Addis Ababa University

College of Health Sciences

School of Pharmacy

Department of Pharmaceutics and Social Pharmacy

Formulation, optimization and *in vitro* characterization of microcapsules of *Cymbopogan martini* essential oil

by

Christina Haile (B. Pharm)

October, 2023

Addis Ababa, Ethiopia

Formulation, optimization and *in vitro* characterization of microcapsules of *Cymbopogan martini* essential oil

by

Christina Haile (B. pharm)

A Thesis Submitted to the Department of Pharmaceutics and Social Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmaceutics.

Under the Supervision of Dr. Anteneh Belete, Department of Pharmaceutics and Social Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University

October, 2023

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES, SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACEUTICS AND SOCIAL PHARMACY

This is to certify that the thesis studied by Christina Haile Shoddo, entitled: **"**Formulation, optimization and *in vitro* characterization of microcapsules of *Cymbopogan martini* essential oil" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmaceutics complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Approved by:

Name	**Signature**	**Date**
Dr. Anteneh Belete (Advisor)	………………………………	
Dr. Admassu Assen (External Examiner)	…………………………….	
Mr. Tesfa Marrew (Internal Examiner)	…………………………….	

Head of Department

…………………………….

Abstract

Complex coacervation is one of the microencapsulation techniques by which encapsulated substances release profile improved. This study was conducted by using complex coacervation to formulate and optimize microcapsules of the *Cymbopogan martini* essential oil. The preliminary study was conducted to define the optimal polymeric ratio for the coating materials (gelatin B and sodium alginate) and the optimal pH for coacervate by taking dry coacervate yield and turbidity as response variable. In screening study eight factors, total polymeric concentration, Palmarosa oil concentration, surfactant concentration (Tween 80), reaction time, temperature, stirring speed, crosslinker concentration (Tannic acid) and crosslinking time were screened by Plackett–Burman design generated by Minitab software using encapsulation efficiency, microcapsules surface property, microcapsules size and distribution as response variables.

The three significant factors from screening design, total polymeric concentration, Palmarosa oil concentration and Surfactant concentration were further optimized by taking encapsulation as response variable, which had significant relationship with factors in screening design. Finally, the optimized microcapsules oil release was fitted to zero and first order kinetics models. The optimal polymeric ratio and optimal pH were 0.3:1 and 3.5, respectively and in the optimization study quadratic mathematical model ($p < 0.0001$) was an excellent fit to analyze the relationship between factors and response variable (R^2, R^2adj and R^2 pred were > 0.9). The optimal encapsulation efficiency was 96.7% and the zero-order kinetics model (where $R^2 = 0.9937$) was defined the oil release from microcapsules.

Keywords; Microencapsulation, Complex coacervation, Palmarosa essential oil, Plackett–Burman screening design, Central composite design, *Invitro* release

Acknowledgements

First and foremost, I would like to thank my saviour LORD JESUS CHRIST, THE SON OF THE TRUE LIVING GOD, for HIS mercy and unconditional love throughout this thesis research development. I wouldn't have made this far without the presence of HIM in my life. Secondly my appreciation goes to my advisor Dr. Anteneh Belete for his kindness and support until the end of this research work. My appreciation also goes to Addis Ababa University, Department of Pharmaceutics and Social Pharmacy, Ethiopia Public Health Institute, Armauer Hansen Research Institute, Ethio Agri-CEFT PLC. Addis Ababa, Ethiopia, Sino-Ethiop Associate (Africa) PLC. Addis Ababa, Ethiopia and last but not least I would like to thank peoples with kind heart who had contribution on this research work.

Table of Contents

List of tables

List of figures

Abbreviations and acronyms

CCD	Central composite design
EE	Encapsulation efficiency
FTIR	Fourier-transform infrared spectroscopy
IRS	Indoor residual spraying
LLIN	Long-lasting insecticidal net
VBD	Vector born disease
WHO	World Health Organization

1. Introduction

1.1 Vector-borne diseases

Vector-borne diseases (VBDs) are one of the public health threats which are known to be a cause of death and disability all over the globe. VBDs are the result of pathogens transmitted by the bites of infected vectors like mosquitoes. VBDs take 17% of the worldwide infectious disease burden and the resulting death exceeds 700,000 every year. Study shows more than 80% of world population is exposed to the risk of VBD among that half of the population live with two or more VBDs. General speaking VBDs more burdened on the poorest population particularly tropical and sub-tropical countries. In those countries, challenge to access health services, adequate housing, drinking water, nutrition, and maintaining sanitation systems on top of that their weak governance structures and socioeconomic underdevelopment favors the transmission of VBDs (Golding et al., 2015; World Health Organization, 2017a)

Climate change affects every creature on the planet Earth. The advancement of technology has been accused of global warming since many factories release greenhouse gases as their byproducts. The greenhouse gases are extensively studied for their global warming effect and the study predicts by 2100 there will have be a 1.0 -3.5 °C increment in global temperature (Watson; et al., 1996). It has been proved that climate change has a great impact on the biology and ecology of vectors and hence it also affects the transmission of VBDs (Lindsay & Birley, 1996). Global warming alter the upper and the lower limit of temperature for vector-borne disease transmission for instance for most diseases transmission the lower temperature range is 14 -18 °C and the upper range is 35-40 °C but warming the lower temperature range can lead to changing the extrinsic incubation period as the result further propagation of the disease transmission occurs (Watts et al., 1987).

