Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 7 Issue 2 February 2023

Research Article

Nano Emulsion Based Pesticides Formulations-A Bioengineering Perspective

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Received: December 07, 2022

Published: January 11, 2023

Abstract

With the recent decline in crop production, caused due to pests and diseases infestation, crop protection measures using modern techniques become imperative. One of the main problems that is being faced in the agricultural industry is the use of traditional agrochemicals that causes various hazard and have shown a negative impact in our environment. This issue has paved way for researchers to develop newer agrochemicals with suitable and effective delivery systems. The promising one is nano technology which is safe, green and eco-friendly. The use of nanotechnology in plant protection is a promising interdisciplinary approach. The opportunity and advancement in developing nano emulsions based plant shielding materials or nano delivery system for agro chemicals in agricultural practices has been the subject of intense research. The unique chemical and biological properties of these nano emulsions have led to the development of a favourable nanoencapsulated pesticide formulation that has shown slow releasing properties with enhanced stability, permeability and solubility. The recent discoveries in the use of nanoemulsion based agrochemicals has shown that these nanoemulsions are capable of enhancing the solubilty of active ingredients, by improving its stability, bioavailability and wettability properties during its application, thus helping in better pest management. These nanoformulations have also helped in reducing or minimizing risks caused by pesticides to human health and environment for sustainable pest management. This article focuses on basic and analytical information for researchers in the field of nanotechnology especially the Nano formulations in integrated pest management.

Keywords: Nano Formulation, Nanoemulsions; Agrochemical; Properties; Pesticide; Agriculture

Introduction

The application of nano formulatins in pesticide delivery system is almost new and in and in its preliminary stage of development. The importance of nano formulation has opened up some the advantages of nano formulation have opened up some alluring possibilities alluring possibilities for boosting technology in agricultural sector. It has been estimated in industrial agriculture that about 70% of conventional pesticides are not so efficient due to its repetitive use at high dosage in order to obtain optimal bio-efficiency thus causing pesticide resistance, environmental hazards by various process including leaching, and volatilization [1]. Some of the traditional agrochemicals that are usually in emulsifiable concentrate (EC) or wettable powder (WP) forms remain embedded in soil or ground water for decades. Due to long degradation period it remains accumulated in food chain that leads to hazardous impact on human and animal health in the environment. There are certain pesticides that are strictly banned by imposing certain regulations, eventually it is still being used in many developing countries [2]. Thus using nanoemulsion systems the water based pesticide formulations can be used as a better alternative and replacing the conventional formulations [3]. Figure 1 explains the advantages of nano emulsion-based pesticides formulation.

Nanoemulsion system

Nanoemulsion, also known as a miniemulsion, is sub-micron emulsion or ultrafine emulsion, the size of which ranges between 20-500 nm. Three different types of nanoemulsions are being widely used: oil in water (OW), water in oil (W/O) and bi-continuous. In the bi-continuous system the surfactant layer is used to separate the oil phase and aqueous phase. Nano emulsions consist of three main parts: oil, surfactants and water. In nanaoemulsion system the organic or oil and water phase are always unmixable

Figure 1: Advantages of nano emulsion-based pesticides formulation.

hence they appear to be separated by interfacial tension that is induced by the surfactants. The nano emulsion structure can be customized as per its need in different application.

Nanoemulsion in pesticides formulations

Nanoemulsion based pesticides formulations are formulation that has active chemicals which can be used in preventing and treating the crops from any pest or diseases. The maximal efficiency can be achieved through Nano emulsion that acts as a vector in carrying and delivering bioactive compounds to the target pest in crops [4]. The beneficial physiochemical properties of nano emulsions is that it has a variable nanosize that results in a larger surface area which helps in releasing, accumulating and uptake of the active ingredients more efficiently as compared to the conventional pesticide formulations. The combination of active ingredients in nanoemulsion formulations has led to proper kinetic stability [5] that increases the dissolution and stability of poorly water-soluble agrochemicals and lowers the surface tension and good wettability leading to highly improved foliage adhesion by which the pesticide are able to adhere to the leaves or any other part of the plants. Protection against photodegradation can be provided through nanoemulsions which act as a protective layer for pesticides.

