 0.1–10 Hz, following application of a constant stress of 1 Pa. Systematic error in frequency was given by the rheometer and was around 0.01 Hz.

Investigation of mucoadhesive properties

Phase behaviour

Phase behaviour was studied to determine the phase progression displayed by the formulations with increasing artificial mucus content. The simulated mucus used was a solution containing 8% (w/v) mucin, 7.45 mg/ml NaCl, 1.29 mg/ml KCl and 0.32 mg/ml $CaCl_2.2H_2O$, with pH adjusted to 5.7.[22] The formulations were mixed with 30, 50 and 100% of simulated mucus (w/w) in relation to initial weight of the formulations; more specifically, 3 g of each formulation was mixed with 0.9 g of simulated mucus (30% of initial formulation weight), 1.5 g of simulated mucus (50% of formulation weight) and 3 g of simulated mucus (100% of formulation weight). A partial phase diagram was constructed to provide a trajectory of the system compositions across the liquid crystal region after the addition of simulated mucus. To identify the samples with added simulated mucus, the percentage added was written after the formulation code (e.g. formulation 1A, mixed with 30% simulated mucus, was named 1A30). Phase transformations after addition of simulated mucus were identified by PLM and SAXS measurements, as described above at 32°C to mimic the nasal temperature.[23]

Rheological analyses of mucoadhesion

Dynamic frequency-sweep measurements were carried out on all the samples, as previously described, but at 32°C to mimic nasal temperature.[23]

Assessment of the mucoadhesive strength of the formulations

The mucoadhesive strength of the formulations under investigation was estimated by measuring the force required to detach the formulations from a crude porcine mucin disc (250 mg, 123 mm diameter) using a TA-XTplus Texture Analyser (Stable Micro Systems, Godalming, UK) in adhesive test mode. The mucin discs were attached with double-sided adhesive tape to the lower end of the cylindrical probe (diameter 12.3 mm). The mucin disc was hydrated by

submersion in 8% solution of mucin for 30 s. Excess surface liquid was removed. At the temperature of 32°C, 3 g of each formulation, packed into shallow cylindrical vessels (4 cm diameter), was placed under the analytical probe, which was then lowered until the mucin disc was in contact with the surface of the sample. A downward force of 0.1 N was applied for 30 s to ensure intimate contact between the mucin disc and the sample. The probe was then moved upwards at a constant speed of 1.0 mm/s and the force required to detach the mucin disc from the surface of each formulation was determined from the resulting force–time plot. All measurements were performed in seven replicates.

Statistical analyses

The mucoadhesive force obtained by texture analyser for each sample was compared using one-way analysis of variance. In all cases, post-hoc comparisons of the means of individual groups were performed using Tukey's Honestly Significant Difference test. $P < 0.05$ denoted significance in all cases. All treatments were performed for seven replicates.

Results

Characterization of the formulations

Samples were prepared by simple mixture of the components in a beaker at room temperature. The appropriate amount of zidovudine (40.0 mg/ml) was dissolved directly in previously prepared formulations, and the samples were mixed for 24 h at a controlled temperature of 25°C. Zidovudine-loaded samples were named 1A, 2A and 3A.

Formulations were analysed by PLM to identify their isotropic or anisotropic behaviour. A drop of each sample was placed on a glass slide, covered with a coverslip and then examined under polarized light. Figure 1 contains the images obtained for formulations 1, 2 and 3 and zidovudine-loaded samples 1A, 2A and 3A showed the same pattern. A dark field was observed in all the samples, which is characteristic of microemulsion systems.[24–26] Microemulsions are thus defined as a system of water, oil and surfactant, which is a single optically isotropic and thermodynamically stable liquid solution.[24] The formulations were transparent and under thermodynamic equilibrium, since they were obtained by simple mixture of the components and no phase separation was observed in 24 months.

