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PHYSICOCHEMICAL CHARACTERISTIC OF PURIFIED LATEX (PL)

(Ciri Fisikokimia Lateks Tertulen (PL))

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Abstract

Purified latex (PL) was prepared via urea incubation of commercial high ammoniated natural rubber latex (HA-NRL) in the presence and absence of sodium dodecyl sulphate (SDS) as surfactants. During this purification process, most of non-rubber substances presences in NRL including proteins, lipids, carbohydrates, are being removed especially at centrifugation stage. Effect of non-rubbers depletion was further elucidated by conducting a comparative study on HA-NRL and PL. Apparently from zeta potential analysis, negatively charged rubber surface over whole pH range and constant zeta potential value after pH 4 for PL suggesting the strong adsorption of surfactant on the surface of purified rubber. In the absence of surfactant, the surface charge of rubber particle also appeared to be a negatively charge but with a reduction trend in its zeta potential magnitude as pH increases attributed to urea residual. It is appeared that the occurrences of strong surfactant adsorption on the surface of rubber particles enable PL to retain their stability. Replacing the non-rubbers with surfactant has affect the rheological properties of PL as it experienced shear-thinning behaviour, increased in viscosity characteristic and distinctive linear viscoelastic region (LVR) manner in comparison to that of the HA-NRL. Obviously, an almost similar rheological characteristic was observed for PL in the absence of surfactant and HA-NRL.

Keywords: non-rubbers, purified latex, rheology, surfactant, zeta potential

Abstrak

Lateks tertulen (PL) telah disediakan melalui inkubasi urea terhadap lateks getah asli komersial tinggi ammonia (HA-NRL) dengan kehadiran dan ketiadaan natrium dodecyl sulfat (SDS) sebagai surfaktan. Semasa proses penulenan ini, kebanyakan bahan-bahan bukan getah yang hadir di dalam NRL termasuk protein, lipid, karbohidrat, dikeluarkan terutamanya pada peringkat pengemparan. Kesan penyusutan bahan bukan getah diperjelaskan dengan lebih lanjut dengan menjalankan kajian perbandingan ke atas HA-NRL dan PL. Jelas daripada analisis potensi zeta, permukaan getah yang bercas negatif terhadap keseluruhan julat pH dan nilai potensi zeta yang malar selepas pH 4 untuk PL mencadangkan penjerapan kuat surfaktan terhadap permukaan getah tertulen. Dalam ketiadaan surfaktan, cas di permukaan zarah getah juga muncul menjadi cas negatif tetapi dengan aliran pengurangan dalam magnitud potensi zeta apabila pH meningkat berpunca daripada sisa urea. Ia kelihatan bahawa keadaan penjerapan surfactant yang kuat pada permukaan zarah getah membolehkan PL untuk mengekalkan kestabilan mereka. Menggantikan bahan-bahan bukan getah dengan surfaktan memberikan kesan terhadap sifat-sifat reologi PL kerana ia mengalami kelakuan ‘shear-thinning’, peningkatan dalam ciri kelikatan dan perilaku rantau viskoelastik linear (LVR) tersendiri berbanding dengan HA-NRL. Jelas sekali, satu ciri reologi yang hampir sama diperhatikan untuk PL dalam ketiadaan surfactant dan HA-NRL.

Kata kunci: bahan-bahan bukan getah, lateks tertulen, rheologi, surfaktan, potensi zeta

Introduction

It is undeniable that NRL is the supreme materials used to manufacture consumer products which necessitating reasonably thin structure with great elasticity. It is also the best pertinent material for intended medical products, such as rubber gloves, catheters, novelty items and condoms which attributable to its high wet gel strength and excellent physical properties [1,2]. The underlying features of films made from NRL, apart from the strength properties is the ease of forming favourably comfort thin smooth film with high level of protection against infectious organisms, virus penetration and blood pathogens. Even so, the presence of natural occurring proteins had brought unpleasant perception to consumers, thus create great limitation in NRL bases industrial growth as a result of fatal incidence report in early 1990's.

