Radiological: Pre-clinical Comparative study of microbial derived surfactants with survanta for treatment of Respiratory Distress Syndrome (RDS)

6.1. Introduction

Lungs are lined by a surface active material called the pulmonary surfactant which prevents alveolar collapse and maintains alveolar stability. Pulmonary surfactant (PS) is a complex mixture of lipids and proteins that coats the interior surface of the vertebrate lung as a film. It consists of about 90% lipids (mainly phospholipids) and 8–10% protein. The main component of native Lung surfactant (LS) is dipalmitoyl-phosphatidylcholine (DPPC) an amphiphilic protein that were recently shown to play an important role in the surface activity of LS (Gerber et al. 2005; Nguyen et al. 2013).

The main function of PS is to maintain normal respiratory mechanism by reducing the surface tension at the alveolar air-liquid interface of lungs to avoid alveolar collapse at the end of expiration. Lack of deficiency or inactivation of PS causes severe respiratory disorders that can be lethal such as breathing problems in newborn babies, pulmonary haemorrhage, pulmonary oedema, and Pneumonia, respiratory distress syndrome (RDS)- a most common causes of death in premature infants (Liggins et al. 1972; Rubenfeld et al. 2005).

However, in general probability for RDS is more prominent in premature infant because lungs are under developed, so the lung surfactant production is less. In Most cases of RDS babies were born before 37 weeks. If a premature baby is lacking surfactant,

artificial surfactant may be given for normal functioning (Merritt et al. 1986; Dunn et al. 1991; Kattwinkel et al. 1993).

Amount of surfactant present in fetal lungs can be estimated by measuring the amount of surfactants in amniotic fluid (Haymond et al. 2006). Treatment of neonates immediately after birth with exogenous surfactant delivered endotracheally can be effective if early delivery cannot be prevented (Kendig et al. 1991). In the medical research, imaging and diagnosis via a infra-red (IR) imaging has been increased due penetration in deep tissue imaging (Aswathy et al. 2010). Diagnosis of RDS noninvasively, primary test is chest radiography which is used as one of the most usual and accessible diagnostic tools in respiratory distress syndrome, which has shown promising results regarding the early diagnosis of respiratory distress (Armstrang et al. 2004). IR spectroscopy is one of a standard clinical analyses test of amniotic fluid assays to assess fetal lung maturity (Shaw et al. 2008).

In a clinical study and radiographic diagnosis matched upto 95% of cases (Kurl et al. 1997), In another study the specificity and sensitivity of the radiographic test was 82.5% and 35.8% respectively (Naghibi et al. 2014) and in the same study author concluded that diagnostic value of RDS based on radiologist opinion were about one-third of all cases and has low sensitivity. Although we have many options/methods to diagnose RDS but still there is not a single non invasive method which can be 100% reliable.

In 1990, the United States Food and Drug Administration (FDA) released colfosceril palmitate for use in preterm infants with RDS. Shortly thereafter, other types of surfactants became available: Exosurf® (synthetic surfactant) and Survanta® (modified

natural surfactant). Also domestically available are Curosurf® (natural surfactant) and Infrasurf® (natural surfactant). Animal derived surfactants are mostly used as drugs for the treatment of respiratory distress syndrome (RDS) in neonates. They also have several disadvantages such as they carry the potential risk of transmission of infectious agent as well as exposure to foreign proteins, proinflammatory mediators and variability in a native surfactant proteins from batch to batch (Logan et al. 2009). In this study we have taken survanta as standard for the comparative analysis because this is most common one, have highest DPPC composition and cost-effective.

The objective of present study was to do comparative study of microbial produced surfactant with FDA approved drug survanta by using Near IR and Far IR spectroscopy this is useful for preclinical test for RDS treatment. Lim et al. (Lim et al. 2003) identified two spectral diagnostic windows for deep tissue imaging in Near IR region of the spectrum (700–900 nm) and for Far IR spectrum 1,200– 1,600-nm, these two IR spectrum region used as "diagnostic window" of tissue imaging, where the absorption coefficients of body fluids are at their minimum (Shaw et al. 2008).

