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RESEARCH ARTICLE

Preparation and FTIR-ATR combined with chemometrics analysis of self-emulsifying loaded sungkai extract from *Peronema canecens*

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ABSTRACT:
The use of immunomodulators is one strategy in maintaining the immune system during the Covid-19 pandemic. Sungkai leaf extract from *Peronema canecens* keeps the immune system in good shape. Therefore, in this study, we formulated a self-emulsifying loaded sungkai leaves extract (SE-SLE) with oleic acid and virgin coconut oil (VCO) oil phases, span 80 and tween 80 as surfactants and co-surfactants in the form of PEG-400 and PG. Chemometric analysis was conducted by observing the typical pattern in each FTIR-ATR spectra. The pattern is divided into several groups based on the wavenumber and analyzed using principal component analysis (PCA) to identify the compounds contained therein. Grouping based on chemical properties via IR spectra on SE-SLE resulted in two large groups. The results obtained are beneficial as initial information in developing and optimizing the self-nano emulsifying drug delivery system formula.

KEYWORDS: Chemometrics, FTIR-ATR, *Peronema canecens*, Self-emulsifying, Emulsion, Sungkai, Principal component analysis, cluster analysis.

INTRODUCTION:
The immune system has an essential role in the Covid-19 pandemic situation. The immune system is intended to protect the body from infection by producing protein molecules (antibodies) that bind to antigens[1]. One of the plants believed to act as an immunomodulator is sungkai (*Peronema canecens* Linn.). An immunomodulatory is any substance that can increase immunity[2]. Sungkai leaf extract contains phenolic, tannin, steroid, saponin, and flavonoid activities as antioxidant, antipyretic, antiplasmodial and can increase the immune system[2,3,4].

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Phytochemical compounds such as alkaloids and flavonoids act as immunomodulators by increasing the activity of interleukin 2 (IL-2) and lymphocyte proliferation[5]. Local people usually use the sungkai leaves directly by boiling them and making them fresh vegetables. This direct use method has several drawbacks: it cannot be stored for more prolonged time consumption and less stable. In addition, the phytochemical compounds are still not specific but using the medicinal plants as herbal immunomodulators have positive effects such as reducing the side effects of using conventional chemical compound[6]. Therefore, the self-emulsifying formulation of sungkai leaf extract is an exciting development innovation.

Self-emulsifying is a delivery system design in an isotropic mixture of oil, surfactant, co-surfactant, and extract. The formulation forms an emulsion spontaneously after being introduced into the aqueous phase by peristaltic motion, producing mild agitation in the gastrointestinal tract[7]. Self-emulsifying can increase oral absorption and dissolution speed so that bioavailability and stability can be increased[8]. In addition, it can reduce the dose and frequency of drug administration when it is formulated with self-emulsifying[9]. This carrier system can be formulated with various oils, surfactants, and co-surfactants. Therefore, preliminary studies related to the composition of the constituents are needed to determine the grouping of formulas based on their constituent components. Self-emulsifying drug delivery systems are classified as superior methods for oral drug delivery[10].

This research formulates self-emulsifying with several different formula compositions. The types of oil used were oleic acid and virgin coconut oil (VCO), surfactants in the form of tween 80 and span 80, co-surfactants selected PEG 400, and propylene glycol. Combining these different constituent components provides a different pattern of interactions with the phytochemical compounds present in the extract. Therefore, it is exciting to evaluate using the FTIR-ATR spectra pattern with chemometric analysis. The chemometric analysis approach aims to classify and study the correlation of the responses generated. The chemometric approach is carried out by multivariate statistical modeling in data processing and evaluation and interpretation with much data[11-13].

MATERIAL AND METHODS:

Materials and Chemicals:

Sungkai leaves (*Peronema canescens*) were obtained from Sekayu, Musi Banyuasin, Sumatera Selatan. Ingredients such as oleic acid oil, virgin coconut oil (VCO), span 80, tween 80, PEG-400, and PG were purchased from Bratachem (Jakarta, Indonesia). Solvents such as alcohol, aqua pro injection, and distilled water were obtained from Embacang (Palembang, Indonesia).