Because of the advancement of transportation, the mobility of people has been increasing over the years. Although the socioeconomic importance of human mobility is unquestionable but its contribution to speeding up the epidemic wave is not undermined. Human mobility, either transcontinental or intercontinental, is without isolating the population at risk. Studies suggested that human mobility is an important driver for vector-borne disease outbreaks. The advanced, fast transportation and the socio-economic urge can worsen the transmission of emerging and remerging vector-borne diseases (Barbosa et al., 2018).

Urbanization is one of the major contributors to VBD transmissions, particularly in developing countries. Human population size has been dramatically changing for instance in only one century, starting from the turning of the 20th century to the end of the 20th century human population number has risen from 1 billion to 6 billion which is predicted even to reach 10 billion by 2050 (Tilman et al., 2001). Likewise, urbanization also has been increasing parallelly with human populations studies show in 1980, 1.7 billion (39%) peoples were lived in the urban area but in 1997, 2.7 billion (46%) people were lived in urban areas it is also estimated that by 2030, 5 billion (60%) human population will live in the urban area (United Nations Population Devision, 2002). Urbanization is also much more pronounced in developing countries different studies estimate by the coming 25 years people living in urban areas of Africa will almost double. And also, in Latin America and the Caribbean urbanization will increase by 50%. The dense populations in addition with inadequate infrastructure and poor health care systems, particularly in those developing countries potentiate disease transmission and provide suitable environment for vectors to breed (McMichael, 2000).

Manmade problems such as deforestation have been shown to have a major impact on vector bone disease transmissions. Studies revealed that deforestation offers suitable breeding sites for different species of vectors. For example, land usage for residential agriculture or industrial purpose followed by deforestation in Amazon has been accused of favoring breeding sites for vectors and by doing so also blamed for accelerating transmission of vector-borne diseases (Tadei et al., 1998). And also, VBDs have a significant impact on national and global economies. Its burden high, particularly on those developing countries with low-income status. The cost of the diseases includes, money spent on prevention and control strategies, for materials used for protection and treatment as well as for wasted time and money spent due to hospitalization of the patient and caregivers during the time of sickness (World Health Organization, 2017b). For instance, in the year 2015 alone, USD 2.9 billion was spent to control and eliminate malaria and it has been reported that on average treatment costs for an individual is nearly USD 3. Unfortunately for people living in an extreme poverty, an estimated 750 million people, income is only USD 1.90 (WHO, 2016).

On the other hand, in the year 2013 only, the global cost of dengue and Chagas diseases were USD 8.9 and 7 billion respectively. Taking a range of years observing the disease's cost also gives an understanding of how the burden of the disease greatly affects the world economy. For instant from 2011-2020, the economic cost for Lymphatic filariasis, schistosomiasis, and onchocerciasis were USD 10.5, 5.5, and 1.19 billion, respectively. But by 2021-2030 the cost will be USD 13.8, 11.9, and 2.11 billion, respectively (L Redekop *et al.*, 2017). However in Ethiopia, the nationwide epidemiologic data for VBD has not been gathered to figure out the depth and severity of disease burden . (Asebe et al., 2021)

1.1.1 Mosquito-Borne diseases

Arthropods are well known for their being a threat to human and animal populations. Deadly bacteria viruses or parasites can be transmitted by infected arthropods and can lead to an epidemic or even to pandemic. Mosquitoes are among the notorious arthropods, that have been a cause for transmission of life-threatening pathogens to people. Unfortunately, this public health threat could invade novel habitats due to human mobility and migration. From mosquito genera, the most clinically important for human population as well as for animals are *Anopheles*, *Aedes*, and *Culex* Achee *et al.*, 2019). The most popular parasitic infection malaria, which is transmitted by *anopheline* mosquitoes, has been reported endemic to more than 80 countries where around 3 billion people live. Sub-Saharan African countries, where 93% of the global malaria burden lies, have been affected more. Studies show the mortality and morbidity for children under 5 among these countries were 85% and 90% respectively.

A wide range of mosquitos' species are blamed to be vectors for the transmission of Wuchereria *bancrofti* and *Brugia*, which are clinically important disease-causing pathogen, responsible for 25 million men suffering from *hydrocele* and for more than 15 million people with *lymphoedema* and currently 36 million peoples have been living with these diseases No matter how the damage seems high but the prevention method still relays on vector control. Dengue disease, caused by the pathogenic dengue virus, is also mosquitos borne disease. Global estimation suggested that around 3.6 billion people are affected and hundreds of million new cases are reported every year for dengue disease even the diseases have been epidemic for many countries (WHO, 2021).