The theory of pesticide delivery system was established based on the drug delivery concept that is usually used in medicine where the therapeutics to target organ is delivered by using the nanoparticles. The specific target at specific concentration and duration to achieve estimated biological efficacy and lowering its harmful effect is also obtained by designing the PDS in a particular way [6]. Previous study shows that controlled delivery plays an important role in increasing the release of essential and sufficient amount of pesticide within a specific time. The use of nanoparticles as nanocariers has been increased due to its highly efficient loading capacity, larger surface area, and faster mass transfer to the target and easy attachment of different pesticide molecules. Fewer application are required by the pesticide molecules as it slowly releases over time and at the same time degradation nanoparticles delay the loss ineffectiveness of the pesticides. Nanoparticles are loaded with pesticide molecule by various methods, viz, adsorption, entrapment inside the nanoparticle, encapsulation and covalently bonded by various ligands.

Components and their characteristics in nanoemulsion

The nano emulsion-based pesticides formulations have various components. One of the important components is surfactants, also called as emulsifiers. There are four types of surfactants: a) cationic, b) anionic, c) amphoteric and d) nonionic. Usually in nanoemulsion based pesticides formulation, non-ionic surfactants are usually encapsulated in nanoemulsion as the pH and ionic strength are less affected. The cohesion between the anionic surfactant and the solution can cause an additional component to change the size and stability of nanoemulsion. The hydrophilic-lipophilic balance (HLB) is associated with the selection of the surfactant. A high HLB value indicated that there is an increase in surfactant solubility towards water which favors oil in water formulations in pesticide formulation. The HLB value ranging from 10-16 is generally used, as HLB VALUE < 10 is considered as oil soluble surfactants as it produces good oil in water formulations and hence it is one of the most commonly method used in agro formulations. To produce kinetic stability the HLB value is considered as one of the most important parameters. Different HLB values can be procured by using single or mixed surfactants. Surfactant ranging from 1.5 to 10% and 5% is generally used as it is most efficient and appropriate in naoemulsion production. The low aggregation is caused due to use of surfactant which is believed to alter the electrostatic charge in nanoemulsion [7]. There are various studies conducted on the influence of single and complex surfactants in nanoemulsion [8]. Mixed surfactants shows greater hydrophilic-lipophilic balance (HLB), as it increases the flexibility of surfactant layer and ability to divide at high levels into the oil-water interface. Emulsion stability was also aided due to the mixing of nonionic surfactants and producing desired HLB vale.

S.no	Type of surfactant	Examples
1	Nonionic	Polysorbate 80, Polysorbate 20, Polyoxyl 40, Polyoxyl 40 stearate, hydrogenated castor oil, cremaphor, sorbitanmonooleate, polyethylene glycol
2	Anionic	Carboxylate groups, soaps, sulfonates
3	Cationic	Amines and ammonium compounds

Table 1: Types of surfactants and examples.

Type of surfactant	Example	Formulation type	Bioengineering method	Mean size (nm)	Reference
Anionic	Polysorbate 80 (Tween-80) and sorbitan monooleate (Span 80)	Mixed with organic phase	Stirring at 2400 rpm for 20 min	<100	[10]
	Polysorbate 80 (Tween-80) and sorbitan monooleate (Span 80	Mixed with organic phase	stirred at 400 rpm for 30 min under controlled temperature, 80 ± 5 °C	Below 500	[11]
	Polysorbate 20	Mixed with organic phase	sonication process	99-170	[12]
	Polyoxyethylene nonyl phenyl ether (TX-10)	Mixed with aqueous phase		200-400	[13]
Nonionic and anionic	Agnique BL1754 (AG54)	Mixed with organic phase,	stirring at 400 rpm for 30 min at 40 °C	250	[14]
	Palm oil-based. anionic: methyl ester sulfonate (MES)/Nonionic: polyethylene glycol dioleate (PDO)/and polyethylene glycol monooleate (PMO)	Mixed with oil phase	1000 rpm for two hours at room temperature	350-480	[15]
	Polyethylene glycol dioleate (nonionic) and toximol (ionic)	Mixing with oil phase	vortexes at room temperature	5-20	[16]

Table 2: List of surfactants used in nanoemulsions pesticides preparation.

