POLY(GLYCEROL-SUCCINATE) OLIGOESTERS AS BIO-BASED SURFACTANTS FOR STABILITY OF O / W EMULSION FORMULATED WITH RICE BRAN OIL

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Abstract

Poly(glycerol-succinate) oligoesters, PGS oligoester, were synthesized and characterized by nuclear magnetic resonance and liquid chromatography mass spectrometer. The objectives of this work are to observe the detailed characterization of PGS's structure and to optimize the conditions. The influence of PGS and chitosan concentration on the physical stability of emulsion was studied in 0.25 – 5 wt. % range by visual inspection and microstructural techniques.

1. Introduction

PGSs resulting from the one pot synthesis of glycerol and succinic acid were expected to be branched [1]. Dendrimers, hyperbranched, highly branched and branched polymers have attracted a considerable attention in recent years because of their singular characteristics in the field of macromolecules and were defined the chemical structure as depicted in the **Figure 1**. They have the hydrophilic structure and could be further grafted with fatty alkyl chains leading to amphiphilic structures [2]. In our work, we using rice bran oil (RBO) grafted onto PGS to promote fully synthesized bio-sourced surfactant. The synthesized surfactants were applied into the applications of microencapsulation to develop a drug delivery system and test of stability in the mixing of water and oil.

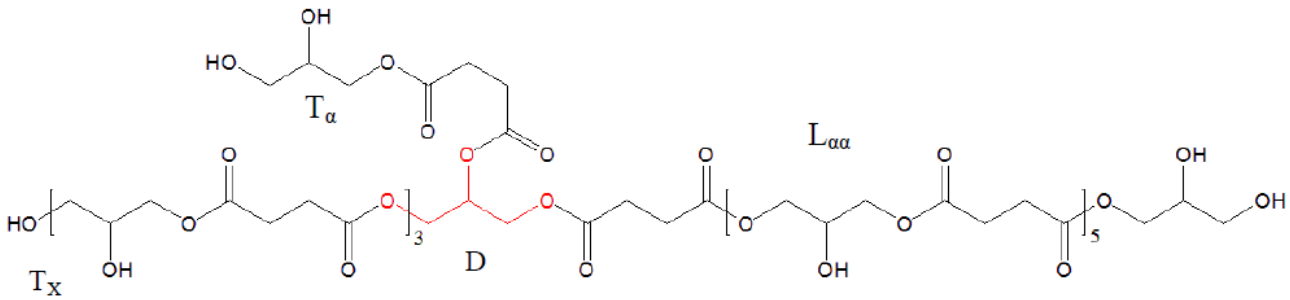

Figure 1. Chemical structure of PGSs, with terminal units (T_X, T_α, T_β), linear units ($L_{\alpha\alpha}$, $L_{\alpha\beta}$) and the dendritic unit (D). $R = CH_2–COOH$ when T_X is a succinyl, $R = H$ when Tx is non-grafted and $R = (CH_2)_n–CH_3$ when T_X is a acyl group of fatty acid.

2. Methods

2.1. One-pot synthesis of non-grafted and grafted poly(glycerol-succinate) oligoester (PGSs)

$PG_{1.5}S$: 0.3 mmol of succinic acid and 0.45 mmol of glycerol were mixed and equipped with a Dean-Stark apparatus. Staring time of reaction was taken when temperature reached 120 °C for 24 h to obtain a brown gel. Grafted PGS oligoester was synthesized following the same procedure as above mentioned with the formulations are described in **Table 1**.

Table 1. Composition of the various molar ratios of functional groups used to synthesis.

PGSs	Alkyl chains	Molar ratios of functional groups			Abbreviations
		G / S	F_R / S	Sor / S	
Non-grafted	–	1.5	–	–	$PG_{1.5}S$
Grafted	Rice bran oil ester	1.5	0.05	–	$PG_{1.5}SF_{R0.05}$
	Rice bran oil ester and sorbitol	1.5	0.01	0.05	$PG_{1.5}SSorF_{R0.01}$

2.2. Characterization of non-grafted and grafted poly(glycerol-succinate) oligoester (PGSs)

Three types of PGS were obtained as one-phase brown gel. The following NMR results indicated whether the α- and β-carbons (primary and secondary alcohol) of glyceride characteristics have been esterified (esterification indicated by D as dendrimer, L as linear and T as terminal) or not and the type of the structural unit (shown in **Table 2**) have been calculated.

Table 2. The succinate and glycerol region ^{13}C NMR spectra of the indicated copolymerized with glycerol.