Zika virus, which belongs to the family of Flaviviridae, is transmitted by *Aedes aegypti* as a primer vector (Diallo et al., 2014) and *Aedes albopictus* is known to be its secondary vector. However, it has been reported that various other species are also involved in the transmission of the virus. Different studies suggest that Zika virus has become one of public health threat (Giraldo et al., 2023). The *chikungunya* virus (CHIKV), a genus *Alphavirus*, a family of *Togaviridae* characterized as positive sense single-stranded RNA virus is responsible for chikungunya fever and it is clinical manifestations include antalgic gait and severe joint pain. After the first report in 1955, there have been several outbreaks of the Chikungunya virus in different countries. Study shows that the infected person with the chikungunya virus could developed chronic illness in the range from 1.4 % to 90%. (Rahman *et al.*, 2019).

The hemorrhagic fatal virus under the family of Flaviviridae called yellow fever virus is responsible for numerous outbreaks in many countries particularly where no vaccine is available (Silva *et al.*, 2020). In the 2016 summer season, the yellow fever virus outbreak, was reported in 47 countries and 42 countries were classified as at risk of spreading the virus. Studies revealed that the virus burden was high particularly in African countries for an instant in 2017, 29 countries identified as at highest risk for virus transmission (Simon LV, Hashmi MF, 2018). Even though the virus's fatal rate is around 33.6%, still several outbreaks have been reported in different countries (Possas et al., 2018; Selemane, 2019). On the other hand, in Japanese encephalitis, which is also mosquitos borne disease, 67,000 new cases have been reported annually and it has been known 20-30 % of cases are fatal and 30-50% of cases have remarkable contributions to neurological disorders (Campbell et al., 2011)

Clinically important bacteria that are a threat to public health have been identified in mosquitoes (Guo et al., 2016; J. Zhang et al., 2019). Even if it is not fully understood whether bacteria were ingested or acquired from the environment or multiplied inside mosquitoes and transmitted. But different species of bacteria have been detected in adult mosquitoes those including, *Ehrlichia*, *Candidatus Neoehrlichia*, *Bartonella*, and *Rickettsia*. Even a very surprising result was revealed in a laboratory experiment which showed that *febrile rickettsiosis* and *Rickettsia felis* species were identified in mosquitoes and proved that they can be transmitted by *Anopheles mosquitoes* (Zhang *et al.*, 2019). It was also reported that *Francisella tularensis* was found in *Ades* mosquitoes and it was also confirmed as a mosquito-borne bacterium (Eliasson et al., 2006).

1.1.1.1 **Mosquitos' borne diseases-control and prevention strategies**

Controlling the mosquitos is one of the best strategies in mosquitos borne disease reduction. the study showed that more than 75% of malaria death has been decreased since the turn of this century because of the vector control measure (Bhatt et al., 2015). As per the WHO vector control is the only alternative for reduction and even for the elimination of mosquito-borne diseases like malaria (World Health Organization, 2011). Different studies also suggested that focusing on vector control could have a significant contribution to the reduction of VBDs for instance It has been reported that controlling vectors such as tsetse flies (Franco et al., 2020) and sandflies (World Health Organization, 2015) led to significant reduction of diseases caused by this vectors. On the other hand, even if a lot of effort has been put into the production of vaccines for mosquito-borne diseases but no potential vaccine is available for the total population at risk so far. Several scientific articles suggest that the best option for controlling and preventing mosquito-borne diseases is controlling the vectors (Mackenzie.V, 2007).

Generally, mosquito-controlling strategies rely on two basic parameters namely, vectorial capacity and vectorial competence where the former studies the interaction of vector and environment, that include biological, chemical control strategies. The latter deals with the interaction of vector and pathogen. The goal of vector control is to minimize diseases by getting rid of vicious mosquitoes or turning down the life span of mosquitoes and inhibiting mosquitoes from propagation (Esvelt, 2016). Moreover the advancement of genomics enable to block of pathogenic entity growth in mosquitoes the good example of this is the utilization of the Wolbachia bacterium and genetic manipulation of mosquitoes (Baldini et al., 2014).

1.1.1.1.1 Chemical interventions -insecticides

Insecticides were originally made as pesticides but because they contain an active chemical that is also toxic to insects, they have been used as insecticides. Chemicals used to control vectors are classified under four major chemical categories which are organochlorines, organophosphates, carbamates, and pyrethroids (Ndiath, 2019). Organochlorines, are made by Diels-Alder reaction through double chlorination and are either cis or trans α- and β-chlordane and both isomers are known to have Insecticidal properties by opening specific ion channels in the nervous system and lead to insect paralysis and death. Since nervous system in insects are more highly sophisticated and robust in functioning than in other mammals and humans insecticide toxicity is even dangerous to humans and other mammals (Costa, 2013).

Even though organochlorine, and chlordane were banned in the USA due to their devastating impact on human and the environment, they are still in several undeveloped countries. Chlordane and other classes of organochlorines such as aldrin and dieldrin have the same mechanism as

GABA in the nervous system. It is well understood that the compounds are much like in structure with picrotoxin which is proven to be competitive $GABA_A$ receptors. Organochlorine are one of the compounds that are exhaustively studied, several scientific papers revealed that these compounds cause different kinds of diseases including chronic diseases, such as Parkinson's disease and some types of cancer (Sharma *et al.*, 2010).