Nanoemulsion formation

Three important steps are involved in preparation of nanoemulsion. Firstly, through mechanical stirring of the nonionic surfactants (E-80 and Span 60), pesticide and ILs that is done by dissolving in the solvent by mechanical stirring at 150rpm. In the second step the water is added drop wise into the oil mixture at a rate of 1ml/min in water bath (323 K) trough mechanical stirring at 150 rpm. At last the samples are cooled down to room temperature after which it is poured into glass vials and placed under suitable temperature. The concentrations of ILs types and ILs are systematically determined after analysis. The composition and preparation used in preparation of nanoemulsion used should be uniform.

Methods of producing nanoemulsion based pesticide

Nanoemusion structure with stable conditions is produced using various advanced techniques in order to ensemble the target application. Oil in water formulation nanoemulsions are most commonly used pesticides formulation. Due to the hydrophobic nature of nanoemulsion they are preferably used in improving the dissolution absorption of agrochemicals. Previous studies show that when polymer was added it supported hydrophobicity and gave better result on oil in water nanoemulsions stability when compared to the hydrolysed polymer. The properties of nanoemulsions are significantly improved due to the presence of a compound with hydrophobic group that enhance the electrostatic interaction on droplet interface [17]. The resulting properties of nanoemulsions are altered based on the concentration of polymer used in nanoemulsion [18].

Preparation method of pesticide based nanoemulsions

In order to initiate stress level to get over Laplace pressure ranging between 10-100 ATM the droplets are ruptured and energy is generated that transforms the nanoemulsion into a highly stable system. The energy used in transforming the nanoemulsion into stable system can either be though high or low energy system.

The above diagram clearly shows a difference between the high energy and low energy method. In the first one a specific device is used to break macroemulsion into nanoemulsion, whereas in the second method the energy is obtained through interaction of components during the emulsification processes.

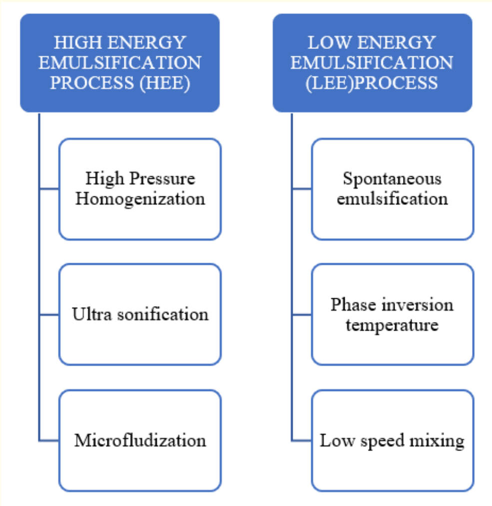

Figure 2: Types of Nanoemulsion formulations methods.

Low-energy method

In the low-energy method the surfactants behaviour during emulsification process is relied on the internal interaction of the components in the system, various studies have been conducted before for the preparation using this method [19,20]. This process includes phase inversion and self-emulsifying methods. In the self-emulsification system the component interacts through fast diffusion of solvent or surfactant without causing any alteration in surfactant curvature in the system. Low energy method is generally performed using aqueous phase or oil phase titration process. The concentration of oil phase and surfactant differ during the preparatory phase and ternary diagram that is generated based on these three components that are oil, water and surfactants. The ternary diagram shows various isotropic regions that represents various combinations of formulation. The phase diagram containing the nanoemulsion region is considered as the optimum formulation with minimum surfactant concentration that is selected for pesticide formulations.

