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CHARACTERIZATION OF MUCOADHESIVE PROPERTIES OF pH RESPONSIVE MESOPHASES BASED ON MONOOLEIN AND OLEIC ACID

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ABSTRACT
Mucoadhesive systems remain in close contact with the absorption tissue, the mucous membrane, releasing the drug at the action site leading to a bioavailability expand and improvement in both local and systemic effects. A group of fatty acid esters capable of forming liquid crystals has been identified as a new class of potential bioadhesive substances. Hexagonal and cubic mesophases are exceptionally of high curiosity in the mucoadhesive drug delivery area because of their excellent prospective as drug carrier. The experimental work was focused on the pH responsive monoolein (MO) and oleic acid (OA) mixture mesophases. The pH responsive mucoadhesive properties of MO and OA have been proven through a flushing in vitro experiment approach and a tensiometric approach using a TA.XT Plus texture analyzer. It has been proven that a combination of monoolein (MO) and oleic acid (OA) ready of self-assembling right into a cubic phase at pH 7.4 and switching into hexagonal phase at pH 1.2. The test methods showed evidently that MO/OA mixture mesophases possess pH responsive mucoadhesive properties. Furthermore we had shown the fact that cubic phase has brilliant mucoadhesive properties as compared to hexagonal phase and for that reason an exciting candidate for a bioadhesive drug delivery system.

KEYWORDS: Mucoadhesive, Mesophase, Monoolein, Oleic acid, Liquid Crystal.

INTRODUCTION
The outcomes of a drug can now be strengthened as a result of the event of latest release techniques. Controlled release consists of systems that build the active agents accessible for a target, supplying an enough release rate and time to provide the preferred effect. The primary controlled medication systems currently accessible cover matrices, pellets, floating techniques, liposomes, microemulsions, liquid crystals, solid dispersions, nano suspensions, transdermal systems, cyclodextrin inclusion complexes, osmotic pumps and bioadhesive methods.[1]

Bioadhesive systems utilized to mucous membrane are as a rule defined as mucoadhesive, however the terms are interchangeable. Bioadhesion is the capacity of a material (synthetic or biological) to hold fast to a biological tissue for an elevated period of time. When activated to mucosal epithelia, a bioadhesive polymer may just adhere notably to the mucous secretion layer by a phenomenon referred to as mucoadhesion.[2] It's possible to design a bioadhesive approach in exclusive dosage varieties, when you consider that the residences of adhesion largely depend upon the features of the material utilized in its preparation. Hence, a few traditional drug delivery techniques already in use can end up bioadhesive after redesign by including bioadhesive supplies of their components.[3,4]

Different highlights related with the advancement of controlled drug delivery systems making use of bioadhesive molecules incorporate a scale back in drug administration frequency and a rise in patient compliance to the medical care. Consequently, a bioadhesive system dominant controlled drug release system might improve the treatment of diseases, serving to keep up an efficient concentration of the drug at the activity site.[5,6]

The abilities use for mucoadhesive systems as biologic carriers lies in its prolongation of the duration at the assimilation site, permitting escalated contact with the epithelial boundary. On the opposite hand, attachment of preparations onto mucous film can be impeded by the mucociliary clearance procedure. This clearance, a characteristic resistance component of the body against the deposition of polluting influences onto the mucous film, can likewise take away the preparation. Along these lines, by way of exploiting bioadhesive molecules, it is conceivable to hold the preparation at the activity site and to guide the medication to a particular site or tissue.[7,8]

Hexagonal and cubic mesophases/liquid crystal phases are particularly of high interest in the mucoadhesive drug delivery field due to their exceptional potential as drug vehicles.[9] They are highly investigated for their ability to control or sustain the release of both hydrophilic and hydrophobic drug molecules having wide range molecular weights. Drugs can be incorporated in these gel-like phases, additionally they have non-toxic, biodegradable and bio adhesive characteristics which incorporate significant value addition in drug delivery function. These characteristics of hexagonal and cubic phases made them one of the favorable means for the researchers to deliver drug through different routes of administration e.g. buccal, gastrointestinal, intravenous, lung, nasal, oral, rectal and vaginal.[10,11,12]

A few methodologies have been used to assess *in vitro* interaction between mucin and mucoadhesive polymers and for measuring the mucoadhesive capability of candidate materials.[13] One such method is to determine the adhesive strength between the polymer and the hooked up substrate. It will be decided via measuring the force required to detach one entity from the other by way of the appliance of an external force in the form of a shearing, tensile or peeling force. Some of these tactics have been accounted for.[14,15]