Functional Group	Chemical shift	PGSs		
		$PG_{1.5}S$	$PG_{1.5}SF_{R0.05}$	$PG_{1.5}SSorF_{R0.01}$
Succinate groups	–$\underline{C}H_2$–COOH	28.70	28.81	28.77
	–$\underline{C}H_2$–COOR	29.48	29.33	29.32
	–CH_2–$\underline{C}$OOR	171.83	172.03	172.04
	–CH_2–$\underline{C}$OOH	-	172.99	-
	–CH_2–$\underline{C}$OO– (sorbitan)	-	-	211.15
Glyceryl groups	$L_{\alpha\beta}$, HO–$\underline{C}H_2$–CHOR–CH_2–OR	60.35	60.37	60.31
	T_β, HO–$\underline{C}H_2$–CHOR–CH_2–OH	60.77	60.71	60.68
	T_α, HO–$\underline{C}H_2$–CHOH–CH_2–OR	62.19	62.22	62.22
	D, RO–$\underline{C}H_2$–CHOR–CH_2–OR	62.55	62.58	62.54
	$L_{\alpha\beta}$, HO–CH_2–CHOR–$\underline{C}H_2$–OR	63.10	63.08	63.08
	T_α, HO–CH_2–CHOH–$\underline{C}H_2$–OR	63.67	63.65	63.56
	$L_{\alpha\alpha}$, RO–$\underline{C}H_2$–CHOH–$\underline{C}H_2$–OR	65.27 & 65.71	65.29 & 65.70	65.27 & 65.59
	T_α, HO–CH_2–$\underline{C}$HOH–$\underline{C}H_2$–OR	67.18	67.14	67.12
	D, RO–CH_2–$\underline{C}$HOR–CH_2–OR	69.44	69.47	69.42
	$L_{\alpha\alpha}$, RO–CH_2–$\underline{C}$HOH–CH_2–OR	69.91	69.93	69.89
	$L_{\alpha\beta}$, HO–CH_2–$\underline{C}$HOR–CH_2–OR	72.61	72.64	72.58
	T_β, HO–CH_2–$\underline{C}$HOR–CH_2–OH	75.96	75.93	75.90

2.3. Applications of PGSs

2.3.1. Formulation of o / w emulsions

The emulsion was prepared by mixing in a ratio 1 : 1 of aqueous phase and oil phase in the presence of $PG_{1.5}SF_{R0.05}$. Aqueous phase were consisted of chitosan (2.5 or 5 wt. %) and gum Arabic (2.5 wt. %) distilled water, added to a final weight of 100 g. The emulsion was mixed at 70 °C and held at 30 °C for 30 min, then immediately homogenised using a homogenizer (Ross High Shear Mixers LSK-I Model, Germany). After homogenisation, the mixture was cooled to 4 °C and aged at this temperature for 4 h. Then, then samples were tested for visual inspection of their stability in 3 conditions: centrifuge 3000 rpm for 5 min, kept at room temperature and at below 5 °C for 7 weeks.

2.3.2. Microencapsulation of curcumin by complex coacervation

For the simple emulsion, active compound (ascorbic acid or curcumin) solution was added to RBO in the presence of $PG_{1.5}SF_{R0.05}$, then slowly added to aqueous chitosan solution to form the double emulsion and followed by adding the above solution to gum arabic solution. pH was adjusted to 4.0 using acetic acid then slow cooling and freeze-dried to obtain the coacervate material for further morphological and FT–IR characterization.

3. Results and discussion

3.1. Synthesis and characterization of PGSs

^{13}C–NMR is a technique for the study of the topology of branched and hyperbranched polymers and oligomers. The degree of branching (DB) has been defined by Fréchet and Frey commonly determined by the relative proportions among the NMR resonance peak areas. All mathematic models were used here to extract these data by quantitative ^{13}C–NMR as shown in **Table 3**. FTIR spectra exhibited a positive peak at 3680 – 3300 cm^{-1} for the –OH stretch, 2932 and 2884.9 cm^{-1} for $–CH_2–$ stretch in core structure, and 1731.6 cm^{-1} for C=O stretch peak of the ester.

Table 3. Conversion and polymerization characteristics of the PGSs.

