Effect of Gelatin/Xanthan Gum Ratios on Jackfruit Leaf Extract Nanoemulsion Gel Stability and Properties

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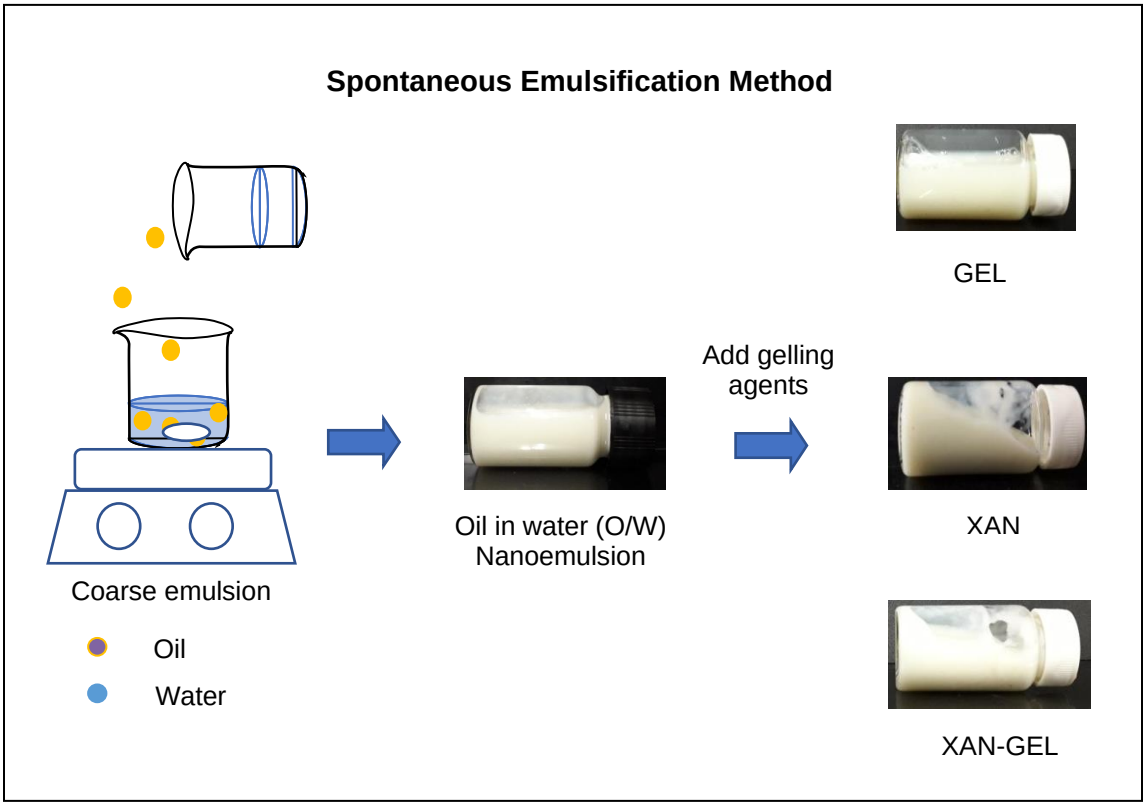
GRAPHICAL ABSTRACT



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Nanoemulsions were formulated as hydrogels, incorporating extract from jackfruit leaf (JLE) and consisting of a blend of xanthan gum and fish gelatin (XAN-GEL). Utilizing the spontaneous emulsification technique, single xanthan gum (XAN), single fish gelatin (GEL), and XAN-GEL blends were hydrated at a mass ratio (0.5 to 2.5% w/v) and were prepared and subsequently assessed for various parameters such as droplet size, polydispersity index (PDI), zeta potential, colloidal stability, and viscosity. Additionally, plain nanoemulsion (NE) and carbomer were synthesized as control samples for comparative analysis. The droplet size, PDI, and zeta potential values of the developed nanoemulsion and gelled nanoemulsion fell within the ranges of 131 to 223 nm, 0.23 to 0.33, and -27.3 to -47.3 mV, respectively. Notably, all formulations exhibited stability except for the lower amount of XAN ratio blends at 1.0% (MD) and 0.5% (ME). Furthermore, all stable nanoemulsion gels demonstrated shear thinning behavior, and the highest amount of XAN ratio blends at 2.0% (MA) enhanced the viscosity of the nanoemulsion by fine tuning the rheological characteristics of the targeted gelled nanoemulsion suitable for future topical drug delivery application.

