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Article

Synthesis and characterization of acetamiprid nanoemulsion by high-energy methods

Mohanad Isawi[1,*], *and Tatiana Dolzhenko*[2,*]
[1]*St. Petersburg State Agrarian University, Peterburgskoe sh. 2, St. Petersburg, 196601 Russia*
* *Correspondence: mohanad_bahar@yahoo.com , dolzhenkotv@mail.ru*
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ABSTRACT

Nanotechnology's application in agriculture has opened up new options for generating nanosized agrochemicals that have the potential to improve efficiency, improve stability, extend the effective duration, and reduce environmental impacts [1]. One of the most pressing difficulties in the agricultural industry is the need to handle pesticide-related issues such as environmental contamination, bioaccumulation, and increases in insect resistance, which necessitates reducing the amount of pesticide sprayed on crops and protecting stored products. Nanotechnology is proving to be an appealing tool for achieving this goal since it provides new ways to synthesize and transport active ingredients known as nanopesticides [1]. Nanoemulsions are particularly well suited to creating lipophilic functional agent delivery systems [2]. The current study uses a high-energy (ultrasound) emulsification approach to create oil-in-water (O/W) acetamiprid nanoemulsions with synthetic and natural additives. The acetamiprid nanoemulsions were spontaneously formed by adding a mixture of acetamiprid and solvent in an aqueous solution containing a surfactant (tween) with continuous stirring. The nanoemulsions were then formed by ultra-sonication. Various characterization techniques for acetamiprid nanoemulsions include particle size analysis (DLS), Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM). Acetamiprid nanoemulsions are further evaluated by studying thermodynamic stability. This includes a Centrifugation assay, Freeze-thaw cycle nanoemulsions, Heating-cooling test, stability at room temperature of 25°C, pH measurement, and viscosity measurement. The droplet size and morphology of the acetamiprid nanoemulsions were measured by dynamic light scattering (DLS) and transmission electron microscopy (TEM). DLS and TEM measurements showed that acetamiprid nanoemulsions had an almost droplet size distribution (PDI < 200 nm). On this basis, an insecticide acetamiprid was incorporated into an optimized nanoemulsion system to demonstrate potential applications in pest control.

Keywords: Acetamiprid nanoemulsion, nanoemulsion characterization, nanoemulsion stability.

INTRODUCTION

Over the last few decades, research into nano-agrochemicals' structure in the form of "nano pesticides" and "nano fertilizers" has been increasingly popular [3,4]. Pesticides are necessary for modern agriculture. However, the development of nano pesticides has received less or delayed attention compared to other food production areas, such as food processing and packaging. Furthermore, numerous studies have raised concerns about the health risks of farmer exposure and population occupational exposure to high pesticide residues 5,6. Many agricultural problems, such as pest management using traditional methods, the toxic effects of chemical pesticides, and the development of improved crops, can be solved with nano pesticides. [7,8]. Nanopesticides comprise minimal active ingredient components or small structures that can enhance agricultural formulations' dispersal and wettability. Stiffness, permeability, crystallinity, thermal stability, solubility, and biodegradability are all valuable features of nanopesticides. Nanoparticles, nanocapsules, and nanoemulsions are some methods for delivering nanopesticides for plant protection that have been discussed. [9,10]. Nanoemulsions are emulsions that contain an oil nanoscale or water droplets (20–200 nm) spread in the outer phase of opposite polarity due to the action of surfactants on the oil-water interface.[11,12]. Nanoemulsions are non-equilibrium systems with excellent kinetic stability, low viscosity, and optical transparency, making them appealing for various industrial applications.[13,14].Depending on the composition, three types of nanoemulsions can be formed. Oil-in-water nanoemulsions in which the oil droplets are dispersed in the aqueous phase. Water in oil nanoemulsions with dispersed water droplets in the continuous oil phase. Bi-continuous nanoemulsions have oil and water micro-domains dispersed throughout the system in the three forms of nanoemulsions. A proper combination of surfactants and co-surfactants stabilizes the interface[15]. Low-energy, high-energy, and mixed processes are used to make the nanoemulsions. High-shear agitation, ultrasonic emulsification, high-pressure homogenization, microfluidics, and membrane emulsification are examples of high-energy processes [16]. The phase inversion temperature method, the emulsion inversion point method, and spontaneous emulsification in non-equilibrium systems are the most extensively utilized low-energy approaches [17]. Ultrasonic emulsification is a high-energy, rapid, and efficient process for creating nanoemulsions with small droplet diameters and narrow size distributions.[18,19]. Nanoemulsions, including external and non-ionic surfactants and chitosan, have recently been employed in agriculture [20].