PGSs	Conversion	DB_{Frey} (%)
$PG_{1.5}S$	94.9	22.61
$PG_{1.5}SF_{R0.05}$	94.4	24.23
$PG_{1.5}SSorF_{R0.01}$	95.7	16.21

3.2. Application

3.2.1. Formulation of o / w emulsions

Simple emulsion showed a thermodynamically unstable system due to flocculation, creaming, coalescence, phase inversion and Ostwald riping [3]. A new synthesised surfactant, $PG_{1.5}SF_{R0.05}$, is introduced as an emulsifier which can stabilise the emulsion by absorption at the

interface, caused lowering the interfacial tension. $PG_{1.5}SF_{R0.05}$ can perform to improve the emulsion stability when used 5 wt. % and combined with5wt% gum Arabic. The emulsion observed to be stable for 2 weeks at room temperature and colorised after 6 weeks as shown in **Figure 2B**.

Figure 2. A) Water: RBO (with $PG_{1.5}SFR_{0.05}$ 2.5 wt. %) in a ratio of 50 : 50; B) Water (with gum Arabic 5 wt. %): RBO (with $PG_{1.5}SF_{R0.05}$ 5 wt. %) in a ratio of 50 : 50.

3.2.2. Microencapsulation of curcumin by complex coacervation

To achieve the coacervation of a core material viable, a water-in-oil emulsion was first prepared using rice bran oil, a 1 % solution of active compound (curcumin or ascorbic acid) and $PG_{1.5}SF_{R0.05}$, as the surfactant. Optimized microcapsule formulations (w / o / w) were prepared containing chitosan, gum arabic and active compound at ratios of 1 : 1 : 0.2, with 0.004 g / mL of the $PG_{1.5}SF_{R0.05}$.

3.2.2.1. Loading efficiency

The quantitative of active compound (ascorbic acid or curcumin) loading efficiency in the complex coacervated was measured following the equation

$$\eta = \frac{C_f}{C_i} \cdot 100\ \%,$$

where C_f and C_i were the final and initial loading contents of curcumin. We have received the standard curve from UV–Vis spectroscopy, which has revealed equation as followed $y = 0.1555x - 0.0027$, $R^2 = 0.9998$ for curcumin and $y = 0.0359x - 0.0956$, $R^2 = 0.9945$ for ascorbic acid. The result of loading efficiency for curcumin was higher than 97.96 % (average of 98.040 ± 0.112 %) but loading efficiency for ascorbic acid was lower than 44 %. It is suggested that the formation of these complex coacervation is in agreement with typical microencapsulation of hydrophobic agents using complex coacervation reported to produce high encapsulation efficiency. In addition, the microencapsulation of hydrophilic active agents by complex coacervation often reported to produce low EE [4].

3.2.2.2. Morphological characterization of the microcapsules by optical microscopy

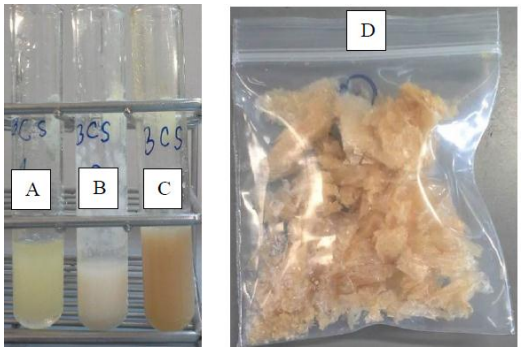

Figure 3. Blank appearance of (A) simple emulsion (w / o); (B) double emulsion by 5 wt. % chitosan solution (w / o / w); (C) complex coacervation and (D) freeze-dry.

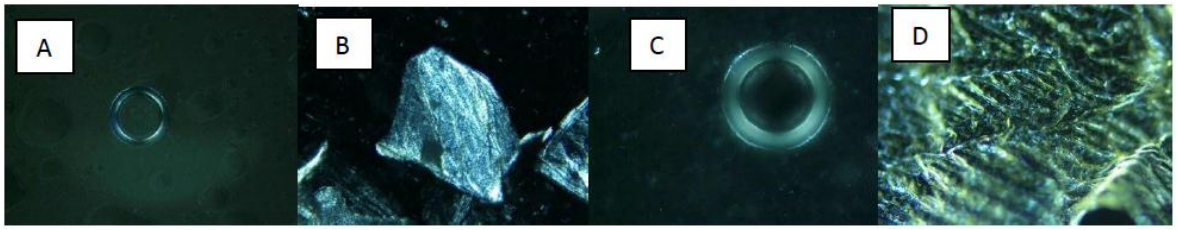

Figure 4. Blank micrograph of A) the simple emulsion (20 ×). B) the double emulsion by 5 wt. % chitosan solution (40 ×). C) the coacervated microcapsule (prior to freeze-drying) (40 ×). D) freeze-dried coacervated microcapsules (10 ×).