It has been accepted since then, that extractable proteins (EP) from NRL products can cause latex allergy among sensitive individuals and this allergy is classified as Type I hyper-sensitivity [3,4] which can be potentially fatal. Although protein is in fact occurred less than 2% in NRL, it has the ability of binding with IgE antibodies, hence regarded as potential contributor in latex allergy. In accordance, a mandatory regulation had been introduced by Food and Drug Administration (FDA) to overcome the arising issue such as labelling all NR medical devices and the extension of biocompatibility tests required currently for wide range of disposable gloves. The American Society of Testing and Materials (ASTM) have also formed a group to study suitable test methods for measuring EP in latex medical devices. Indeed, a regulation for latex medical device may be introduced as early as 1995 by the PDA of U.S.A [3].

This stringent directive, posed a serious challenge to all latex manufacturers that effective measures need to be taken to reduce the EP content in latex products. Since scarce data on the duration of exposure and the minimum amount of EP remain on the product which could bring about sensitisation prevailed, the approach to eliminate the culprit specifically extended starting from its preliminary stage – by producing latex with low extractable protein content. This event shall ensure that latex products of excessively high protein content will disembark the market and that medical devices are safe for use since products with low protein content are likely to give low allergic response. Generally, low protein latex can be prepared via few approaches namely mechanical [5], enzymatic treatment [1] or chemical [6].

The mechanical process usually involves multiple centrifugation or membrane filtration of dilute latex. The dipped films made from such lattices are expected to have a lower EP but it's occurred that owing to its added cost with negligible reduction in EP content, this method is less favoured. There is also other approach which involves combination of proteolytic enzyme [1] that was claimed to be the most effective method to deplete the protein. However, controlling enzymatic reactions and maintaining consistent protein level of such products concurrently remain as major hurdle. While in chemical treatment, chemicals such as urea are used to unfold proteins from the surface of the latex particle and its removal is expedited by the action of surfactant. Displacement of adsorbed proteins in latex is compensating by the presence of surfactant and subsequent purification of the treated latex by centrifugation or possibly through creaming process.

It is clarified in earlier report that types of surfactant assist in substantial removal of proteins latex and improves the properties of dipped product simultaneously such as surface morphology and mechanical stability as well as enhance the film strength [6]. Moreover, the distinctive behaviour of surfactant in NRL system also relies comparatively on its alkyl chain length. Apparently, the tendency of anion surfactant to be adsorbed at the rubber-water interface improves with increasing of its alkyl chain length until certain extent of length. Evidently, latex with surfactant containing 9 to 11 carbon atoms appeared to be the most effective in enhancing mechanical stability [7]. While those that contains more than 13 carbon atoms is indeed giving an equivalent performance as those containing 5 or 7 carbon atoms.

In most studies, the effect of protein removal in latex is generally scrutinized based from technological aspect – evaluating its mechanical stability test (MST) value and also its film strength. However this information is inadequate to upscale the value of NRL in order to retain its sustainability within rubber industry. Despite extensive reports were made on its remarkable physical properties, nevertheless, purified latex (PL) is still poorly accepted by industry. Former generation of PL mass production encountered several issue such as long term stability, excessive foaming and premature coagulation [8]. Therefore further investigation on the after-protein-removal latex properties should be elucidates since protein latex is regards as an essential component not only in latex colloidal stability. As such, the aim of this paper is to conduct a fundamental analysis via latex profiling study of PL in comparison to that of common latex commercial, high ammoniated natural rubber latex (HA-NRL).

Materials and Methods

High ammoniated natural rubber latex (HA-NRL) a commercial sample, preserved with a 0.7 wt. % ammonia was used for the preparation of purified latex throughout this study. All chemicals were used as supplied without further purification.