Phase inversion method is the method in which the changes in surfactant curvature take place during the emulsifying process. The composition or temperature are important factors in producing phase transitions, hence it is named as phase inversion temperature (PIT) and phase inversion composition (PIC) respectively. In the PIC method any surfactant can be used wheras in PIT method only surfactants that are sensitive to temperature can be used [21]. PIT method is a well-chosen method as the use of organic solvents can be exempted in it [22]. *In situ* phase inversion is one of the used advanced in which the oil is mixed with surfactant and solvent simultaneously without using any equipment, an extra emulsifier can be added in the aqueous phase to obtain the small size of nanoemulsions [23]. Another eco-friendly method also known as solvent free low energy method is also suggested as it has less impact on environment and can be set up at lower cost. The use of essential oil in the nanoemulsion formulation is also suggested which is known as non-heating process. The mortality rate of *Aedes aegypti* is also being controlled using this method [24].

Figure 3: Bioengineering of nanoemulsions based pesticides using Low Energy Emulsification (LEE) method.

High-energy method

In this method one or more devices is required to generate intense forces to produce smaller droplets in the formulations. Some methods like high shear stirring, ultrsonication and high pressure homogenization is used in this method. The size of nanoemulsion is inversely proportional to the amount of energy applied. Ultrasonication is the eastiest method used in nanoemulsions among the other high-energy methods. The ultrasonic processor produces a strong uncontrolled force that causes These strong disruptive forces produced by the ultrasonic processor cause utmost shear that breaks the droplets into smaller size to produce nanoemulsion. The particle size is further reduced due to high kinetic energy that is generated due to high sonication time [30]. In high pressure method homogenizer is a mechanical device that is usually used. In high energy method a good dispersion is seen as a result of applied energy that forces the liquid through a specified valve that generates high energy speed that causes the droplet to rupture. In the high shear stirring method shear force is produced by velocity of droplet that flow through a small gap between the rotor and stator. This method is mostly used as it consumes low power and has a simple operation. This high energy method for nanoemulsion preparation has been previously described elsewere [25-29]. To maintain the stability of nanoemulsion in the initial preparatory phase there are sequence of steps that has to be followed. Two phases are usually involved in all preformulation process: the organic and aqueous phase. In the former oil and dispersant are present whereas a mixture of surfactant with water is present in aqueous phase. In the previous studies it has been proved that maximal stability can be attained by altering the additional sequence of organic and aqueous phase [30]. Treatment in final stage plays a crucial role in conversion of coarse emulsion to nanoemulsion [31].

Optimization and characterization of nanoemulsion-based agrochemicals

The formulation must go through two important steps a) preformulation and b) parametric optimization during the preparatory phase. The formulations must initially pass-through screening after which it is chosen for further chemical and biologic characterizations.

Characterization of preformulation

The HLB value and critical micelle concentration (CMC) of the surfactant should be obtained during the preformulation process as these values are important in determining the starting point in optimizing process. The CMC value is the point at which self-assembly occurs after this point the addition of surfactant does not cause any change in the surface tension. Based on the type of nanoemulsion produced O/W, W/O or other types the HLB value acts as an indicator here. The CMC value can be revealed using various method, some methods used are fluorescene probe, surface plamon resonance, dye solubilisation, tensiometer, viscometry, calorimetry and electrical conductivity. According to the studies conducted before one of the most convenient and precise method used in obtaining the CMC value is the pyrene fluorescene probe. This technique could not be used as an exact method in calculating a system with low CMC value as minimum concentration of pyrene solution must be atleast 6×10^{-6} M to get a good pyrene fluorescent spectra. An alternative system called as diluting concentration with very low CMC value can be used [32]. Due to the charge and functional group of the selected probe molecule the CMC value may be affected [33]. A range of nanoemulsions with various parameters are developed at the time of optimization, after which it undergoes initial screening process that include centrifugation assay, freeze thaw cycle, heating cooling test and stability of nanoemulsion at room temperature of 25 °C. The Nanoemulsion selected for futher characterization are the ones having maximum stability in which the phase separation does not occur along a certain period of time. This stage is utmost important in determining the thermodynamic stability studies.