In this study we analysed the Near IR and Far IR spectrums of three surfactants (survanta, surfactin produced from *B. subtilis* MTCC2423 and lipopeptide (Biosurfactant) produced from an acclimatized strain produced from *C. tropicalis* MTCC230). Spectrums were obtained from different concentrations of surfactant were compared and analyzed. The results showed that there is a specific set of frequencies for all three surfactants and these set of frequencies can be used to detect the presence of surfactant. Survanta (FDA approved drug used for RDS treatment) was used as a standard for the comparative radiological study of surfactin produced from *B.subtilis* MTCC2423 and lipopeptide

(Biosurfactant) produced from an acclimatized strain produced from *C. tropicalis* MTCC230 that was characterized as surfactin in previous chapter. This study reveals that microbial surfactant may be considered for treatment of RDS in an infant.

6.2. Materials and Methods

We analysed the spectrum of Survanta (manufactured by: M/s AbbVie Inc. 1401, Sheridan Road, North Chicago, IL, 60064, USA) , Surfactin (*B. subtilis* MTCC 2423) and Lipopeptide (*C. tropicalis* MTCC230) were produced in Protein Engineering Laboratory, School of Biochemical Engineering, IIT (BHU), Varanasi (Ashish et al. 2011; Verma et al. 2015).

A spectroscopic measurement was performed by using Aventes Spectrometer system which consists of:

1. Light source- AvaLight-HAL Tungsten Halogen Light Source, The AvaLight-HAL is a compact stabilized halogen fan-cooled light source that can be used for the visible and the near infrared range. Its wavelength range is 360-2000 nm.

2. Detectors: AvaSpec-2048 Fiber Optic Spectrometer, it is a 2048 pixel CCD Detector Array. Usable wavelength range is 300-1100 nm. AvaSpec-NIR256-2.2TEC NIR Line Near-Infrared Fibre-optic Spectrometer, its wavelength range is 1000-2200 nm.

3. Cuvette sample holder

4. Fiber optic cables

5. Cuvette

6. Micropipette

7. Avasoft 8(software installed in a personal computer) was used to process data received from above detectors.

Sample preparation

Samples of different concentration of each surfactant (survanta, surfactin and lipopeptide) were prepared. Survanta was obtained from pharma market of varanasi, india (manufactured by: M/s AbbVie Inc. 1401, Sheridan Road, North Chicago, II, 60064, USA), Surfactin was produced from *B. subtilis* MTCC2423 and lipopeptide (Biosurfactant) was produced from an acclimatized strain of *C. tropicalis* MTCC230 (Ashish et al. 2011; Verma et al. 2015). There supernatants were used as a pure sample for Near IR and Far IR spectrum studies.

Comparative Study

Radiological Comparative studies of survanta, surfactin and lipopeptide (Biosurfactant) were performed by using Near IR spectrum of wavelength range is 300-1100 nm. Survanta act as a standard for the comparative study, further comparative studies were performed by using Far IR spectrum of wavelength range is 1000-2200 nm.

Concentration Variation

Radiological study of survanta, surfactin and lipopeptide (Biosurfactant) was performed at different concentration maintaining the final volume 2ml via a different dilution rate with distilled water.

Fermented broth of Supernatant containing surfactin and lipopeptide (Biosurfactant) used as pure sample then gradually increases the dilution with distilled water at dilution rate i.e., 50%, 62.5%, 75%, 87.5% respectively. Pure survanta is highly viscous so initial

dilution rate was started from 0.20ml survanta + 1.80ml distilled water, then gradually increases the dilution rate as same in case of surfactin and lipopeptide. All samples were measured by using VIS-NIR spectrum of wavelength range is 300-1100 nm and Far IR spectrum of wavelength range is 1000-2200 nm.