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Keywords: Artocarpin; Fish gelatin; Jackfruit leaf extract; Nanoemulsion gel; Xanthan gum

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INTRODUCTION

Nanoemulsion gels are innovations in designing drug delivery systems to enhance the delivery of bioactive compounds, especially those that are lipophilic compounds, since they are poorly soluble and tend to suffer from low bioavailability. Enhanced performance has been demonstrated by integrating nanoemulsion formulation with gelling agents, thereby combining the advantage of small droplet size and enhanced stability with better application properties for topical delivery. The integration of characteristics not only facilitates enhanced solubility and bioavailability of lipophilic drugs but also provides a controlled release mechanism and ease of application. This results in significant improvement of drug delivery systems and addresses the challenges in conventional drug delivery systems such as poor solubility, low absorption, rapid degradation, hypersensitivity reactions, epidermal thinning, and inadequate drug concentration at the action site, resulting in poor patient acceptance (Coondoo *et al.* 2014; Irfan and Gouda 2024). Previous work by Atanase *et al*. (2015) reported that their synthesized novel triblock copolymers as gelling agents significantly enhanced stability and solubility of Miglyol 812/PEG 400 nanoemulsion by optimizing their concentration.

There have been several reports on the development of topically administered nonsteroidal anti-inflammatory drugs (NSAIDs), which have drawbacks such as poor aqueous solubility and bioavailability (Elmateeshy *et al*. 2018; Anita *et al.* 2021). Poor drug permeability may lead to expensive therapy costs and reduced patient compliance in relation to the requirement for long term use of topical medications (Tan *et al.* 2012). The efficacy of a topical product should include desirable and optimum consistency of formulation, which helps deliver and safeguard a suitable dose applied to the target application site. Unfortunately, the protective integrity of the human skin limits the permeability of many active agents in some topical formulations. One of the most important drawbacks of nanoemulsions designed for topical use is their low viscosity, which leads to a propensity for rapid dislodgement from the skin surface (Froelich *et al.* 2017).

Xanthan gum and fish gelatin are widely recognized natural biopolymers in food and pharmaceutical applications due to their excellent gelling, emulsifying, and biocompatible properties. Adding these gelling agents into conventional nanoemulsion enhances the structural integrity and stability of the formulation, making it better for sustained release applications. The synergistic action of xanthan gum and fish gelatin in nanoemulgel formulations offers distinct advantages. Xanthan gum, a microbial type of polysaccharide, contributes excellent viscosity control and stabilizing properties, making it ideal for ensuring the structural integrity of the gel matrix during application. Fish gelatin, derived from natural sources, enhances biocompatibility and provides additional gelling and stabilizing properties to the nanoemulgel formulations. Together, both biopolymers improve the mechanical strength and water retention capacity of the formulation that is essential for the prolonged release of active compounds (Wang *et al.* 2018; Yin *et al.* 2021).

Jackfruit, known scientifically as *Artocarpus heterophyllus* Lam, (Moraceae family) is a widely favored tropical fruit with a rich history of consumption in Malaysia. The edible components, encompassing the flesh and seeds, are typically consumed fresh, cooked through boiling or frying, and incorporated into a diverse array of food items such as purees, juices, freeze-dried snacks, and flavors for ice cream. Conversely, the inedible segment, notably the leaves, has been identified to harbor medicinal properties characterized by antioxidant, anti-inflammatory, antimicrobial, anticancer, hypoglycemic, and hypolipidemic attributes. Its incorporation into an edible formulation could leverage these benefits for skin applications, including wound healing and protection against microbial infection (Chan *et al.* 2018).

Despite the advancements in nanoemulsion-based delivery systems, the potential of combining xanthan gum and fish gelatin for stabilizing nanoemulgels containing the plant-based bioactives jackfruit leaf extract (JLE) remains underexplored. Furthermore, while JLE offers many health benefits, its efficient delivery through a gelled nanoemulsion formulation to enhance topical therapeutic efficacy lacks comprehensive investigation. Therefore, this study aimed to develop polymer blends comprising xanthan gum and fish gelatine-enhanced nanoemulsions, with the goal of achieving improved and efficient gelled nanoemulsion properties. The effects of the polymer blends on the characteristics of the formulation were investigated.

EXPERIMENTAL

Materials

Fresh jackfruit leaves were obtained from the Organization of Farmers plantation in Maran, Pahang Darul Makmur, Malaysia. The Palmester 3575 (caprylic/capric triglyceride) oil was provided by KLK Oleo (Palm Oleo Sdn. Bhd). Local purchases were made for coconut and palm kernel oils. The procurement of surfactant Tween 80 (polyethylene glycol sorbitan monooleate, T80), Tween 40 (polyoxyethylene sorbitan monopalmitate, T40), Span 80 (sorbitan monooleate, S80), and phosphate buffer was carried out from R&M Chemicals. Fish gelatin, xanthan gum and artocarpin standard were acquired from Sigma Chemical Co. (St Louis, MO, USA), Merck (Darmstadt, Germany) and Chemfaces (Wuhan, China), respectively.

