Food Nanoemulsion System
Yanjing Yin\textsuperscript{1} and Dejian Huang\textsuperscript{2}

\textit{Food Science and Technology Programme, Department of Chemistry, National, University of Singapore, 3 Science Dr. 3, Singapore 117543, Republic of Singapore}

ABSTRACT
Methyl Linoleate, phosphate buffer, Tween-20/SDS (emulsifier), butanol (cosurfactant) was mixed to form non-ionic/ionic nanoemulsions. The microstructure of Tween-20 nanoemulsion was found to be oil-in-water micelle with particle size around 88nm; that of SDS nanoemulsion was suggested to be bicontinuous lamellar structure. Antioxidant effects of vitamin C and vitamin E in both systems were examined by polarographically monitoring the oxygen consumption initiated by hydrophilic (AAPH) and hydrophobic (AMVN) radical initiators in methyl linoleate oxidation at 37°C. Synergism was calculated using area under curve (AUC) approach. Significant synergism was only observed in the SDS nanoemulsion methyl linoleate oxidation induced by AAPH, possible synergism may be found in other systems with increased vitamin C or vitamin E concentrations.

INTRODUCTION
The \textit{in vitro} mechanism of vitamin E regeneration by vitamin C has been known for some time. However, contradictory results were obtained for \textit{in vivo} experiments: a high intake of vitamin C increased tissue vitamin E concentrations in some (Liu et al., 1998) but not all (Burton et al., 1990) (LH, 1981) animal studies. Possible reasons could be the limited contact surface area as hydrophobic vitamin E and hydrophilic vitamin C are separated in two phases. On the other hand, synergism has been found in w/o fish oil nanoemulsions using lecithin as surfactants. (Jakobsson et al., 1994, Yi, 1991).

With the two vitamins still separated in two phases, the enlarged oil-water interface area in nanoemulsion may solve the problem and result in synergism. Reaction efficacy for other food grade substances can also be enhanced if this is found to be true. Therefore, this experiment was designed to test the synergistic inhibition effect of vitamin C and vitamin E in ML oxidation in both ionic and nonionic nanoemulsion systems. Possible interaction mechanism is suggested:

\begin{center}
\begin{tikzpicture}
  \node [text=black] at (0,0) {Lipid/water interface};
  \node [text=black] at (0,-1) {\textit{LOO}\textsuperscript{-} vitamin E \rightarrow \textit{vitamin C}};
  \node [text=black] at (0,-2) {\textit{LOOH} vitamin E \rightarrow \textit{vitamin C}};
\end{tikzpicture}
\end{center}

MATERIALS AND METHODS
Chemicals were commercially obtained with high purity except styrene was distilled before use. Phosphate buffer (75mM) was prepared and adjusted to pH=7.14. Oxygen concentration was monitored by Biological Oxygen Monitor, Model YSI 53 (Yellow Springs, Yellow Springs Instrument Co., U.S.A). The Ziploc membrane for electrode was bought from Jonhson. The particle sizes of both nanoemulsions were determined using a Photon Correlation Spectroscopy laser light scattering system 90Plus (Brookhaven, Brookhaven Instruments Co., U.S.A.) Images of polystyrene were collected with field-emission transmission electron microscopy (FETEM) (JEM 2010F, JEOL, Japan).

\textsuperscript{1} Student
\textsuperscript{2} Supervisor
Nanoemulsion preparation Nanoemulsions were prepared by mixing the appropriate components and stirring vigorously until clear. The basic composition for nonionic Tween-20 nanoemulsion, which was modified after work from Sandra(Sandra, 2006), is ML (12.0 wt%), (PSB, 44.0 wt%), Tween-20 (29.0 wt%), butanol (15.0 wt%). The basic compositions for ionic SDS nanoemulsion, which was selected from four composition of SDS mixture with the lowest viscosity and highest transparency, is ML (21.0 wt%), PSB (44.0 wt%), SDS (20.0 wt%), butanol (15.0 wt%). Vitamin C stock solution(0.008M) was prepared in PBS buffer and vitamin E stock solution(0.008M) was prepared in butanol. Vitamin C nanoemulsion and vitamin E nanoemulsion were obtained by adding 10μL of vitamin C stock or 20μL of vitamin E stock to 2ml nanoemulsion, respectively. Vitamin C vitamin E nanoemulsion was obtained by adding 10μL of vitamin C stock and 20μL of vitamin E solution to 2ml nanoemulsion.