However, forming stable nanoemulsions over a long period is a crucial but difficult feature to achieve. The specific methods by which nanoemulsions arise and how their characteristics can be regulated are still being studied at the fundamental level. Compared to traditional emulsions, nanoemulsions exhibit significantly superior gravitational separation and aggregation [17]. Ostwald ripening, flocculation, and coalescence are the additional Physico-chemical processes that break down nanoemulsions. To produce a stable nanoemulsion formulation, several parameters must be regulated, such as component composition, sequence of addition of components, and application of shear or surfactants in a way that efficiently ruptures droplets [14]. According to one study, increased surfactant concentrations resulted in more stable nanoemulsions. A combination of surfactants can sometimes give better stability than a single surfactant (Wang et al. 2007). The emulsifiers or surfactants act as a shear, which breaks down a dispersible liquid into small droplets (typically nanometers in size). This process is known as emulsification, where surfactants reduce the surface tension, and precipitation is no longer likely to occur. Nanoemulsions are an excellent choice for delivering poorly water-soluble active ingredients. However, nanoemulsion formulations that improve the bioavailability of active chemicals can distribute and encapsulate hydrophilic and hydrophobic pesticides.

Nanoemulsions have considerable economic and safety advantages for pesticide delivery in agrochemicals.[21].The main objective of this study was to prepare oil in water nanoemulsion containing an Acetamiprid insecticide. Moreover, a Nanoemulsions system was prepared with surfactant (Tween 80 or lecithin). The nanoemulsion preparation method was high shear agitation, which is widely used for pesticide preparation. The mixing ratio of the three levels of the active ingredient, solvent, co-solvent, surfactant, sonication time, and sonication cycle and power. The effect of these factors on the long-term stability of the nanoemulsions, droplet size distribution (PDI) by dynamic light scattering (DLS), (FTR)Fourier-transform infrared spectroscopy, (TEM)Transmission Electron Microscopy, viscosity, pH.

MATERIALS AND METHODS

Chemicals and reagents

Acetamiprid grade (95%) was obtained from the Ministry of Agriculture National Center for Pesticides Control. (Abu Grabe, Baghdad, Iraq). (Polyethylene glycol 6000 PEG-6000), (Chitosan), (Sodium Tripolyphosphate Light Density STPP), (Linseed oil), (Acetic acid), surfactant (Tween 80 or lecithin), and Emulsifier SPAN 20 purchased from the chemical bureau(Bab Al Moatham, Baghdad, Iraq). Deionized water was used for all the experiments throughout the study. All other commercially available solvents and reagents were used without further purification.

Preparation of acetamiprid nanoemulsion

Preparation of active ingredient Acetamiprid (Nanoemulsion): For the preparation of nanoemulsion (active ingredient Acetamiprid), Many combinations with different proportions of materials were prepared; after passing all tests, this emulsion was chosen, which gives the greatest stability. The nanoemulsion was prepared according to the method described previously by Sugumar and co-authors with some modifications [22] .As well as the procedure with some modifications [23].

The nanoemulsion was prepared by taking 10 ml of Tween solution into a flask (surfactant) and adding 100 ml of thawed water. Added 30 ml of linseed oil, and the oil phase was slowly added to the aqueous phase with stirring at 4000 rpm for 30 minutes. Added 3 grams of the active ingredient, dissolved in 97 ml of solvent (ethanol), and added to the mixture. The formed emulsions were then sonicated with an ultrasonic probe (ultrasonic homogenizers). These combinations were tested to find out their stability.

Preparation of commercial insecticide (nanoemulsion): The commercial insecticide nanoemulsion was prepared by taking 200 ml of the commercial pesticide and dissolved in 400 ml of deionized water to obtain a commercial emulsion with a high concentration. The formed emulsions were then sonicated with an ultrasonic probe (ultrasonic homogenizers). These combinations were tested to find out their stability [23].

Thermodynamic stability studies to screen nanoemulsions.

Centrifugation assay: The samples were centrifuged for 30 minutes at 5000 rpm, and phase separation, foaming, and cracking were observed. Nanoemulsions should have maximum stability not associated with phase separation (foaming and cracking). The successful formulations were subjected to other thermodynamic stability tests. The measurements were carried out in triplicate [24].

Freeze-thaw cycle nanoemulsions: This test was performed to determine the increased stability of the nanoemulsion formulations. The formulations were exposed to two temperatures (-21 ° C and 21 ° C) for at least 24 hours for each temperature test. The measurements were carried out in triplicate [24].

Heating-cooling test: It is used to demonstrate the radiation effect of heating and cooling on the stability of prepared nanoemulsions, where the prepared nanoemulsions were held at 4 and 40 ° C with exposure for 48 hours for each temperature test. Formulations that are stable at this temperature are subject to further study. The measurements were carried out in triplicate [24].

Stability at room temperature of 25°C: A glass tube was filled with around 20 mL of freshly manufactured nanoemulsions. During an a4-week storage period, the shift from steady-state to creaming and coalescence was studied [24].

PH measurement: The pH value is one of the main parameters of nanoemulsions. Observation of the pH value follows from the determination of the stability of the nanoemulsion due to the change in pH in the presence of chemical reactions. In the present study, a digital pH meter was used to determine the pH values of the prepared nanoemulsions. The samples were repeated three times.