6.3. Results and Discussion

Comparative Study

Comparative Studies of surfactin and lipopeptide (Biosurfactant) with the survanta were performed by using Near IR spectrum of wavelength range is 300-1100 nm as shown in Figure 6.1, result shows that the absorbance peak frequencies for surfactin, lipopeptide (Biosurfactant) and survanta were 396nm and 967nm, So the absorbance peak for surfactin and lipopeptide (Biosurfactant) was fall in same wavelength as survanta shows, Absorbance units (AU) were different for each surfactant due to concentration difference. Similarly for Far IR spectrum, wavelength range 1200nm-2200nm used for comparative study of surfactin and lipopeptide (Biosurfactant) with the survanta, Figure 6.2 shows that the least absorbance frequency was obtained at 1249nm for all the three surfactants. These results reveal that microbial produced surfactin from *B. subtilis* MTCC2423 and lipopeptide (Biosurfactant) from an acclimatized stain of *C. tropicalis* MTCC230 showing the same Spectroscopic properties for Near IR and Far IR as shown by drug survanta. This result is very much helpful to pass preclinical trials for the development of drug used in RDS (respiratory distress syndrome). Surfactin and lipopeptide (Biosurfactant) may considered as drug for the treatment of respiratory distress syndrome (RDS) in premature infants, as it clears the primarily preclinical test i.e., radiological study.
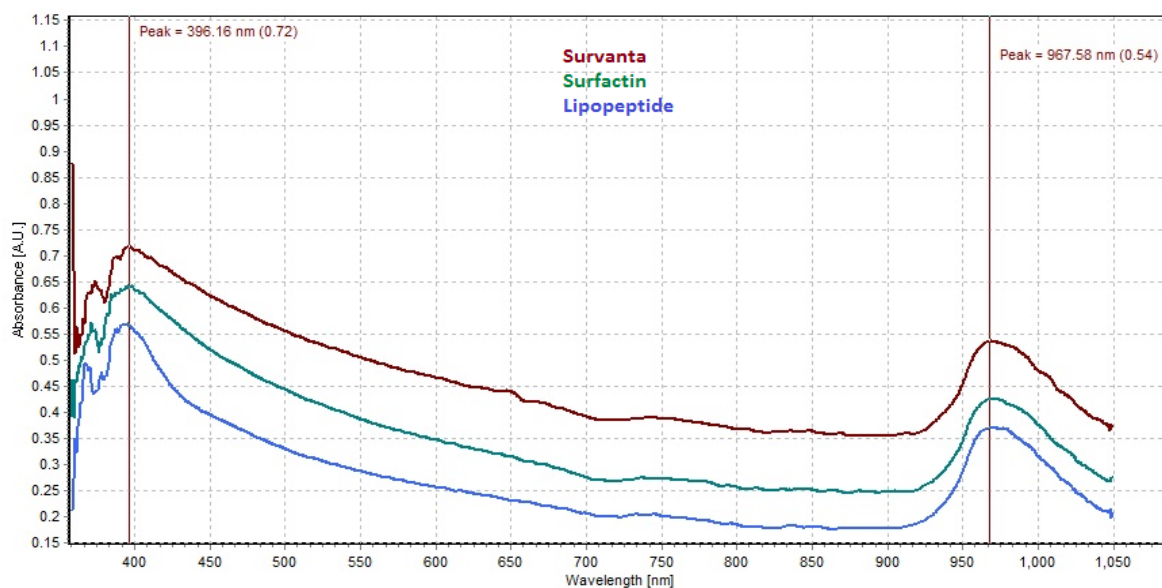
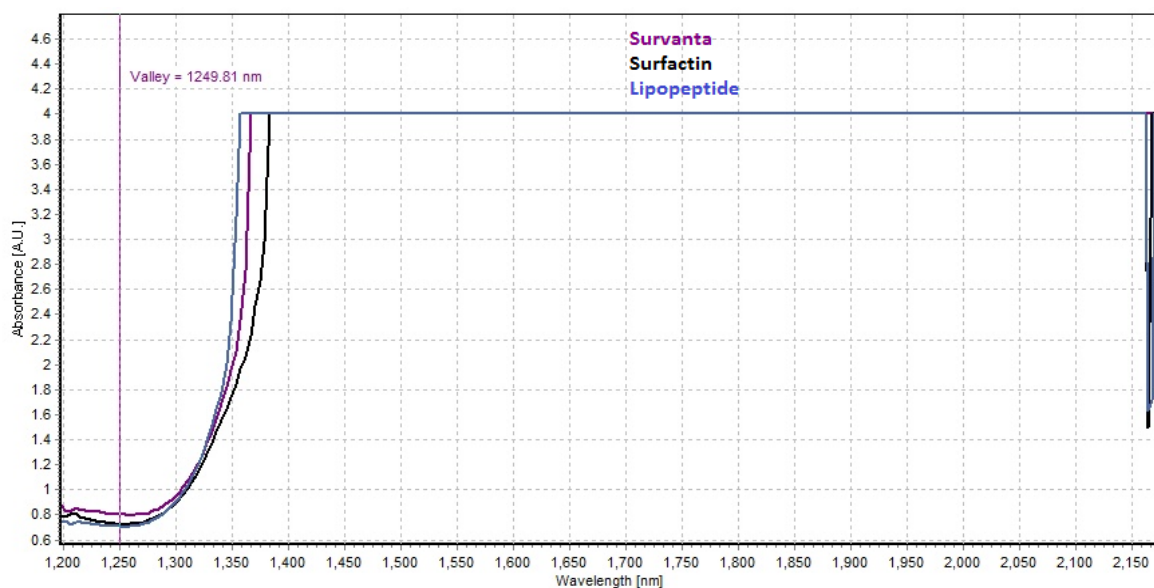**Figure 6.1**. Near IR spectrum of surfactin and lipopeptide (Biosurfactant) with the survanta.