Preparation of sungkai leaf extract (SLE):

Sungkai leaves were dried in the sun covered with black cloth for 3 x 24 hours. Dried leaves are converted into powder by a grinding process. Sungkai leaf powder was extracted using the ultrasound-assisted extraction (UAE) method with 70% ethanol solvent in a ratio of 1:10.[14]

Preparation of self-emulsifying loaded sungkai leaf extract (SE-SLE):

A total of 16 formulas were designed using the fractional factorial design method[15]. Self-emulsifying (SE) preparation was started by dissolving the extract with a carrier oil, then vortexed and ultrasonicated for 5 minutes at room temperature. Then added surfactant and co-surfactant in the oil-extract solution and vortexed until homogeneously mixed.

Evaluation of SE-SLE:

The SE-SLE viscosity was determined at 25-30 °C using Ostwald viscometer instrumentation[13]. The emulsification time was measured by adding SE-SLE in 500 mL of distilled water at a temperature of 37 °C using a magnetic stirrer with a speed of 120 rpm[16]. Density was measured with a pycnometer by weighing an empty pycnometer using an analytical balance, filling the pycnometer with microemulsion preparations to the brim, and weighing with an analytical balance[17]. SE-SLE pH determination uses a universal pH indicator. A total of 10 µL of the sample was dropped evenly on the surface of the universal pH indicator. The color pattern formed is matched with the standard pH color that has been provided in the pH indicator box. The uniform color pattern indicates the pH of SE-SLE[18].

Clarity using spectrophotometer:

Self-emulsifying nanoemulsion of Sungkai leaf extract was taken 1 mL and diluted 100 times using distilled water. The transmission percentage was measured with a UV-Vis spectrophotometer Biobase BK-UV1000 (Shandong, China) at 638 nm with distilled water as blank[19].

FTIR-ATR fingerprinting:

The analysis was carried out by observing the interaction between the constituent materials of SE-SLE qualitatively using Fourier transform infrared-attenuated total reflectance (FTIR-ATR) fingerprinting. The FTIR-ATR uses the Nicolet iS10 series instrumentation (Thermo Scientific, USA) equipped with Omnic software. The FTIR-ATR spectrum was analyzed based on the vibrations of the functional groups of each component of the material. The analysis was carried out at 4000 cm^{-1} to 500 cm^{-1} with three times replication[20]. ATR crystal has to be cleaned with ethanol p.a before used to analyze the sample for minimize the noise[21].

Chemometrics analysis:

Data analysis used a chemometric approach with principal component analysis (PCA) and cluster analysis (CA) methods. The PCA-CA method was processed using Minitab[12,13].

RESULT:

SE-SLE was designed into 16 formulas using various carrier oils in the form of oleic acid and VCO, variations of surfactants tween 80 and span 80, and co-surfactants with types of PEG-400 and PG. Each formula is composed of different components and different concentrations. SE-SLE preparation was performed by mixing the oil and extract until homogeneous using a vortex at a constant speed. The next process is sonication with an ultrasonicator which aims to reduce the particle size and homogenize the self-emulsifying mixture so that its solubility can increase[22]. Visualization of SE-SLE can be seen in Figure 1. Each formula has a different color starting from light yellow to brownish-yellow. These different colour influenced by the type of oil, the amount of extract and the concentration of each component. Self-emulsifying has light yellow color indicated by F1, F4, F6, F8, F13, and F15, a formula with the dark yellow color indicated by F2, F5. F7, F10, F12, and F14, while F3, F11, and F16 are yellow-brown.

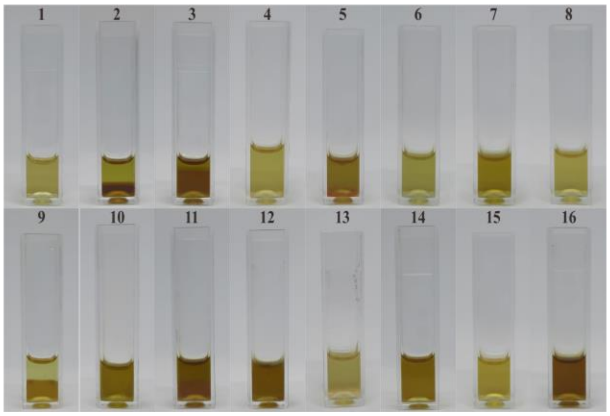

Figure1. SE-SLE visualization from formulas 1 to 16

The self-emulsifying characterization of sungkai leaves included viscosity tests, specific gravity, pH, and transmittance (Table 1). The viscosity in this study is related to the ability of the SE-SLE formula to flow. The viscosity is influenced by several factors such as molecular size, solution concentration, attractive intermolecular forces, and temperature. Viscosity measurements on self-emulsifying sungkai leaves showed that F12, F7, and F8 were the three formulas with the largest viscosity values. These three formulas were surfactants span 80 (F7 and F12) and tween 80 (F8). The co-surfactant used is propylene glycol (PG). The type of surfactant and co-surfactant used to affect the viscosity of a self-emulsifying agent.