Viscosity measurement: The nature and concentration of surfactant, oil phase components, droplet size, and the viscosity of the essence of oil production all influence the viscosity of nanoemulsions [25,26]. BROOKFIELD CAP 2000+viscometer at 25C was used to assess the nanoemulsion's dynamic (absolute) viscosity. The viscosity of the formulations was determined without the use of any additional dilution.

Characterization of the prepared nanoemulsions

Particle Size Analysis DLS: This technique is commonly known as dynamic light scattering (DLS) but is also called photon correlation spectroscopy (PCS) and quasi-elastic light scattering (QELS). The latter terms are more common in older literature. DLS is most commonly used to analyze nanoparticles. Examples include determining nanogold, protein, latex, and colloid sizes. The technique is generally best used for submicron particles and can be used to measure particles with sizes less than a nanometer. [27]. Nanoparticles may differ in physical properties, such as composition and concentration, as well as in size, shape, surface properties, crystallinity, or dispersion state. These properties are usually assessed by several methods, aiming for the complete characterization of the nanoparticles; dynamic light scattering (DLS) is one of the most commonly used [28,29].

The particles' size and polydispersity index (i.e., size distribution) were determined through Dynamic Light Scattering Technology (DLS) using a HORIBA model SZ-100 to determine particle size. All evaluations were performed in triplicate. Tests were carried out for samples in the Faculty of Biology, University of Tehran, Science Campus, Enghelab St., Tehran, Iran.

Fourier-transform infrared spectroscopy FTIR

Determination of the active groups contributing to the preparation of particles (Nanoemulsion): Infrared spectroscopy (FTIR) is an essential means of determining the effective groups that can be reducing agents, capping agents, and stabilizers for the prepared nanoparticles. In the current study, several peaks of energy units were obtained when analyzing the preparation of active ingredient acetamiprid nanoemulsion, preparation of commercial insecticide nanoemulsion, and standard commercial insecticide.

Transmission Electron Microscopy TEM: Transmission electron microscopy is, without a doubt, one of the most essential techniques for characterizing nanoparticles. TEM produces micrographs of nanoscale materials with a high lateral spatial resolution by focusing an electron beam on a thin (usually less than 200 nm) sample [25,26]. By eliminating picture distortion with aberration correctors, current electron microscopes may attain resolutions as low as 0.05–0.1 nm, resulting in high-resolution images with atomic resolution. [32,33] By spatially restricting and concentrating the impinging beam and measuring the resulting electron diffraction pattern, TEM may also analyze the crystalline structure of specific tiny sections of crystalline materials [34] TEM allows for the analysis of size, shape, and crystal structure at the single-particle level due to its great spatial resolution and selectivity [35].

The TEM technique is frequently used to characterize nanoemulsions as a supplemental tool for observing lipid particles directly and obtaining trustworthy data about the system's shape [36]. Tests were carried out for samples in the Faculty of Biology, University of Tehran, Science Campus, Enghelab St., Tehran, Iran.

RESULTS

Table 1 summarizes the pH measurements of pesticide nanoemulsions, which indicated that the pH ranged from 5.00 to 5.60 for all formulations. Insecticides are more vulnerable to alkaline degradation than fungicides or plant growth regulators. Furthermore, insecticides are the most vulnerable to alkaline degradation or "high" pH solutions.

The results in Table 1 show that all nanoemulsions were less than 25mPas.Each reading was collected after the sample had reached equilibrium for 2 minutes. Nanoemulsions of (Commercial Insecticide Acetamiprid Nanoemulsion), (Active ingredient acetamiprid Nanoemulsion), and (particles pesticide Acetamiprid) had low viscosity values (7.10, 6.10, and 24.23mPa.s, respectively).

№	**preparation**	Viscosity (mPa.s.)±	pH ±
1	Commercial Insecticide Acetamiprid Nanoemulsion.	7.10±0.11	4.63±0.02
2	Active ingredient Acetamiprid Nanoemulsion .	6.10±0.12	4.97±0.01
3	particles pesticide Acetamiprid.	24.23±0.19	5.01±0.02

Table 1: Dynamic viscosity and pH of (Commercial Insecticide Acetamiprid Nanoemulsion) (Active ingredient acetamiprid nanoemulsion and pesticide Acetamiprid).