Figure 6.2. Far IR spectrum of surfactin and lipopeptide (Biosurfactant) with the survanta

Further surfactin and lipopeptide (Biosurfactant) comparative studies with survanta were done on account of varying the concentrations, absorbance peak of each surfactant shows the concentration and presence of that surfactant so it's important to measure the absorbance peak by varying the concentrations.

Concentration Variation study

Results of Comparative study reveal that Surfactin and Lipopeptide (Biosurfactant) showing the same absorbance spectrum of Near IR and Far IR as shown by survanta then their concentration variation studies were performed, this study was helpful to establish the sensitivity of NIR and Far IR absorbance spectrum with these surfactants.

Lipopeptide (Biosurfactant) produced from an acclimatized strain *C. tropicalis MTCC230,* was serially adapted from low to high concentrations of hydrocarbons (petroleum waste) (Ashish et al. 2011; Verma et al. 2015). Supernatant of fermented broth of *B.subtilis* MTCC 2324 *and C. tropicalis* MTCC 230 containing surfactin and Lipopeptide (Biosurfactant) respectively, These Supernatants were used for analysing Near IR spectrometry and Far IR spectrometry with different dilutions.

Initially 2ml of supernatant was used for Near IR spectrum (range 300nm-1100nm), Supernatant containing Lipopeptide (Biosurfactant) showing absorbance 1.35 AU at 396nm (Figure 6.3) and Supernatant containing Surfactin show absorbance 0.59 AU at 369nm as (Figure 6.4), this signifies that acclimatized strain *C. tropicalis* MTCC 230 producing high amount of lipopeptide (Biosurfactant) then known strain of *B. subtilis* MTCC 2324 producing surfactin.

Absorbance Unit (AU) of 2ml supernatant containing the Lipopeptide (Biosurfactant) is nearly equal to pure drug survanta when diluted as 0.20ml of survanta + 1.80ml of distilled water at 369nm as shown in Figure 6.5.
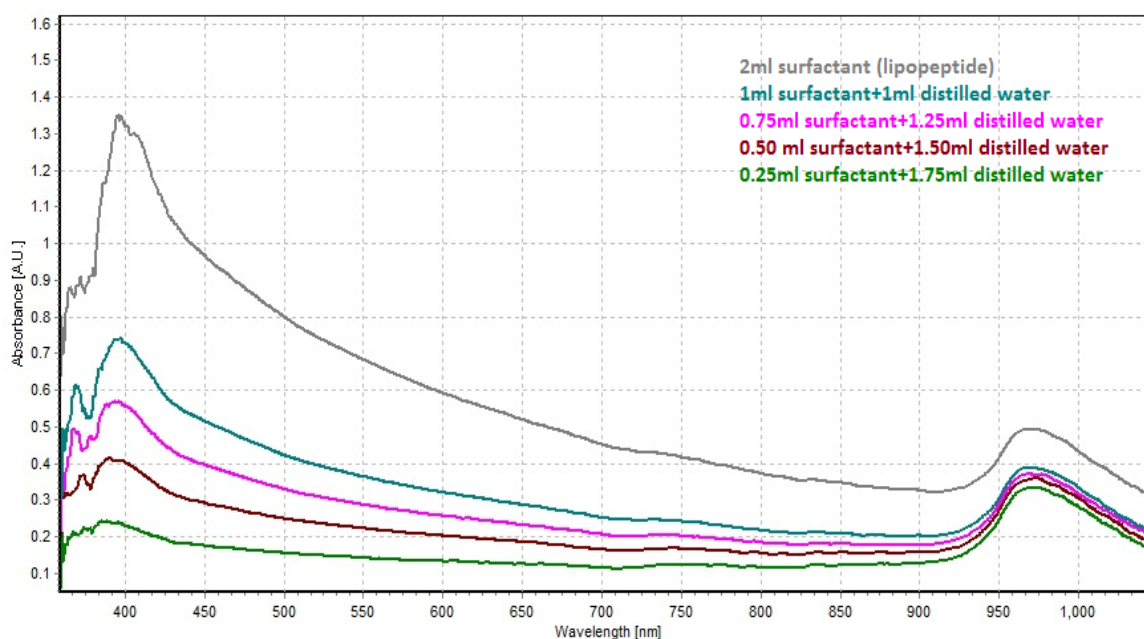