Observation of transmittance of SE-SLE at a wavelength of 638 nm shows a formula with high transmittance, namely F9, F16, F10. Emulsions with a transmittance value close to distilled water, namely 100, indicate that the emulsion is getting clearer and has smaller droplets with an estimated size of 10-200 nm[23]. The pH value ranges from 5-6, which does not follow the intestinal pH requirements of 6.8, and the stomach has a pH of 2.0 [24].

Spectra of FTIR-ATR:

The FTIR data is processed based on the resulting pattern to obtain data in numbers. Absorption regions with peaks will be grouped by wavenumber and analyzed using chemometrics. The obtained vibrations are then read to determine the functional groups present at the peak of the spectra. Fundamental vibrations are generally in the 4000-2500 cm^{-1} area with O-H, C-H, and N-H stretching clusters. Absorption in the area with a wavenumber of 2500-2000 cm^{-1} indicates a triplicate group. The bands in the 2000-1500 cm^{-1} area are caused by C=C and C=O stretching.

Spectra data obtained from 16 formulas that have been analyzed based on certain wavenumbers are shown in Table 2. Infrared spectra in 16 formulas show medium-intensity absorption in the 3580-3207 region, indicating O-H functional groups (free alcohol). The second absorption is at wavenumber 3001-2953 cm^{-1} with a strong intensity indicating the C-H group of stretched alkane. C-H absorption is also found in wave numbers 2890-2811 cm^{-1}, with weak intensity indicating the presence of aldehydes. Absorption at wavenumbers 858-816 cm^{-1} with strong intensity indicates aromatic groups (bend, out of plane).

Table 1. Evaluation results of SE-SLE and the emulsion formed

Formula	Oil	Surfactan	Co-surfactan	Color	Viscosity (N/m^2)	Density (g/cm^3)	pH	Clarity (%T)
1	Oleic acid	Span 80	PEG-400	Light yellow	1.76	0.62	5	66.40
2	VCO	Tween 80	PG	Dark yellow	1.23	0.43	6	46.10
3	VCO	Tween 80	PG	Brownish yellow	1.38	0.29	6	82.30
4	Oleic acid	Tween 80	PEG-400	Light yellow	0.53	0.56	5	73.30
5	Oleic acid	Span 80	PG	Dark yellow	0.38	0.20	6	87.20
6	Oleic acid	Span 80	PG	Light yellow	0.49	0.52	6	47.90
7	VCO	Span 80	PG	Dark yellow	1.95	0.69	6	86.10
8	Oleic acid	Tween 80	PG	Light yellow	1.93	0.68	6	41.90
9	VCO	Tween 80	PEG-400	Light yellow	0.76	0.80	6	92.20
10	VCO	Span 80	PEG-400	Dark yellow	0.41	0.43	6	90.80
11	VCO	Span 80	PEG-400	Brownish yellow	0.35	0.37	5	78.30
12	VCO	Span 80	PG	Dark yellow	2.07	0.43	6	19.30
13	Oleic acid	Span 80	PEG-400	Light yellow	0.54	0.57	6	73.70
14	Oleic acid	Tween 80	PEG-400	Dark yellow	0.94	0.25	6	80.30
15	Oleic acid	Tween 80	PG	Light yellow	1.73	0.60	6	88.70
16	VCO	Tween 80	PEG-400	Brownish yellow	2.84	0.49	6	92.00

Table 2. Wavenumbers and functional groups based on FTIR-ATR spectra from SE-SLE

Functional groups	Wavenumbers (cm^{-1})	Type of vibration	Intensity
O-H	3580 – 3207	Alcohol (free)	Medium
C-H	3001-2953	Alkane (stretch)	Strong
	2890-2811	Aldehyde	Weak
	858-816	Aromatic (bend, out of plane)	Strong
C ≡ N	2379-2314	Nitrile	Medium
C=O	1750-1733	Ester	Strong
	1724-1702	Ketones	Strong
N=O	1475-1428	Nitro	Strong
C-O	1269-1222	Alcohols, ethers, esters, carboxylic acids, anhydrides	Strong
	1133-1001		
C-X	739-696	Chloride	Strong