Preparation of Purified Latex

To prepared purified latex, HA-NRL was initially diluted to approximately 30% total solid content (TSC) with 25% NH_3 solution and incubate for at least 20 hours with a known amount of 10% SDS, 10% urea and 20% potassium laurate before underwent centrifugation process. The centrifuged latex was then diluted again to 30% TSC with 25% NH_3 solution before incubate with a known amount of 25% potassium laurate and centrifuged.

Physicochemical Characterization of Prepared Purified Latex: Typical Latex Properties

The latex properties were determined in accordance to the relevant ISO test methods for NR centrifuged latex concentrates. Total extractable protein (EP) content was determined according to the ASTM D5712-99 test method. The water soluble proteins of specimens (6 X 6 cm^2) were first extracted in phosphate-buffered saline (PBS) (1 g / 5 mL) at 25ºC for 3 hours before precipitated. Protein precipitated was then dissolved and quantified colorimetrically.

Determination of Proteins Molecular Weight

The purified latex was firstly centrifuged at 21 K RPM for 1 hr (T=5^{o}C) to have a clear serum fraction. The sample from serum fraction was then subjected to SDS - Polyacrylamide Gel Electrophoresis (SDS-PAGE) which contains 4% stacking gels and 15% separating gels. The SDS-PAGE analysis was conducted at a constant current of 0.25A for 1 hr. The reference marker was run concurrently in all experiments. The SDS-PAGE gels were stained with Coomassie Blue R-250 staining system afterwards before destained in destaining solution.

Particle Size and Zeta Potential Measurement

The particle size distributions of latexes were measured by 90Plus/BI-MAS (Brookhaven Instruments Corporation). The technique employed photon correlation spectroscopy of quasi-elastically scattered light which is based on correlating the fluctuation about the average, scattered, laser light intensity. Approximately 0.01wt% of latex was prepared by diluting 0.1ml of 60wt% latex with 1L of distilled water prior to the test.

Zeta potential was determined by ZetaPlus (Brookhaven Instruments Corporation) with measurement run at T = 25^{o}C. Each experiment was repeated five times to give an average value of zeta potential. The pH value of system was adjusted with 0.1 M H_2SO_4 and NaOH and pH were recorded using a calibrated pH meter.

Rheological Behaviour

The rheological behaviour of each samples were measured by Discovery Hybrid Rheometer (DHR) from TA Instruments. All experiments were performed at 25^{o}C and using a peltier concentric cylinderical geometry. Concentric cylinder geometries are commonly used device for testing low viscosity fluids, dispersions or any liquids that are pourable into a cup. It is necessary to calibrate all devices each time before running the experiments. The viscosity parameters and shear stress in a function of shear rate (0.001 s^{-1} – 900 s^{-1}) were determined by using flow ramp protocol. To study the viscoelastic properties of the compounded latex, oscillation tests were carried out at angular frequency = 10 rad/s and strain % at 0.001 to 100%. Of each experiments, approximately about 23 ml of samples were poured into the cylindrical geomotery.

Results and Discussion

Effect of Protein Removal and Surfactant on Properties of Latex

Apparently, removal of protein in latex did not significantly alter latex typical properties for instance their DRC, VFA, etc as tabulated in Table 1. Nonetheless, a high MST value with below detection limit of EP value was obtained for PL. It implied the effectiveness of SDS in proteins removal and at the same time confirmed its' role as stabilizer in retaining the stability of the purified latex system. This below detection EP limit of PL as compared to that of HA-NRL confirmed the attainment of low protein latex or purified latex.