Organophosphates are compounds that are characterized by containing phosphate radicals as the center. Because of their lipophilicity they act on the central nervous systems and several studies proved that organophosphates inhibit the acetylcholinesterase enzyme, which is known for the termination of acetylcholine by hydrolyzing the neurotransmitter and thus resulting in overstimulation and death of insects (Ndiath, 2019). Also, it has been reported that they have significant toxicity to humans because of their free radicals. Several studies also showed Organophosphates lead to chronic illness (Manthripragada AD, Costello S, Cockburn MG, Bronstein JM, 2010). Carbamic acid ester which is also known as carbamates have been used as insecticide due to their competitive inhibition of acetylcholinesterase through such mechanism, they are proven to be toxic for both insect and humans.

It has been reported in various scientific journals that exposures to carbamate have a significant impact on vital physiologic systems of human like neuron, respiratory, immune, renal, and reproductive system. And also different studies showed that carbamates cause different kinds of diseases such as diabetes, cancer, asthma, rhinitis, neurological disorder, sleep apnea and renal diseases (Patel and Sangeeta, 2019).Pyrethroids are synthesized compounds from the naturally existing plant called *Chrysanthemum cineraria folium*. Pyrethroids have been used extensively as indoor spray. They have ability to increase the oping time of Voltage-gated sodium channels and thus lead to paralysis and death of insects. But like other chemical derivates insecticides pyrethroids have also negative health impacts on humans and animals (Giron et al., 2019; Moran et al., 2017). Insecticides, besides their toxicity to humans and other animals, have been reported to show resistance by different kinds of mosquitoes. Several studies have confirmed that even a single mosquito species has various mechanisms for resistance. It has been well studied that increasing metabolic degradation of insecticides by mosquitoes and lowering in sensitivity have contributed to insecticides resistances (Li & Liu, 2010; Yang & Liu, 2014).

1.2 Natural product for vector control

Using natural products as insecticidal remedies is not a recent practice, there has been documentation that showed the usage of botanical remedies by humans as insecticides at around 8000 BC. In the 17^{th} century, natural products such as pyrethrum extracts, rotenone derivative, and nicotine were widely utilized as insecticides in Europe. A study showed that thousands of plant materials have been identified as an insecticide.(Rimando & Duke, 2006)

1.2.1 Essential oils as mosquitos' repellents

Essential oils (EO) are volatile compounds that are differentiated by their aroma and found in different parts of plant materials. Essential oils contain plants' secondary metabolites and thousands of them have been identified so far and licensed as safe to use for different purposes by US-FDA. It has been reported that essential oils fight against several diseases like cancer, hypertension, cardiovascular disorder, bacterial infection, allergies, depression, gastric illness, diabetes, thrombosis, ulcers, respiratory disorders, and fungal infections. Besides their use for medicinal purpose, they also have mosquitos' repellant activities. Among essential oils, *cymbopogon* species have been exhaustively studied for their repellant activities (Nerio et al., 2010).

1.2.1.1 Palmarosa essential oil

Palmarosa grass, native to India, which is also known as rusa grass, is classified under the Poaceae family genus of *Cymbopogon* species of *Cymbopogon martini* which has a 2n=20 chromosomal number. Palmarosa is a perennial aromatic grass can grow from (1.3 – 3) meters and planted widely for its aromatic use. The extracted essential oil from Palmarosa has a rose-like fragrance and is commercially recognized as Palmarosa oil. Tropical and subtropical area of the forest have been a great habitat for Palmarosa. The prim exporter of Palmarosa oil is India, report showed an estimate of USD 4,964,850 in revenue for 147 tons of Palmarosa essential oil export followed USD 1,819,552 by France and Germany USD 524,620 revenue. Extracted essential oil from Palmarosa leaves and shoots have been used in the making of personal care, cosmetics, and pharmaceutical products (Kumar et al., 2021; Verma et al., 2010).

The major compound in leaves of Palmarosa are geranial (53.1%) and neral (36.1%) where as in root part of Palmarosa, the major compounds are 31.5% of α-elemol, 25% of geranial and 16.6% of neral (Dangol et al., 2023). Because of its chemistry Palmarosa essential oil does have several kinds of pharmacological response such as antifungal, antibacterial and anthelmintic activities. The genus *Cymbopogon* has an ancient history among indigenous peoples for instant there have been practices of using it as a mosquito repellant in the Amazon jungle by Bolivian. The genus *Cymbopogon* is the most utilized plant in the world for its repellant properties. For instance, *Cymbopogon* excavates showed 100% protection for 2 hours against laboratory-reared *Anopheles arabiensis* (Govere et al., 2000) *Cymbopogon winterianus* essential oils have 6 hours of 100% repellant activities towards *Aedes aegypti*, *Anopheles dirus*, and *Culex quinquefasciatus* (Tawatsin et al., 2001). *Cymbopogon martinii* also has 12 hours of full protection against *Anopheles* mosquitoes (Ansari & Razdan, 1994). Although several essential oils have been proven for their insect repellent activities, their potential is limited due to their high vapor pressure. Microencapsulation is one of the novel technologies which can be utilized to overcome the above-mentioned problems (Sousa et al., 2022).

