MS Editions

Blacpma

BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
19 (1): 65 - 76 (2020)
© / ISSN 0717 7917

Artículo Original | Original Article

Preparation of polymer nanoparticles loaded with *Syzygium aromaticum* essential oil: An oral potential application

[Preparación de nanoparticulas polimericas cargadas con aceite esencial de *Syzygium aromaticum*: Una potencial aplicación oral]

Osvelia Rodríguez-Luis, Julia Verde-Star, Azucena González-Horta, Gabriel Báez-González, Rocío Castro-Ríos, Eduardo Sánchez-García & Abelardo Chávez-Montes

[1]*Department of Microbiology, College of Odontology, Universidad Autónoma de Nuevo León, México*
[2]*Department of Chemistry, College of Biological Sciences, Universidad Autónoma de Nuevo León, México*
[3]*Department of Analytical Chemistry, College of Medicine, Universidad Autónoma de Nuevo León. Monterrey, Nuevo León, México*
Contactos | Contacts: **Abelardo CHÁVEZ-MONTES** - *E-mail address:* **abelardo.chavezm@uanl.edu.mx**

Abstract: Due to the biological activities of *Syzygium aromaticum* essential oil, its incorporation in methacrylate polymeric (Eudragit E100) nanoparticles (NP), physical characterization, and antimicrobial essays were evaluated. The clove bears great potential for applications in dentistry. The oil was obtained by hydrodistillation and oil loaded NP using the nanoprecipitation method. Particle size and polydispersity index were determined by photon correlation spectroscopy, and physical morphology by electron microscopy. Loading capacity and *in vitro* eugenol release were evaluated by gas mass chromatography, and the antimicrobial activity of oil loaded-NP was calculated against *Streptococcus mutans*. Different chemical ingredients were characterized, and eugenol was the principal compound with 51.55%. Polymer content was directly related to NP homogenous size, which was around 150 nm with spherical morphology. A 73.2% loading capacity of eugenol was obtained. Oil loaded NP presented a fickian-type release mechanism of eugenol. Antimicrobial activity to 300 µg/mL was obtained after 24 h.

Keywords: Clove essential oil; Polymeric nanoparticles; *Streptococcus mutans*.

Resumen: Debido a las actividades biológicas del aceite esencial de *Syzygium aromaticum*, se evaluó su incorporación en nanopartículas (NP) de metacrilato polimérico (Eudragit E100), su caracterización y ensayos antimicrobianos. El clavo tiene un gran potencial para aplicaciones en odontología. El aceite se obtuvo por hidrodestilación y las NP cargado de aceite utilizando el método de nanoprecipitación. El tamaño de partícula y el índice de polidispersidad se determinaron mediante espectroscopia de correlación fotónica y su morfología por microscopía electrónica. La capacidad de carga y la liberación de eugenol *in vitro* se evaluaron mediante cromatografía de gases en masa, y la actividad antimicrobiana se evaluó contra *Streptococcus mutans*. Se caracterizaron diferentes ingredientes químicos, siendo el eugenol el principal compuesto con 51.55%. El contenido de polímero se relacionó directamente con el tamaño homogéneo de NP, que fue de alrededor de 150 nm con morfología esférica. Se obtuvo un 73,2% de capacidad de carga de eugenol. El aceite cargado en NP presentó un mecanismo de liberación de eugenol de tipo fickiano. La actividad antimicrobiana a 300 µg/mL se obtuvo después de 24 h.

Palabras clave: Aceite esencial de clavo; Nanopartículas poliméricas; *Streptococcus mutans*.

Recibido | Received: April 9, 2019
Aceptado | Accepted: August 8, 2019
Aceptado en versión corregida | Accepted in revised form: August 25, 2019
Publicado en línea | Published online: January 30, 2020
Este artículo puede ser citado como / This article must be cited as: O Rodríguez-Luis, J Verde-Star, A González-Horta, G Báez-González, R Castro-Ríos, E Sánchez-García, A Chávez-Montes. 2020 Preparation of polymer nanoparticles loaded with *Syzygium aromaticum* essential oil: An oral potential application. **Bol Latinoam Caribe Plant Med Aromat** 19 (1): 65 – 76.

