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To,

Prof. A U Digraskar,
Central Project Advisor
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Subject: Action Plan of Center of Excellence on "Applied Research, Training And Education In Lipid Science" Established At H.B.T.I., Kanpur, (U.P.)

Sir,

This has reference to your letter no. AC/TEQIP-II/CoE/2014/446 dated July 10, 2014 in connection with CoEs Review Meeting held at New Delhi on June 02, 2014. In this regard kindly find attached herewith the details of the research activities under taken at the institute in the thematic area. The committee had recommended to do more characterization and experimentation with surfactants, which is depicted in the annexure containing the details of the projects under taken in the thematic area. A lot of instrumentation is involved in the characterization part of projects in the thematic area. It is worth mentioning that the work is still in progress and some other modern instruments will also be involved in due course of time. Besides this, the center is also planning to hold workshops and seminars in the thematic area in due course of time.

Hope you will find it in order.

Thanking you

Sincerely yours

(V.K. Tyagi)

Coordinator, CoE

Annexure

RESEARCH ACTIVITIES UNDER THE CENTRE OF EXCELLENCE ON "APPLIED RESEARCH, TRAINING AND EDUCATION IN LIPID SCIENCE" ESTABLISHED AT H.B.T.I., KANPUR, (U.P.)

The center of excellence established at H.B.T.I., Kanpur is focused on the research activities related to the following thematic areas:

- ***Novel Surfactants, eco-efficient soaps and detergents***
- ***Nutraceuticals, bioactive compounds***
- ***Oleochemicals and Advance Oil Processing Technologies***
- ***Renewable feedstock based technologies for lubricants and fuels(biofuels)***
- ***Eco- efficient polymers and coatings***

Details of research work done on the thematic area under the activities of the CoE are as given below:

Thematic Area	***Novel Surfactants, eco-efficient soaps and detergents***
Research Project-1	
Name of the research project	***Amino acid based Gemini surfactants derived from Lysine and fatty acids***
Objective of the work	Gemini surfactants derived from renewable sources not only possess excellent surface active & performance properties but also have excellent biodegradability. Amino acid based gemini surfactants are future alternative for conventional surfactant on account of above mentioned facts. The objective of this study is : • To synthesize the amino acid based Gemini surfactant by reaction between chloride derivative of fatty acid(lauric & Myristic) and Lysine (amino acid) • Evaluation of physio-chemical and surface active properties of synthesized amino acid based surfactants.
Methodology	• Conversion of fatty acids into their respective chlorides • Synthesis of amino acid based Gemini surfactant by reaction between chloride derivative of fatty acid and Lysine (amino acid). • Optimization of reaction variables like temperature, molar ratios of reactants and duration for the synthesis of amino acid based surfactants.
Instrumentation and characterization	• The synthesized lysine based gemini surfactants were characterized for surface active properties viz. CMC, Krafft temperature, surface Tension & Interfacial tension • Characterization of synthesized amino acid based surfactants by modern instrumentation viz. FT-IR, ^{1}H