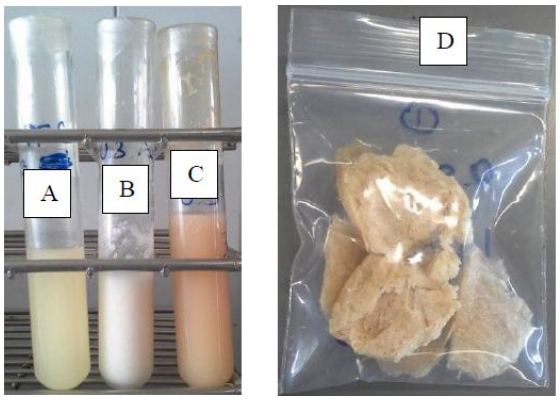

Figure 5. Ascorbic acid in (A) simple emulsion (w / o); (B) double emulsion by 5 wt. % chitosan solution (w / o / w); (C) complex coacervation and (D) freeze-dry.

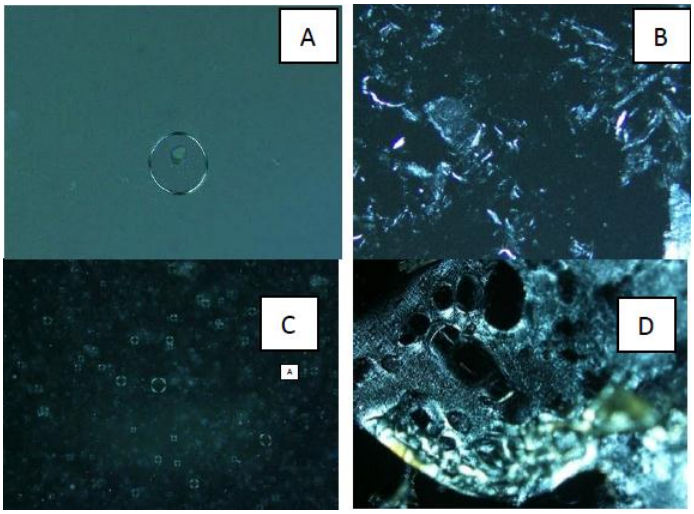

Figure 6. Micrograph of ascorbic acid in A) the simple emulsion (20 ×). B) the double emulsion by 5 wt. % chitosan solution (20 ×). C) the coacervated microcapsule (prior to freeze-drying) (10 ×). D) freeze-dried coacervated microcapsules (10 ×).

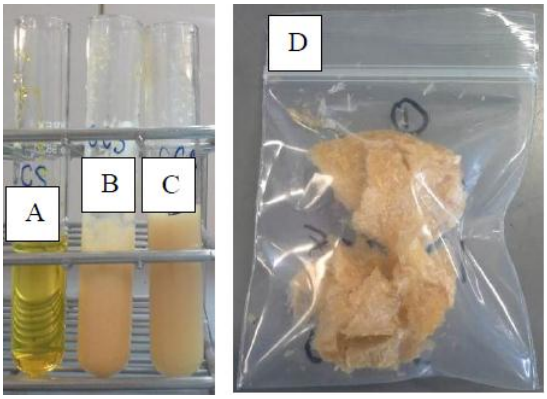

Figure 7. Appearance of Curcumin in (A) simple emulsion (w / o); (B) double emulsion by 5 wt. % chitosan solution (w / o / w); (C) complex coacervation and (D) freeze-dry.

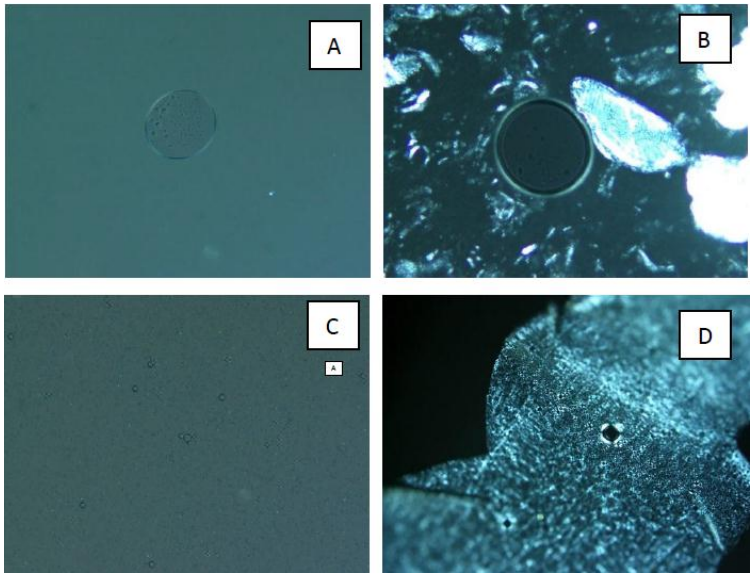

Figure 8. Micrograph of Curcumin in A) the simple emulsion (20 ×). B) the double emulsion by 5 wt. % chitosan solution (20 ×). C) the coacervated microcapsule (prior to freeze-drying) (10 ×). D) freeze-dried coacervated microcapsules (10 ×).