The structural identification of zidovudine-unloaded and -loaded formulations was confirmed by SAXS measurements and the SAXS curves are presented in Figure 2. Micellar systems exhibit SAXS curves with a broad band or very wide peaks associated with the short-range 3D spatial correlation of the micelles or microemulsion droplets.[27] SAXS patterns determined for each sample (Figure 2) show such wide peaks associated with microemulsion systems, confirming the isotropy visualized in PLM. It can be seen that zidovudine incorporation has not changed the structural features of the formulations. In SAXS curves of formulations 3 and 3A there is a second small and wide peak (Figure 2, arrow), which may be related to a higher degree of structural organization. The correlation distance between the scattering objects is calculated as $d = 2\pi/q_{max}$, where q_{max} is the q value at the peak of the intensity I(q). For samples 3 and 3A, it was observed that the correlation distance of the first wide peak was 69.77 Å and for the second small and wide peak it was 34.88 Å. The ratio between the first and second correlation distance is 2 : 1, characteristic of lamellar liquid crystals.[28] These results suggest that there is a lamellar phase mixed with the microemulsion droplets. Given that the

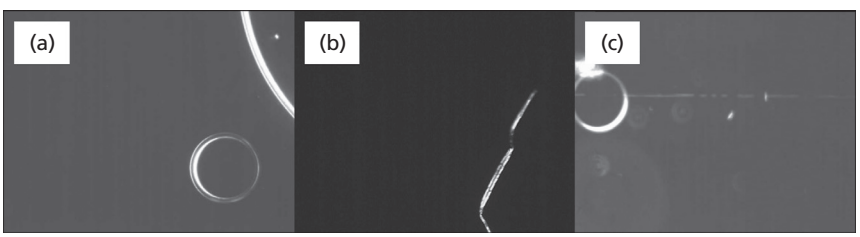

Figure 1 Photomicrographs of samples 1 (a), 2 (b) and 3 (c) obtained by polarized light microscopy. Isotropic field. Magnification 20 000×

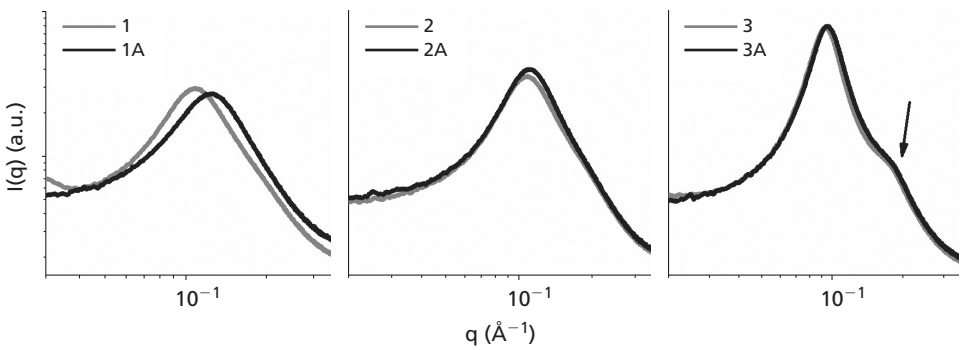

Figure 2 Small-angle X-ray scattering curves of zidovudine-unloaded and zidovudine-loaded formulations. Data were collected at 25°C

two peaks observed in the SAXS patterns for formulations 3 and 3A are very wide and there is an absence of Malta crosses in the PLM, there is probably only a small amount of lamellar phase coexisting with this microemulsified system. It is possible that a phase transition may take place in samples 3 and 3A, since they contain higher water contents and smaller amounts of oleic acid than formulations 1 and 2 (see Table 1). Thus, the increase in the water content may induce the microemulsion to undergo a liquid crystal transition. In light of this observation, it was expected that these microemulsions transit to a liquid crystal phase when brought into contact with the mucus layer.