Preparation of Jackfruit Leaf Extract (JLE)

Jackfruit leaf extract was prepared using a maceration method as mentioned by Mustafa *et al*. (2020). The sample was macerated in ethanol/water (80%, v/v) and left in an incubator shaker with stirring at 200 rpm continuously for three days. The specimens underwent filtration, followed by solvent removal using a rotary evaporator, resulting in the formation of a dense green residue.

Selection of Oils

The solubility of JLE in different types of oils, namely Palmester 3575, coconut oil, and palm kernel oil, was determined by Hussain *et al*. (2016). Excessive JLE was combined with 1 mL of oil in sealed vials for duration of 72 h at 37 °C in an isothermal water bath shaker (Memmert, type 3047, Germany). Subsequently, the solvent-drug mixture underwent centrifugation at 5000 rpm (Centrifuge-5804R, Eppendorf, Germany) for 15 min. The resulting supernatant was diluted with ethanol, and the concentration of the solubilized extract was assessed using an ultraviolet visible (UV-VIS) spectrophotometer (Shimadzu UV1800; Shimadzu Scientific Instruments, Columbia, MD, USA) at a wavelength of 265 nm with ethanol as the blank.

Selection of Surfactant Ratios

The hydrophilic lipophilic balance (HLB) is a numerical value that is specific for a particular emulsifying agent expressing the strength of the hydrophilic and lipophilic part of the molecule (emulsifying agent) that is required to produce a physically stable emulsion. The HLB of the surfactant blends was calculated using equation in Eq. 1,

$$HLB_{mix} = x(HLB_A) + y(HLB_B) \quad (1)$$

where *A* is surfactant polysorbate 80 (Tween 80;T80), *B* is surfactant sorbitan monooleate 80 (S80), x is the ratio of T80 and *y* is the ratio of S80. To develop nanoemulsion, the concentration ratios of surfactant components were subjected to initial screening. The surfactant components were blended in different ratios ranging from 10:0 to 6:4 (Table 1). Different ratios of surfactant components were titrated by adding the oil phase (oils and surfactants) dropwise into the water phase until all components dissolved. All mixtures that formed transparent oil in water system were checked for particle size. The smallest particle size was used to prepare the nanoemulsion. The turbidity of each formulation was observed visually.

Table 1. Mass Ratio of Surfactant Blends Polyethylene Glycol Sorbitan Monooleate 80 and Sorbitan Monooleate 80 (T80 : S80)

Surfactant	Mass Ratio				
T80	6	7	8	9	10
S80	4	3	2	1	0

Construction of Pseudoternary Phase Diagram

The phase behavior of the mixed surfactant system (T80:S80) was investigated by constructing a pseudoternary phase diagram (PTD). The pseudoternary phase diagram was plotted using the Ternary Plot Chemix School version 8 software. The phase state was classified according to physical appearance, which was categorized into isotropic, homogeneous, and two or multiphase regions (Mahdi *et al.* 2011).

Preparation of Nanoemulsion

Jackfruit leaf extract (JLE) was dissolved in the selected oil phase based on the selection of oils and surfactants that have the highest solubility reading measurements determined by UV-Vis spectrophotometer. Nanoemulsion was prepared using spontaneous emulsification method with modifications (Mehrnia *et al.* 2016). Seventy milligrams (70 mg) of JLE was added to the surfactant mixture (smix) at the determined ratio and mixed continuously using a hotplate stirrer until fully dissolved. Finally, the oil phase (JLE, smix, and Palmester 3575 oil) was incorporated into the water phase by drop-wise addition (ratio 2:8) at 50 °C operating at 700 rpm to obtain a homogenous JLE nanoemulsion using the spontaneous emulsification method. This nanoemulsion served as the control and labeled NE.

Development of Nanoemulsion Gel

A single polymer, xanthan gum (XAN), fish gelatin (GEL), and blends of xanthan gum – fish gelatin (XAN-GEL) were hydrated at a mass ratio (0.5 to 2.5% w/v) with maximum polymer(s) added at 2.5% (w/v) in a sufficient quantity distilled water overnight on a hotplate stirrer operating at 700 rpm at 50 °C. The complexes were kept for 24 h to allow complete swelling. The nanoemulsion gel formulations were formulated by incorporating JLE nanoemulsion into the prepared gels in a 1:1 ratio, resulting in a nanoemulsion-based gel (Elmataeeshy *et al.* 2018). The samples were labeled XA -XC for nanoemulsion containing xanthan gum, GA-GC for nanoemulsion incorporated with fish gelatin, and MA-ME for nanoemulsion blends with XAN-GEL. Carbopol was prepared using the identical procedure and served as the positive control.