Another system for mucoadhesion testing is the utilization of the TA.XT plus texture analyzer and porcine stomach and intestine. The utilization of the strategy gives a further developed and exact technique for assessing the mucoadhesive properties of substances. The impact of various instrumental parameters on candidate mucoadhesive polymers has been studied and the outcome demonstrate that variables equivalent to contact drive, contact time and the pace of removal of the probe from the mucosal tissue layer can have an impact on the mucoadhesive performance of a process.[15,16]

The aim of the present study was to characterize the mucoadhesive properties of Monoolein (MO)/Oleic acid (OA) mixture mesophases. A flushing *in vitro* test system and a tensiometric method using a TA.XT Plus texture analyzer were used.

MATERIALS AND METHODS

Apparatus

A TA.XT plus Texture Analyzer ((Stable Micro Systems, Surrey, UK)) equipped with a 5 kg load cell interfaced with an Dell PC compatible computer running XT-RA dimension software was used in the tensiometric mucoadhesion experiments.

The liquid crystalline phases were determined in polarized light microscope (Leica DM750 with camera DFC295, Leica Microsystems) interfaced with a Dell PC compatible computer running Leica Application Suite Version 4.3.0 software.

Materials

Monoolein (MO) and Oleic acid (OA) were purchased from TCI Co. Ltd., Japan. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer was purchased from HiMedia Lab. Pvt. Ltd., India. Buffer substances and all other chemicals were of analytical or reagent grade.

Preparation of MO/OA mesophases

Lipid dispersions were prepared by adding appropriate amounts of various pH buffers to the MO and OA blend. The dispersion was vortex blended for 5 minutes at room temperature; the vortex blending was rehashed a few times to accomplish a homogenous state.[17] The blend was equilibrated at room temperature for 48 hours to acquire mesophases. For pH 1.2 HCl buffer and for pH 6.8≤pH≤pH7.4 HEPES buffer were used, these buffers contained 0.05 M NaCl. Milli-Q-grade water was utilized for the preparation of mesophases.

Table 1: Composition of different mesophase formulations.

Batches	Monoolein % w/w	Oleic Acid % w/w
P1	90.00	10.00
P2	80.00	20.00
P3	70.00	30.00
P4	60.00	40.00
P5	50.00	50.00
P6	40.00	60.00
P7	30.00	70.00

Determination of the liquid crystalline phase formed

The liquid crystalline phases had been analyzed utilizing polarized light microscope. (Leica DM750 with camera DFC295, Leica Microsystems). A drop of every mesophase was once placed on a glass slide, blanketed with a cover slip, after which examined beneath polarized light. The microscope was used to investigate a few fields of each and every sample at 25°C. Photomicrographs have been obtained at a magnification of 200x. The determined patterns had been when compared with the average textures of liquid crystals fashioned by way of different surfactants.[18,19]

Flushing *in vitro* method to assess mucoadhesion

The bioadhesive test system described in the following is a modification of the method described by Rango Rao and Buri, 1989.[14] The stomach, small intestine and large intestine from healthy freshly slaughtered pigs (either sex, 60–80 kg) were purchased from the slaughterhouse in Dibrugarh, Assam, India. The stomach and intestines had been kept on ice except washed with 0.9% w/w sodium chloride solution, which was once achieved within 2 h. The lumens were gently rinsed with the saline until the intestines had been cleaned. The intestines were reduce into strips of three–four cm, immediately frozen (-20°C) and saved for as much as 2 months before use. Earlier than testing, the segments have been gently thawed out. The intestine segment used to be opened

along the mesenteric border and the serosa and muscularis layers were removed via stripping with a pair of tweezers, taking care to preserve the integrity of the mucus layer. The initially folded mucosal surface was hence flattened. Earlier than use, the tissue used to be equilibrated in the test medium for about 10 min, an interval lengthy enough for the tissue to accomplish the investigation temperature and pH equilibrium, which used to be measured by means of pH paper.

The gut strip, reduce longitudinally, was positioned on a glass tube (2 cm in distance across cut length routes at its middle) with the mucosa layer confronting upwards, spread out and held in position by tying both favor string on the posterior of the tube. The aid with the tissue used to be tilted at an altitude of -25° in a cylindrical cell thermo statically controlled at 37°C. A schematic delineation of the cell is given in Fig. 1.