The nitrile group C N was obtained at wavenumber 2379-2314 cm^{-1} with medium intensity. Wavenumbers 1750-1733 cm^{-1} indicate an ester group with a strong intensity, and wavenumbers 1724-1702 cm^{-1} indicate a ketone group with strong intensity. The band in wavenumbers 1475-1428 cm^{-1} indicates a strong intensity nitro group. The absorption in the 1269-1001 cm^{-1} region indicated the presence of alcohol, ether, ester, carboxylic acid, anhydride groups of strong intensity, and absorption at 739-696 cm^{-1} indicated the presence of a strong chloride group.

Chemometrics analysis:

Figure 3 shows the results of PCA and CA from the overall absorbance data of the FTIR-ATR spectra on the SE-SLE sample. Chemometric analysis using PCA resulted in a score plot (Figure 3a), scree plot (Figure 3b), loading plot (Figure 3c), and dendrogram (Figure 3d) of CA. The score plot shows the classification of formulas grouped at different distances from each other. The score plot results show that 15 of the 16 existing formulas have similarities. The score plot analysis on PCA showed that there were two different groups. Group A has many groups, and group B consists of F15 only. The close distance between group A evidences this, while group B has a considerable distance from the other formula points. The further apart a sample is in the score plot analysis, the less similarity of the sample [25].

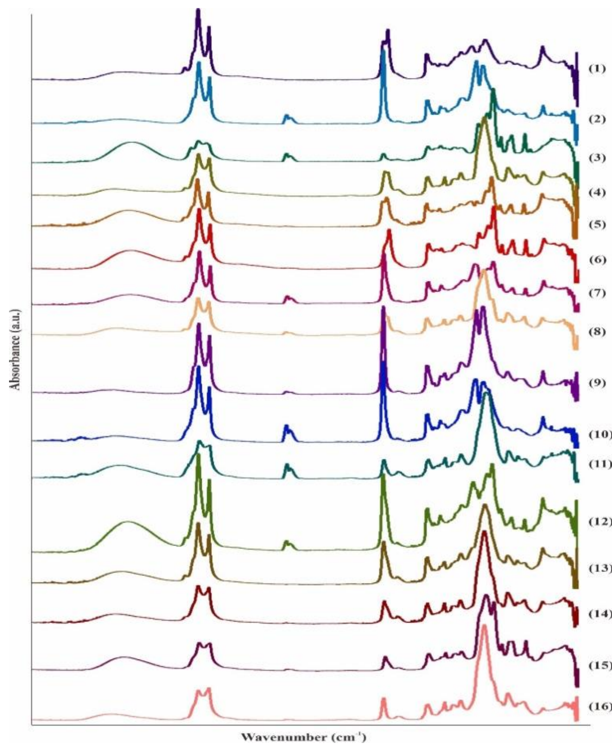

Figure 2. FTIR-ATR Spectra of SE-SLE(1) F1, (2) F2, (3) F3, (4) F4, (5) F5, (6) F6, (7) F7, (8) F8, (9) F9, (10) F10, (11) F11, (12) F12, (13) F13, (14) F14, (15) F15, (16) F16

Tabel 3. Absorbance value at selected wavenumber of SE-SLE

Formula	Absorbance at wavenumber										
	1	2	3	4	5	6	7	8	9	10	11
1	0.021	0.035	0.163	0.068	0.044	0.108	0.093	0.055	0.054	0.045	0.047
2	0.027	0.160	0.127	0.160	0.070	0.180	0.064	0.055	0.060	0.053	0.055
3	0.078	0.118	0.097	0.073	0.075	0.302	0.110	0.116	0.065	0.103	0.101
4	0.030	0.138	0.125	0.083	0.066	0.253	0.078	0.071	0.081	0.069	0.074
5	0.059	0.124	0.090	0.065	0.054	0.148	0.066	0.069	0.091	0.078	0.063
6	0.040	0.118	0.087	0.054	0.050	0.131	0.064	0.065	0.071	0.053	0.050
7	0.043	0.169	0.120	0.151	0.077	0.180	0.071	0.070	0.087	0.073	0.061
8	0.023	0.130	0.119	0.083	0.058	0.237	0.084	0.072	0.069	0.060	0.047
9	0.020	0.149	0.133	0.068	0.057	0.134	0.126	0.059	0.054	0.050	0.032
10	0.021	0.135	0.156	0.040	0.145	0.077	0.167	0.109	0.055	0.047	0.044
11	0.043	0.157	0.129	0.045	0.098	0.091	0.089	0.075	0.083	0.087	0.087
12	0.021	0.035	0.163	0.068	0.044	0.108	0.093	0.055	0.054	0.045	0.047
13	0.027	0.160	0.127	0.160	0.070	0.180	0.064	0.055	0.060	0.053	0.055
14	0.078	0.118	0.097	0.073	0.075	0.302	0.110	0.116	0.065	0.103	0.101
15	0.030	0.138	0.125	0.083	0.066	0.253	0.078	0.071	0.081	0.069	0.074
16	0.059	0.124	0.090	0.065	0.054	0.148	0.066	0.069	0.091	0.078	0.063