Table 1. Properties of purified latex in comparison with high ammonia latex

Latex Properties	Purified Latex	High ammonia Latex
Total solids content / % m/m	60.0	60.9
Dry rubber content / % m/m	59.3	60.3
Alkalinity / % m/m on latex concentrate	0.6	0.6
Mechanical stability time / s	1423	1450
Volatile fatty acid (VFA) number	0.02	0.03
pH	10.1	9.9
Viscosity / cps	88	90
Extractable Protein (EP) content	BD*	146

*BD: Below detection range

Particle Size Distribution

Type of diameter obtained with photon correlation spectroscopy (PCS) is the hydrodynamic diameter; which measure the particle diameter with existence of double layer thickness. The effective diameter was measured when a distribution of sizes appears showed an average diameter which is weighted by the intensity of light scattered by every particles presents in the suspension. As such, a monomodal particle size distribution was obtained for each sample besides a noticeable shifting effect on particle size distribution as latex proteins are removed from the NRL system (Figure 1). Apparently, a narrower distribution was observed as a result of protein removal in the absence of SDS. While presence of SDS had remarkably shifted the size distribution to larger particle size ranges larger than to that of HA-NRL. The main reason for a considerable distribution curve shifting behaviour could be assigned due to removal of proteins adsorbed rubber, multiple centrifugation procedure and the displacement of protein layer with SDS during PL preparative routine.

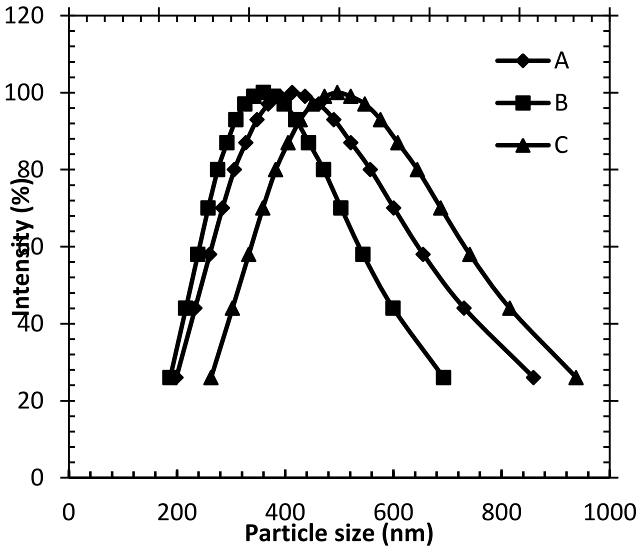

Figure 1. Particle size distribution of A) HA-NRL B) PL in the absence of surfactant and C) PL in the presence of surfactant.

It is describe elsewhere that larger NRL particle size is accepted to comprise of smaller primary particle which is clustering with each other; that addition of strong alkali to latex enhanced the clustered appearance [3]. During the incubation process of the purified latex, proteinaceous substances in the latex were unfolded by urea, and further centrifugation will eliminate proteins as well as the protein that adsorbed as smaller primary latex on the rubber surface. This whole purification process affected the width of particle size distribution. Apparently, non-uniform shifting on particle size distribution from curve A (HA-NRL) ranges from 198.8 - 859.5 nm to curve B (PL in the absence of surfactant) ranges from 187.4 – 693 nm was observed. A significant reduction on larger particle size distribution, which is about 166.5 nm than about 11.4 nm for lower range indicates that the rubber particles were surrounded with smaller primary particles that were easily eradicated through centrifugation process.

Meanwhile, broad particle size obtained for PL in the presence of SDS showed SDS ability to disperse and adsorbed at latex particle interface; producing a balance electrostatic repulsion. The SDS molecules are presuming enfolded latex and form a layer that encapsulated the rubber particles. Since higher amount of SDS was used, it was deduced that thicker layer will encircle the rubber particles. These layers will restrain from PCS penetration thus resulting to larger particle size distribution. Again a non-uniform shifting of particle size distribution was observed for PL with the presence of surfactant (curve C) ranges from 315.7 – 1126 nm as compared to that of PL in the absence of surfactant (curve B).