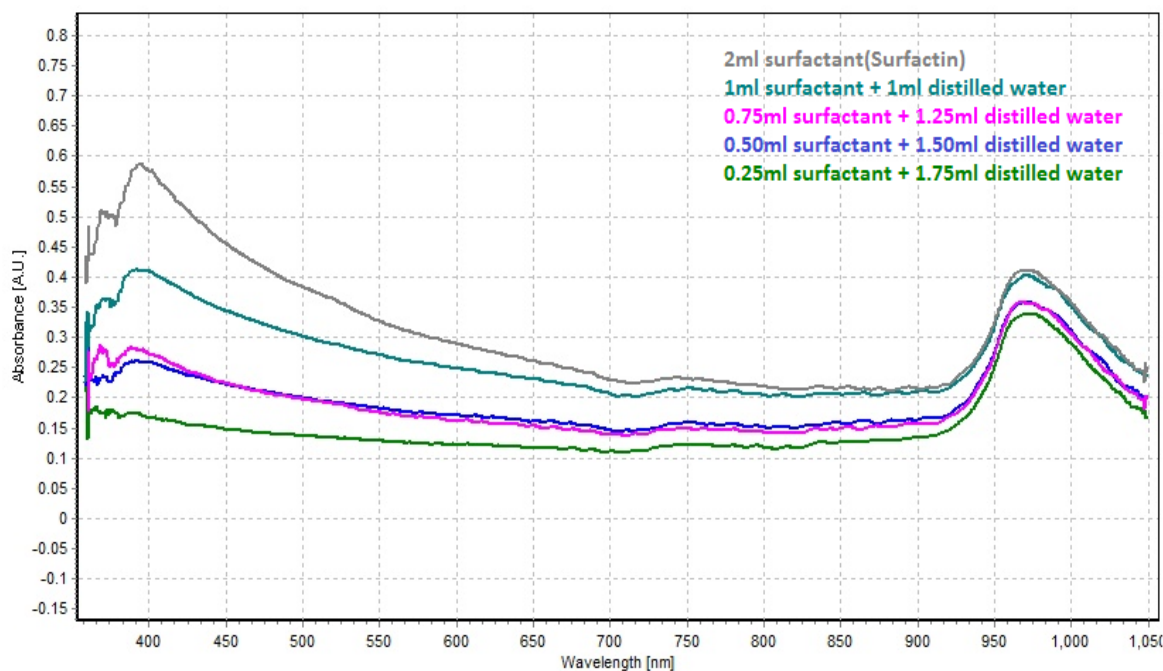**Figure 6.3**. Near IR spectrum of lipopeptide (Biosurfactant) at different dilutions.