Characterization of Nanoemulsion and Nanoemulgel

Droplet size, polydispersity index and ζ-potential

The determination of particle size, polydispersity index (PDI), and zeta potential (ζ potential) of the specimens was performed using a dynamic light scattering (DLS) droplet size analyzer (Zetasizer Nano ZS90, Malvern Instruments, UK). The samples were diluted 100 times with deionized water before being introduced into the cuvette capillary cells to avert multiple scattering effects. Measurements were taken at an angle of 173° at room temperature (25 ± 0.5 °C) (Elmataeeshy *et al.* 2018). Measurements of each sample are reported in triplicate with the mean ± SD.

Stability study

The nanoemulsion and nanoemulgels produced were subjected to various thermodynamic stability assessments, including freeze-thaw cycles, centrifugation, and heating and cooling, as outlined by Elmataeeshy *et al.* (2018). All formulations underwent three freeze-thaw cycles between -13 °C and 25 °C for 24 h at each temperature to observe phase separation. Centrifugation tests were executed on the samples for 30 min at 5000 rpm. All formulations were exposed to six cooling and heating cycles between 4 °C and 45 °C by storing them at each temperature for 48 h. Subsequent observation was carried out to detect any precipitation or separation.

Viscosity

The measurement of viscosity or steady state flow was conducted to investigate the viscosity of the formulations (Barradas *et al.* 2018).The viscosity of the optimized nanoemulsion was analyzed using a dynamic rheometer (AR-G2; Thermo Electron Corporation, TA Instruments, USA). The measurements were performed with a 1° angle/60 mm cone and plate geometry (steel) with a gap set at 30 µm at the rheometer base (Peltier plate) at a temperature of 25 °C controlled by a temperature control unit (AWC 100, Julabo, Germany). The experiments were carried out in the continuous mode at a shear rate at a controlled rate range between 0.01 and 100 s^{-1}. Prior to measurement, the sample was allowed to stand for 10 min after loading to reach equilibration. Measurements were performed in triplicate. Viscosity was calculated as a measure of the shear rate. The power law equation (Eq. 2) was used to fit the association between the shear stress and shear rate for each formulation.

$$\tau = \kappa \gamma^{n} \quad (2)$$

where τ is the shear stress, κ is the flow consistency index (Pa.s) is the shear rate or the velocity gradient perpendicular to the plane of shear, and n is the flow behavior index (dimensionless).

Statistical Analysis

All data were evaluated employing the Minitab 16 software (Minitab Inc., State College, PA, USA). Analysis of variance (ANOVA) was used to compare significant differences between samples. The information is displayed as mean ± standard deviation. At p-values < 0.05, differences were deemed statistically significant. All analyses were carried out in triplicates.

RESULTS AND DISCUSSION

Selection of Oils

The selection of caprylic/capric triglyceride (commercially known as Palmester 3575), coconut oil, and palm kernel oil as excipients was based on their biocompatibility and low toxicity. The solubility of JLE in various excipients is presented in Fig. 1. Among them, JLE exhibited the highest solubility in caprylic/capric triglyceride, followed by coconut oil and palm kernel oil. The solubilization capacity depends on the type of oil. Caprylic/capric triglyceride is classified as a medium chain triglyceride (MCT) with the ability to dissolve a variety of drugs. It is commonly used in nanoemulsion systems and exhibits improved stability in formulations (Severino *et al*. 2011; Zulfakar *et al*. 2024).

Selection of Surfactants

As shown in Fig. 1, JLE demonstrated the highest solubility in the T80:S80 blends (8.39 ± 0.01 mg/mL). The combination of T80:S80 surfactants were chosen due to their biodegradability, biocompatibility, and stability in various applications. The 8:2 ratio of T80:S80 blend solubilized slightly more JLE (8.39 ± 0.01 mg/mL) than T80 (7.99 ± 0.13 mg/mL) in the formulation, resulting in a stable nanoemulsion. This performance is attributed to the polyoxyethylene, a hydrophilic head group in the surfactant used. The Tween 80 surfactant product is derived from polyoxyethylene sorbitan (head group) and oleic acid (tail group), with the tail group comprising a lengthy chain C18 unsaturated oleic acid. The maximum water solubilization capacity is influenced by the oxyethylene chain and configuration of the polar head group and hydrocarbon moiety of nonionic surfactants. Moreover, the presence of unsaturated trioleate affects the solubilization capacity, with a lipophilic tail group structurally resembling the octyl group of the caprylic/carpic triglyceride, aiding in the efficient packing of the surfactant at the interface. Caprylic/capric triglyceride exhibits less interaction with high amounts of lipophilic surfactants such as sorbitan monooleate 80 or surfactant blends with low HLB values. In this study, a desired HLB mix of T80:S80 blend at 12.86 was used, enabling higher solubilization of JLE compared to other surfactant blends. This emphasizes the importance of carefully selecting surfactant blends with strong solubilization capacities for enhanced stability.