In order to equilibrate the tissue with the buffer and to rinse off loose mucus and impurities, the tissue used to be flushed with washing solution, most often buffer solution at 37°C for 5 minutes, at a flow rate of 10 ml min-1, utilizing a peristaltic pump. An adequately weighed quantity of the sample to be tested for mucoadhesiveness about 200 mg used to be spread evenly over the mucosal segment with the aid in a horizontal position. The segments were left for 10 min within the cell to have interaction with the glycoproteins in the tissue. After that the support was tilted at a perspective of -25° and the segments have been evenly flushed with the washing answer at a constant waft rate of 15 ml min^{-1} for 60 min. The tip of the tube carrying the buffer solution was once placed 3–4 mm above the tissue to make certain a good flow of liquid over the mucosa. The amount of sample final on the tissue was calculated.

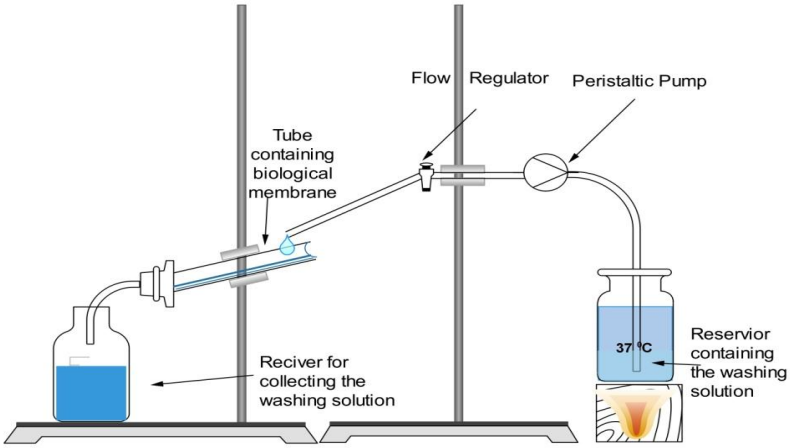


Figure 1. Schematic delineation of an *in vitro* model for testing bioadhesion.

Tensiometric method

A tensiometer, TA.XT plus texture analyzer, was used to measure the force of adhesive bonding of the MO/OA blend when introduced into contact with porcine intestinal mucosa and stomach (the principle of the experiment equipment is shown in Tobyn *et al*., 1995).[16] To experiment adhesion, a section of gauze was positioned instantly on the tissue holder and the tissue (3cm×3cm) used to be put onto the gauze with the mucous layer upwards. This precaution used to be taken to stabilize the contact force. To moisten the tissue, about 1ml of buffer was brought to the tissue.

The cubic and hexagonal phases of MO/OA have been smeared onto the probe in a thin, tender layer (200 mg). The instrument probe with sample was brought down onto the tissue at a test speed of 0.1 mm s^{-1}so as to bring the tissue and sample into contact under a constant drive. The experiments have been carried out at room temperature (25°C). The contact area was 1.27cm^2 (probe). The contact force used to be set at 5.0g and the contact time to 10 min. After 10 min the probe used to be withdrawn at a rate of 0.1 mm s^{-1} (post test speed) for 15 mm. Initial experiments confirmed that this was well past the place where the sample and mucus separated in the course of withdrawal.[20]

The peak detachment force and the area underneath the force/time curve used to be calculated routinely with XT-RA dimension application. The work of adhesion (kg.Sec), said to be the most accurate predictor of mucoadhesive execution was ascertained.[13]

RESULTS AND DISCUSSION

Effect of oleic acid (OA) and monoolein (MO) concentrations on the structure of MO/OA mesophases at different pH level

Different mesophases can be distinguished by the visual inspection of the characteristics texture through the crossed polarizer in polarized light microscopy (PLM). The results indicated that the selected formulations formed different liquid crystalline mesophases depending on the ratio of the MO and OA, since the amount of aqueous phase was the same for all formulations.

All the formulations (P1-P7) at pH 1.2 were classified as hexagonal liquid crystalline mesophase because it showed streaks on the photomicrographs (Fig. 2). At pH 6.8 P2 and P6 were in hexagonal phase but P1, P3, P4, P5 and P7 were isotropic formulation because it presented a dark field in the photomicrographs, and was therefore classified as a cubic liquid crystalline mesophase owing to its high viscosity. At pH 7.4 all the formulations (P1-P7) were classified as cubic liquid crystalline mesophase because it showed dark field in the photomicrographs.