Fig 1.1 picture of Palmarosa grass

from: Internet sours - https://wendysgarden.com.au/product/palmarosa-cymbopogon-martinii-indian-geranium-gingergrass-rosha-grass-palm-rose-parmrosa-herb-seeds/

1.3 Microencapsulation as a novel drug delivery platform

Microencapsulation is an advanced technique by which small materials either, liquids, solids, or gas are incorporated within a coating polymer and gradually diffused out in a controlled fashion. Microencapsulation offers a lot of benefits including lowering the vapor pressure of essential oils thus helping to prolong the efficiency of the product, manipulating the release profile to desired form and since microencapsulation can change the liquid product to free-flowing solid this improves handling property of the products. Because of its importance several production companies have applied microencapsulation technique in their production processes that include food, agriculture pharmaceuticals, chemicals, cosmetics, and the like (Sharma et al., 2010).

When it comes to pharmaceutical application, microcapsules are considered one of the novel drug delivery systems. Several studies by far confirmed microcapsules as excellent drug carriers. Microencapsulation utilized to improve the organoleptic property of the drug and to decrease irritation of the drug due to local effects. Incompatibility between the drugs and excipients can be prevented by applying microencapsulation. There have been numerous studies done so far to microencapsulate the existing drugs, hormones, and vaccines to enhance their physico-chemical and mechanical nature. Some of these include, gentamicin (Y. Y. Huang & Chung, 2001), erythromycin (S. J. Park & Kim, 2004) pactitaxel (Ruan & Feng, 2003), diclofenac sodium (Sajeev et al., 2002), isoxsuprine hydrochloride ((Shashikala et al., 2012), growth factor (U. Park et al., 2019) and anti-diabetic b-cells (Mooranian et al., 2016).

1.3.1 Microencapsulation techniques

Several techniques have been developed so far in the making of microcapsules. But all methods fall under three broad categories which are physico-mechanical methods, the physico-chemical methods, and chemical methods (Jyothi et al., 2010).

1.3.1.1 Physico mechanical methods

These methods are well known in pharmaceuticals industries, the methods include spray drying, spray congealing, fluid bed coating, pan coating, and solvent evaporation methods. Based on the encapsulated material properties methods can be applied (Ghosh, 2006).

1.3.1.1.1 Spray drying and spray congealing methods

This technique is well known for its easiness and lower cost. However, due to the involvement of lots of heat in the drying process, it is not a favorable technique In addition, due to the process of evaporation microcapsules prepared by this technique are irregular in shape and porous. Generally, the method follows four basic steps in making microcapsules. The first step starts by emulsifying the active ingredient in the coating material then homogenizing the emulation and atomization of the homogenized dispersion and finally drying the atomized dispersion (Shahidi & Han, 1993). The spray congealing process is when a molten atomized mixture of core and coating material is sprayed in a cold chamber by which hot molten desperations congeal to dry microcapsules. Unlike spray drying this technique offers smooth and uniform shape microcapsule because of the absence of evaporation and can be prepared without solvent requirement which makes it ideal for environmental protection, especially by avoiding organic solvents in the process. Moreover, it is also convenient for moisture liable core materials. In addition to that microcapsules are readily harvested which offers the absence of further procedures. However, the spray congealing process like any other method has its limitations. It is not suitable for heat-sensitive core materials (Swarbrick & Boylan, 2003).

1.3.1.1.2 Fluidized-bed method

Microencapsulation by fluidized-bed technique mostly encapsulates solid particles. The method involves the nuzzling of liquid coating material to the core substance. Then, the fast evaporation process provides a formation coating layer. The fluidized bed method is generally classified into three systems which are top, bottom, and tangential fluidized bed spray coating Top fluidized spray system offers the coating material to spray down to the fluid bed so that the core material can have a chance to come forward to the coating region to be uniformly encapsulated. The opposite flow of core and coating material spray maximizes the encapsulation yield and this method has better encapsulation efficiency by far compared to the others. Wurster's coater' or the bottom spray operates by moving the core material upward and the coating material goes in the same direction through the perforated bottom via nozzles to the top then the coating material will encapsulate the core material and microcapsules can be collected after evaporation. Even if this method is time-consuming, the multilayer formation lowers particle defects. The third method is known as tangential spray which contains a rotary disk having a diameter the same as the chamber. In this process, the gap is created when the disc is inclined this makes a tangential nozzle on the top of the rotary disk by which the coating material is sprayed thus whichever core material passes through the gap will be encapsulated. (Bui, 2012; Jyothi et al., 2010).