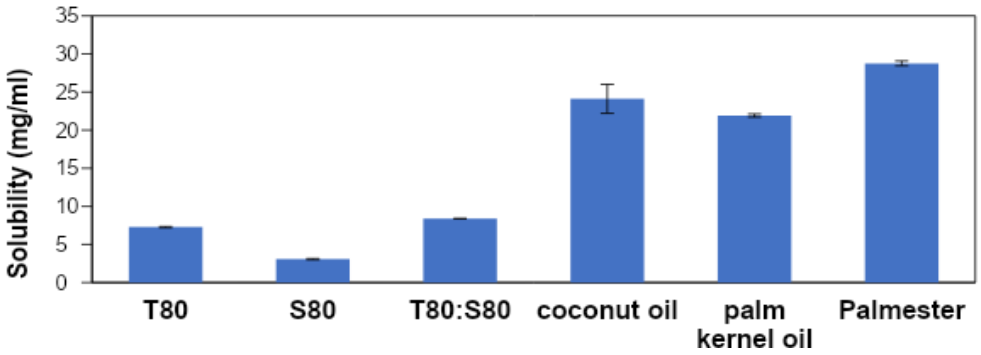

Fig. 1. Solubility of JLE in selected surfactants, surfactant mixtures and selected oils

The choice of surfactant plays a crucial role in emulsion formulation, as optimal surfactant selection or blends can reduce surface tension and enhance droplet stability against recoalescence. Mahdi *et al.* (2011) reported that a blend of T80:S80 at a 9:1 ratio resulted in increased solubility of the extract studied. Tavano *et al.* (2011) compared different amounts of polyethylene glycol sorbitan monooleate 80/sorbitan monooleate blends with HLB values ranging from 6 to 15, noting stable nanoemulsions produced at higher HLB values (HLB 10 and above) with no separation, clear and isotropic characteristics, particularly at an HLB of 12. Generally, small molecular surfactants such as polyethylene glycol sorbitan monooleates and sorbitan monooleate are employed in nanoemulsion preparation using low-energy methods such as spontaneous emulsification (Walia *et al.* 2022). The combination of T80 and S80 has been shown to improve the stability of oil in water (O/W) emulsion systems (Salim *et al.* 2016).

Construction of Pseudoternary Phase Diagram

Analyzing the region of NE in the phase diagram is imperative for the successful advancement of an optimal NE (Sharma and Tailang 2020). Therefore, the construction of

a PTD is crucial in ascertaining the concentration variation of components for the existence of NEs. The PTD (Fig. 2) was established utilizing caprylic/carpic triglyceride as an oil phase, polyethylene glycol sorbitan monooleate 80 and sorbitan monooleate 80 as a surfactant mixture (smix), and water as the aqueous phase to determine the most appropriate composition for nanoemulsion formation. To achieve a desired formulation, the selection of surfactants is of critical importance. Each surfactant offers a specific HLB value. The combination of surfactants of T80:S80 in an 8:2 ratio resulted in an oil-in-water nanoemulsion region depicted as a single phase. The formulation was transparent, translucent, and exhibited easy flow. The creation of a single-phase system within the diagram manifested as a clear solution without any separation, indicating isotropic behavior, implying that the surfactant blend effectively reduced the surface tension between the aqueous and oily phases, thereby facilitating nanoemulsion formation. The determination of the phase boundary was based on the observation of sample appearance transitioning from turbid to transparent or vice versa. The presence of liquid crystalline was confined to the monophase region. It has been documented that the existence of liquid crystalline phases along the emulsification pathway significantly influenced nanoemulsion formation. The creation of a liquid crystalline layer is attributed to hydrogen bonding between the hydroxyl group of the oxyethylene group of T80 and the hydroxyl group of water, leading to the formation of a gel network, as discussed by Caldero *et al.* (2016). Typically, the hydrogen bonding is disrupted upon dilution with water. The breakdown of the liquid crystalline system with increased water addition emphasizes the importance of hydrogen bonding in the development of the liquid crystalline structure, especially in cases where there is a lack of OH- group with the oxyethylene moiety compared to the rising water content.

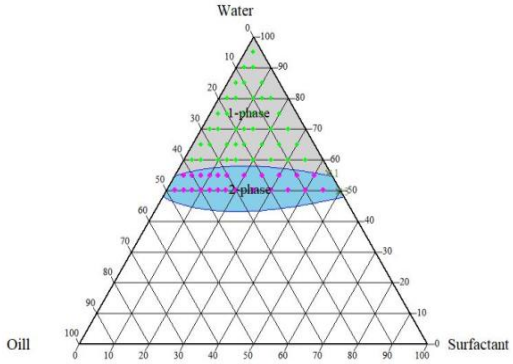

Fig. 2. Pseudoternary phase diagram of T80 and S80 at the ratio 8:2 dispersed in water

Preparation of Nanoemulsion

According to the construction of the pseudoternary phase diagram, an optimum JLE nanoemulsion was prepared by mixing components of water phase and oil phase at ratio of water (80%), surfactants blend (10%) and caprylic/capric (10%). It was found that after mixing all the components, the nanoemulsion (NE) visibly appeared to be translucent.