The results of Fig. 2 clearly showed that increasing concentration of oleic acid (OA) in the MO/OA mixture at near neutral pH i.e. at pH 6.8 caused the transition of liquid crystalline phases from hexagonal to cubic phase. At neutral pH in a fixed salt concentration environment (0.05M NaCl), the OA has a negative charge and thus, in the OA/MO membranes, the large electrostatic repulsion between the head groups stabilizes the cubic phase. The carboxyl group of the OA has an intrinsic pK ~5. In the negatively charged membranes, pK values of phosphate groups and carboxyl groups at the membrane interfaces are larger than their intrinsic pK values.[23] As pH of the solution decreases, the protonation of the carboxyl group occurs, decreasing the surface charge density of the OA/MO membranes. The decrease in the surface charge density will decrease the electrostatic repulsive interaction in the membrane interface causing transition to hexagonal phase.

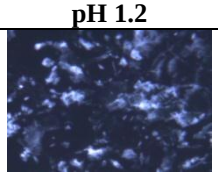
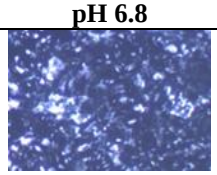
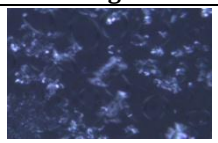
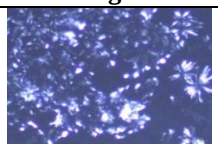
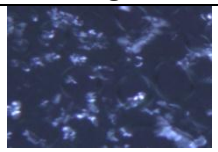
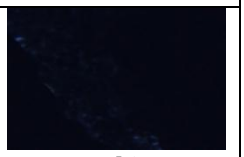
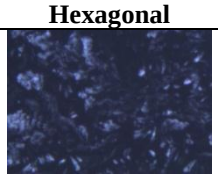
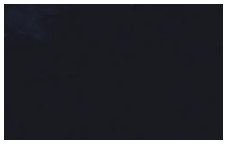
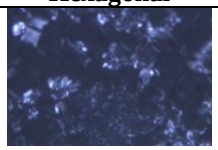
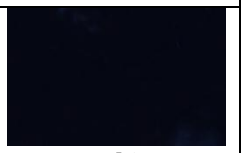
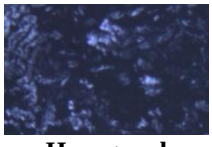
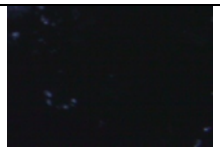
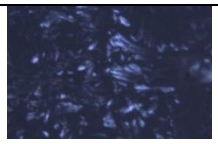
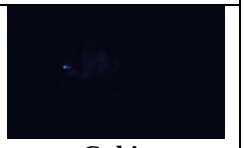
Batch	pH 1.2	pH 6.8	pH 7.4
P1	 Hexagonal	 Hexagonal	 Cubic
P2	 Hexagonal	 Hexagonal	 Cubic
P3	 Hexagonal	 Cubic	 Cubic
P4	 Hexagonal	 Cubic	 Cubic
P5	 Hexagonal	 Cubic	 Cubic
P6	 Hexagonal	 Cubic	 Cubic
P7	 Hexagonal	 Cubic	 Cubic

Figure 2. Polarized light microscopy of the batches from P1 to P7 at pH 1.2, pH 6.8 and pH 7.4.

Effect of oleic acid (OA) and monoolein (MO) concentrations on the *in vitro* mucoadhesion of MO/OA mesophases