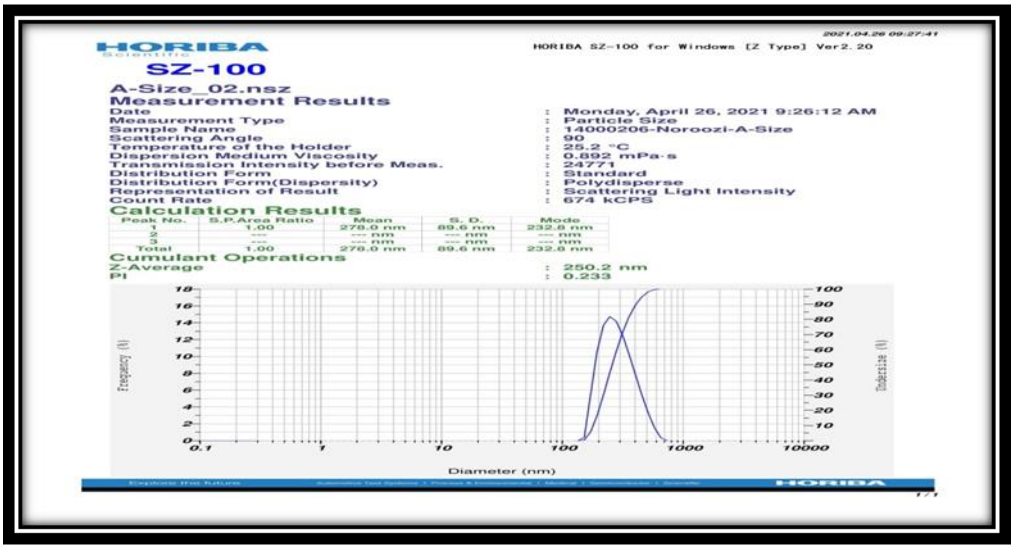
Particle Size Analysis DLS Fig 1 shows the DLS graph of *Active ingredient acetamiprid nanoemulsion, with an average size of particles 278.0nm, While the size of the nanoparticles prepared by * Commercial insecticide acetamiprid nanoemulsion in Fig 2 DLS graph, which an average size of particles 300.6nm., The size of standard pesticide acetamiprid particles in Fig 3 DLS graph shows an average size of particles of 730.3nm.

In the current study of infrared spectroscopy (FTIR), the result has several peaks of energy units obtained when analyzing * Commercial insecticide acetamiprid

nanoemulsion*Active ingredient acetamiprid nanoemulsion*pesticide acetamiprid. Also recorded values at 2179.01 1/cm, which represents the group aliphatic isonitrile s; CN stretching vibrations, well as recording peaks for the region of 1648.00 1/cm, which represents isolated group w-m; C=C stretching vibration, While the peak at 660.59 1/cm represents a Vinyl hydrocarbon compound w; C-H wagging vibration. Spectroscopy (FTIR) has shown *Active ingredient Acetamiprid Nanoemulsion, shape 2 having peaked at 3360.89 1/cm, which represents Imines group m; N-H stretching vibration (associated), Also recorded values at 2979.79 1/cm, which represents the group Ethers s-m; CH3 asym. Stretching vibration, As well as recording peaks for the region of 1644.26 1/cm, which represents isolated group w-m; C=C stretching vibration, While the peak at 686.72 1/cm represents a group of Vinyl hydrocarbon compound w; C-H wagging vibration. As shown by spectroscopy analysis, * particles pesticide acetamiprid in Figure 3 have peaks at 2962.51 1/cm, representing Ethers group s-m; CH3 asym. Stretching vibration, Also recorded values at 1662.66 1/cm, which represents the isolated group w-m; C=C stretching vibration, As well as recording peaks for the region of 1089.17 1/cm, which represents sat. Prim alcohol group s; C-O stretching vibration. Tests were carried out for samples in the Faculty of Biology, University of Tehran, Science Campus, Enghelab St., Tehran, Iran.
http://www.science-and-fun.de/tools/
The micrograph of three nanoemulsions Figure 4,5,6 shows that the droplets have a spherical shape, typical of an oil-in-water nanoemulsion. According to TEM analysis, the compositions' droplet diameters are nanometric in size.
The particle size of prepared nanoemulsions produced by Transmission Electron Microscopy (TEM), Figure 7, showed droplet size values of (Commercial insecticide acetamiprid nanoemulsion) with an average size of 61.5nm as well as recording droplet size values of Figure 8 (Active ingredient acetamiprid nanoemulsion) which has an average size of particles of 94.66nm. As shown by the microscope (particles pesticide acetamiprid) in Figure 9, the average size of particles is 84.3nm.

DISCUSSION

The optimal pH level should be between 5 and 7 [37]. However, the (particle pesticide Acetamiprid) had the highest value (27.23 mPa.s). From these results, it can be concluded that viscosity increased slightly in the low water-loading nanoemulsion [38]. The size of emulsion droplets was estimated at an average of three measurements and was shown as an average diameter in nanometers. (DLS) values indicated a restricted size distribution providing good stability of nanoemulsions [39,40]. Spectroscopic analysis has shown *Commercial insecticide acetamiprid nanoemulsion, shape 1 Many peaks of energy units; it recorded peaks at 3366.53 1/cm, which represents the Imines group v; N-H stretching vibration (free) to the spectroscopic values table (FTIR table) [41]
While the peak at 657.52 1/cm represents a group of Vinyl hydrocarbon compound w; C-H wagging vibration [41], TEM analyses also confirmed that the droplet diameter of the formulations falls on a nanometric scale. The average size of a drop of oil-in-waterer type nanoemulsions is typically between 20 and 200 nm [42]. Several authors verified the nanodroplet size measurements, reporting that the microstructure and size distribution were produced with nanoemulsions containing specific pesticides [23,43,22]. Droplet size in nanoemulsions has been shown to improve water delivery of insoluble compounds and active compounds [43,44]. Understanding the physics of nanoemulsion generation is crucial for managing droplet volume [45]. It should be mentioned that previous research [46,23] surface concentration, type of oil, ultrasonic energy, time on drop diameter.