Droplet size measurements

Dynamic light scatter (DLS) and Malvern Zetasizer Nano-ZS90 (Malvern Instruments, UK) at 298 K is generally used in measuring droplet size (z-average diameter) and size distribution (PDI) of nanoemulsions. The instrument has varying intensity argon laser (λ = 633nm). In order to avoid multiple light scattering the emulsions are diluted about 1000 times with Milli-Q water before every measurement and a minimum of three readings per samples are made.

Zeta potential measurement

Malvern Zetasizer Nano-ZS90 (Malvern Instruments, UK) at 298 K is used in measuring the zeta potential of nanoemulsion droplets. The emulsions are diluted about 1000 times with Milli-Q water before each measurementand the results are reported as average reading of three measurements.

Transmission electron microscopy (TEM)

The detail information about the morphology and particle size of nanoemulsion partcicles is obtained using a transmission electron microscope**.** The samples are prepared using 10μL of diluted nanoemulsion sample (100 times) that is dropped on the copper coated carbon grid for 1 minute, while the remaining sample is wiped off using a capillary tube. The copper is usually kept at room temperature for overnight being placed in the electron microscope.

Determining of critical micelle concentration (CMC)

The critical micelle concentration (CMC) values of aqueous surfactant solutions is usually calculated these two methods: a) surface tension measurement and b) conductivity method. Surface tensiometer is generally used in measuring surface tension using wilhelmy plate method. A conductometer having platinized electrode is used to perform the conductivity at 298 K.

Rheological measurements

Rheological measurements are usually carried out using a rheometer at 298 K. A spindle of DG3-DIN under shear rate control condition ranging between 1s-1 to 200 S-1 is used in performing all measurements.

Long-term stability test

The stability of nanoemusion for long term is achieved by measuring the change in droplet size along with the time of storage at different storage temperature by DLS.

Application of nano emulsion-based pesticides

Various studies have been carried out to prove the potential of different nanoemulsion based pesticides. In the previous studies conducted it was shown that the nanoemulsions prepared using cinnamon essential oil (CEO) showed higher inhibition zone against *Aspergillus niger*, *Rhizopus arrhizus*, *Pencillium species* and *Colletotrichum gloeosporiodes* when compared to CEO coarse emulsion [34]. In another study conducted using peppermint oil-based nanoemulsion showed to decrease the biomass of *Alternaria solani* which causes early blight in tomato plants [35]. The nanoemulsion prepared with neem and citronella oil has shown to reduce the effective dose (ED50) towards *Rhizoctonia solani* and *Sclerotium rolfsii* [36]. The nanoemulsions prepared by using garlic oil has shown to degrade the protein in *Pencillin italicum* by lowering its minimum inhibitory concentration, MIC value of 0.23%, lower than pure garlic oil with value upto 3.7% [37].

Nanoemulsion containing microbes as active ingredients for treating crop disease is of greater importance. It was shown that *Talaromyces flavus* when combined with nanoemulsion inhibited the growth of *Fusarium oxysporium* [38]. The Downy mildew disease was controlled by using nanoemulsions that were encapsulated with *Trichoderma species* [39]. In previous work done before on fungus *Aspergillus niger* and *Rhizopus stolonifer* it was noticed that it showed resistance towards microbial diseases such as *Erwinia carotovora* [40] there was a increase in number of fungi *Colletotrichum* when the polymer was added in nanoemulsion [41]. In studies conducted before it has been seen that chitosan nanoemulsion shows better result with lower EC_{50} in fighting against plant pathogen.

Nanoemulsions are usually used in controlling the growth of unwanted plants such as weeds and grass that compete with the crops for space and essential nutrients. This situation is seen mostly in case of smaller plants that are more prone to than the larger ones. In the studies conducted previously on *Convolvulus arvensis* and *Setaria virdis* the use of nanoemulsions of *Majorana hortensis* and *Thymus capitatus* has shown to inhibit the growth of bindweeds by reducing the fresh and dry weight of both to more than 50% when compared to the control [50]. In the study conducted on pretreatment of slender button weed, *Diodia ocimifolia* seeds with palm oil derived nanoemulsions have exhibited toxic effect that was seen at the time of germination at low concentration of $5gL^{-1}$ [42].