Development of Nanoemulgel

Nanoemulgel was developed by enhancing the nanoemulsion (NE) system with xanthan gum (XAN), fish gelatin (GEL) as well as xanthan gum-gelatin (XAN-GEL) blends and Carbopol® as a control system. The prepared JLE incorporated with nanoemulgel was found to exhibit smooth, clear, and homogenous texture.

Characterization of Nanoemulsion and Nanoemulgel

Droplet size, polydispersity index and ζ-potential

Table 2 illustrates the droplet size, polydispersity index (PDI), and ζ potential of the developed nanoemulsion and nanoemulgels. The nanoemulsion displayed a droplet size of 131 nm and a PDI of 0.23, while the zeta potential was found to be -27.3 mV. In the case of the nanoemulsiongel formulated with a single polymer, droplet sizes ranged from 172 to 230 nm for XA-XC and 138 to 142 nm for GA-GC, with corresponding PDI values of 0.30 to 0.33 and 0.25, and zeta potential values varying from -47.3 mV to -31.2 mV. XAN exhibited larger droplet sizes compared to GEL and NE, which was attributed to its high molecular weight (2×10^6 to 2×10^7) linear molecular properties, resulting in greater hydrodynamic volume and increased viscosity at lower concentrations. The addition of XAN in the nanoemulsion gel enhanced stability by providing higher charge densities and forming a dense polymer network. Conversely, fish gelatin (GEL) is characterized by a high molecular weight polypeptide polymer and differs from mammalian gelatin due to its lower proline (Pro) and hydroxyproline (Hyp) contents, leading to lower gel modulus, as well as lower gelling and melting temperatures. The PDI serves as a measure of particle homogeneity, ranging from 0.0 to 1.0, with lower values indicating greater homogeneity within the formulation (Danaei *et al*. 2018). In this study, NE and GA-GC exhibited lower PDI values compared to other formulations, suggesting a more uniform distribution of droplet sizes in these systems than in XAN and XAN-GEL blends. The ζ potential, reflecting surface charge interactions and repulsion forces within the emulsion system, influences its physical stability. Notably, the ζ potential of XAN formulations surpassed that of NE and GEL, indicating enhanced stability and well-separated emulsion globules.

Table 2. Size, PDI, ζ Potential of NE, XAN, GEL and Blends of XAN-GEL (MA-ME) Formulations

Composition (%) XAN	GEL	Formulation	Size (nm)	PDI	ζ-potential (mV)
-	-	NE	130.9 ± 0.21	0.23 ± 0.01	-27.32 ± 0.01
-	-	Carbopol	461.1 ± 3.45	0.31 ± 0.02	-41.21 ± 0.01
1.5	-	XA	171.6 ± 2.69	0.30 ± 0.01	-46.33 ± 2.77
2.0	-	XB	199.7 ± 4.81	0.31 ± 0.01	-46.97 ± 2.03
2.5	-	XC	222.9 ± 2.66	0.33 ± 0.01	-47.34 ± 1.39
-	2.5	GA	137.5 ± 1.20	0.25 ± 0.02	-31.24 ± 2.39
-	5.0	GB	140.0 ± 2.06	0.25 ± 0.03	-32.92 ± 1.23
-	7.5	GC	142.1 ± 1.69	0.25 ± 0.03	-34.57 ± 1.45
2.0	0.5	MA	214.5 ± 2.33	0.32 ± 0.01	-46.39 ± 0.22
1.5	1.0	MB	185.8 ± 1.45	0.31 ± 0.01	-45.72 ± 0.33
1.25	1.25	MC	167.6 ± 1.22	0.31 ± 0.01	-43.09 ± 0.77
1.0	1.5	MD	164.5 ± 1.13	0.30 ± 0.01	-40.76 ± 0.25
0.5	2.0	ME	155.0 ± 3.26	0.25 ± 0.01	-38.62 ± 0.13