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HORIBA SZ-100 for Windows [Z Type] Ver2. 20

HORIBA Scientific

SZ-100

A-Size_02.nsz

Measurement Results

Date : Monday, April 26, 2021 9:26:12 AM
Measurement Type : Particle Size
Sample Name : 14000206-Noroozi-A-Size
Scattering Angle : 90
Temperature of the Holder : 25.2 °C
Dispersion Medium Viscosity : 0.892 mPa·s
Transmission Intensity before Meas. : 24771
Distribution Form : Standard
Distribution Form(Dispersity) : Polydisperse
Representation of Result : Scattering Light Intensity
Count Rate : 674 kCPS

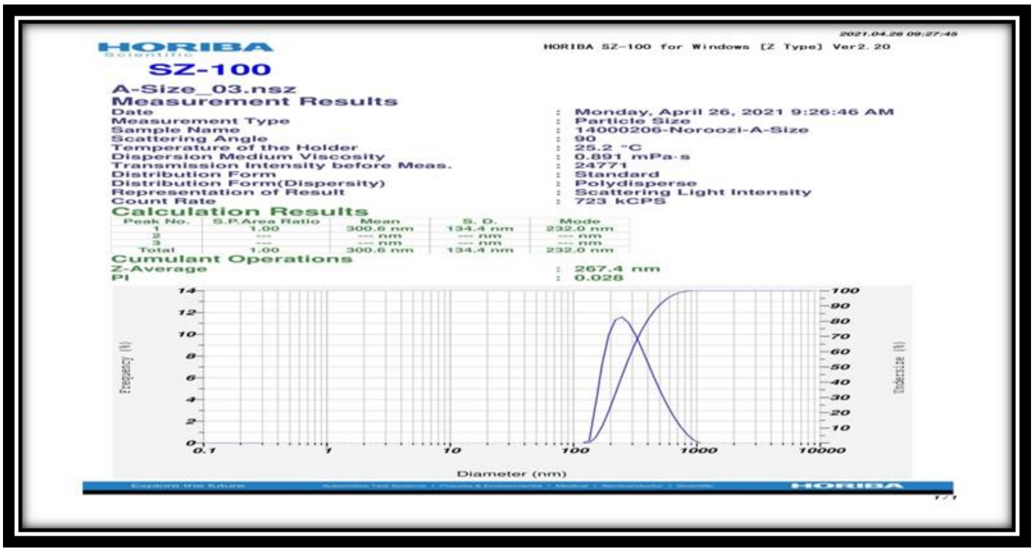
Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	278.0 nm	89.6 nm	232.8 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	278.0 nm	89.6 nm	232.8 nm

Cumulant Operations

Z-Average : 250.2 nm
PI : 0.233

Figure 1. DLS graph of Active ingredient acetamiprid nanoemulsion



2021.04.26 09:27:45

HORIBA SZ-100 for Windows [Z Type] Ver2. 20

HORIBA Scientific

SZ-100

A-Size_03.nsz

Measurement Results

Date : Monday, April 26, 2021 9:26:46 AM
Measurement Type : Particle Size
Sample Name : 14000206-Noroozi-A-Size
Scattering Angle : 90
Temperature of the Holder : 25.2 °C
Dispersion Medium Viscosity : 0.891 mPa·s
Transmission Intensity before Meas. : 24771
Distribution Form : Standard
Distribution Form(Dispersity) : Polydisperse
Representation of Result : Scattering Light Intensity
Count Rate : 723 kCPS

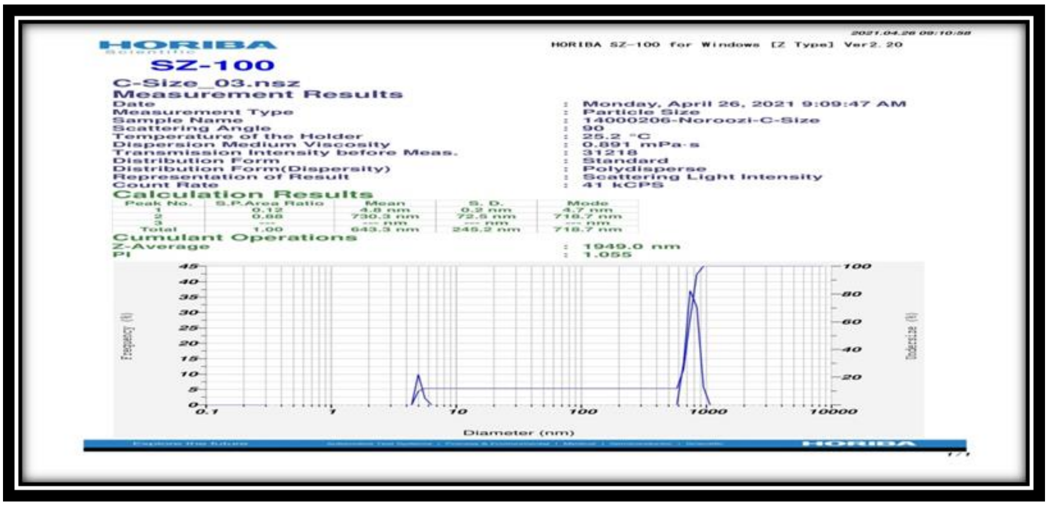
Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	300.6 nm	134.4 nm	232.0 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	300.6 nm	134.4 nm	232.0 nm