	NMR & ESI-MS.
Results and Discussion	• FT-IR spectral data of the synthesized surfactants : C_{12}SDLL : 2955(C-O-H bending), 1557(N-H primary amide), 1317(-CH_2 bending), 1194 (C-N), 922(C-O), 724(-CH_2 rocking), 539(S-S disulphide) C_{14}SDML : 2954(C-O-H bending), 1557(N-H primary amide), 1320(CH_2 bending), 1190(C-N), 904 (C-O), 722(-CH_2rocking), 549(S-S disulphide) • 1H NMR spectral data of the synthesized surfactants : C_{12}SDLL : δ4.46(d,1H,CHCOONa), δ3.74(d,1H,SCH_2), δ2.57(d,2H,$COCH_2CH_2$), δ1.26(S,2H,$COCH_2CH_2$), δ1.11(S,16H(CH_2)10), δ0.72(t,3H,CH_3) C_{14}SDML : δ4.64(d,1H,CHCOONa), δ3.77(d,1H,SCH_2), δ2.59(d,2H,$COCH_2$), δ1.28(S,2H,$COCH_2CH_2$), δ1.17(S,16 H(CH_2)$_{12}$), δ0.75(t,3H,CH_3) • ESI-MS base peak(m/z) of the synthesized surfactants: C_{12}SDLL : 527.25 C_{14}SDML : 446.32 • Based on the evaluated surface active properties, the amino acid based surfactants derived from lauric acid (SDLL) proved to be superior as compared to amino acid based surfactant surfactants derived from myristic acid (SDML). Besides this, yield of the C_{12}SDLL surfactant was also more than that of C_{14}SDML.
Conclusion	• The optimum molar ratio of Lysine and lauroyl/ Myristoyl chloride was found to be 1:2.5 for the synthesis of C_{12}SDLL surfactants as well as C_{14}SDML surfactants. • The reaction temperature of 10-15^{0}C was found to be more appropriate for the synthesis of both C_{12}SDLL and C_{14}SDML surfactants. • The yield of the synthesized C_{12}SDLL surfactant and C_{14}SDML surfactants was found to be 79.5% and 72.2% respectively. • Based on the evaluated surface active properties, C_{12}SDLL surfactant has better surface active properties in comparison to C_{14}SDML surfactant.
Research Project-2	

Name of the research project	***Studies on novel methyl diethanolamine based esterquats***
Objective of the work	• Synthesis of methyl diethanolamine based esterquats and study of their surface active properties. • Evaluation of surface active and performance properties of synthesized esterquats.
Methodology	• Procurement of raw materials viz. palm fatty acids and methy diethanolamine. • Synthesis of diesters by esterification of palm fatty acids usin methyl diethanolamine. • Optimization of reaction variables like temperature, molar ratios of reactants and duration during synthesis of diesters. • Conversion of synthesized diesters into esterquats by quaternization of diesters. • Evaluation of physiochemical and surface active properties of synthesized esterquats.
Instrumentation and characterization	• Characterization of synthesized esterquats by modern instrumental techniques, viz. FT-IR, ^{1}H-NMR, ^{13}C-NMR. • Properties of diester obtained at 160°C Acid value : 2.91 Saponification value : 136.05
Results and Discussion	Under processing.
Conclusions	Under processing.
Research Project-3	
Name of the research project	***Studies on novel phosphate anionic gemini surfactants***
Objective of the work	Gemini surfactants are promising surfactants of the future as they have excellent surface active & performance properties as compared to conventional surfactants. They find wide industrial applications and are used in minute quantities. The basic objective of the present work is : 1.Synthesis of phosphate anionic gemini surfactant and study of their surface active properties 2.Study of synergism in mixture of phosphate anionic gemini surfactant with anionic(SDS) and non-ionic surfactant(Triton 100X).
Methodology	• Procurement of raw materials viz. dihydroxy alkanes(neopentyl diol), phosphorus oxychloride and fatty alcohols such as dodecanol. • Synthesis of bisphosphate gemini surfactant by