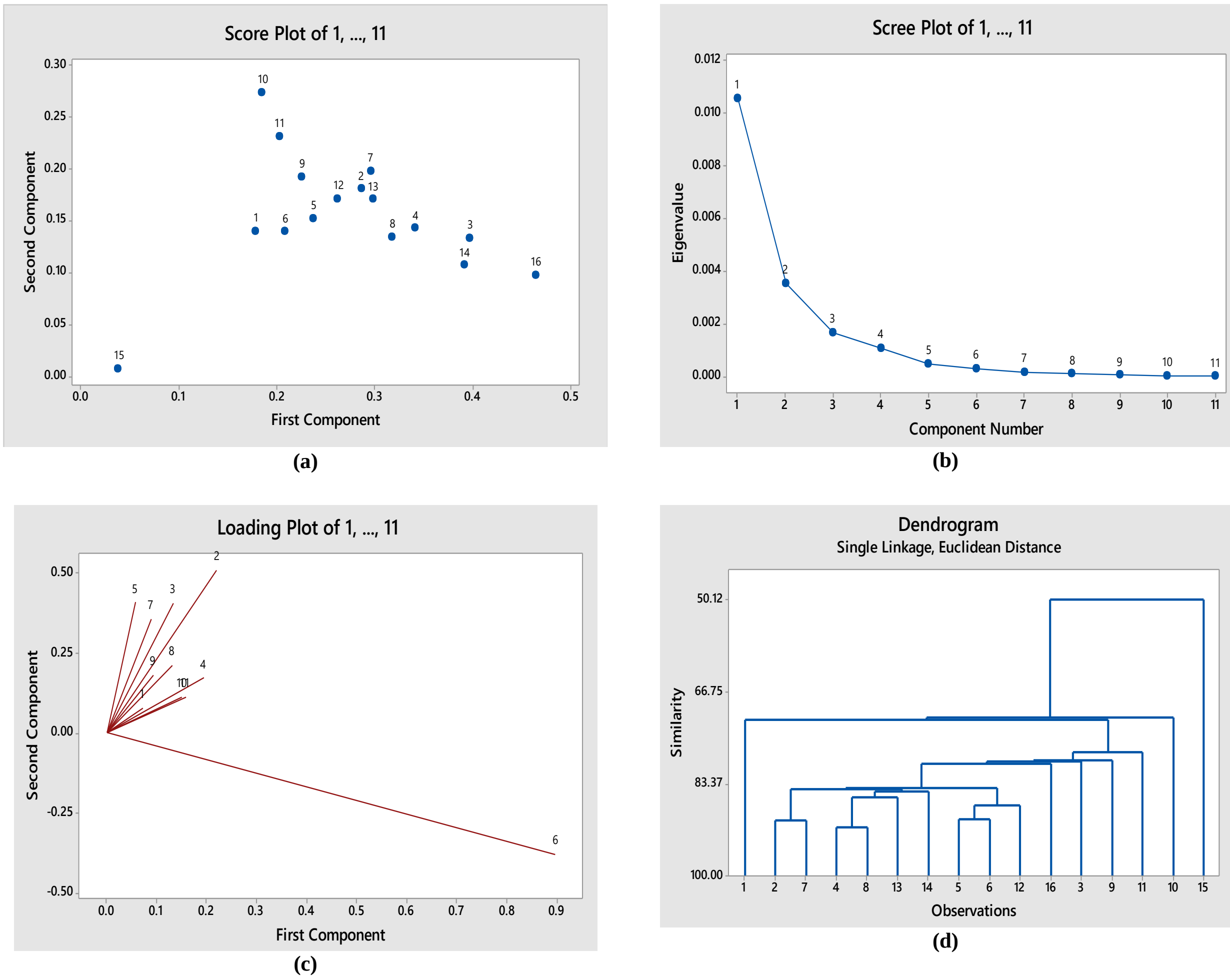

Figure 3. Result of chemometric analysis using PCA method *self-emulsifying* sungkai leaves extract, (A) Score plot, (B) Scree Plot, (C) Loading Plot, (D) Dendogram