Figure 6.4. Near IR spectrum of Surfactin at different dilutions.
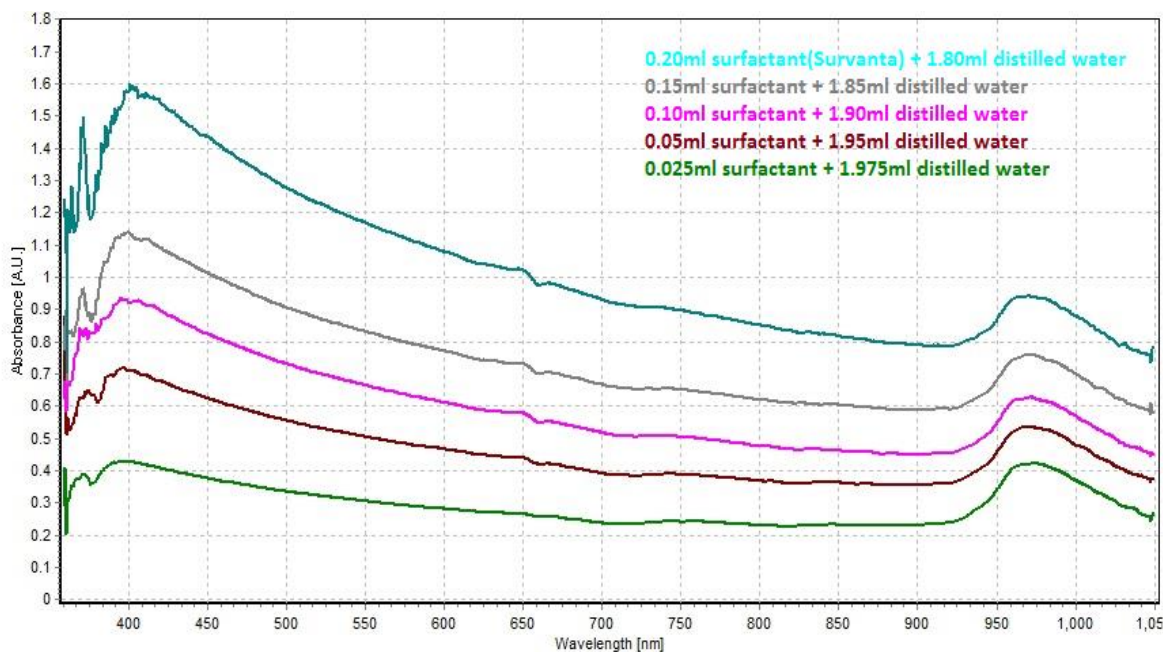

Figure 6.5. Near IR spectrum of Survanta at different dilutions.

To estimate the lowest absorbance unit for analysing the sensitivity and efficacy of

Near IR and Far IR spectrum for microbial surfactants, dilution was increases gradually for

lipopeptide (Biosurfactant) and surfactin containing supernatant, and also for survanta with the same dilution rate respectively. Different dilution rate shows different absorbance unit, as dilution rate increases absorbance unit decreases respectively. Surfactin shows minimum absorbance unit in highest dilution then followed by lipopeptide (Biosurfactant) supernatant then survanta i.e., 0.17, 0.24 and 0.42 respectively. Further Far IR spectrometry studies were also performed for each surfactants (Lipopeptide, surfactin and survanta) for different dilution rate as similar in case of Near IR, all the surfactants shows least absorbance at 1250nm for Far infrared spectrum range 1200nm-2200nm, this absorbance is showing very small change with change in concentration of Lipopeptide, surfactin and survanta as shown in Figure 6.6, 6.7, and 6.8 respectively.
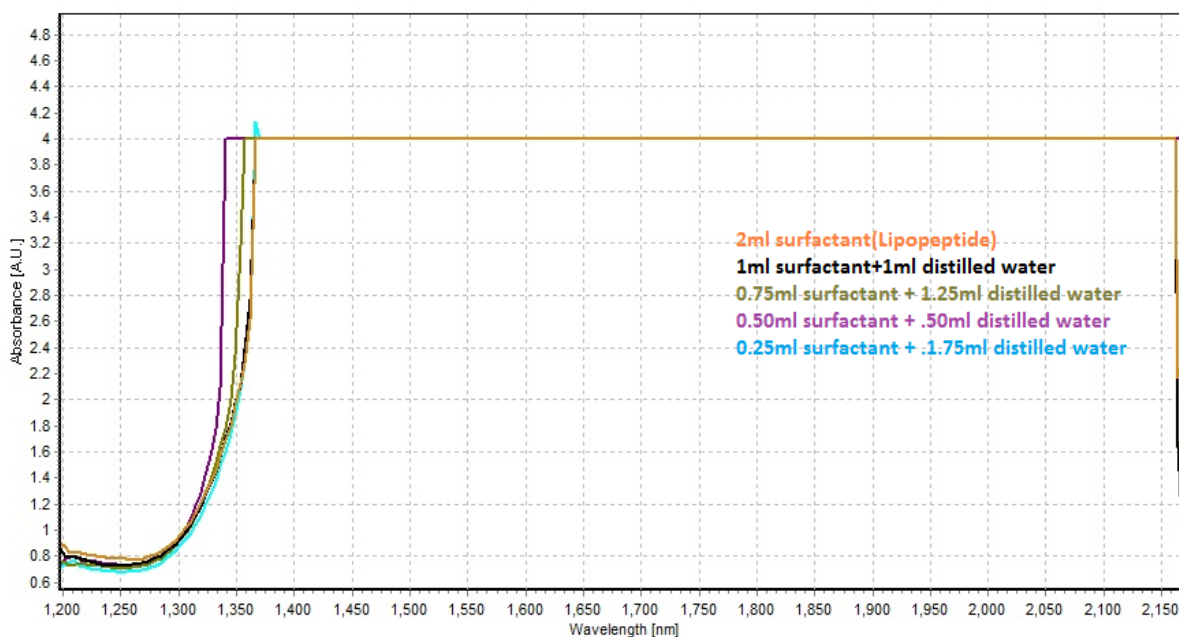