	phosphorylation of dihydroxy alkanes using phosphorus oxychloride and further alkylation with long chain alcohols viz. dodecanol. • Optimization of reaction variables like temperature, molar ratios of reactants and duration of the synthesis of bisphosphate Gemini surfactants. • Characterization of synthesized bisphosphodiester gemini surfactant • Evaluation of properties of synthesized gemini surfactant and their comparison with conventional anionic and non ionic surfactants.
Instrumentation and characterization	• The synthesized gemini surfactant was analysed using FT-IR, ^{1}H NMR and ^{31}P NMR • The Gemini surfactant was characterized for Critical Micelle Concentration(CMC) & surface tension • The surface active properties and performance properties of mixed surfactant systems were evaluated.
Results and Discussion	• The most appropriate temperature for obtaining higher yield of Neopentyl-bis(dodecyl phosphate) surfactants was found to be $30 \pm 1^{o}C$. • Surface tension and Critical Micelle Concentration of Neopentyl bis(dodecyl phosphate) were found to be 26.2 ± 0.2 mN/m and 0.028 mM/L, respectively • FT-IR spectral data of the synthesized gemini surfactant : 2924 (-CH_2 bending), 2854(CH_3 bending),1699 (P-OH), 1012 (P-H), 721(P-O) • ^{1}H NMR(500MHz, $CDCl_3$)spectral data of synthesized gemini surfactant, δ ppm: 0.80(t,6H,2CH_3) 1.73(m, 4 H, 2CH_2) 3.32-3.97(m,8H, 4CH_2-O)
Conclusions	• The yield of the synthesized Neopentyl-bis(dodecyl phosphate) surfactant was found to be optimum at 2:1 molar ratio of dodecanol & neopentyl diol at $30 \pm 1^{o}C$ reaction temperature and was amounted to 47.5% • Based on the evaluated surface active properties of mixture of anionic conventional surfactant SDS and Neopentyl-bis(dodecyl phosphate), the mixture showed synergistic effect as it further reduced the surface tension as compared to SDS alone.On the other hand, there was no significant effect of mixing of Neopentyl-bis(dodecyl phosphate) with SDS on foaming. • The surface active properties of mixed solution of non-ionic surfactant (Triton X 100) and neopentyl-bis(dodecyl phosphate) showed marginal reduction in surface tension. On the other hand, there was marginal effect of mixing of C_{12}PGS with Triton X 100 in lowering the foaming ability.

Thematic Area	***Nutraceuticals, bioactive compounds***
Research Project-1	
Name of the research project	***Natural antioxidants and synthetic antioxidants: a comparative study***
Objective of the work	The objective of this research project is to deeply study the antioxidant activity of the antioxidants present in peanut seed coat and dried tulsi(basil) leaves in order to examine their impact on the stability of a specified oil.
Methodology	▪ Collection of samples from natural sources like tulsi (basil) leaves and peanut skin coat. ▪ Samples were first dried at normal room temperature till the entire moisture gets released and then crushed using the conventional grinder. ▪ The grounded extracts were stored separately for later analysis in different bottles. ▪ Samples were mixed separately in alcohol preferably ethanol for the extract preparation and kept overnight in first method and in second method, stirred on orbital shaker at a particular rpm . ▪ Entire solution containing sample and alcohol was filtered using Wattman Filter paper and the extract was separated out using condenser assembly. ▪ A pasty mass was obtained in case of tulsi and dried flakes were obtained in the case of peanut skin and the extracts were stored separately in amber colored bottles for further analysis. ▪ The total phenolic component was also calculated separately using spectrophotometer. ▪ Oxidation Stability Index (OSI) of oil was studied using Rancimat by comparing it with a control sample and sample containing synthetic antioxidant for a period of 20-25 days. ▪ Other parameters like peroxide value, p-anisidine value were also monitored for the desired period of days keeping control samples (blank) as reference. ▪ The calibrations were done according to the readings achieved. ▪ Then the TOTOX Value was calculated as per the peroxide and p-Anisidine values measured
Instrumentation and characterization	For comparing the antioxidant activity of Natural Antioxidants with the Synthetic Antioxidants, various analysis for measuring the antioxidant activity of sample on basis of storage time was done. ▪ Total Phenolic Component (TPC)at 760 nm absorbance using Spectrophotometer in each of the natural source was measured in order to calibrate the concentration required to

monitor the peroxide value, p-anisidine value, OSI Values and TOTOX Values.

- Calibration of OSI Values was done using 743 Rancimat (Herisau, Switzerland) at 120º C following the AOCS Official Method Cd 12b-92 (1999) (2) .The test was performed to study the induction period (oxidative stability) of soybean oil with the addition of two Natural extracts i.e., Peanut skin and tulsi leaves and calibrate their comparative analysis with a Synthetic Antioxidant(BHA).The induction time readings were recorded at an interval of 5 days for a total period of 25 days.
- In the Analysis of Peroxide Value the hydroperoxides formation in all four oil samples each of peanut skin and tulsi leaf extract were kept for a storage period of an interval of 5 days for 30 days in contrast to the samples of BHA and control which were monitored using the formulae:

$$PV = \frac{(S-B)*N*1000}{M}$$

where,
S = reading of Sample
B = reading of Blank
N = Normality of Sodium thiosulphatesolution
M = Mass of the oil sample