Recent studies have displayed the enhancement of toxic effect of pesticide nanoemulsions towards the target pests. In the study conducted on *Ephestia Kuehniella* larva the use of *Mentha longifolia* nanoemulsion shows moderate release of essential oil and prolonged contact toxicity [43]. In the nanoemulsions containing Tasmanian blue gum essential oil the use of surfactants like span 80 and Arabic gun has shown greater toxic and ovicidal effect against *Callosobruchus maculatus* adults [44]. A good acaricidal activity was seen in garlic essential oil nanoemulsion in which water was used as surfactants against the erophyid olive mites that had no toxic effect towards the albumin and total protein content in the rats [45].

Previous studies has shown the evidence of morphological and histological damages caused to insects after nanoemulsion treatment. It was examined that nanoemulsions are capable of entering the cuticle of insects and significantly causes harm to them. *Pimpineela anisum* essential oil nanoemulsions causes necrosis and blackening on Tribolium castaneum which has been shown during SEM investigation [46]. The cuticle of insect was damaged in such a way that it was hard to distinguish between the exocuticle and endocuticle. The decompression of internal structure compared to the control due to lesser regenerative cells causes the death. The bacillus spore in rats could be treated upto 98% when treated with nanoemulsions containing soyabean oil, tri-n-butyl phosphate and Triton X-100, BCTP it was noticed in the study done between the control and the treated ones in which the later showed inflation and inflammation in its cellular structure and the former showed serious tissue necrosis.

Nanoemulsions have shown a significant effect towards the plant seedling either as stimulating growth factor or in the treatment of seeds. In the study conducted in soyabean plant the application of nanoemulsions derived from saponin and thymol essential oil helped in inhibiting the growth of bacteria while assisting its growth [47]. The fusarium wilt disease in the cotton seed plant was prevented by usuage of nanoemulsions produced from eugenol oil that helped in promoting the germination process and by producing high tolerance towards the disease. In the study conducted in maize seedlings, the nanoemulsion produced from methylcellulose stimulated the growth by increasing the root and shoot length by 18% and 33% respectively [48]. Turmeric nanoemulsion with seed priming has resulted in better germination and growth in watermelons [49]. The neem oil nanoemulsion in combination with pectin exhibit growth promotion to the soybean seeds.

Conclusion

This article focuses on the potential of nanoemulsions in developing lipophilic active-loaded products for pesticide application. Previous studies have shown a significant advantage of nanoemulsions in the pesticides delivery system. In addition the physiochemical properties and stability can be improved by enabling their water dispersibility, reducing the volatility and protecting them from external environment through nanoencapsulation. The Antimicrobial activity towards the microbial pathogens increased based on the nature of the nanoemulsions used in agriculture by changing the concentration and components constituent that is achieved by change in size and stability of nanoemulsion system.

Figure 4: Bioengineering of nanoemulsions based pesticides using High Energy Emulsification (HEE) method.

Figure 5: Difference between normal emulsion and nano emulsions on the leaf surface of crops.

The antifungal and insecticidal activity was well exhibited in nanoformulations form with small size particles as the constituent or molecule trapped were optimized and surface area was high. The increased solubility due to week absorption and quicker degradation effected the nanodispersion. Researches have to be carried out to evaluate the threat to nanopesticides based on the methodology established in nanotoxicology and nanomedicine. Research needs to be carried to access the toxicological effect, environmental behaviour and pharmacokinetics of nanoparticles. A conceptual basis for the development of nanopesticides and the continuous application of nanotechnology in agriculture should be provided apart from studying the interlinkage activity between the nanopartcicles and the plants exhibiting beneficial effect on quality and safety in agricultural products.

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