INTRODUCTION

For centuries, essential oils have been applied in pharmaceuticals and alternative medicine, since they have been proven to be strongly effective as antibacterial, antifungal, antiviral, anti-inflammatory, anti-oxidant, and as anti-cancer (Qaralleh, 2014). Clove essential oil is obtained from the flower buds of *Syzygium aromaticum* (L.), which belongs to the *Myrtaceae* family; some of its chemical components include β-caryophyllene, phenols, and sesquiterpenes, among others. The major constituent of the oil is the phenylpropene eugenol, which is responsible for the plant's characteristic aroma (Chaieb *et al*., 2007). Traditionally, clove has been used worldwide (Rahmatulla *et al*., 2013; Mahomoodally, 2014), revered for its properties as an antioxidant, hypotensive, dental analgesic (Keene *et al*., 1998), antibacterial (Sessou *et al*., 2012), anti-inflammatory (Muruganadan *et al*., 2001), and antifungal (Fu *et al*., 2007). Due to its biological activity, the clove bears great potential for applications in dental practice.

Streptococcus mutans is one of the main causes of dental cavities and tooth decay worldwide, and it is considered as the most cariogenic streptococci (Ajdic *et al*., 2002). It is the primary pathogen in dental cavities etiology; not only in children but also in adults (Struzycka, 2014). The search for molecules derived from plants with antimicrobial activity is a promising research line to provide new active ingredients as a treatment for various dental pathologies. Moreover, in order to improve the function of active metabolites, an interesting option is to incorporate them into new dosage forms (Patel *et al*., 2017).

Conventional drug delivery systems like tablets, capsules, suspensions, exhibit specific problems. For example, many drugs potencies and therapeutic effects are limited or otherwise reduced because of the partial degradation that occurs before they reach its desired target in the body. The goal of all drug delivery systems is to deploy intact medications in a specific target part of the body through a medium that can control the therapy's administration by means of either a physiological or chemical trigger. To achieve this goal, researchers are turning to advances in the worlds of micro and nanotechnology (Kumari *et al*., 2010; Mukhopadhyay *et al*., 2014). During the past decade, polymeric microspheres, micelles, and more recently, nanoparticles, have all been shown to be effective in enhancing drug targeting specificity, lowering systemic drug toxicity, improving treatment absorption rates, and providing protection for pharmaceuticals against biochemical degradation (Mănescu *et al*., 2015). Among all existent polymers, Eudragit®, a cationic polymer based on dimethylaminoethyl methacrylate, is one of the most used as drug carrier (Guzmán *et al*., 2012). Eudragit E 100 is widely used in oral and topical formulations and is generally regarded as nontoxic, nonirritant, and essentially safe in humans (Madgulkar *et al*., 2008). This polymer is used especially in pharmaceutical applications due to the positive nature of its surface charge, since it allows it to interact effectively with bacteria of dental interest, which is known to have a surface with negative net charge (Dillen *et al*., 2007).

In this work, the clove essential oil was obtained and incorporated into polymeric nanoparticles (NP). Characterization of the prepared nanoparticles regarding physical morphology, particle size, polydispersity index, content of eugenol encapsulated, and in vitro eugenol release was performed. In addition, the activity of clove oil loaded-NP was evaluated against *S. mutans*. The results will provide NP as a novel alternative to delivery system active ingredients from some plant extracts.

MATERIALS AND METHODS

Plant material

For this study, dry cloves (*Syzygium aromaticum* Fam. Myrtaceae) were purchased from a local market in Monterrey, Nuevo León and identified by the Department of Botany, School of Biological Sciences, Universidad Autónoma de Nuevo León (U.A.N.L.), with identification No. 025574.

Preparation and Extraction of essential oil

Clove essential oil was obtained by hydrodistillation, by steam distillation using the glass Clevenger-type trap, using a 250 g sample of dried and ground flower buds and 500 mL of distilled water. This solution was then treated with Na_2SO_4, filtered, transferred to amber screw cap tubes, and stored in refrigeration until use.

Thin-layer chromatography (TLC) analysis

TLC was carried out on silica plates (10 x 2.5 cm; GF254). The oil sample was applied, and plates were developed using a 9:1 hexane-acetone mixture as a

mobile phase. After drying, bands were observed with UV light (320 nm) and developed by spraying $CoCl_2$. In all cases, experiments were made in triplicate. A comparative TLC analysis was carried out using different standard compounds to identify the main components. This method was also used for the oil-loaded NP.