DISCUSSION:

The analysis of SE-SLE spectra with FTIR-ATR aims to determine the vibration of functional groups and interactions between spectra in each formula. FTIR spectra were read at wavenumbers of 4000 cm^{-1} to 400 cm^{-1} [17]. A total of 16 self-emulsifying formulas of sungkai leaf were tested using FTIR-ATR, with each formula being run for three replications. This replication aims to obtain more accurate data based on similarities or differences in peaks, wavenumbers, and absorbance of each run. The peak at a certain wave number will be shown by FTIR spectra based on the vibration of the functional group of a material in the formula.

Chemometric analysis was carried out by observing the typical pattern in each FTIR spectra to identify a compound conveniently. A distinctive pattern is what distinguishes one compound from another[26]. The resulting FTIR spectra pattern will be divided into certain groups based on the wavenumber for further analysis using PCA chemometric method. Principal component analysis (PCA) is used to process multivariant data with unknown samples to simplify the uncertain elements by reducing their dimensions[27]. PCA analysis was conducted to determine the characteristics and characteristics of each formula and evaluate the degree of similarity[21,22]. The chemometric analysis method was carried out by grouping all the formulas into several small groups based on the similarity of characteristics and the closeness of the responses possessed by each formula. Chemometrics becomes an important method for analysing active compounds in pharmaceutical formulations[28]. Chemometric analysis can work as a new analytical tool for many studies, combining formulas with the desired parameters[29].

The dendrogram in PCA analysis shows the formula group with the same variable and has a bond in a group based on the similarity of the value [30]. The results of the calculation of the dendrogram similarity are shown in Figure 3d. SE-SLE formulas are grouped based on their similarity. F2 and F7 have a similarity of 89.95%, F4 and F8 91.27%, F1 and F11 71.86%, F13 and F14 84.79%. The loading plot determines the formula that has the most role in forming the principal component[31]. The loading plot analysis describes the angle that shows the correlation between the responses in the 16 formulas.

SE-SLE is an isotropic mixture of oil, surfactant, and cosurfactant that spontaneously forms nanoemulsions when mixed with water. Surfactants and cosurfactants that can mix well with the oil phase will increase the formation of the nanoemulsion system. Surfactants and cosurfactants in the nanoemulsion system work together to form a good and flexible interface system and reduce the surface tension value to near zero to support the formation of stable nano-sized globules[31]. SE-SLE is designed in several 16 formulas with eight variations of the constituent components, namely from oil, surfactant, and co-surfactant. The differences in the composition of the SE-SLE make each formula have differences both visually and the results of characteristic testing. The carrier oils used in preparing SE-SLE were oleic acid and VCO. The surfactants used were tween 80 and span 80 and co-surfactants PG and PEG-400.

VCO belongs to the group of triglycerides with medium-chain fatty acids and is an oil suitable for manufacturing nanoemulsions. SE-SLE made with VCO carrier oil showed a light yellow color with surfactant components such as Tween 80 and co-surfactant PEG-400. PG and PEG are organic solvents suitable for oral delivery, which in some range concentrations can be hydrophilic surfactant[32,33]. This phenomenon indicates good results visually and is evidenced by the highest transmittance value of 92.20. Using VCO as a carrier oil with tween 80 as a surfactant and PEG-400 as a co-surfactant can produce nanoemulsions with nanometer particle size[25]. Solubility of the drug in the oil phase is influential in the formulation of self-emulsifying and affects the process of absorption in the gastrointestinal area. Each oil in the formula has different solubility and physicochemical properties[32,34].

CONCLUSION:

FTIR-ATR spectra and chemometric analysis were successfully applied in the initial evaluation of the self-emulsifying formula. There are two major groups based on the similarity of the FTIR-ATR spectra pattern. Formula 15 into its separate group.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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