- The chemical analysis method for p-Anisidine Value determines the amount of aldehydes present in vegetable oils and fats by reaction of these compounds with the p-Anisidine. This reaction highlights the concentration of the quantity of **aldehydes and ketones**, giving the dimension of the secondary oxidation of the fat matrices. The lower **the p-Anisidine Value**, the better the quality of fats and oils analyzed.The official method (AOCS Official Method Cd 18-90), a spectrophotometric analysis method measuring the absorbance at 350 nm, requires the use of two different reagents. The p-Anisidine Analysis was carried out for the two natural extracts using the formulae:

$$pAV = \frac{25*(1.2*A_1-A_2)}{m}$$

where,
A_1 = absorbance of test solution (b) at 350 nm,
A_2 = absorbance of test solution (a) at 350 nm,
m= mass of the substance to be examined in test solution (a), in grams

	▪ TOTOX value is used to describe the total oxidation to which an oil has been exposed. TOTOX Value is a combination of Peroxide value and p-Anisidine Values. These values are summed up to compare with the values appearing for BHA and Control as a contrast. It is calibrated as:
	PV x 2 + pAV = TOTOX
Result and discussions	▪ In the readings calibrated each for Tulsi and Peanut Skin it was seen that the OSI values tend to decrease on storage for each sample but it was noted in both the cases that as the conc. of antioxidant increased, the values tend to increase in contrast to the values of the Synthetic Antioxidant (BHA) and control sample. ▪ In the plot of both the graphs for p-Anisidine Value and Peroxide Value it was concluded that as the conc. of natural antioxidant increase both the values increases as per a particular day analysisin contrast to the values of Synthetic Antioxidant and when monitored on storage basis it was also found that the values for Natural Antioxidant increases gradually than that of Synthetic Antioxidant(BHA). ▪ In the readings calibrated each for Tulsi and Peanut Skin it was seen that the TOTOX values tend to increase on storage for each sample but it was noted in both the cases that as the conc. of antioxidant increased the values tend to decrease than that of the Synthetic Antioxidant (BHA) as per day analysis
Conclusions	The use of added natural antioxidants like extracts of tulsi leaves extract and peanut skin extract in vegetable oils have proved to sustain the oil for a much longer period of time without causing any harmful effect as it was seen in the case of Synthetic Antioxidants. Though they are costly but when looking for a long term remedy to protect the oil from deterioration then to meet the customer satisfaction Natural Antioxidants are considered far much better than the Synthetic ones. Natural Antioxidants as they show no carcinogenic effect on human body on consumption even after being added in larger amounts as compared to the lesser quantity of Synthetic Antioxidant as permitted by the Food Department.
Research Project-2	
Name of the research project	***Isolation of nutraceuticals from crude palm oil***
Objective of the work	To isolate nutraceuticals by saponification process followed by column chromatography method and analysis by HPLC.
Methodology	• Collection of crude palm oil from industry. • Characterisation of crude palm oil (Physico-chemical,