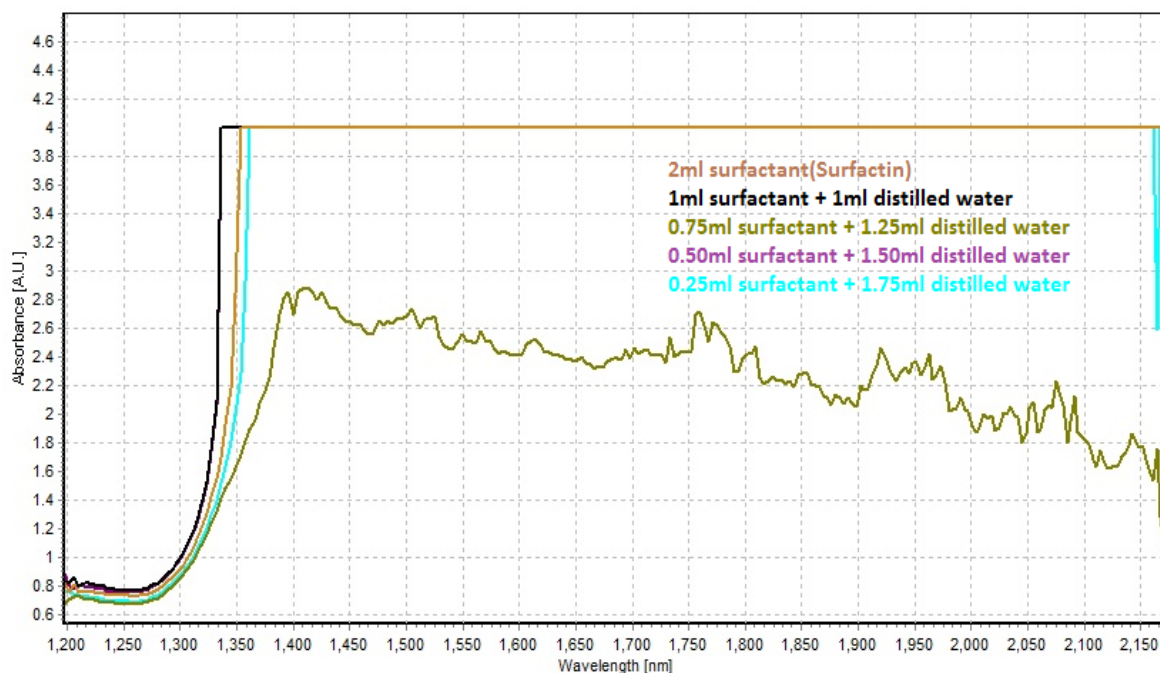**Figure 6.6**. Far IR spectrum of lipopeptide (Biosurfactant) at different dilutions.