Analysis of essential oil by Gas Chromatography-Mass Spectrometry (GC-MS)

Identification and quantification of the components of the essential oil were carried out using an Agilent Technologies 6890N gas chromatograph coupled to a 5973-quadruple mass spectrometer. Helium was used as the carrier gas at an average flow rate of 1 mL/min. The capillary column used was an HP 5MS (30 m x 0.25 mm x 0.25 µm of thickness). Oven temperature was programmed as follows: 1 min at 80°C, raised to 200°C at 10°C/min, held for 3 min, ramped at 15°C/min up to 320°C and kept finally at 320°C for 8 min. The injection was made at 270°C and transfer line temperature was set at 250°C. Data acquisition was carried out in the full-scan mode and electron ionization mass spectra were recorded in the range of 35-550 (m/z). This method was also used for the content of components of oil-loaded NP.

Antimicrobial activity

The minimum inhibitory concentration (MIC) was determined, which is defined as the lowest active concentration that can inhibit the visible growth of a microorganism after incubation for 24 hours. It was tested by two assays, first by measurement of growth inhibition halos in petri dishes with Muller Hinton agar where 100 µL of inoculum were adjusted to 0.5 of the Mc Farland scale, corresponding to a growth of 1.5×10^8 CFU/mL (Cona, 2002) and then seeded by extension. Sterile filter paper of 6 mm diameter was placed with 30 microliters of methanolic oil solution at different concentrations. In the second assay, essential oil was evaluated by the tube dilution method against *S. mutans*. Tubes of 13 x 100 mm were used, strain was activated and adjusted to 0.5 of the Mc Farland scale. Muller Hinton medium was used and inoculated with 100 µL of the oil solubilized in DMSO at different concentrations (1000 to 31 µg/mL), as well as 100 µL of adjusted inoculum, to evaluate the activity of the extract, comparing with the controls chlorhexidine 0.12% as a positive control, and negative DMSO, valued in triplicate for each sample. Subsequently, they were incubated at 37±2°C and compared with the growth and sterility controls. It was read qualitatively in addition to measuring the turbidity by visible spectrophotometry at 540 nm at 24 h. The MIC was determined qualitatively and corroborated with the lowest absorbance between the tested concentrations, after subtracting the turbidity of the extract controls and their concentration to be evaluated.

Finally, 100 µL of each tube were seeded with the driblasky loop with the plate technique and incubated at 37°C for 24 h, counting the CFU to corroborate the antimicrobial activity shown by the tube dilution method.

Oil-loaded NP preparation

The NP loaded with the essential oil obtained from *Syzygium aromaticum* was prepared using the nanoprecipitation technique as proposed by Fessi *et al.* (1989). The polymer Eudragit® E100 at different concentrations and clove essential oil were dissolved (50 mg) in 5-mL methanol (MeOH) to form the diffusing phase. This phase was then added to water using a syringe with moderate magnetic stirring. MeOH was removed under reduced pressure (Rotavapor Laborota 4003) to obtain an aqueous suspension of purified oil-loaded NP. In order to obtain NP sizes below 200 nm, the nanoprecipitation process was optimized, and the effect of the polymer concentration and the aqueous-organic phase ratio was studied. Polymer contents between 40 and 200 mg were tested; while aqueous-organic phase ratios of 5:1, 3:1, and 2:1 were evaluated.

Size determination

Particle size and polydispersity were determined by photon correlation spectroscopy, using a Zetasizer Nano ZS90. An aliquot of each NP batch was diluted in double-distilled water. Mean size and polydispersity were measured in triplicate each time.

NP morphology

Scanning electron microscopy (SEM) was used to analyze NP morphology and to confirm particle size. Samples were prepared by spreading concentrated NP suspensions over slabs, and then drying them under a vacuum. The samples were then coated in a cathodic evaporator with a fine gold layer and observed by SEM using a LEO-435VP scanning electron microscope.

Encapsulation efficiency and oil loading capacity

To evaluate the oil encapsulation efficiency and oil loading capacity, oil-loaded NP was centrifuged at 25,000 rpm for 4 h and 5°C (Allegra 64 R Centrifuge). The pellet of NP was lyophilized at −48°C and 120 mbar for 16 h (Free Zone 2.5 L Freeze Dry System). A known amount of the pellet was dissolved using MeOH and analyzed by the GC-MS method described above. The encapsulation efficiency (% EE) of any component loaded in NP was determined by using the following formula:

$$\% \, EE = \frac{amount \; of \; encapsulated \; eugenol \; (g)}{total \; amount \; of \; eugenol \; in \; the \; organic \; phase \; (g)} \, x \, 100 \text{..........Equation 1}$$

On the other hand, the percentage oil loading capacity (% OLC) was determined using the following formula:

$$\% \, OLC = \frac{amount \; of \; encapsulated \; eugenol \; (g)}{amount \; of \; eugenol \; in \; loaded \; NPs \; (g)} \, x \, 100 \text{ .... Equation 2}$$