The nanoemulsion gel blends of XAN-GEL demonstrated variations in droplet size, PDI, and ζ potential values, with a discernible increasing trend across the studied properties. Higher xanthan gum additions, from ME (0.5%) to MA (2.0%), led to larger droplet sizes, slightly elevated PDI values, and more strongly negative ζ potential charges. This behavior may be linked to the bridging of the polymer and effects of the charge

neutralization. When the fish gelatin content was higher in ME (2.0% GEL) in comparison to MA (0.5% GEL) formulations, the viscosity of the system was predominantly influenced by GEL-GEL interactions. Conversely, by incorporating xanthan from MD to MA, the XAN-XAN interactions became the primary factor. The lower concentration of xanthan gum could potentially result in a decreased adsorption of available cationic molecules to the negatively charged droplets. As a consequence, these cationic molecules are dispersed among the gelatin droplets, where they are anticipated to independently adsorb onto each individual droplet. Additionally, the cationic molecules tend to neutralize the negative charge, and an escalation in xanthan gum concentration results in partial or complete charge neutralization. This neutralization of droplet charges diminishes repulsive forces, prompting the droplets to come closer to each other, possibly leading to aggregation and an enlargement in droplet particle size (Abbas *et al.* 2015). A study by Yahoum *et al.* (2023) has noted that increasing xanthan gum concentration results in larger droplet sizes. In the present investigation, carbomer 940 usage resulted in larger droplet sizes at a 2% (wt/wt) concentration compared to other formulations. This may be attributed to agglomeration at higher concentrations using low-energy methods, which may necessitate prolonged mixing and vigorous agitation to achieve full dispersion of the polymer. However, excessive exposure to high shear forces could potentially damage the polymer, leading to reduced viscosity. Nonetheless, the particle sizes of carbomer 940 remained within the nano size range of 20 to 500 nm (Arianto *et al.* 2020).

The PDI values of MA-ME indicated a relatively narrow size distribution of globules, with a PDI value below 0.4, suggesting successful production of nanoemulsions and nanoemulsion gels through spontaneous emulsification. The results showed that all formulations maintained nanoscale sizes throughout the storage period. Alhamdany *et al.* (2021) also observed that PDI values below 0.4, reflecting the uniformity and homogeneity of formulations. Higher xanthan gum content in XAN and XAN-GEL blends decreased the zeta potential of both single and mixed formulations due to the strongly negative charge of xanthan gum compared to the weakly positive charge of fish gelatin. The reduced ζ potential in the mixture containing GEL and XAN was attributed to electrostatic interactions, potentially between the negative surface charges of xanthan gum's polar groups (such as carboxyl: COO- and glucuronic acid residues containing pyruvate groups) and the positive charges of amino acid residues, thus affecting the zeta potential values of the systems (Yin *et al.* 2021). The negative charges of xanthan gum's polar groups enhance the overall negative charge at the surface of the nanoemulsion, leading to increased negatively charged ζ potential values.

Colloidal stability

Results of stability tests of nanoemulsion and nanoemulgel are delineated in Table 3. The outcomes suggest that all formulations remained physically stable without any indications of precipitation, sedimentation, cracking, or phase separations, except for MD and ME nanoemulsion gels, which exhibited instability during the storage stability period. Both formulations displayed signs of phase separation and cracking, possibly due to an excess of GEL compositions (1.5% in MD and 2.0% in ME) compared to XAN compositions, rendering them kinetically unstable. The hydrophilic group's attraction to water may lead to excessive water penetration into the oil bulk, causing interfacial disruption of droplets and subsequent phase separation. A phase separation system phenomenon occurs when biopolymer complexes become thermodynamically incompatible, resulting in the emergence of a protein-rich phase (Maphosa and Jideani

2018). Only formulations passing the thermodynamic stability tests proceeded to the subsequent evaluation study.

Viscosity

The investigation was conducted on the viscosity of formulations that remained stable throughout the storage period, revealing an increase in viscosity with increasing XAN content, as depicted in Fig. 3. The results indicated that carbomer 940, XAN, and XAN-GEL blends exhibited shear-thinning characteristics. The addition of GEL to XAN-GEL blends (MA – MC) improved the nanoemulsion gel systems and significantly impacted the formulation by promoting a more homogeneous structure (Yin *et al.* 2021). This was achieved through a synergistic gelation mechanism involving the formation of interpolymer complexes between XAN-GEL (Wang *et al.* 2018). The integration of biopolymer blends led to a lower flow index, suggesting an enhancement in the flow properties of the gel compared to single xanthan gum formulations (XA-XC). Notably, the incorporation of 0.5% GEL in MA nanoemulsion gel formulation resulted in viscosity outcomes similar to those of a single xanthan gum (XC) formulation with a 2.5% w/v concentration.

The optimal ratio of XAN/GEL in MA formulation positively influenced the viscosity of the produced nanoemulsion gel. The addition of xanthan gum was found to strengthen the network formation by establishing stronger bonds around the oil droplets, thus ensuring droplet stability and increasing the viscosity of the nanoemulsion gels (Syed Azhar *et al.* 2018). The shear-thinning behavior of the nanoemulsion gel suggests the presence of network entanglements among the natural polymer chains. These entanglements act as physical nodes that hinder the free flow of the systems, consequently leading to increased viscosity. A similar trend was observed in a previous study on nanoemulsions stabilized with saponin (Nejatian and Abbasi 2019). The findings indicated that nearly all concentrated nanoemulsions exhibited shear-thinning behavior that conformed well to the power law model. Conversely, NE and GA-GC displayed Newtonian behavior, characterized by a flow behavior index (n) of 1, as shown in Table 4.