The formation of an oil drop in water was occurred and revealed simple emulsion with low stability supported by the picture (**Figure 3A**) and microscope image (**Figure 4A**). Loadings of ascorbic acid and curcumin were investigated with homogenous appearance (**Figures 5A** and **7A**) and showed the uptake images in **Figures 6A** and **8A**, respectively. **Figures 4B** and **6B** showed no optical microscope images of the double emulsion after adding chitosan solution as wall material. The presence of droplets on the microscope slide of figure 8B indicated the successful formation of the double emulsion when loading the o / w emulsion with curcumin. All pictures of complex coacervation revealed pink creamy emulsions according to gum Arabic used in the coacervation process and showed in **Figures 3C**, **5C** and **7C**. **Figure 4C** revealed thicker wall image of microcapsule successfully formed. The freeze-dried microcapsules were observed and showed the agglomerated microcapsules formed connections with each other through solid bridges in their microscope images (**Figures 6D** and **8D**).

3.2.2.3. Fourier transform infrared spectroscopy (FT–IR)

The spectrum of microcapsule was obtained in the range from 4000 to 400 cm^{-1} (**Figure 9**). During complex coacervation, the carboxyl groups of polysaccharides interact with the amino groups of proteins in gum Arabic to form a complex containing an amide bond. The spectrum of chitosan and $PG_{1.5}SF_{R0.05}$ showed intense vibrations between 3663 and 3166 cm^{-1}. The peak that appears the intensity at approximately 1749 cm^{-1} for the gum Arabic is characteristic of carboxylic groups that are negatively charged. The binding of positive and negative charges (i.e., amino and carboxyl groups) is expected to promote the process of coacervation and the formation of amides. Major peaks can be observed at approximately 1661 and 1457 cm^{-1}, which could indicate the presence of an amide, confirming the formation of this complex. The peak appearing at approximately 1108 cm^{-1} for the gum Arabic was characteristic of the amine groups, which can be positively charged.

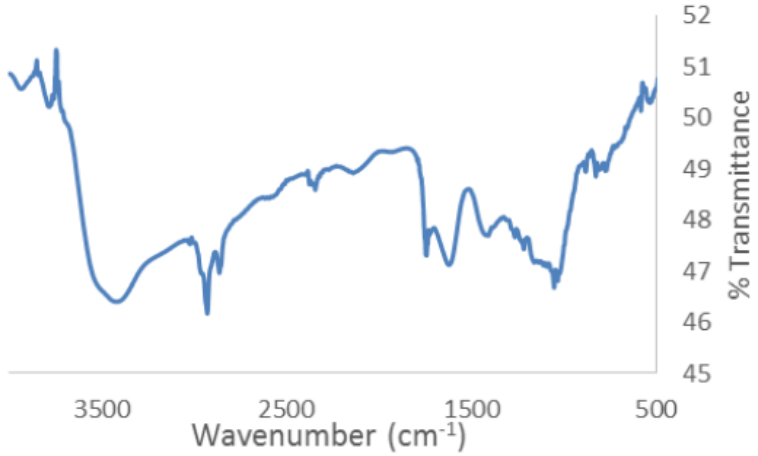

Figure 9. FT–IR spectra of the freeze-dried coacervated microcapsules loading of curcumin.

4. Conclusions

The one pot PGSs synthesis in various molar ratios of functional groups showed that grafted $PG_{1.5}SF_{R0.05}$ was successfully synthesized and had a good in physico-chemical behavior of the surfactant. $PG_{1.5}SF_{R0.05}$ has been successfully applied in emulsion formulation to improve the

textural characteristics and stability of emulsion, while gum arabic is usually added to increase the viscosity and gelling properties. The application in formulation of the microencapsulation was confirmed by FT–IR, which could indicate the presence of amide (to promote the process of coacervation). In a drug delivery system, $PG_{1.5}SF_{R0.05}$ can be applied for complex coacervation formed by chitosan solution (see [5]) with highly loading efficiency of curcumin.

References

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