In-vitro release study

Oil release from NP was studied using a series of 24 flat-bottom glass vials filled with 5 mL acetate buffer (100 mM, pH = 5) and NP containing 1 mg oil, under slow stirring, since the streptococcus that cause caries as *S. mutans*, for its metabolism of carbohydrates, its capable of down the pH of its environment to 5 or less (Ojeda-Garcés *et al*., 2013). At specific time intervals, the content of one of the vials was taken, extracted with chloroform, and the released eugenol was determinate by the GC-MS method described above for oil content. Experiments were conducted in triplicate over a period of 24 h.

Release Kinetic Modelling: To find out the mechanism of drug release, first 60% drug release data was fitted in various kinetic models used to describe the release kinetics. Zero order rate equation describes drug release rate is independent of its concentration in the systems. The first order equation describes the release from system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion. Korsmeyer - Peppas derived a simple mathematical relationship which described molecules release from a polymeric system. Equations used are shown in Table No. 1.

Table No. 1

Mathematical equations for the models used to describe release characteristics of eugenol from NP. Mt / M∞ is the fraction of solute that has been released at time t, and K is the rate of release constant; Co is the initial concentration and n is the exponent that indicates the release mechanism of the active.

Model	Equation
Zero-order kinetics	$\frac{Mt}{M\infty} = K \times t$
First order Kinetics	$logC = logCo - Kt/2.303$
Higuchi	$\frac{Mt}{M\infty} = K \times t^{1/2}$
Korsmeyer-Peppas	$\frac{Mt}{M\infty} = logK \times t^{n}$

Antimicrobial activity of NP

Evaluation of the activity of oil-loaded NP against *S. mutans* was performed at 24, 48, and 72 h at 37°C. *S. mutans* was added to 3 mL of Müller-Hinton medium and incubated at 37°C for 24 h. Inoculums were adjusted to 0.5 McFarland turbidity standards, which corresponds to a growth of 1.5 x 10^8 CFU/mL. Purified oil-loaded NP suspensions were centrifuged for 3 h at 25,000 rpm and 5°C in order to eliminate non encapsulated oil. Pellets of oil-loaded NP were resuspended in sterile water and sterilized by filtration through 50 mL Steriflip filter units (0.22 µm *Millipore* Express PLUS membrane; Millipore Corporation, Billerica, MA, USA). An aliquot of the sterilized suspension of oil-loaded NP was added to the 3 mL bacterial culture tubes, obtaining different concentrations (75-600 µg/mL). Different systems were inoculated at time 0, 24 and 48 h and incubated at 37°C for 24 h to complete the release period of 24, 48 and 72 h. Finally, absorbance at 530 nm was measured. Oil-free NP and chlorhexidine (0.12% w/v) were used as negative and positive control, respectively.

RESULTS AND DISCUSSION

Nowadays, there is an extensive knowledge of the dental cavities process and the main factors that cause it. The microbiological factor being the main determinant in the progress of this type of pathology (Featherstone, 2000). As cavities become progressive and more aggressive, the environment around it becomes more acidic, promoting the proliferation of aciduric bacteria. This in turn produces acids such as lactic, formic, acetic, and propionic, that quickly dissolve the dental mineral (Featherstone, 2004). This, along with other factors such as poor oral hygiene and regular consumption of carbohydrate-rich foods and beverages, has made dental cavities the most prevalent chronic disease worldwide (Petersen, 2003). In Mexico, the prevalence of dental cavities in 2015 was 93.2% in the adult population (age 20 and above) (Mejía-González *et al*., 2015). A promising line of research for the treatment of this disease is the incorporation of molecules with antimicrobial properties into new dosage forms such as nanoparticles (Patel *et al*., 2017).

The objective of this study was the analysis of the essential oil obtained from clove samples that are commonly found in Mexican markets by GC-MS, prior to its incorporation into NP. It is known that the chemical composition of the volatile fraction of essential oils is influenced both by the genetic constitution of the plant, and by the environmental conditions of the region where it is found; such as temperature, rainfall, altitude, hours of sunshine, etc. (Islam *et al*., 2010).

In the present study, only some volatile ingredients were identified in clove essential oil, since during the time of processing the samples, most of the more volatile chemical components evaporated due to the hot extraction.

Essential oil extraction

A deep-yellow oil was obtained from bud cloves with a percentage yield of recovery of the essential oil of 2.20%, a common result according to Ayoola *et al*. (2008) with a yield of 2.20%.