1.3.1.1.3 Solvent evaporation method

Microencapsulation by solvent evaporation is one of the methods which help to control the release of core material and it is well exercised by numerous pharmaceuticals companies. This technique contains three major constituents which are material subject to be encapsulated, coating material, and liquid manufacturing vehicle. The process starts by dissolving the core material in a volatile solvent that is not soluble in a liquid manufacturing vehicle. Then, the mixture is dispersed in the liquid manufacturing vehicle and there will be constant agitation in the system. Finally, the system is heated to evaporate the solvent thus the coating material which surrounds the core gets the chance to encapsulate the core material (Jyothi et al., 2010).

1.3.1.1. 4 Pan coating

The pan coating is also one of the techniques under the physico mechanical category in microencapsulation. Even though this method is well known, it is only applied to solid core material with a minimum of 600µm size diameter. Generally, the process involves tumbling of the core material in the coating pan and gradual spraying of atomized coating material to encapsulate the core. Then, the pan can be cooled down to solidify the coating material surrounding the core or hot air is blown in the pan to dry up the microcapsules (Jyothi *et al.*, 2010).

1.3.1.2 Chemical Methods

1.3.1.2.1 Interfacial polymerization and in situ polymerization

This technique involves the chemical interaction of two different monomers, one is contained in the organic phase the other in the aqueous phase, and polymerization takes place at the interface of the system. In the organic phase core material and reactive monomers which can be dissolved by organic solvent are present, in the aqueous phase surfactant and water-soluble reactive monomers are contained. Finally, the two reactive polymers come together at the interface and react to yield polymer which encapsulates the core material. This method helps to obtain small microcapsules However, the limitation of the method is that the development of a layer between reactive material can prevent further polymerization reaction thus fragile microcapsules can be produced. On the other hand, the reactive polymer may not be compatible with the core material, and the addition of reactive polymer may end up with the formation of microspheres and not microcapsules (Cho et al., 2002; Nguon et al., 2018).

In situ polymerization is not like any other polymerization. The reactant is exclusively added to the continuous phase thus the polymerization reaction only takes place in the continuous phase. The process starts by making an emulsion of core material and homogenizing the emulsion after homogenized emulsion is prepared, the reactant is added and by adjusting the pH or temperature or any other factor contributing to polymerization. Finally, the microcapsule shell is formed by polymerization. Essential oils of peppermint, tea tree, and thymus have been used in situ polymerization technique to produce microcapsules (Jun-Seok et al.,2006).

1.3.1.3 Physico-chemical processes

1.3.1.3.1 Microencapsulation by supercritical fluid technology

Supercritical carbon dioxide has been known for its characteristics like environmental compatibility, and low viscosity which make it an excellent choice for mass transfer, non-flammability, antimicrobial activity. Added advantages include its cheap price compared to others, its critical point (31.1 ◦c and 72.83 atm) and inertness which makes it ideal for heat labile and oxidation susceptible compound. Advancement in technology also brings supercritical fluid in the making of microencapsulation. The rapid expansion of supercritical solution, method involves high pressure to dissolve the coating material and the core with supercritical fluid then letting them through the small nozzle to atmospheric pressure to make desolvation by dropping the pressure thus the core material shelled by coating material precipitate. The limitation of this method is that most polymers are not soluble in supercritical fluid. However, effort has been done to overcome this problem by adding cosolvent to enhance the solubility of polymers in supercritical fluid (Jennifer Jung, 2001).

Another technique that involves supercritical fluid is the supercritical antisolvent (SAS) method which is helpful for those compounds which are not soluble in the supercritical fluid. The process involves, first dissolving the coating and core material in suitable organic solvent and pumping it into the compartment where the pressure and temperature are set to supercritical fluid condition, and the organic solvent is pumped to the compartment dissolved with supercritical fluid in this case, carbon dioxide, which results in loss of the solvent power and solute perspiration. However, this method has its own limitation when it comes to water-soluble compounds because water has poor solubility in the supercritical fluid. Lastly, the particle form gas saturated solutions process takes the supercritical fluid as a solute rather than as a solvent. Microcapsules are made by loading the coating material in a high-pressure compartment to let the supercritical fluid diffuse into the coating material to liquefy the coating material thus after lowering the pressure the coating material will deposit on the surface of the core material (Varona et al., 2013).

1.3.1.3.2 Complex coacervation

The word coacervation comes from Latin, and is coined from two words 'acervus' and ‘co,’ acervus standing for aggregation and co taking the meaning of being together. The study the word was first utilized in 1623 and Tiebackx, F.W.Z. explained the process of coacervation in 1911. Furthermore, intensive studies were done for a better understanding of complex coacervation for two decades (1920 -1940) by Bungenberg de Jong and Kruyt by using the gelatin/ gum Arabic complex as coating materials. In the 1950s, the technique was used in the industry to make a carbon-free duplicate as it was reported by Barrette Green. Currently, this technique has taken increasing attention from pharmaceutical and food sectors and it has been reported by several studies that it is an efficient method to encapsulate a wide range of core materials (Glomm et al., 2023; Sukri et al., 2023).The complex coacervation method involves the interaction of two oppositely charged polymers (basically proteins and carbohydrates) to form coacervate as a coating material to the core by manipulating different factors such as temperature, pH, polymeric concentration, and the like. In the formation of coacervate two phases are formed within the system one is named as ‘polymer-rich’ phase which has the precipitated coacervate and the other phase is called the ‘solvent-rich’ phase which mainly contains the solvent of the system. The main interaction force that triggers the coacervation is electrostatic interaction, hydrogen bonding, and hydrophobic. Even though the interaction has not been fully understood, two-step nucleation and growth-type kinetics (Klassen, 2010).

