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(Cationic + nonionic) mixed surfactant aggregates for solubilisation of curcumin

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ABSTRACT

Curcumin is a potential drug for variety of diseases. Major limitations of curcumin are low water solubility, rapid hydrolytic degradation in alkaline medium and poor bioavailability. To overcome these limitations, highly potential mixed micellar system has been prepared. In order to reduce inter ionic repulsion and precipitation of surfactants, (cationic + non-ionic) mixed system have been chosen that directly influence its applicability. Hydrophobic chain of non-ionic surfactant significantly influences the cmc of mixed surfactant system as indicated by fluorescence and conductivity data. UV–visible spectroscopy analyses show that solubility, stability and antioxidant property of the curcumin is remarkably improved depending on cmc and aggregation number (N_{agg}) of mixed surfactants, where N_{agg} plays crucial role. Generally, curcumin undergoes complete degradation in slight basic medium, but stability has been maintained up to 8 h at pH-13 using formulated mixed micelles (only (20 to 25)% degraded). Location of curcumin which is monitored using emission spectroscopy, fluorescence quenching and ^{1}H NMR spectroscopy techniques play the most important role. Observed results show that the major population of curcumin is located at the polar region and some are in hydrophobic region of the mixed micelles. To ensure the effect of mixed surfactants and curcumin loaded mixed surfactants on DNA, the interaction parameter indicates non-interclative interactions.

1. Introduction

Bioavailability of hydrophobic drugs has always been a challenging task. Two main approaches are commonly used when inventing a new drug carrier. Firstly, the bulk material is dispersed in fine structures and secondly, molecules capable of self-assembly are used. Self assembly of surfactants is an interesting option due to its number of applications and easy formulations. Single surfactant systems have been extensively explored. However, binary systems are preferentially studied due to their high pliability.

Curcumin, a hydrophobic polyphenol with yellow colour, is one of the most important constituents extracted from dried rhizome of turmeric, a plant which grows in the Indian subcontinent and in tropical countries *i.e.* South East Asia [1]. It is the main ingredient in Asian food stuffs. In addition to its anti-oxidative and anti-inflammatory properties, it possesses pleiotropic pharmacological effects on neural, cardiovascular, pulmonary, metabolic, autoimmune, anticancer and neoplastic diseases [2–4]. However, the applications of curcumin have been impeded due to its enormously low water solubility and poor oral bioavailability [5]. To increase curcumin bioavailability, several carriers including hydrophobically modified starch [6], cyclodextrin [7], liposomal [8], polymeric nanoparticles [9] and bovine whole casein micelles [10] have been investigated. Mixed micellar systems are also effective carrier for hydrophobic drugs.

The present work focuses on the aggregation properties of the mixed surfactant system *i.e.* (cationic + non-ionic) having HLB (figure S1). Formulated mixed micelles have been used to enhance the solubility and stability of curcumin. Aggregation properties of micelle formulations have been characterised by specific conductivity measurements and fluorescence spectroscopy with anilinonaphthaline-8-sulfonic acid (ANS) as the probe. The stability of curcumin in basic medium has been evaluated up to eight hours. Location and mode of interactions of solublizate within micelles are evaluated using fluorescence quenching and ^{1}H NMR methods. To understand the biocompatibility of formulations, the interactive behaviour of ct-DNA in micellar media and curcumin loaded micelles has been explored through fluorescence spectroscopy.

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2. Experimental

2.1. Materials

See table 1.

2.2. Methods

2.2.1. Critical micelle concentration (cmc) determination

The cmc of mixed surfactants was obtained from conductivity data. Conductivity measurements were made with a Pico conductivity metre from Labindia. The glass cell was connected to the RE320 Ecoline thermostat controlled to better than ±0.01 K temperature variations. The cell constant of the cell used was 1.11 cm^{-1}. Conductivity was measured with absolute accuracy up to ±3%. Further, the cmc of mixed surfactants was also confirmed from fluorescence data measured using a fluorescence spectrometer (Hitachi-F7000) with quartz cells; ANS was used as a probe with excitation at 330 nm, scan rate 1200 nm · min^{-1} and emission collection range of (340 to 650) nm. Excitation and emission slit width was fixed at 5 nm.

2.2.2. Aggregation number determination

The aggregation number (N_{agg}) of the mixed surfactant systems was determined by fluorescence spectroscopy (Hitachi-F7000). Quenching experiments were performed using cetylpyridium chloride (cpc) as quencher within the concentration range of (70 to 700) μM and by keeping the probe (pyrene) concentration constant (1 μM). The excitation wavelength, scan rate and slit width were fixed at 380 nm, 1200 nm · min^{-1}, 10 nm, respectively. The emission data were collected over the (400 to 700) nm range.