Chromatographic analysis

The chemical ingredients identified in the essential oil were mono and sesquiterpene, normal and cyclic hydrocarbons, and phenolic derivatives, as well as their oxygenated derivatives reported by several authors (Keene *et al*., 1998; Sartoratto *et al*., 2004). According to other authors, we have identified some chemical constituents of the essential oil, eugenol being the main chemical (Hossain *et al*., 2014). This, among caryophyllene, humulene, and acetyl-eugenol, are the predominantly bioactive ingredients used in perfumery, flavorings, essential oils, and medicines such as local antiseptic and anesthetic, analgesic, antibacterial, antifungal, and antimutagenic (Moura-Mendes *et al*., 2012). In addition, it has been reported that, among the chemical compounds in clove oil, eugenol is considered the main substance responsible for the numerous biological activities of clove extract (Alma et al., 2007).

TLC analysis established the potential presence of eugenol as the major component at extract by the retention factors (Rfs) comparison with an eugenol standard (Figure No. 1). GC-MS analysis showed that the oil contained among other compounds: α-farnesene, α-cubenene, δ-cadinene, α-amorphene, α-humulene, α-pinene, and β-E-caryophyllene, with α-eugenol as the predominant component with 51.55%.

Antimicrobial activity of essential oil

For the MIC determination, antimicrobial activity was identified for the oil at the concentration of 125 µg/mL after 24 h of incubation, showing the highest inhibitory effect against *Streptococcus mutans* with a

halo of average inhibition of 17.5 mm at 1,000 µg/mL Table No. 2).

Figure No. 1
Comparative TLC of the standard eugenol (A1) and eugenol in the oil obtained by hydrodistillation (A2).

Table No. 2
Antimicrobial activity of the essential oil of *S. aromaticum* against *Streptococcus mutans* at 24 h of exposure

Microorganism	Average inhibition (mm)							
	DMSO	Chlorhexidine 0.12%	*S. aromaticum* oil (µg/mL)					
			1000	500	250	125	62	31
S. mutans	0	19.00±0.5	17.50±0.4	16.93±0.2	13.33±0.29	10±0.87	0	0

Seeding results in agar were confirmed by the technique of serial dilution in liquid medium. Table No. 3 show the growth inhibition at different concentrations of the essential oil. By measuring the turbidity by visible spectrophotometry at 540 nm, it was observed a MIC of 125 µg/mL (Table No. 3).

Other studies report the antibacterial activity of *S. aromaticum* essential oil extracted from the plant leaves with a MIC of 250-500 and >4000 µg/mL for *S. mutans* and *Streptococcus sanguinis,* respectively (Freires *et al*., 2015).

Table No. 3
Minimum inhibitory concentration (MIC) of essential oil without encapsulating against *Streptococcus mutans.*

Plant / *Microorganism*	*Syzigium aromaticum (clove)*								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
S. mutans	-	-	-	-	+	+	+	+	-

Concentrations (µg/mL): 1 (1000), 2 (500), 3 (250), 4 (125), 5 (62), 6 (31), 7 (15), 8 (DMSO) and 9 (chlorhexidine 0.12%). Growth (+), no growth (-).

Therefore, based on the previously mentioned criteria established by Sartoratto *et al*. (2004), essential oils with a MIC of 50 to 500 µg/mL are considered to have a strong antimicrobial activity. Considering the experimental conditions analyzed, clove essential oil, obtained by hydrodistillation, has shown strong antimicrobial activity against *S. mutans*.

NP preparation

For NP preparation, in a first stage, the main variables of the technique were evaluated. It was found that by modifying experimental variables, such as the concentration of the polymer and volume of organic phase, particle size can be directly influenced; thus, polymer content at the organic phase is directly related to particle size. Mean sizes from 65±0.15 to 280±7.00 nm were obtained using 40 to 200 mg of polymer, respectively (Figure No. 2A). Also, an inverse relationship was encountered between NP size and aqueous-organic phase ratio. Mean sizes of 180 and 70 nm were obtained when using aqueous-organic phase ratios of 5:1 and 2:1, respectively (Figure No. 2B).