The mucoadhesion of MO and OA mesophases were investigated having a modified flushing bioadhesion check system first reported by Rango Rao and Buri, 1989[14]; it is schematically illustrated in Fig. 1. The amount of substance remaining around the tissue was taken as a measure of mucoadhesion. The *in vitro* test system was used to examine the mucoadhesion of MO/OA on different kinds of mucosa. 0.1M HCl, pH 1.2; HEPES buffer solution, pH 6.8 and pH 7.4 was used as the washing medium for pig stomach, pig small intestine and pig large intestine respectively (Fig. 3). The specific period of time is important to allow the MO/OA to interact with the glycoproteins inside the mucosa. For least a few min was needed to attain maximum mucoadhesion. Table 2 offers example of the influence of numerous ratios of MO and OA in the bioadhesion of the mesophase batches. About 94% of the applied mesophase containing 80% w/w MO/ 20% w/w OA (P2) remained on the porcine large intestine at the end of the experiment. Moreover in case of the porcine stomach and small intestine, the formula with higher amount of Monoolein (MO) showed higher sum of % residual in comparison to formulation with higher share of Oleic acid (OA). The MO maintains its mucoadhesive properties when OA is undoubtedly added in low amount, but ruins them with higher proportions (Table 2). The polarized light microscopy study revealed that the presence of OA in the mesophases makes the system pH sensitive causing the transition from hexagonal phase at lower pH to cubic phase at a higher pH. As reported cubic phases are stickier than hexagonal phases having more contribution to the mucoadhesive properties which can be observed in the Table 2. At pH 1.2 all the batches were in the hexagonal phase despite of higher OA proportion and showed limited % residual after 60 minute, this may be due to the decrease the electrostatic repulsive interaction. At pH 6.8 batches from P3 to P7 were in cubic phases due to increase in electrostatic repulsive interaction OA at higher pH causing transition from hexagonal to cubic phase. The % residual of batches from P3 to P7 showed higher values compared to P1 and P2 at pH 6.8 despite of lower proportion of MO due the transition from hexagonal to cubic phase which adds to the mucoadhesivity. At pH 7.4 all batches were in cubic phase due to higher pH level sufficient for OA to cause transition of the batches from hexagonal to cubic phases. The % residual were increased in all the batches due to formation of cubic phases adding to the mucoadhesive properties of MO.

Table 2. *In vitro* mucoadhesion testing of MO/OA mesophases in porcine stomach at pH 1.2, small intestine pH 6.8 and large intestine pH 7.4.

Batch	% Residual amount ± SD (n=3)		
	Porcine stomach (pH 1.2)	Porcine small intestine (pH 6.8)	Porcine large intestine (pH 7.4)
P1	21.17 ± 1.26	6.5 ± 1	91.5 ± 3.5
P2	18 ± 1.5	16.67 ± 2.26	94.33 ± 1.26
P3	12.5 ± 1.80	55.67 ± 1.61	85.83 ± 3.06
P4	6.67 ± 1.53	47.67 ± 1.76	79.5 ± 1.5
P5	6.67 ± 1.26	40.5 ± 1.81	70.5 ± 1
P6	4.33 ± 0.77	61.17 ± 5.62	67.33 ± 2.08
P7	2.83 ± 0.77	35.83 ± 1.26	54 ± 6.56

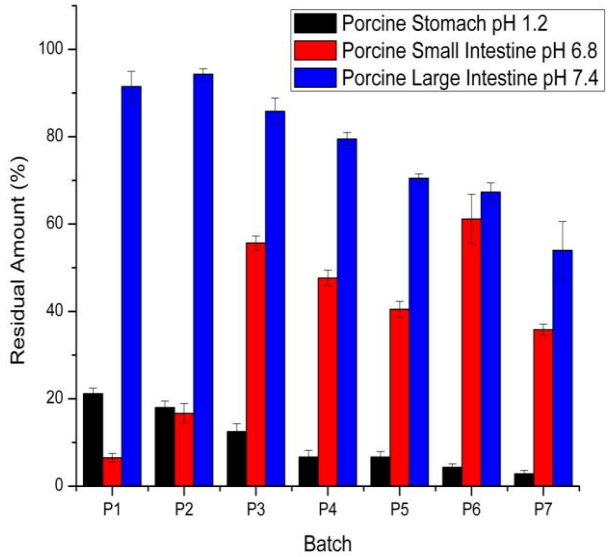


Figure 3: Influence of pH in the bioadhesion of the mesophase batches (P1 – P7).