The oscillatory rheological data of zidovudine-loaded microemulsions was represented as storage modulus (G′) and loss modulus (G″) at a selected oscillatory frequency of 1.5 Hz in Table 1. The storage modulus is a measure of energy stored and recovered per deformation cycle and reflects the solid-like component of a viscoelastic material. The storage modulus is large if a sample is predominantly elastic or highly structured. The loss modulus is a measure of the energy dissipated per cycle and reflects the liquid-like component.[22] In general the rheological behaviour of microemulsions is typically of low viscosity and Newtonian flow.[29] The frequency sweep analyses demonstrated that microemulsions 1A, 2A and 3A are more viscous than elastic in the selected frequency, because $G'' > G'$ and both moduli are frequency dependent. This property is desirable in nasal formulations, since the systems must have adequate liquid-like behaviour to achieve the nasal cavity.

Mucoadhesive properties

The development of new mucoadhesive systems has expanded in the last few decades, but no standard test method has been specifically designed for mucoadhesion analysis.[11] Almost all tests reported in the literature are conducted *in vitro*, the commonest techniques being rheological assessment of mucoadhesion and measuring the force of attachment.[11]

Since the phase transition is a very important property for the successful mucoadhesion of the systems proposed in this work, the phase behaviour was also studied to gain a better understanding of the mucoadhesive mechanism of these systems. A partial phase diagram was constructed by addition of simulated mucus to the microemulsions, progressively increasing the mucus content by 30, 50 and 100% (w/w) in the samples. This dilution procedure simulates, as closely as possible, the in-vivo situation that develops after nasal administration of the microemulsions. The resulting mixtures are indicated in the phase diagram of Figure 3, which shows the modified percentages of the original components.

Figure 4 shows the polarized light photomicrographs for microemulsions in contact with different amounts of simulated mucus. The Malta crosses exhibited in Figure 4 in all the samples suggest the formation of a lamellar liquid crystal structure, irrespective of the mucus content.

Figure 5 shows the evolution of the SAXS patterns of the microemulsion systems with successive increases of the simulated mucus ratio. From the scattering patterns in Figure 5, the values of the correlation distances $d = 2\pi/q_{max}$, where q_{max} is the value of the scattering vector q at the maximum

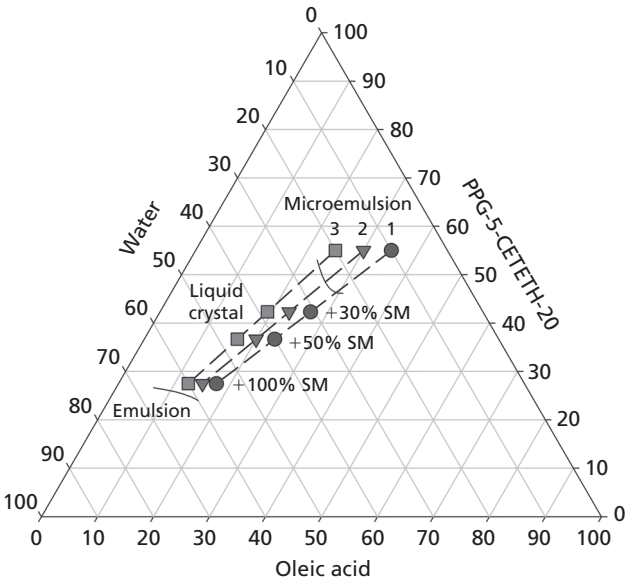

Figure 3 Phase diagram of PPG-5-CETETH-20–oleic acid–water

intensity I (q_{max}), were calculated and are given in Table 1. The lamellar or hexagonal structures of the lyotropic liquid crystal phases were ascertained from the relative positions of the SAXS diffraction peaks on the scattering vector (q) axis. Diffraction peaks with relative q_{max} positions (with respect to the first and most intense peak) of 1 : 2 : 3 are well known to be characteristic of lamellar periodicity,[30] indicating that after addition of simulated mucus the microemulsions (1A30, 1A50, 1A100, 2A30, 2A50, 2A100, 3A30, 3A50 and 3A100) are lamellar liquid crystals, as suggested by the observation of Malta crosses in the PLM analysis.