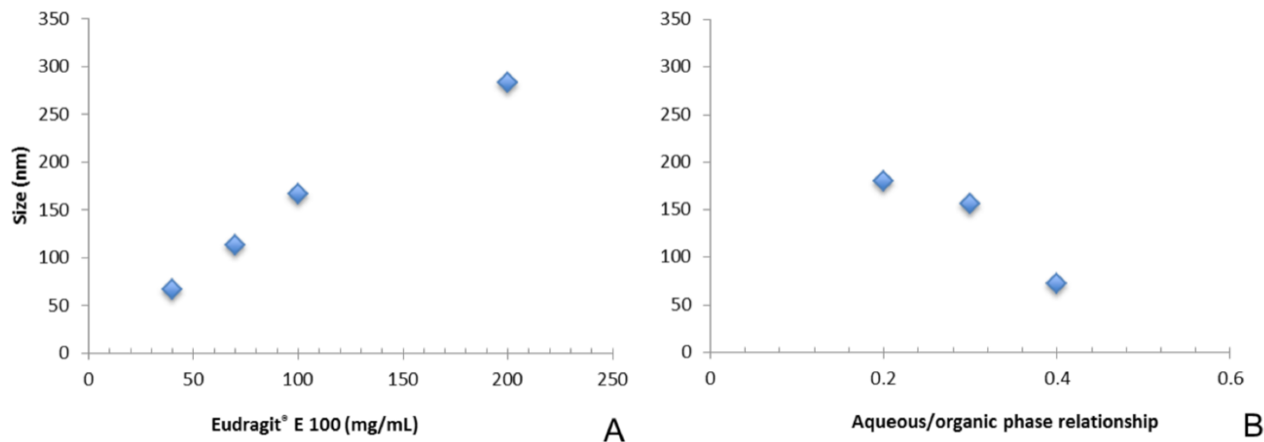

Figure No. 2

Effect of polymer content (A) and aqueous-organic relationship (B) on mean particle size (n=3).

The presence of a large amount of polymer in the organic phase produced larger particles due to the availability of more polymer chains during its aggregation. On the other hand, by increasing the volume of diffused organic phase, smaller particles were obtained. Finally, the formulations with 100 mg of polymer and with an aqueous-organic phase ratio of 3:1 showed homogeneous particle sizes less than 200 nm characteristics desired for microbiological studies; being these the conditions of the variables used for the development of the NP formulation with encapsulated oil. These parameters allowed formulations with optimal characteristics for biological studies including sterilization by filtration (Desai, 2012).

NP Size and morphology

NP characterization showed that formulations were homogeneous in size, with a mean polydispersity index of 0.082 in photon correlation spectroscopy (Figure No. 3A). Scanning electron microscopy analysis confirmed NP size and showed that NP formulations had a homogeneous size and a spherical morphology (Figure No. 3B).

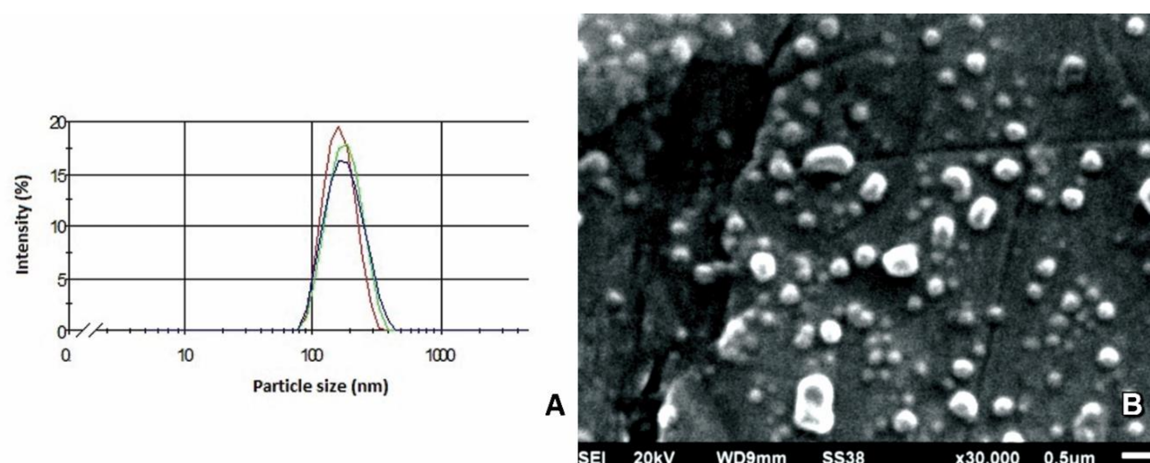

Figure No. 3
Physical characterization of NP size and morphology by photon correlation spectroscopy (A) and scanning electron microscopy (B).