The complex coacervation procedure follows five basic processes. The first step starts by dissolving the protein and carbohydrate polymers in an aqueous media by fixing the temperature and pH above the gelling point and isoelectric point of the protein polymer respectively. The next step is followed by the emulsification of the core material with the dissolved polymers. The third step began by lowering the pH of the system below the isoelectric point of the protein polymer to give the protein polymer cationic nature which triggers electrostatic attraction between the two polymers, and causes phase separation of the system. The two distinguishable phases of the systems are the polymer-rich phase, which is insoluble with the aqueous media, and 'the solvent-rich phase. The fourth step is all about cooling down the coacervate by maintaining the temperature below the gelling point for the formed coacervate to serve as a shell by being deposited on the surface of the core. The final step is hardening the formed microcapsules by using a crosslinking agent and drying the microcapsule (Lemetter, C., F. Meeuse, 2009).

1.3.1.3.2.1 Factors considered in the complex coacervation methods

The polymers involved in the coacervation process and their interaction with each other and the nature of contained core material have a significant impact on the shape, size, and texture of the coacervate, encapsulation efficiency, and stability of the formed coacervate. Because of that, finding out the critical factor and understanding their role in the coacervation process is very crucial. The major accountable force for the formation of coacervate is a free electrostatic reduction. So the charge density of the reactant polymers governs the complex coacervation even though it is determined by pH and ionic strength of the system media since the polymeric interaction occurrence depends on the isoelectric point of the protein and pKa of the carbohydrate. And, the ionic strength of the system highly affects the formation of coacervate. Different studies showed that when the ionic strength of the system is increased, the coacervate formation is suppressed or dissociated (Huang *et al.*, 2012).

The polymeric ratio between the protein and the carbohydrate has also a remarkable effect on net charge balance, the reactivity of polymers, and the ability of self-aggregation in the reaction process (Aryee & Nickerson, 2012). On the other hand, it was also observed that the total polymeric concentration has a significant effect on coacervation formation, and the highest concentration was reported as not favoring the coacervate formation (Schmitt & Turgeon, 2011). Although extensive studies have been done on complex coacervation for almost a century, understanding the effect of the process factors, like stirring speed, reaction time, and temperature of the system, on coacervate is still one of the limitations of the area (Lemetter, C., F. Meeuse, 2009). Complex coacervation can not only be defined by its formulation and process factors. This is because the formed coacervate does not have enough thermos-mechanical stability so crosslinking agent is needed for the robustness of the technique (Burgess & Carless, 1985). Chemical Crosslinkers, those having aldehydes as a functional group, have been used for hardening and dehydrating the coating wall to enhance the robustness of the formed coacervate. This Crosslinking chemical interacts with the amino group of the protein polymer by their aldehyde functional group to form non-soluble networked coating material which strengthens and dehydrates the shell to avoid swelling. Even if the chemicals have been reported as efficient methods for crosslinking the microcapsules however because of their toxicity it is not recommended to use them as crosslinking agents for human use. In the journey of search for safe and efficient crosslinking agents it has been reported that some of the promising nontoxic crosslinking agents among those natural-based products, tannic acid takes the lead due to its numerous functional phenyl groups to interact with the amino group of the protein polymer on top of its wide safety margin for human use (Chen et al., 2022).

1.3.1.3.2.2 Gelatin and sodium alginate complex as coating material.

Gelatin is one of the predominant polymers which is extracted from collagen by hydrolytic degradation of acid or alkaline treatment. There exist two types namely, type A and type B. Type A gelatin is made by hydrolysis of pig skin with the help of acid digestion while type B comes from alkaline degradation of cattle bones or cattle hide. The difference in extraction treatment makes them both have different isoelectric points which are important for their physicochemical property. Gelatin A has an approximate isoelectric point of 8-9 whereas gelatin B's isoelectric point is around 4-5 the lower isoelectric point occurring due to the cleavage of amide groups by alkaline degradation. Gelatin is amphoteric by nature as it has both carboxylic and amino functional groups. Gelatin's chemical property depends on the pH of the media if it is kept above the pH of its isoelectric point, it is characterized as an anionic polymer whereas it shows cationic nature at pH below its isoelectric point. (Xiao et al., 2014).

Alginate, the second abundant anionic polysaccharide polymer next to cellulose, is predominately found in brown seaweed which contains α-L-guluronic acid and β-D-mannuronic acid as repetition-building block attached by 1,4-glycosidic linkage. Alginate geometrically liner and each sugar molecule have a functional carboxyl group (P. W. Ren et al., 2010; Thies, 2007). Alginate is harvested from seaweed by alkaline extraction, which uses NaOH as an extractant. In the process alginic acid is converted to sodium alginate and Sodium alginate is the most widely existing form of alginate in the market. Because of the plenty of functional carboxyl groups in it and its geometrical linearity, alginate is an excellent candidate for complex formation with another cationic polymer (Ahmad, 2008).