	spectroscopic and chromatographic evaluation). • Recovery and purification of crude palm oil by saponification followed by column chromatography method. • Evaluation of final purified nutraceuticals by HPLC.
Instrumentation and characterization	For Vitamin E : Waters HPLC equipped with an isocratic solvent delivery system and Waters 470 Scanning Fluorescence Detector with excitation and emission wavelengths set at 295 nm. A Zorbax analytical silica column (25cm× 4.6mm ID, stainless steel, 5μm) was used with the mobile phase of hexane : tatrahydrofuran : isopropanol (1000 : 60 : 4 v/v/v) at a flow rate of 1 ml/min. For Carotene : Waters 486 HPLC (Millipore Corporation, Milford, MA) equipped with a variable wavelength (190–900 nm) and Crest-Pak C18S MLC-01-250465 (250 × 4.6 mm i.d.) column (JASCO Corporation, Tokyo, Japan). The isocratic mobile phase was acetonitrile/dichloromethane (8:2, vol/vol). The flow rate was 1.0 mL/min.
Results and Discussion	ETHANOL FRACTIONS: Carotene and Vitamin E concentration was obtained as 15110 ppm and 26145 ppm, respectively. ACETONE FRACTIONS: Carotene and Vitamin E concentration was obtained 14997 ppm and 25947 ppm, respectively. HEXANE : ETHANOL FRACTIONS : Carotene and Vitamin E concentration was obtained 18612 ppm and 25174 ppm, respectively. HEXANE : ACETONE FRACTIONS- Carotene and Vitamin E concentration was obtained 18213 ppm and 24802 ppm, respectively. HEXANE FRACTIONS : Carotene and Vitamin E concentration was obtained 18094 ppm and 16262 ppm, respectively.
Conclusions	Concentration and recovery of Vitamin E was found to be in following order: Ethanol > Acetone >Hexane : Ethanol >Hexane : Acetone > Hexane. Concentration and recovery of Carotene was found in the following order: Hexane : Ethanol > Hexane : Acetone> Hexane > Ethanol > Acetone.
Thematic Area	***Oleochemicals and Advance Oil Processing Technologies***
Research Project-1	
Name of the research project	***Refining of crude rice bran wax***
Objective of the work	Upgradation and value addition of crude rice bran wax.
Methodology	• Refining of crude rice bran wax in two steps, first one is defatting of crude rice bran wax using solvents (Hexane and

	Isopropanol) and second one is bleaching of defatted wax by using sodium hypochlorite as bleaching agent. • Analysis of properties of refined rice bran wax.
Instrumentation and characterization	• Analysis of properties such as moisture content, melting point, specific gravity, iodine value, acid value, saponification value and unsaponification value of crude rice bran wax. • Refined wax was characterized by chromatographic method.
Results and Discussion	• Crude RBW contains about 55-60% oil with slip melting point 65°C. It has moisture content 2.8, specific gravity 0.8625, acid value 34.65, saponification value 110.25, unsaponifiable matter 47% and iodine value 17.06. • In this study, the optimal conditions are wax: hexane ratio is 1:6 (w: v), refluxed temperature maintained at 67°C for 40 min. After that the mixture was cooled to 20°. It was again dissolved in iso-propanol in1:7 (w:v) ratio to remove remaining oil and impurities. Deoiled wax was then bleached with sodium hypochlorite (15% solution) to prepare light colored wax. It yielded 32-36% pure wax. • After refining melting point of wax was obtained 85℃. The moisture content of purified wax reduces to 0.048, specific gravity 0.8943, acid value to 12.5, saponification value 99.11, unsaponifiable matter 90.4 and iodine value 6.52.
Conclusions	The purified wax obtained was in light yellow color crystal form with melting point 85°C. The acid value of purified RBW reduces to three times of crude RBW.The properties of refined wax show that it has very good potential to be used in pharmaceutical, cosmetic industries and food industries.
Research Project-2	
Name of the research project	***Enzymatic synthesis of monoglycerides from soybean oil using lipase***
Objective of the work	• Main objective of this work is to provide a solvent free process for the synthesis of monoglycerides which is acceptable to the food industry. • Another objective of the invention is to provide a process that can enhance selectivity for monoglyceride over

	diglyceride.
Methodology	• Collection of raw materials viz. Refined soybean oil, and glycerol. • Enzymatic synthesis of monoglycerides from **LIPEX:** *Thermomyces lanuginosus*(solvent-free system) by glycerolysis. • Separation and analysis of monoglycerides from sample mixture of mono and diglycerides.
Instrumentation and characterization	Characterization of synthesized monoglycerides by modern analytical techniques, viz. GC, HPLC,HPSEC.
Results and Discussions	Under processing
Conclusion	Under processing
Thematic Area	***Renewable feedstock based technologies for lubricants and fuels (biofuels)***
Research Project-1	
Name of the research project	***Growth optimization of algae for biodiesel production.***
Objective of the work	The objectives of this studies are as follows: • Optimization of culture parameter that affects the growth of algae. • Extraction of algal oil. • Conversion of algal oil to biodiesel.
Methodology	• Harvesting of algal growth. • Production of Biodiesel from algal biomass by following methods : (1) Oil Extraction from Algal Biomass followed by transesterification (2) Direct Transesterification from Algal Biomass • Optimization of Algae Growth by variation in culture parameters such as temperature, growth media, pH, light.
Instrumentation and characterization	Characterization of Biodiesel by modern analytical technique i.e. FT-IR, GC-MS .
Results and Discussion	• The optimal growth rates of this *Spirulina* strain were obtained at pH levels between 8-9, temperature regimes of 28-32°C , light intensities of 1500-2500 lux. • The Spirulina medium was found to be best for *Spirulina*