Cumulant Operations

Z-Average : 267.4 nm
PI : 0.028

Figure 2. DLS graph of Active ingredient acetamiprid nanoemulsion



2021.04.26 09:10:58

HORIBA SZ-100 for Windows [Z Type] Ver2. 20

HORIBA Scientific

SZ-100

C-Size_03.nsz

Measurement Results

Date : Monday, April 26, 2021 9:09:47 AM
Measurement Type : Particle Size
Sample Name : 14000206-Noroozi-C-Size
Scattering Angle : 90
Temperature of the Holder : 25.2 °C
Dispersion Medium Viscosity : 0.891 mPa·s
Transmission Intensity before Meas. : 31218
Distribution Form : Standard
Distribution Form(Dispersity) : Polydisperse
Representation of Result : Scattering Light Intensity
Count Rate : 41 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.12	4.8 nm	0.2 nm	4.7 nm
2	0.88	730.3 nm	72.5 nm	718.7 nm
3	---	--- nm	--- nm	--- nm
Total	1.00	643.3 nm	245.2 nm	718.7 nm

Cumulant Operations

Z-Average : 1949.0 nm
PI : 1.055

Figure 3. DLS graph of Active ingredient acetamiprid nanoemulsion

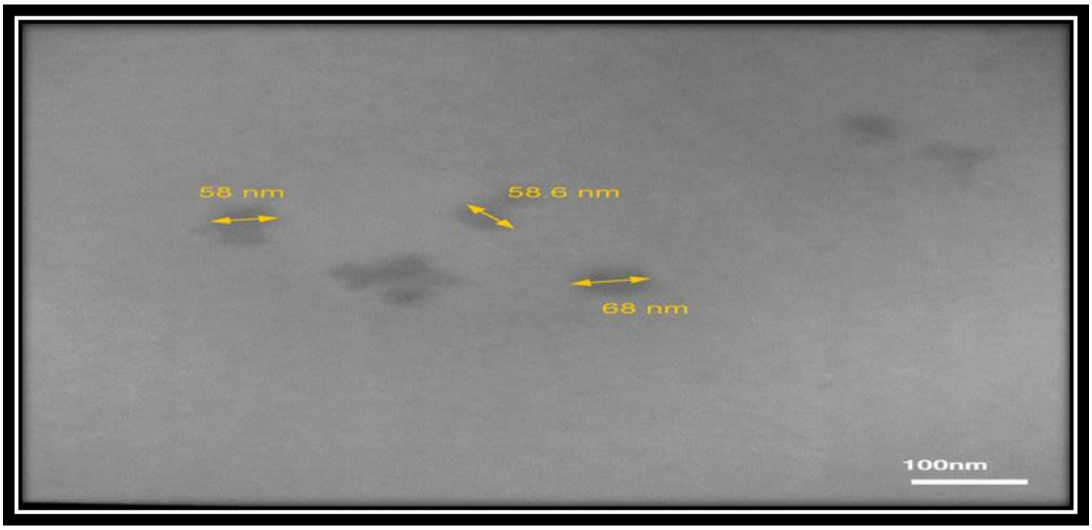


Figure 4. The micrograph of three nanoemulsions