Summarizing, the phase behaviour studies indicated that the microemulsions are transformed into a lamellar phase on addition of simulated mucus, which is attributed to an increase in the packing constraint in the hydrophilic core, and consequently, a reduction in interfacial curvature of the aggregate. The correlation distances (d) in Table 1 gradually increase as the weight fraction of simulated mucus in the system rises. This can be attributed to greater hydration of the PEO chains, which increases the repulsion between the head groups, increasing the distances between lamellae. The phase behaviour study was of great value in foreseeing the possible phase transitions that formulations would undergo in the nasal cavity. The phase identification may offer further support for the understanding of the mucoadhesive properties of these systems.

Mucoadhesion can produce changes in the rheological properties of the interfacial region of the mixture of the formulation and the mucus layer. This has led several authors[14,22,30–33] to suggest that rheological measurements can be used as an in-vitro parameter to determine the mucoadhesive properties of a material. The measurement of the capacity of the systems to increase their viscosity when they interact with mucus is commonly reported in the literature as the rheological synergism or interaction parameter.[32] The rheological profile of the systems can provide

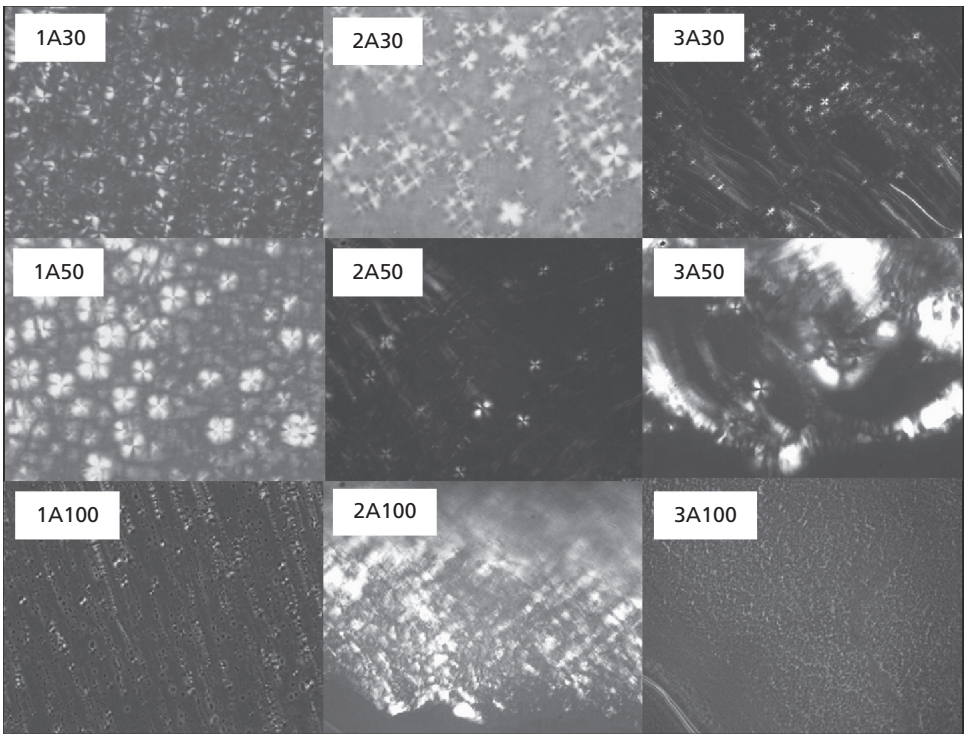

Figure 4 Polarized light microscopy patterns of the microemulsions after dilution with 30, 50 and 100 wt % of simulated mucus at 32°C. Magnification 20 000×

an acceptable in-vitro model of the in-vivo behaviour of the mucoadhesive system.[10,11] This is based on the idea that when a mucoadhesive system is mixed with simulated mucus, there should be a synergistic increase in viscosity, in the sense that the rheological response of the formulation–simulated mucus mixture should be greater than the sum of the contributions from the components.[32,34]