Effect of oleic acid (OA) and monoolein (MO) concentrations on the tensiometric measurement of mucoadhesion of MO/OA mesophases

The commercially available equipment TA.XT plus texture analyzer continues to be approved to become well suited for mucoadhesion measurements lately. Table 3 and Table 4 revealed the adhesiveness (Peak force) and work of adhesion of all the batches at distinct test conditions respectively (Fig. 4, 5 and 6). The tensiometric analysis was in conformation of the values obtained in the *in vitro* mucoadhesion evaluation. At pH 1.2 batch P2 containing 80 percent w/w MO and 20 percent w/w OA showed more 0.1395 kg.sec work of adhesion planning major contribution of MO in mucoadhesion while failure of batch P1 (90% w/w MO and 10% w/w OA) having largest amount of MO could be explained by the contribution of OA inside the mesophase development In case of porcine small intestine at pH 6.8 work of adhesion of P1 and P2 were minimum as a result of remaining in hexagonal stage. As the cubic phases are very sticky when smeared onto skin

it is very challenging to remove, the work of adhesion parameter explained the same consequently (Table 4). In case of porcine large intestine at pH 7.4 batch P2 showed optimum 0. 9315 kg.sec work of adhesion, the values of other batches were also heightened due constituent effect of both cubic phase and mucoadhesivity of MO.

Any explanation of the results acquired with tensiometric test strategy is that the amount of mucoadhesion boost with the rise with quantity of MO in the mesophases.[21] The mucoadhesion of MO was as well influenced by presence OA in the program as noticed in the Table 3 and 4. The batches with 10 to 20% w/w OA were remained in the hexagonal phase by pH 6.8 demonstrating clearly the influence of OA inside the transition of mesophases via heaxagonal to cubic phase. The type of biological tissue and pH used also impacted the mucoadhesion of the unique batches.

Table 3. Mucoadhesiveness of MO/OA mesophases in porcine stomach at pH 1.2, small intestine pH 6.8 and large intestine pH 7.4.

Batch	Peak Force (kg) ± SD (n=3)		
	Porcine stomach (pH 1.2)	Porcine small intestine (pH 6.8)	Porcine large intestine (pH 7.4)
P1	0.054 ± 0.008	0.015 ± 0.005	0.36 ± 0.013
P2	0.031 ± 0.014	0.034 ± 0.007	0.207 ± 0.016
P3	0.031 ± 0.002	0.227 ± 0.010	0.206 ± 0.012
P4	0.019 ± 0.005	0.104 ± 0.018	0.127 ± 0.011
P5	0.017 ± 0.006	0.099 ± 0.011	0.113 ± 0.007
P6	0.016 ± 0.004	0.109 ± 0.025	0.107 ± 0.009
P7	0.013 ± 0.003	0.08 ± 0.006	0.086 ± 0.006

Table 4: Work of Adhesion of MO/OA mesophases in porcine stomach at pH 1.2, small intestine pH 6.8 and large intestine pH 7.4.

Batch	Work of Adhesion (kg.sec) ± SD (n=3)		
	Porcine stomach (pH 1.2)	Porcine small intestine (pH 6.8)	Porcine large intestine (pH 7.4)
P1	0.0189 ± 0.004	0.0087 ± 0.003	0.486 ± 0.009
P2	0.1395 ± 0.009	0.17 ± 0.005	0.9315 ± 0.056
P3	0.0325 ± 0.013	0.1816 ± 0.011	0.2163 ± 0.008
P4	0.0418 ± 0.017	0.2208 ± 0.007	0.2794 ± 0.015
P5	0.0377 ± 0.005	0.1767 ± 0.014	0.2508 ± 0.018
P6	0.1184 ± 0.017	0.1085 ± 0.013	0.7918 ± 0. 023
P7	0.0087 ± 0.003	0.0568 ± 0.010	0.0577 ± 0.007

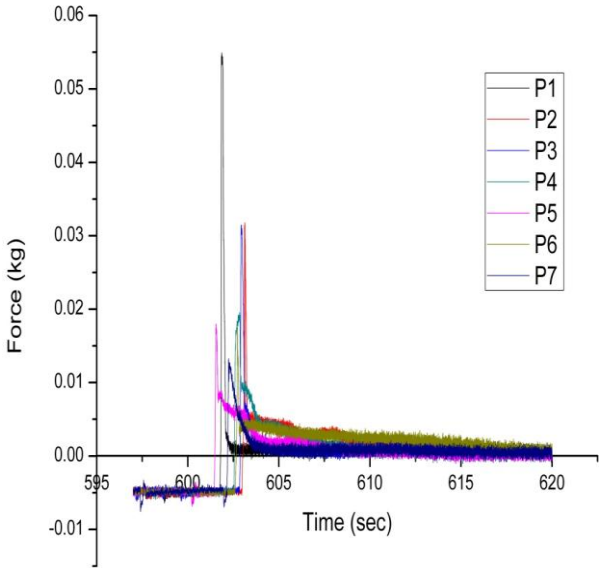


Figure 4. Adhesion force profile of force versus time for mesophases P1 to P7 in porcine stomach pH 1.2.