The viscosity of NE and GA-GC remained unaffected by the applied shear rate, indicating Newtonian properties. The flow behavior properties of the samples aligned with the power law model, with the k-value theoretically correlating with the viscosity trend. Analysis in Table 4 revealed a consistent pattern between the effects of independent variables on k-value and viscosity (Fig. 3). Higher k-values (>12) were observed in XAN and MA formulations, indicating superior nanoemulsion structure (Musa *et al.* 2017). In summary, Carbopol®, XA-XC, and MA–MC nanoemulsion gels with an n-value below 1 exhibited shear-thinning or non-Newtonian behavior, while NE and GEL formulations displayed a flow behavior index of 1, indicative of Newtonian properties regardless of the applied shear rate.

Table 3. Stability Tests of NE, XAN, GEL and blends of XAN-GEL (MA-ME) Formulations

Formulation	Centrifugation	Heating and cooling	Freeze thaw cycle
NE	√	√	√
Carbopol	√	√	√
XA	√	√	√
XB	√	√	√
XC	√	√	√
GA	√	√	√
GB	√	√	√
GC	√	√	√
MA	√	√	√
MB	√	√	√
MC	√	√	√
MD	X	X	X
ME	X	X	X
* Note √: Stable; X: Unstable (phase separation, creaming or cracking) occur			

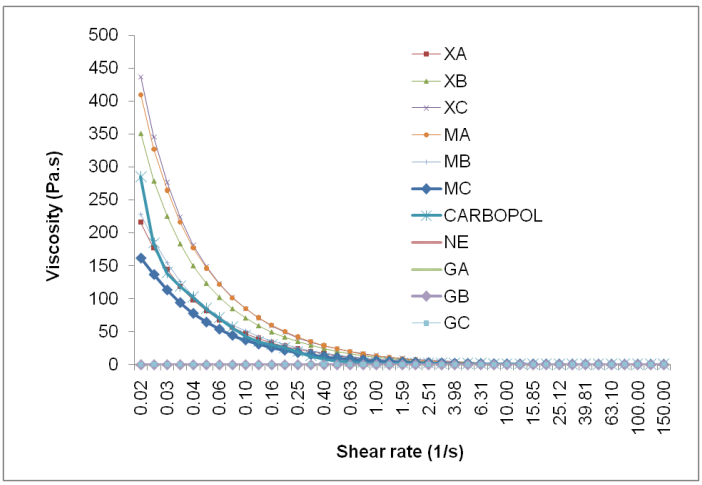

Fig. 3. Viscosity of Carbopol®, NE, GEL, XAN and XAN-GEL blend (MA-MC) formulations

Table 4. Power Law Model Parameters for NE, Carbopol, XAN, GEL, XAN-GEL Blends (MA-MC) Formulations

Formulation	Consistency Coefficients *k*(Pa s^n)	Flow Behavior Indices n
Carbopol	9.96	0.14
NE	1.29 x 10^{-3}	1.00
GA	1.63 x 10^{-3}	1.00
GB	1.75 x 10^{-3}	1.00
GC	2.41 x 10^{-3}	1.00
XA	6.86	0.14
XB	11.95	0.14
XC	12.69	0.12
MA	12.33	0.13
MB	7.26	0.14
MC	5.42	0.16

CONCLUSIONS

1. The nanoemulsion gel formulations successfully resulted in nano-size range (below 250 nm) narrow size distributions of globules (polydispersity index values below 0.4) and more negative ζ potential, showing that all formulations were stable. Formulation with xanthan gum ratio less than 1.25% denoted as MD (1.0% XAN/1.5% GEL) and ME (0.5% XAN/2.0% GEL) nanoemulsion gels exhibited instability within storage stability period,
2. Carbomer 940 (Carbopol®), XA-XC, and MA–MC nanoemulgels with an n-value below 1 exhibited shear-thinning or non-Newtonian behavior, The MA (2.0% XAN/0.5% GEL) nanoemulgel demonstrated its capability to improve the stability and viscosity of nanoemulsion, akin to xanthan gum (XAN), while NE and GEL displayed a flow behavior index of 1, indicative of Newtonian properties regardless of the applied shear rate,
3. It is suggested that the combinations of the xanthan gum and fish gelatin are able to work as a rheology adjuster for the targeted nanoemulgel suitable for subsequent implementation in topical drug delivery systems.

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