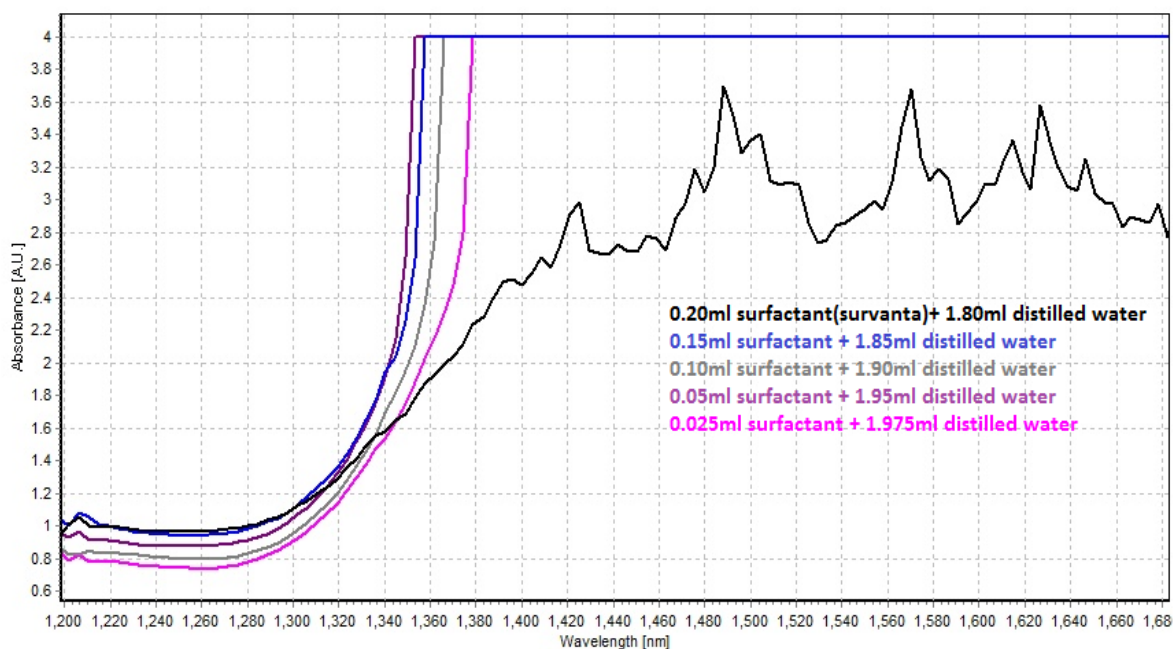**Figure 6.7**. Far IR spectrum of Surfactin at different dilutions.

Figure 6.8. Far IR spectrum of Survanta at different dilutions.

Near IR and Far IR spectrum are the two diagnostic windows considering how light interacts with living tissues, penetration of these IR into the living tissues can helps to

diagnose that whether the lung contains normal or sufficient surfactants (Pogue, Leblond et al. 2010), this is one of most useful method for the determination of lung surfactant in neonates. IR spectroscopic analysis having number of practical advantages like, No reagents are required, there is generally no need to dilute very concentrated specimens, very little sample is required (Shaw et. al. 2008).

Absorbance of IR at particular wavelength shows the presence and concentration of surfactant inside the lungs, microbial synthesized surfactants i.e., surfactin and lipopeptide (Biosurfactant) showing the absorbance at 396nm and 960nm for Near IR and least absorbance is shown at 1250nm for Far IR spectrum is as same as survanta shows.

6.4. Conclusion

This study reveals that microbial surfactants can be considered as drug for the treatment of respiratory distress syndrome (RDS) in neonates, as these surfactants are eco-friendly and non-hazardous for body many of microbial synthesized surfactants are used for many diseases as a drug (Gudiña et al. 2013).

Surfactin and Lipopeptide (Biosurfactant) produced from *B. subtilis MTCC2423* and *C. tropicalis MTCC230* respectively, can be considered for RDS treatment as it clear the primarily study of radiological property of drug by comparable study with FDA approved drug survanta, showing the same IR pre-clinical spectrometric results. Animal derived surfactants and chemically synthesized surfactants used for RDS treatment having some limitations like limited scale of production when animally derived and may hazardous when chemically synthesized over these limitations microbial synthesised surfactants having some advantages, it can be produced in large scale and found to be non-

hazardous for human body. This work explore the potential use of microbial synthesized lipopeptide for the treatment of RDS as it passes the pre-clinical trial of radiological study and so this information and data open the path for clinical and *invivo* study of microbial derived surfactant for RDS treatment in neonates.