	sp. • Further results are under processing.
Conclusions	Under processing
Research Project -2	
Name of the research project	***Winterization of rice bran oil to separate stearine and its use in manufacturing of metal cutting Lubricant***
Objective of the work	The main aim of this study is to make metal cutting lubricant from RBO stearine and mineral oil to improve the kinematic viscosity, lubrication property, frictional property and cooling effect during machine operations.
Methodology	• Hundred gram of the processed RBO was mixed with the same volume of hexane, and the solution was heated at 65 °C until the solution became transparent. • It was cooled to room temperature, and then placed in an incubator at 20 °C for 1 hr. • After the winterization was finished, the yellowish crude rice bran stearine was recovered after the solution was centrifuged at 10 °C for 20 min. • The weight of the stearine was measured, and the yield from the processed RBO was calculated. • RBO stearine and mineral oil in different ratio was mixed and also anticorrosion, viscosity improver biocidal agent is added.
Instrumentation and characterization	Analysis of physiochemical properties such as acid value, iodine value, saponification value, flash point, pour point & kinematic viscosity & pour point of rice-bran oil, rice-bran stearine, & corrosion test of metal cutting lubricant.

Results and Discussion	• Rice-bran oil has acid value 5, saponification value 185 & flash point 120. • Rice-bran olein has Iodine value 102 and stearine acid has Iodine value 5. • Metal cutting lubricant has saponification value 8 & iodine value 5, acid value 3, flash point 165 °C, pour point 1°C and kinematic viscosity at 40 °C is 35 cst.
Conclusions	• The yield of stearine was 3.53% • Rice-bran oil has good biodegradability thus induces the potential for long term pollution control of the environment. • The mixing of RBO stearine in mineral oil make it sustainable for long duration and it has recycling property making it more valuable. • Mineral oil based lubricants are limited and steadily decreasing resource whereas the vegetable based oils are sustainable.
Thematic Area	***Eco- efficient polymers and coatings***
Project -1	
Name of the research project	***Synthesis and characterization of polyurethane resin from vegetable oil***
Objective of the work	The objective of this study is to develop bio-based poly urethane from vegetable oil as renewable resource. To achieve this goal the experimental work was focused on exploiting the reactivity of unsaturated fatty acid compound to prepare asset of polyol for polyurethane synthesis by synthetic methodology.
Methodology	• Synthesis of epoxy from an edible oil i.e. soybean oil. • Synthesis of polyol from prepared epoxy • 3. Synthesis of polyurethane resin from polyol

Instrumentation & Characterization	Fourier transform infrared spectroscopy
Results and Discussion	Under processing
Conclusions	Under processing
Project-2	
Name of the research project	***Use of Rice Bran Olein in Surface Coating***
Objective of the work	The basic objective of the research is to assess the suitability of rice-bran olein for surface coating
Methodology	• Collection of rice bran-oil • Characterization of rice-bran oil • Separation of rice-bran olein by winterization • Epoxidation of rice-bran olein • Blending of epoxidised rice-bran olein and novolec resin in different ratios and curing of blends with DETA • 6. Evaluation of chemical resistance and mechanical properties of cured films.
Instrumentation and characterization	• All physico-chemical properties were determined according to standard methods of AOCS • Epoxidised rice-bran olein was characterized by FTIR.
Results and Discussions	Under processing
Conclusion	Under processing