Zeta Potential Measurement

Zeta potential measurement of latex as depicted in Figure 2 revealed the surface charge behaviour of latex particle in different pH medium. HA-NRL exhibited a typical characteristic zeta-potential curve with isoelectric point (IEP) at about pH ~5 of which IEP is the pH at which a particular molecule or surface carries no net electrical charge. A positive zeta potential region for pH below 5 and negatively charged zeta potential region with an increased in its absolute value for pH above 5 was observed. Since adsorbed protein attached to the surface of latex contain both acidic and alkaline functional groups the protein will carries net positively charged at lower pH and negatively charged when at higher pH [9]. The increased in zeta potential absolute value indicates the stability manner of HA-NRL as pH increases.

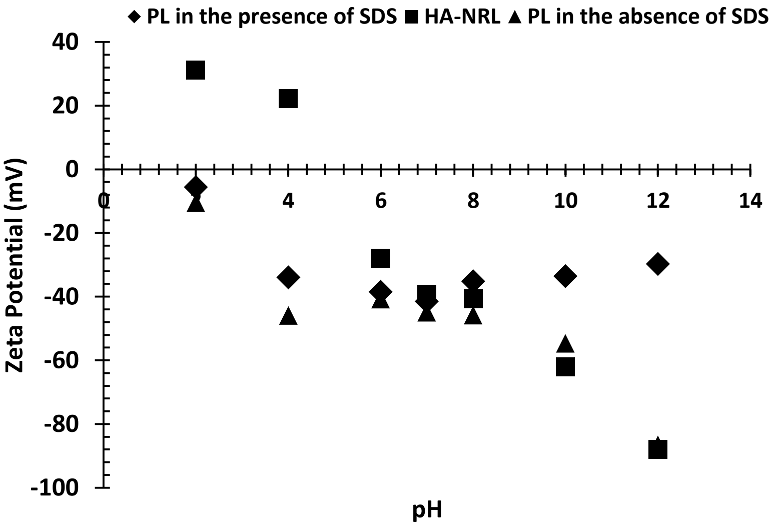

Figure 2. Zeta potential measurement of latex

Removal of adsorbed proteins from latex particle had apparently changed surface charged behaviour of latex. Net negatively surface charged of latex particle with no IEP were observed for both purified latex with or without the presence of SDS. A net surface charge can be attained by the imbalanced adsorption of contrary charged ions. Moreover, it is stated elsewhere that a surface which is in contact with aqueous media are preferentially negatively charged than positively charged [9]. A reduction of zeta potential absolute value for PL in the absence of SDS at lower pH is due to more H^+ that carried +ve charged presence on the latex surface. However, this H^+ is insufficient to neutralize the existing negative charged presence on the surface of latex but high enough to enable it approaching point of zero charge or IEP. As pH increases, more OH^- presence, thus more negative charge exist on the latex particle surface, hence larger zeta potential magnitude observed. Clearly, 12 alkyl chain length of SDS adsorbed strongly on the latex particle surface and determined its zeta potential curve [9]. As anticipated, the anionic surfactant, SDS is expected to impart a negative charge to the surface of latex particle, hence enhanced the colloidal stability of purified latex. As such, the zeta potential became more negatively charged as the pH value increases before giving a constant zeta potential value at pH above pH 4. The constant zeta potential value observed for pH above 4 of PL in the presence of SDS suggesting the saturation adsorption of SDS molecules on the negatively charged latex surface *via* hydrophobic effect [10].

Characterization of Extracted Proteins in Latex

Figure 3 represents SDS-PAGE gel image, whereby lane 1 is the reference containing standard proteins of 10-260kDa. As the image shifts from left to right, it was observed that the blue color intensity receded. The image obtained supported the above justification where presence of surfactant in the system enhanced the clustered appearance; which these clustered particles can be easily removed during centrifugation process.