The rheological mucoadhesive analyses were performed by the oscillatory technique, which is a non-destructive test that measures both the viscous and elastic behaviour of a sample simultaneously and can characterize the network structure of the systems in contact with the mucus layer. The effect of increasing the simulated mucus concentration on the viscoelastic properties of the microemulsions can be derived from Figure 6, in which the storage modulus (G') of the lamellar phases is plotted against frequency, and in Table 1, where the values of G' and G'' are shown at 1.5 Hz.

After addition of 30% simulated mucus to the microemulsions, the log–log plot of G' versus frequency in Figure 6 showed a considerable dependence of G' on frequency. The slopes of these curves for samples 1A30, 2A30 and 3A30 are higher than those of the curves for samples with 50 and 100% simulated mucus and indicate the low elasticity of formulations with 30% simulated mucus, which correlates with values of G'' being higher than G' values for samples 1A30 and 2A30 (Table 1). The addition of 50% and 100% simulated mucus decreased the slopes of the curves of storage modulus against oscillation frequency, showing that G' was relatively independent of frequency and confirming an increase in the elasticity of the mixtures with higher amounts of simulated mucus, as can be seen from Table 1, where values of $G' > G''$. For samples 1A50, 1A100, 2A50, 2A100 and 3A50, an almost constant and high value of G' was observed over the whole frequency range (Figure 6), showing the highly structured character of the systems after mixing with simulated mucus. In the 3A100 mixture, the elasticity was lower than in 3A50 and this is corroborated by the absence of Malta crosses in the corresponding PLM image (Figure 4), which suggests a phase transition to a simple emulsified system. The loss of liquid crystal matrix structure is a necessary event, since it is of great importance that the mucociliary clearance is not impaired, to avoid lower respiratory tract infections.[35] Thus, the formulation should stay in position for a period of time no longer than 20 min, which is the expected half-life for mucociliary clearance.[35]

Hagerstrom and Edsman[36] recommend that the rheological method should not be used on its own for the study of mucoadhesion. One of the limitations of this method is that a positive response is only seen for viscous systems.[34] The rheological method does not give direct information about interface phenomena, because the two phases – simulated mucus and formulation – are mixed before the experiment.[37] With a view to comparing the results from the rheological approach with direct measurement of the adhesive strength at the point of failure of the mucoadhesive joint,[38] a mucoadhesive strength method was used in this study.

The mucoadhesive strength of the formulations was evaluated by measuring the force required to detach the formulation from a mucin disc, in a TA-XTplus Texture