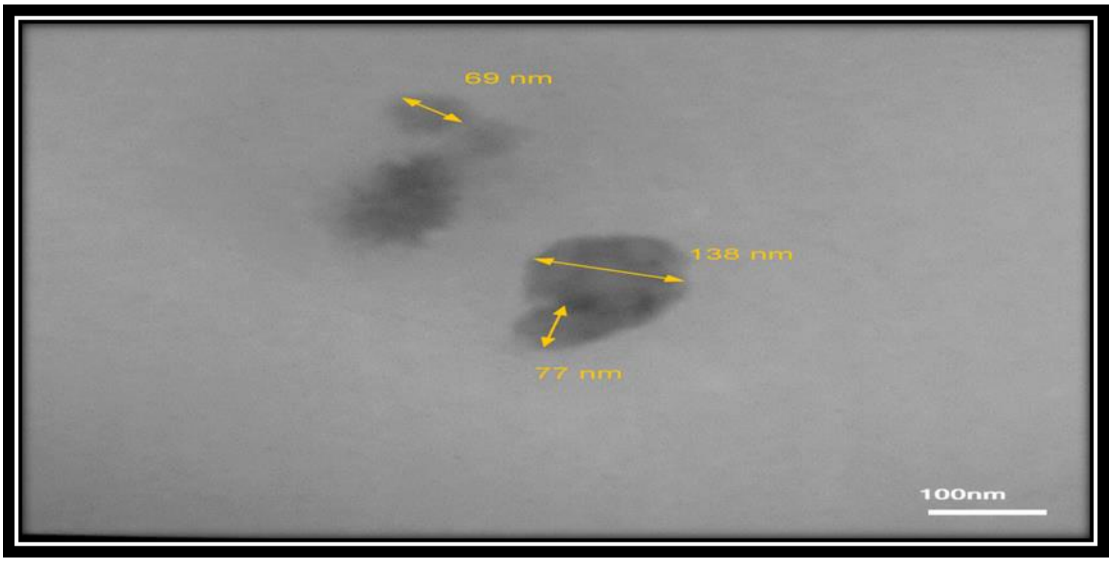


Figure 5. The micrograph of three nanoemulsions

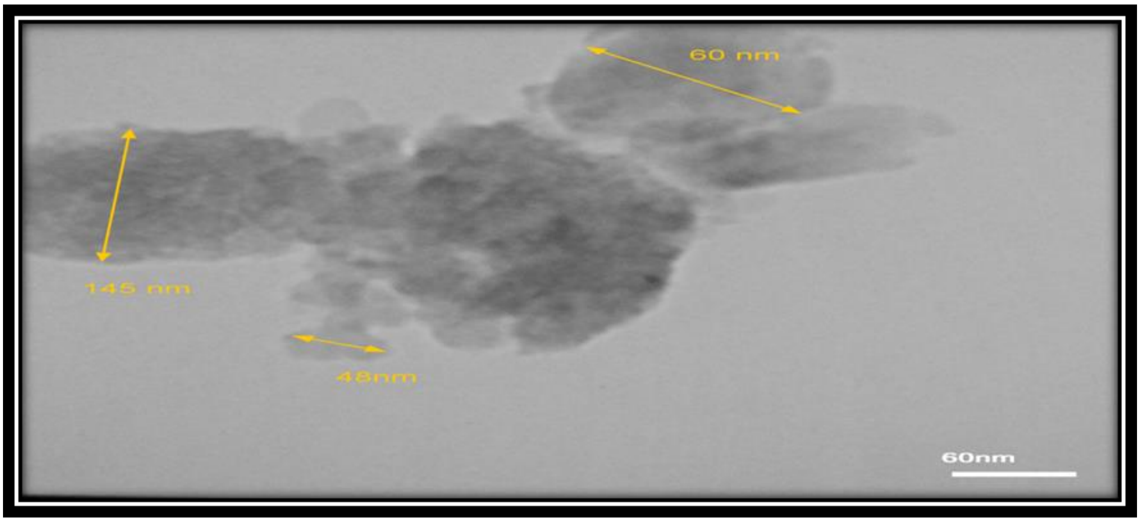


Figure 6. The micrograph of three nanoemulsions

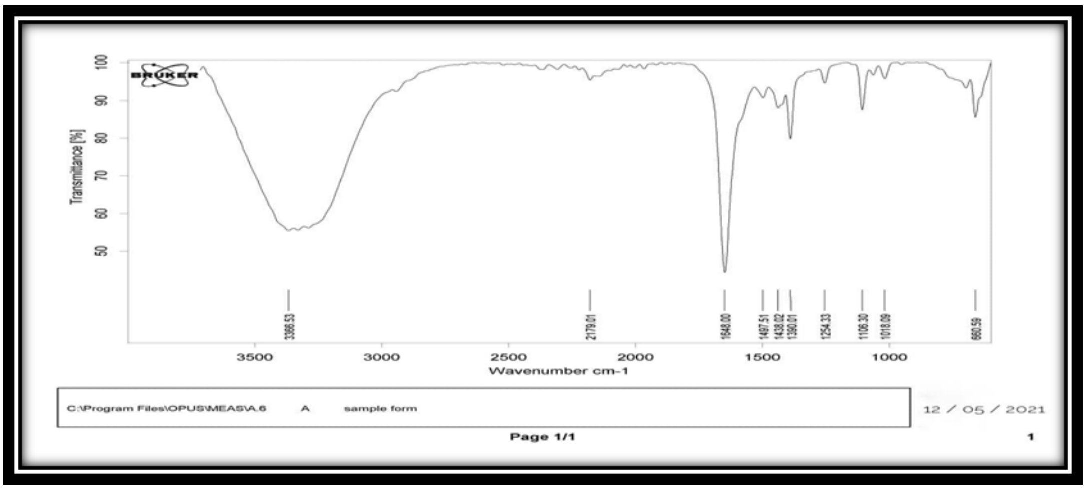


Figure 7. Fourier-transform infrared spectroscopy

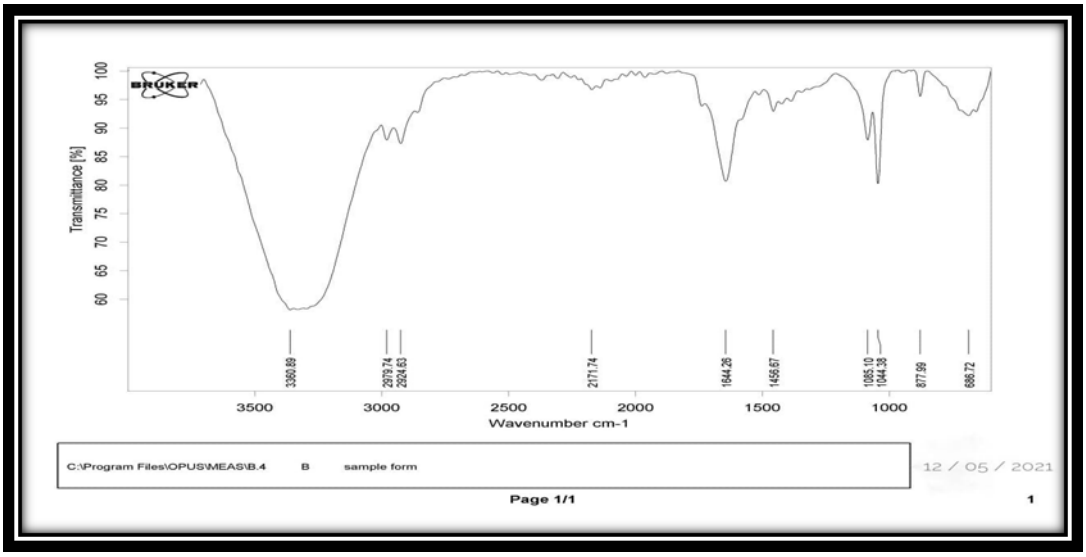


Figure 8. Fourier-transform infrared spectroscopy

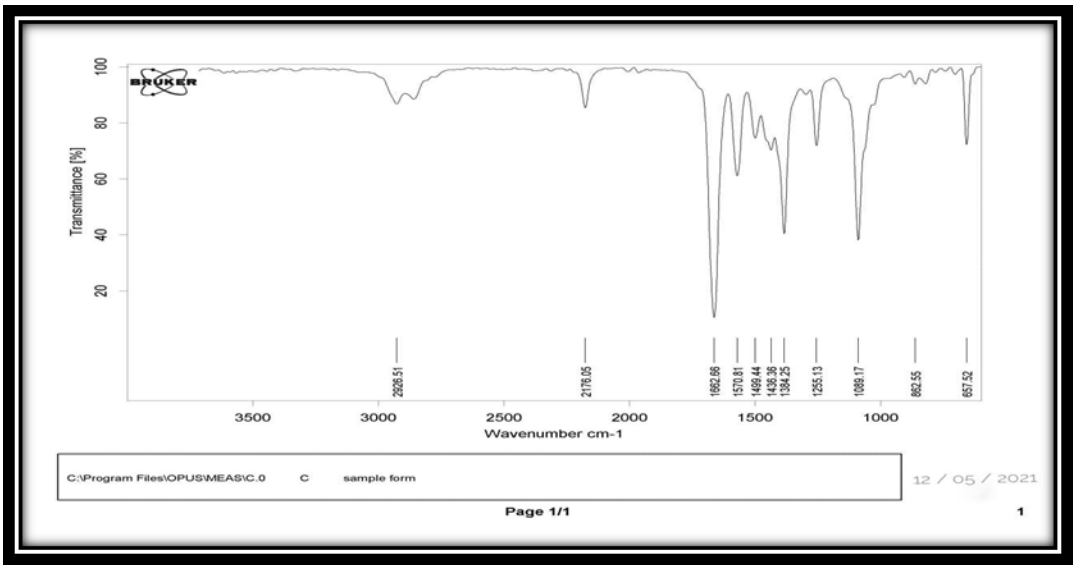


Figure 9. Fourier-transform infrared spectroscopy

CONCLUSION

Oil-in-water (o/w) nanoemulsions containing different insecticides were prepared by ultrasonic emulsification. The active ingredient, solvent, co-solvent as oil phase, surfactant, and water as water phase were used. The conditions of ultrasonic emulsification were applied at a power of 10 kHz for 20 min and sonication pulses of 9 cycle/s. The Particle Size Analysis (DLS), determination of the active groups (FTIR), TEM, viscosity, and pH of the nanoemulsions were studied and confirmed that the products were found in the nanometric range (20-200 nm). Stable nanoemulsions are stable against degradation processes, such as interference, deposition, flocculation, and cohesion. Regarding cost, manufacturing safety, transport, and usage, nanoemulsions containing pesticides offer more substantial benefits than emulsifiable concentrations. These also aid in solubilizing lipophilic active substances by reducing organic solvents, widely used in agricultural applications. Nanoemulsions used at the nanoscale will have a high level of pest control effectiveness while posing no danger to the environment. Nanoemulsions containing pesticides, on the other hand, may hold promise for the creation of pesticide formulations, and further study is needed in this field. Future research will focus on assessing the toxicity of these pesticide nanoemulsions against several major agricultural pests, as well as comparing their efficacy to that of traditional and commercial formulations already in use.

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