2.2.3. Location of curcumin within micellar system

Location of the curcumin in mixed surfactant systems was evaluated by fluorescence spectra of curcumin and the fluorescence quenching technique, where excitation wavelength was fixed at 425 nm, with slit widths of 5 nm and collection range was between (430 and 700) nm. Two different quenchers were used, *viz.* a hydrophilic quencher (potassium iodide) and hydrophobic quencher (acrylamide). The quencher concentration was varied from (0 to 0.15) M. Further, to verify the results, ^{1}H NMR spectra of the mixed surfactants and curcumin loaded mixed surfactant systems were recorded using a Bruker avance II 400 MHz with D_2O as locking agent. Transmission electron microscopy (TEM) images were taken to confirm the location of curcumin by a Hitachi (H-7500) 120 kV.

2.2.4. Solubility, stability and scavenging activity of curcumin

The solubility, stability and scavenging activity of curcumin were analysed with UV–vis. spectra using a Thermo Scientific Evolution 160 UV–vis. spectrophotometer and quartz cells. Absorbance spectra were collected over the range (350 to 650) nm. All the experiments were performed in duplicate to determine the reproducibility.

2.2.5. Sample preparations

Firstly, mixed surfactant aggregates were prepared by sonication method at T = 298.15 K. Solubilisation tests for curcumin in equimolar combination of DDAB with changing non-ionic surfactants (Brij 96, Tyloxapol and Tween 80) were made. Batches of equimolar concentrations of mixed surfactant in the concentration range above their cmc values were prepared. Excess amount of curcumin was separately added to each vial to make sure that the maximum solubilisation occurred. The sample vials after sealing with screw caps were agitated on magnetic stirrer for a period of 24 h at 850 rpm and constant temperature (298.15 K). The vials were left for a period of (2 to 3) h to permit the unsolubilized curcumin to settle down and then they were decanted. The decanted samples were subjected to centrifugation for 15 min at 500 rpm to remove the undissolved solid curcumin. Samples were then filtered through 0.45 μm micro filter. The concentration of dissolved curcumin was measured spectrophotometrically (λ_{max} = 425 nm) followed by proper dilution of an aliquot of the supernatant with the respective concentrations of the surfactants. The equal surfactant concentration was maintained in both reference and measurement cells to eliminate the effect of surfactant on absorbance. For stability studies, surfactant solutions were prepared in 0.1 M phosphate buffer of pH-13 and Stock solution of curcumin (1 mM) was prepared in methanol (MeOH). Then a small volume of stock solution was added to the surfactant solutions to reach the final curcumin concentration of 20 μM. UV–vis. spectra were observed with respect to time. Scavenging radical experiments were carried out with preparation of DPPH stock solution in ethanol (1 mg · mL^{-1}). Further dilutions were made in surfactant solutions, where curcumin concentration was fixed at 20 μM.

2.2.6. Ct-DNA binding studies

Binding of ct-DNA to mixed surfactant systems (0 to 2) mM and curcumin loaded mixed surfactant systems (0 to 25) μM were studied using fluorescence quenching spectroscopy with ethidium bromide (EB) as a probe at constant concentration of EB = 4 μM and ct-DNA = 4 μM. The concentration of the mixed surfactant was fixed at 2 mM for the second experiment. Excitation

TABLE 1
Provenance and mass fraction purity of chemicals used in this study.

Chemical name	Source of supply	State	Mass fraction purity
Curcumin	Sigma Aldrich	Powder	0.99
Dodecylethyldimethylammonium bromide (DDAB)	Sigma Aldrich	Powder	0.98
Brij 96	Sigma Aldrich	Liquid	0.98
Tyloxapol	Sigma Aldrich	Liquid	0.98
Tween 80	Sigma Aldrich	Liquid	0.98
Sodium hydroxide (NaOH)	Sigma Aldrich	Pallets	0.98
Cetylpyridium chloride (CPC)	Sigma Aldrich	Powder	0.98
2,2-diphenyl-1-picrylhydrazyl (DPPH)	Sigma Aldrich	Powder	0.98
1-Anilinonaphthaline-8-sulfonic acid (ANS)	Sigma Aldrich	Powder	0.99
Acryl amide	Sigma Aldrich	Powder	0.99
ct-DNA	Sigma Aldrich	Fibres	0.98
Ethidium bromide (EB)	Sigma Aldrich	Powder	0.95
Deuterium oxide (D_2O)	Sigma Aldrich	Liquid	0.99
Potassium dihydrogen phosphate (KH_2PO_4)	Merck	Powder	0.99
Disodium hydrogen phosphate (Na_2HPO_4)	Merck	Powder	0.99
Potassium iodide	Sarabhai M. Chemicals, Limited	Crystalline solid	Ultrapure
Water[a]			

[a] Water used for the preparation of samples was de-ionised and double distilled (conductivity ⩽ 3 μS at T = 298.15 K).