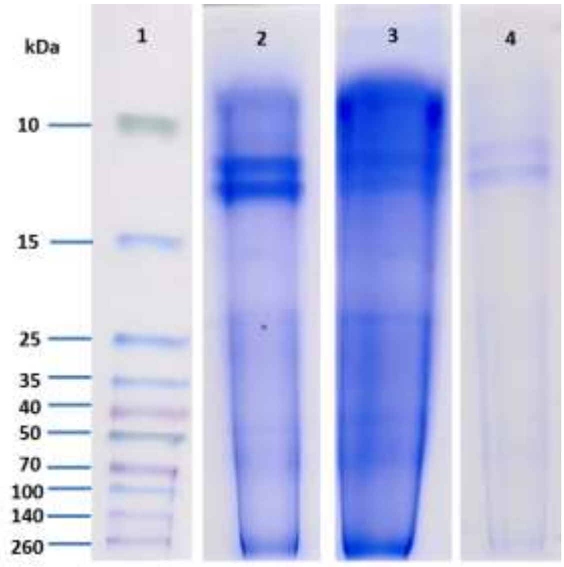

Figure 3. SDS–PAGE images (15% gel) of proteins from the serum fractions; (1) standard protein, (2) HA-NRL, (3) PL in the absence of SDS, (4) PL in the presence of SDS

Rheological Study

Comparative flow behaviour of PL and HA-NRL was scrutinized through steady shear flow procedures in order to further understand their physicochemical properties. It was observed that curve B experienced higher viscosity at lower shear rate (rest state) which almost three times higher than to that of curve A viscosity, while A is more viscous than C as depicted in Fig 4. This higher viscosity characteristic of B is perhaps due to the saturated amount of SDS presence in the latex system which is concurrent to the constant zeta potential value. Shear thinning with Newtonian region behaviour of each system was observed as shear rate increase. Domination of the surfactants molecules in the PL system has increased the steric stabilization, hence contributed to the higher viscosity of the PL in the presence of surfactant. It was reported that presence of suspended materials in certain system may affect the viscosity [11], and therefore altered the rheological performance.

In latex technology, stability of latex is considered as important, therefore to do so, the oscillatory measurements that gives valuable information of loss and storage modulus of the lattices was performed. The length of liner region at linear viscoelastic region (LVR) of which no changes observed on their modulus is an indication for stability (Figure 5). As such, the LVR region of PL in the presence of surfactant is longer than the rest. Plus, it was also found that G' for PL in the presence of surfactant is higher than G" meaning that they experienced changes in their viscoelastic characteristic. For both systems, it was found that once the critical strain reached, the G' start to decrease simultaneously with G" while maintaining a practically constant G'-G" gap. Beyond that critical strain, the viscoelastic properties were converted from solid-like behaviour to liquid-like behaviour. Apparently, the intersection between G' and G" for PL in presence of surfactant is presumed as a completely structure destroyed [11,12] and the system will start to flow. Contradict trend which is no crossover between G' and G" was observed for HA-NRL system. Moreover, G" was larger than the G' for A was obtained indicates a stable latex system, that was easily to be disturbed. Therefore, optimization of surfactant utilization in the PL system shall be further investigated in producing PL that at least has comparable viscoelastic performance as the HA-NRL.