Encapsulation efficiency and eugenol-loading capacity

Chromatographic separation and Rf standards comparison of the previously dissolved components loaded in the NP showed the presence of eugenol. Results of the gas-mass chromatography analysis confirmed the presence of eugenol as the main compound incorporated into NP. A 47.1% encapsulation efficiency and a 73.2% loading capacity were obtained using the gas chromatography data and equations 1 and 2.

In-vitro release study

The *in vitro* release profile of the formulation was evaluated to determine oil release from nanoparticles at pH 5. Figure No. 4 shows that the formulation released around 10% eugenol incorporated from the first hour of exposure to the medium, showing a gradual release until 24 h, time when a release of eugenol close to 48% is observed. Release kinetics was characterized by testing several models. To find out the mechanism oil release data was fitted to each mathematical model and evaluated linearity. The coefficient of determination to each kinetic model is shown in Table No. 4. The best result of the experimental data is achieved using Higuchi's release model (r^2=0.984).

Table No. 4
Mathematical Model used to describe the drug release.

Model	R^2
Higuchi	0.984
Order-Zero	0.629
Primer-order	0.886
Korsmeyer-Peppas	0.712

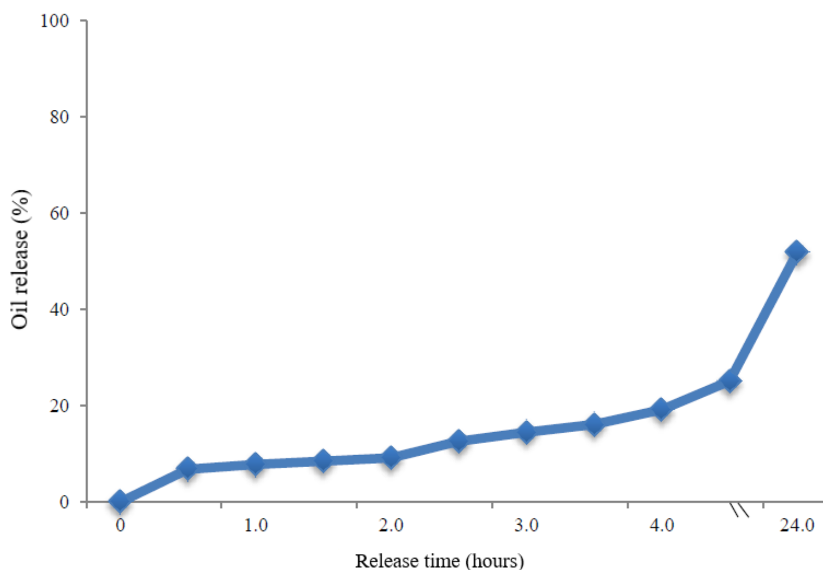

Figure No. 4
Kinetics of release of the eugenol present in the S. aromaticum oil encapsulated in NP at pH 5 (n=3).

It was possible to establish concordance between the system of administration of clove oil incorporated in NP, and a release with diffusion Fickian type mechanism. This diffusion phenomenon implies the relaxation of the polymer chains in the NP once they come into contact with the acid buffer, allowing its release over time (Nokhodchi *et al.*, 2002; Pinto *et al.*, 2006).

Antimicrobial activity of NP

Clove essential oil showed antimicrobial activity against *S. mutans* (1.5 x 10^8 CFU/mL) with a MIC of 125 µg/mL. The biological activity of oil loaded NP formulations was assayed as a function of time and concentration. A growth diminution was observed after 24 h for oil loaded NP to 300 and 600 µg/mL, after 48 h to 150 µg/mL and 72 h later, to 75 µg/mL (Figure No. 5).