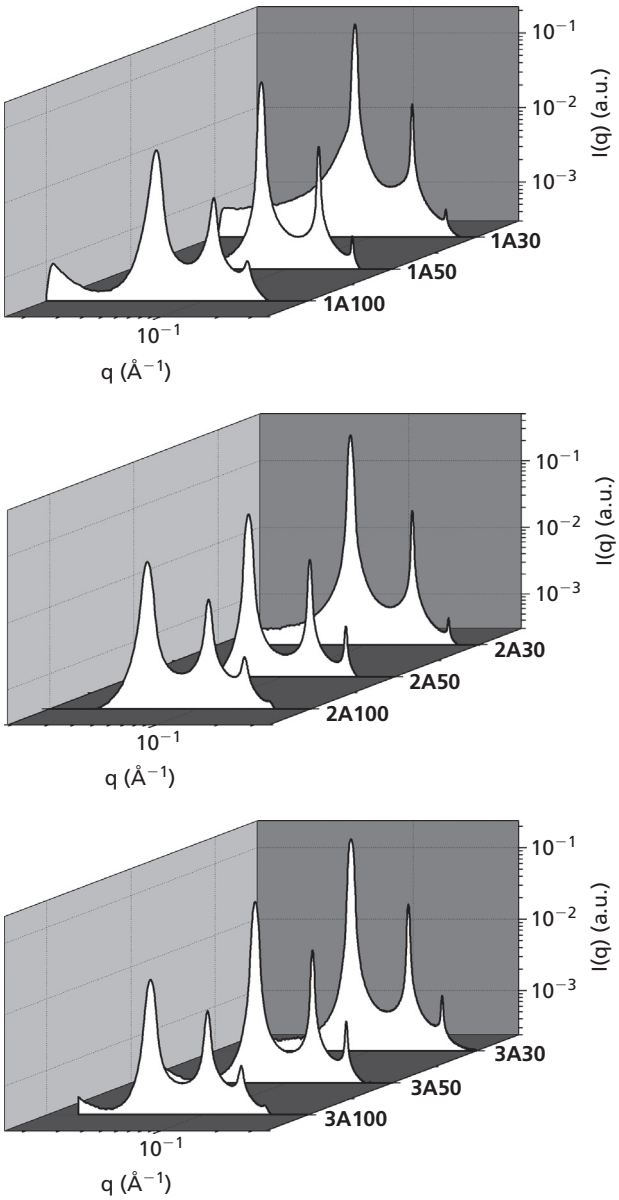

Figure 5 Development of the small-angle X-ray scattering patterns of the samples derived from mixtures of formulations and successive additions of simulated mucus. Data were collected at 32°C

Analyzer.[14] This commercially available apparatus has been proved to be suitable for bioadhesion measurements.[18] In the mucoadhesive strength test, the adhesive bond between surfaces is found by measuring the force required to separate the two surfaces from one another.[11] Table 1 shows the mucoadhesive strength of microemulsions 1A, 2A and 3A and the same microemulsions with 50% simulated mucus added (1A50, 2A50 and 3A50), which results in lamellar phases. The values of adhesive force obtained are between 0.306 N and 0.247 N and these values do not have significant difference when compared by one-way analysis of variance and Tukey's test. The statistical analysis showed that microemulsions 1A, 2A and 3A and lamellar phases 1A50, 2A50 and 3A50 have similar mucoadhesive forces. It was expected that the lamellar phases, due to their more viscous structure, would increase the adhesive force, but the contact of the microemulsions with 50% simulated mucus did not change the adhesive properties of the systems. So, for systems composed of PPG-5-CETETH-20–oleic acid–water, the results suggest that the adhesive force is not influenced by mesophase type. Even so, the adhesive forces presented by lamellar phases (0.304–0.262 N) are similar to well-known mucoadhesive polymers. It is known that carbopol exhibits great mucoadhesive properties.[14,39] Bruschi and collaborators[14] performed a study to evaluate the mucoadhesive force of systems composed of carbopol and poloxamer. In a similar test using a texture analyser, the adhesive force obtained was 0.256 ± 0.026 N. So, the sum of the mucoadhesive force presented by PPG-5-CETETH-20–oleic acid–water systems and the rheological behaviour of lamellar phases can potentially improve the retention time of formulations on nasal mucosa.

Discussion

The aim of this study was to develop a zidovudine mucoadhesive delivery system composed of PPG-5-CETETH-20–oleic acid–water which was able to form liquid crystal phases when in contact with the mucus layer in the nasal cavity, to avoid problems related to mucociliary clearance.

The structural studies characterized these systems as microemulsions, which have generated considerable interest as potential drug delivery systems. Advantages associated with microemulsions include their thermodynamic stability, optical clarity and ease of preparation.[24] Furthermore, it is also possible to increase their viscosity through composition changes, since surfactants in the presence of water and oil can form a wide variety of self-association structures.[24] The phase behaviour studies showed that the microemulsions developed in this work were capable of forming the lamellar liquid crystal phase when in contact with simulated mucus, at nasal temperature. It is important to remember that it is desirable for the lamellar phase to form inside the nasal cavity, because the relatively high viscosity of the lamellar phase may make nasal administration difficult, but this could be circumvented by using less viscous liquid crystal precursor formulations, such as microemulsions. These desirable rheological properties were confirmed by oscillatory measurements. When the PPG-5-CETETH-20–oleic acid–water systems incorporated the simulated mucus, the system passed through the semi-fluid micellar phase to the lamellar phase. As all the collected data showed, the formation of the liquid crystal phase depended on the aqueous phase content and the addition of zidovudine did not alter the phase behaviour.