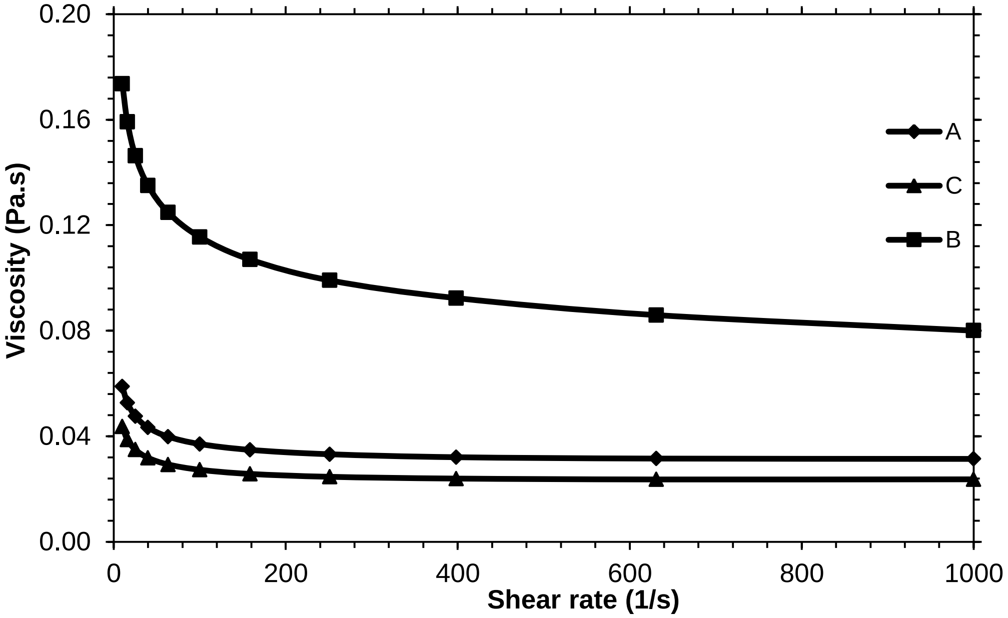

Figure 4. Viscosity as a function of shear rate for A) HA-NRL, B) PL in the presence of surfactant C) PL in the absence of surfactant.

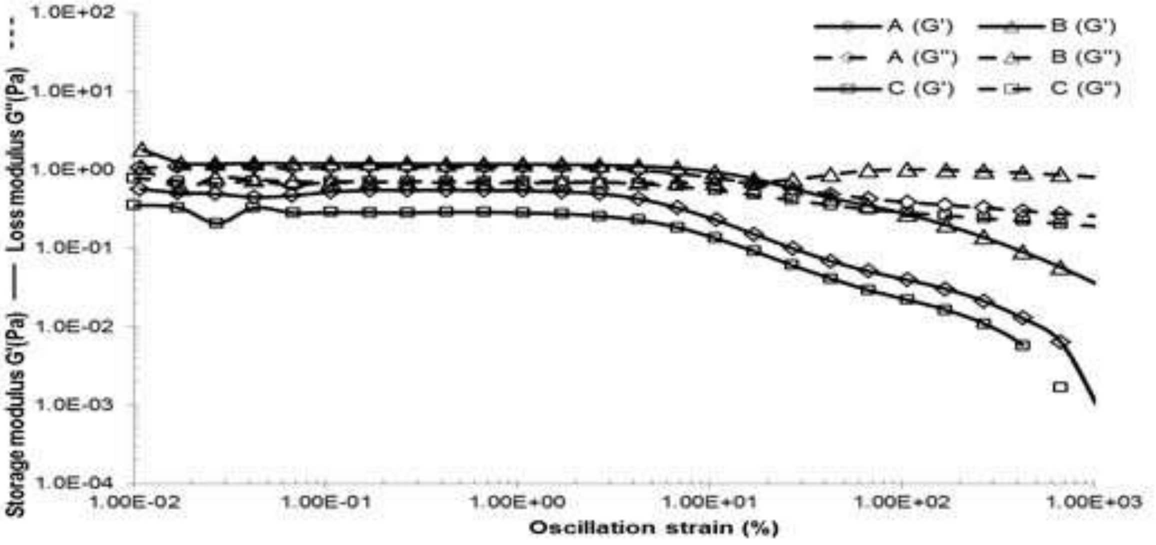

Figure 5. Complex modulus curves versus oscillation strain for HA-NRL and PL

Conclusion

On whole, anionic surfactant namely SDS assist total removal of adsorbed proteins in HA-NRL during urea deprotenization process and at the same time stabilized the latex system. Apparently, viscosity of latex is very much dependent on the purity of latex system; that presence of proteins and also SDS caused an increase in viscosity compared to that of purified latex in the absence of surfactant. Effect of an excessive amount of SDS presence in the latex system can be distinguished from its zeta-potential curve and rheological behaviour. Hence it is suggested that such alteration (i.e protein removal; surfactant addition) should be well monitored.

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