Antioxidant activity assay The reaction vessel, in which a magnetic rod-shaped bar was stirring, was connected to an oxygen electrode and was maintained at 37°C by circulating warm water. Firstly 500μL of the nanoemulsion and 50μL of PBS were loaded, after being incubated for 10 minutes, 50μL AAPH solution (0.2g/ml in PBS) or 50μL AMVN(50mg/ml in butanol) was added to initiate the oxidation. The oxygen percentage readings from the electrode were recorded every 30s until the reading reached zero. Triplicates were taken until reproducible results were obtained (RSD<10%). The measurement was repeated with vitamin C nanoemulsion, vitamin E nanoemulsion and vitamin C vitamin E nanoemulsion. It should be noted that immediately after the addition of 50μL PBS, or the addition of 50μL AAPH dissolved in PBS, local cloudiness was observed. However, the reaction mixture turned back to clear nanoemulsion after being stirred for around 30s. The AUC was calculated from the plot of normalized oxygen against time as:

\[
AUC = 1.5([O_2]_0 + [O_2]_1 + \ldots + [O_2]_{200}) - 0.5 \quad (1)
\]

where \([O_2]_0\) = initial oxygen concentration at 0 minute; \([O_2]_i\) = oxygen concentration at time \(i\) and \([O_2]_{200}\) = oxygen concentration at 200 minutes.

The percentage of synergy was calculated as:

\[
\% \left[ (\text{net AUC}_{\text{mixture}} - \text{net AUC}_{\text{sum}}) / \text{net AUC}_{\text{sum}} \right] = \% \left[ \Delta (\text{net AUC}) / \text{net AUC}_{\text{sum}} \right] \quad (2)
\]

where net AUC_{mixture} = net AUC when both vitamin C and vitamin E are present; net AUC_{sum}=addition of net AUC contributed by vitamin C and vitamin E when present alone in nanoemulsion.

RESULTS AND DISCUSSION

A linear relationship with net AUC (AUC-Blank AUC) and vitamin E concentration was observed. The vitamin E concentration of 4.0x10^{-5} M was selected because at that concentration an obvious lag time is observed and a relative short time frame was required.
Synergy of vitamin C and vitamin E
Positive synergy was observed only in SDS nanoemulsion initiated with AAPH.

For the negative synergism observed, several reasons can be suggested. First, higher concentration of Vitamin C or vitamin E may be required for significant synergism to take place since vitamin C can only interact with vitamin E radical at the lipid/water interface. It has been demonstrated that the synergistic effect is dependent on the antioxidant concentration even in homogeneous methyl linoleate solution. (Shi et al., 2007) Also, positive synergism is observed in an increased vitamin C concentration in AAPH Tween-20 system as shown in Figure 2. Second, peroxides generated from Tween 20 may interfere the vitamin C vitamin E interaction and inhibit the synergism since they were shown to participate in oxidation of vitamin E(Mancuso et al., 1999).
Identification of the SDS system From the DLS and polystyrene SEM results (Sandra, 2006), the Tween-20 system has been determined to be a nanoemulsion system with micelle of around 80nm. However, the DLS results for the transparent SDS system suggested an average particle size around 6000 nm, which lies away from the normal nanoemulsion range. The flat crack of polystyrene obtained from the SDS system suggested a layered microstructure, to which spherical assumption of DLS is not valid. The layered microstructure may provide larger lipid/water contact area compared with micelle microstructure, which also explains the significant synergism observed in SDS nanoemulsion. Further study need to be done to confirm the microstructure.

This SDS layered structure mimics biological membrane environment, in which phospholipid (ionic surfactant) layer acts as the interface. Thus, the SDS nanoemulsion may be used as a simulation of in vivo condition for other similar studies.

FUTURE WORK
In order to further examine the synergism of the other three systems, higher vitamin C or vitamin E concentration can be used. Also, for more quantitative comparisons to be done, trolox standards can be measured in four systems, the antioxidant effects can subsequently be quantified to trolox equivalents. In order to confirm the regeneration of vitamin E by vitamin C in nanoemulsion system, HPLC can be used to monitor the disappearance of both vitamins in the oxidation. Nanoemulsion characterization techniques can be applied to further investigate the SDS system. Viscosity, conductivity, and dielectric measurements can be used for macroscopic level characterization, and NMR, SANS, Freeze-fracture electron microscopy can be utilized for microscopic level characterization.

CONCLUSION
Synergistic effect was found in ionic SDS nanoemulsion system, with possible lamellar microstructure, but not necessarily nano-sized particles inside. This may be a piece of evidence to vitamin C regeneration of vitamin E for in vivo systems as SDS nanoemulsion may be a good imitation of biochemical membrane conditions. Further studies need to be done to investigate the mechanism of vitamin C and vitamin E synergism in such system.

REFERENCES