There are several theories proposed to elucidate the mechanisms of interaction between the mucin or mucus layer and the formulations.[9] Mucoadhesive systems can adhere to mucus by means of secondary surface forces, such as hydrogen bonds, or polymer chains of the adhesive may penetrate into the mucus substrate by diffusion, to create a greater area of contact.[40] In the case of PPG-5-CETETH-20 systems, this surfactant has a high density of available

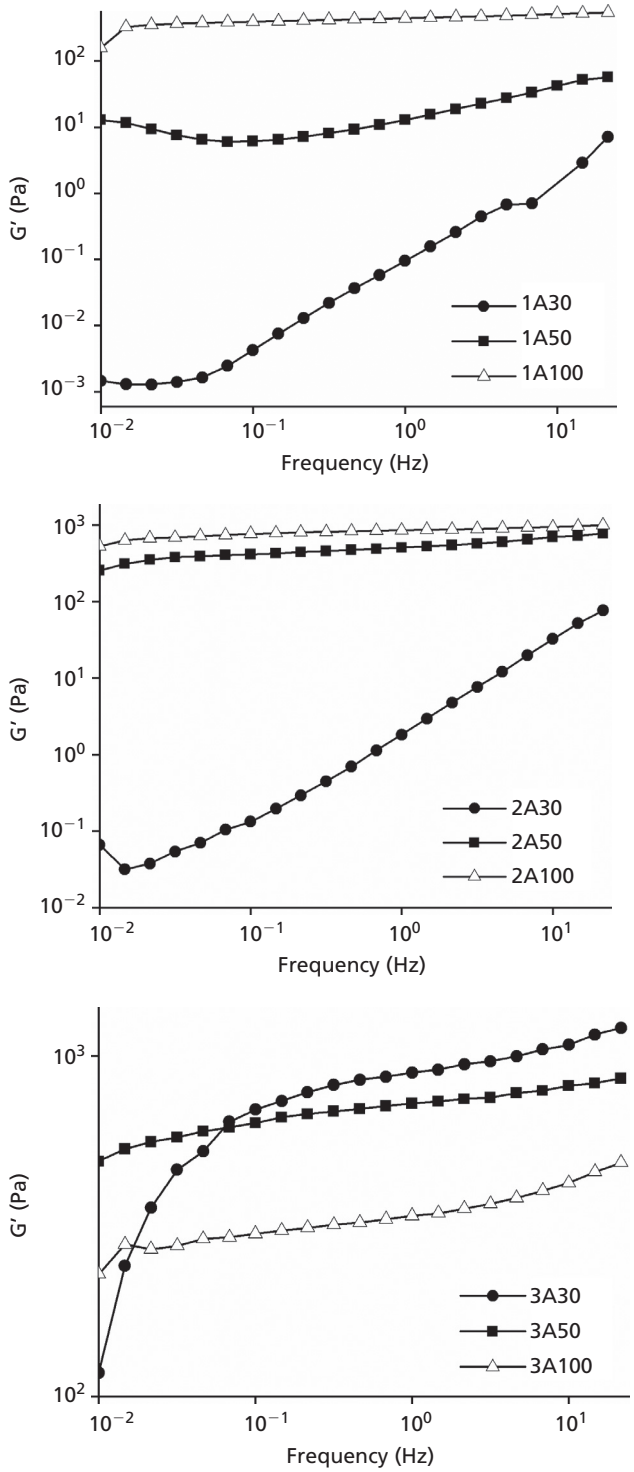

Figure 6 Frequency sweep profile of storage modulus of samples mixed with simulated mucus at 32°C