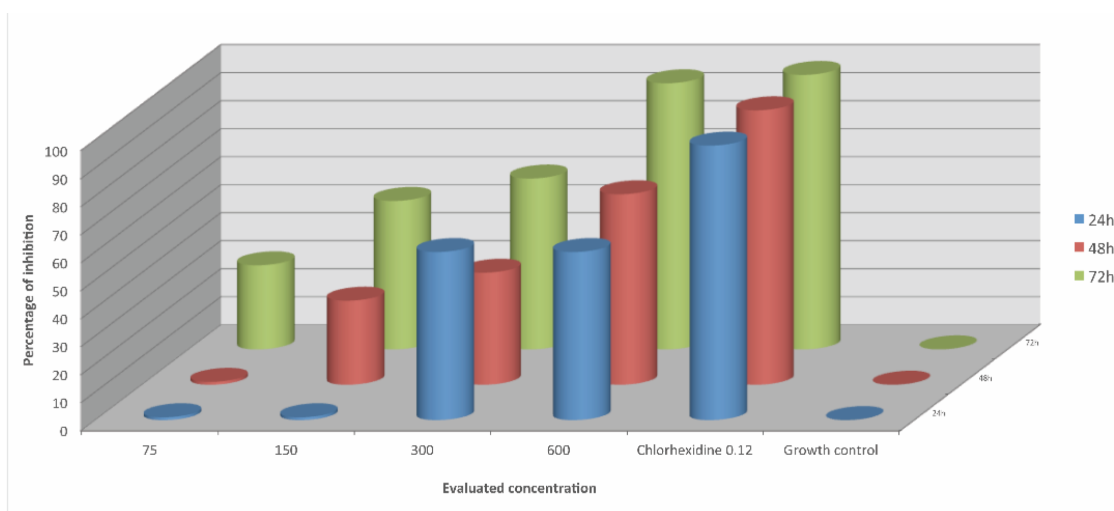

Figure No. 5
Antimicrobial activity of *S. aromaticum* oil loaded in polymeric nanoparticles of Eudragit® E100 against *S. mutans* by dilution method (n=3).

Several studies have established that the release of active compounds is slow (Lollo, 2012). In the first hours of the study, no activity of the oil incorporated in the NP formulation was observed in lower concentrations. However, as time passed, we observed the activity of the NP formulations, which showed the same behavior described in reported studies of prolonged release (Dixit *et al*., 2013). At the end of the 24-hour study, only 50% of the encapsulated oil was released, so based on the results obtained, it can be stated that when the oil is incorporated into the polymeric NP, its effect is immediate, and it is also sustained for at least 24 hours. Other authors have reported similar results with analogous polymers, Dillen *et al*. (2006), prepared nanoparticles with the polymers Eudragit® RS100 and RL100/PLGA loaded with ciprofloxacin. The antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* was determined and the results showed a prolonged release of the drug from all the formulations.

Added to this, Gupta *et al*. (2010) developed PLGA nanoparticles loaded with sparfloxacin using the nanoprecipitation technique for ophthalmic administration, which was shown to improve ocular penetration and precorneal residence time; and Cheow *et al*. (2010) examined the efficiency of the antibacterial activity, and the physical characteristics of NP, PLGA, and PCL loaded with different fluoroquinolone antibiotics (ie, ciprofloxacin and levofloxacin).

Also, it has been shown, that the MIC values of extracts with antimicrobial activity decreased significantly when incorporated into NP compared to the non-encapsulated compound (Kashi *et al*., 2012). This indicated that the antibacterial activity of a compound can be improved effectively by a nano encapsulation process. In this study, clove oil loaded NP formulations showed a markedly antimicrobial activity even to a low concentration than 75 µg/mL Because this behavior was depended on time, so it can be confirmed that the NP act as active carriers and represent a drug delivery system.

Due to demonstrated advantages, such as the improvement of the therapeutic effect, the prolongation of the biological activity, the controlled speed of drug release, and the decreasing of the administration frequency, it is possible understand the great advance made in the formulation of polymeric nanoparticles (Ezhilarasi *et al*., 2013). Moreover, it could be possible to identify these polymeric carries as the potential ideal formulation for carrying antibacterial assets due to the increased effectiveness of the active once encapsulated. We propose the standardization of a formulation to obtain NP with a size that, being within the submicrometric range, increases the surface-volume ratio (Ezhilarasi *et al*., 2013), which facilitates its interaction with the membrane of the bacterial cell, enhancing the antimicrobial action of the essential oil of *S. aromaticum* that it carries, which is widely reported with strong antimicrobial activity by various authors (Moura-Mendes *et al*., 2012).

CONCLUSIONS

In this study, it was possible to develop a reproducible formulation for preparing polymeric NP that incorporates essential oils with acceptable sizes for biological evaluation. Under the experimental conditions tested, the polymeric NP formulations loaded with clove oil showed that the oil is not released immediately. This confirms the operating mechanism of the NP as a reservoir of active substances of plant origin, to provide a potentiated extended release antimicrobial effect. Such formulations show promise for the investigation of naturally occurring, active substances, incorporated in NP, and may facilitate improvement in dental therapy.

ACKNOWLEDGMENTS

We wish to express our gratitude to the National Council for Science and Technology (CONACyT) for grant 256972, the program "short visits to the consolidation of research groups", application No. 246349, and SEP-CONACYT 11314, and CB176853 for the support provided.

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