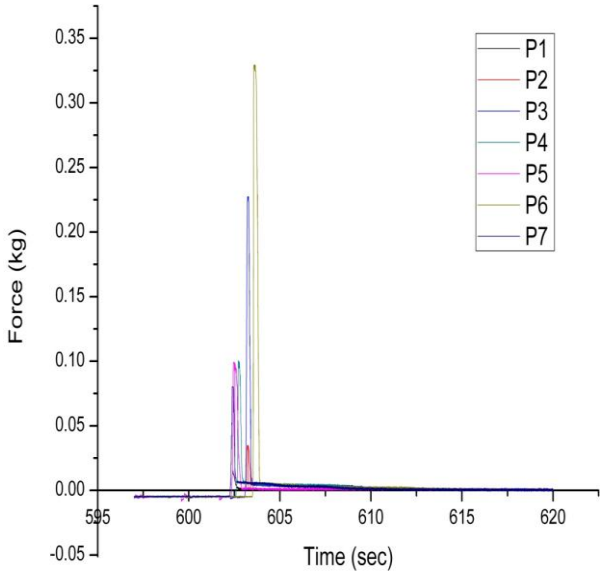


Figure 5. Adhesion force profile of force versus time for mesophases P1 to P7 in porcine small intestine pH 6.8.

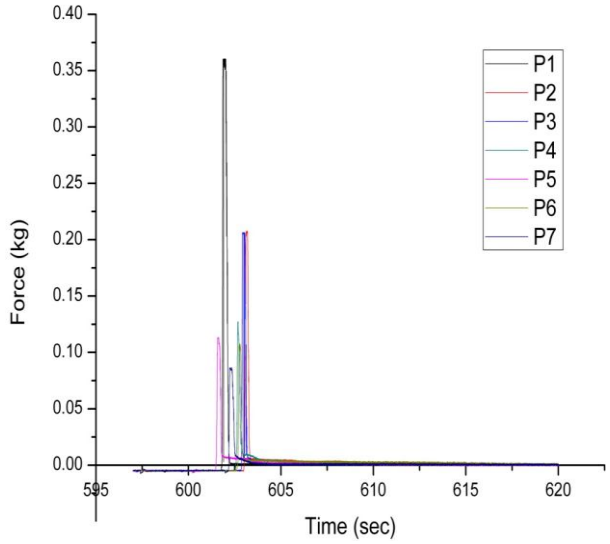


Figure 6. Adhesion force profile of force versus time for mesophases P1 to P7 in porcine large intestine pH 7.4.

CONCLUSION

A pathway has been presented by us to pH-responsive liquid crystalline system. The system includes a blend of monoolein (MO) and oleic acid (OA) capable of self-assembling into a cubic phase at pH 7.4 and switching into hexagonal phase at pH 1.2. An *in vitro* flushing test system and a tensiometric method have showed clearly that MO/OA mixture mesophases possess mucoadhesive properties and as demonstrated in the evaluations the batches were pH sensitive responding to pH level changes of GIT (stomach pH 1.2, small intestine pH 6.8 and large intestine pH 7.4). Because of the 3D organization of the water channels inside the cubic phase, it enables the controlled release of hydrophilic drugs through the water channels of the mesophases. Therefore, in contrast to hexagonal phase, release of hydrophilic drug from the cubic phase might be faster at pH 7.4 than the hexagonal phase at pH 1.2. Furthermore we had shown the fact that cubic phase has extremely good mucoadhesive properties and thus an interesting candidate for a bioadhesive drug delivery system. The cubic phase provides a sustained release system for drug molecules with different physicochemical properties.[9,12]

Yet formulation nonetheless presents a couple of challenges, for example control of the release of medication from the cubic phase is restrictive.[9] The fairly high viscosity of the cubic phase could make the administration of the platform to the anal, vaginal, nose or oral mucosa hard but that can be circumvented with a pH sensitive formulation which is less viscous at lower pH i.e. hexagonal phase but will transform to highly viscous system i.e. cubic phase once comes in contact higher pH. The other factors which might influence the approach could be the digestive tract motility, quick turnover of mucous and adherence of bioadhesive formulation to stomach content.

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