hydrogen bonding groups that are able to interact strongly with the large number of side-groups in mucus. Moreover, it has been demonstrated that PPG-5-CETETH-20–oleic acid–water systems undergo a phase change from a liquid (microemulsion) to a semisolid (lamellar) phase, and the accompanying viscosity change results in increased drug residence time and possibly enhances drug absorption via the nasal mucosa.[41] These results suggest that the phase transition of the microemulsions to the lamellar phase caused by simulated mucus contact may increase the stiffness and viscosity of the systems. Park and collaborators[13] demonstrated that the nasal absorption of plasmid DNA can be effectively and safely enhanced by using an in-situ gelling system. Similarly, the higher elasticity of lamellar phases can be considered a potential factor in relation to bioavailability. For nasal administration, increasing the viscosity of a formulation was observed to decrease the mucociliary clearance and prolong the residence time of the formulation and absorption time of the drug in the nasal cavity.[42]

It is important to quantify mucoadhesion by complementary techniques. For an in-situ gelling formulation capable of forming such a strong liquid crystal matrix as PPG-5-CETETH-20–oleic acid–water systems, it is very likely that the rheological performance of the formulation is as important for achieving a long contact time as the possible interactions with the mucin or mucus layer at the system–mucus interface. Hence, through the combination of the formation of the liquid crystalline matrix with the mucoadhesive force shown by texture analyser measurements, PPG-5-CETETH-20–oleic acid–water systems can be designed for prolonged retention of the formulations in the nasal cavity. Although rheological and tensile strength tests are very important in the research of new mucoadhesive systems, the mucociliary clearance is a natural defence mechanism of the lower respiratory tract against infection and other noxious substances, and an excessive mucoadhesive force would damage this mechanism.[43] Therefore it is essential to augment this research with nasal toxicity studies.

So, the next step of this study will be to evaluate the drug release and permeation on the nasal mucosa after application, analyse the mucosal irritability of the systems and proceed with an adequate pharmacokinetic and drug stability study. These consecutive studies are important to evaluate whether the concentration of zidovudine released before the mucociliary clearance removal of the formulation from the nasal mucosa is enough to obtain the pharmacological effect. However, results presented in this work indicate that these novel drug delivery systems clearly present an opportunity for formulation scientists to overcome the many challenges associated with antiretroviral drug therapy.

Conclusions

The main aim of this study was to develop a zidovudine delivery system, based on the polymeric PPG-5-CETETH-20 surfactant, capable of forming a liquid crystal phase when in contact with the mucus layer in the nasal cavity, so as to avoid its rapid elimination by mucociliary clearance. The phase behaviour study identified the PPG-5-CETETH-20–oleic acid–water formulations as microemulsions, which were able to form a lamellar phase when brought into contact with artificial mucus. A rheological analysis of mucoadhesion proved that these microemulsions had low storage moduli, permitting easy nasal administration, while the phase transition to a liquid crystal matrix provided a high storage

modulus for the systems, indicating that the phase transition was associated with an increase in the viscosity of the formulations. The mucoadhesive strength test demonstrated that all the systems had an adhesive force similar to recognized mucoadhesive polymers, irrespective of their phase behaviour. In conclusion, this study demonstrates the potential use of PPG-5-CETETH-20 surfactant to produce viscous liquid crystalline mucoadhesive matrices that are formed in contact with mucus in the nasal environment, allowing a longer contact time between the zidovudine and the absorbing epithelial membrane, which appears to be the important factor for good zidovudine nasal bioavailability.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

The authors thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPQ (Conselho Nacional de Desenvolvimento Científico e Tecnológico) of Brazil for financial support.

Acknowledgements

The authors thank the National Laboratory of Synchrotron Light (LNLS), Campinas-SP, Brazil, for the SAXS measurements.

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