Using Gelatin-alginate complex as coating material has shown a fascinating result and it has been investigated by several studies. The chemical nature of both polymers contributes to a high yield of robust coacervate. The chemical bonds responsible for biopolymeric interaction are hydrogen and ionic bond due to the interaction of amino and carboxyl groups from gelatin and hydroxyl and carbonyl groups of sodium alginate (see Fig 1.2). In comparison with other anionic polymers, alginate has been an excellent fit to interact with gelatin to form coat material for microcapsules. For instance, in a study carried out to figure out which polyanion best fits for gelatin as a coating material in the process of complex coacervation. Degree of coacervation (ρ) and Enrichment (ε) were used as response variables to judge the efficiency of the anionic polymer (gum Arabic, alginate, polyphosphate) to interact with gelatin. The study showed alginate has the property of an excellent match to gelatin compared to the other polyanions due to the arrangement of its functional groups. For instance, if one compares the presence of carboxylic functional moiety of gum Arabic and alginate in alginate every sugar has a carboxylic functional group whereas in gum Arabic only 20% of sugars contains a functional carboxylic group. On top of this, gum Arabic contain its functional group as branched while alginate functional carboxylic group exist linearly (Thies, 2007)..

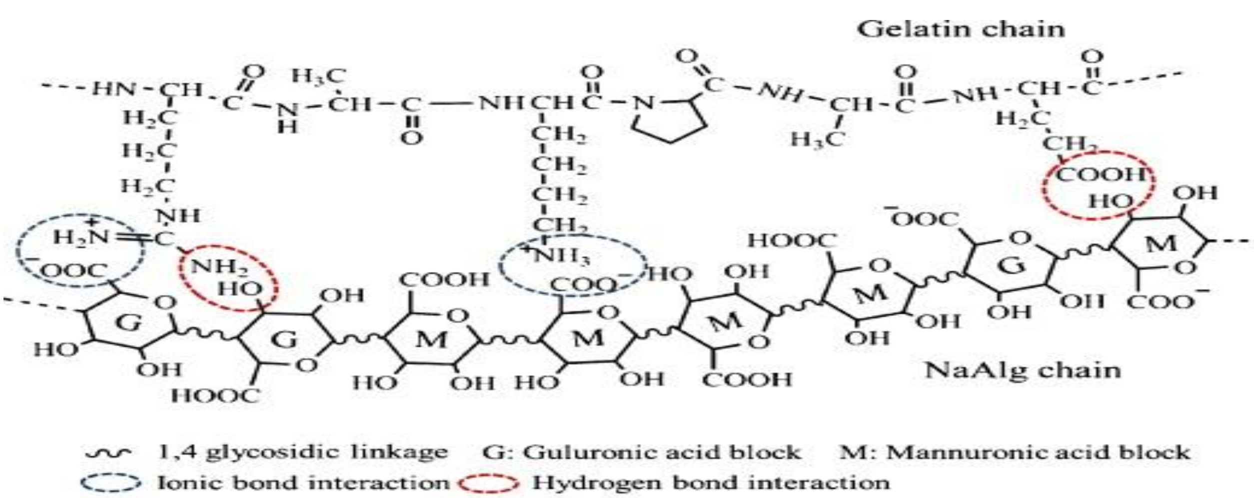


Fig 1.2 Gelatin and sodium alginate complex interaction

1.4 Statement of the problem

VBD are accountable for more than 17% of infectious diseases in the globe and the resulting death exceeds 700,000 every year. Among the VBDs, mosquito-bite takes the largest portion even though the burden is high in the tropical and subtropical poorest populations The current situation shows the severity and the damage has been increasing. However, the prevention and control strategies almost all rely on the usage of chemicals (WHO, 2020). Chemical-based mosquito repellents and mosquitocides are well known for their distractive effects on the ecosystems and human health on top of which some of the products have already been resisted by mosquitos (Jiang and Ou, 2011).

Natural remedies have been used for so many years by mankind as mosquito repellants. Of course, many studies have proven essential oils as potential mosquito repellents. In addition to their repellent properties, their compatibility with the environment and safety profile for human use make essential oils the best alternative for mosquito-borne disease prevention and control (Pavela and Benelli, 2016). However, due to their high vapor pressure, they lack effectiveness against different kinds of mosquito species (Chung et al., 2013; Velasco et al., 2003). So, improving their release profile is very helpful to fully utilize the benefit from essential oil.

1.5 Significance of the study

Microencapsulation technology is one of the current advanced technologies which helps to manipulate the releasing kinetics of essential oils (Sousa et al., 2022). Complex coacervations is believed to be inexpensive and efficient microencapsulation technique (X. Ren et al., 2023). The limitation of using Palmarosa essential oil for its mosquito-repellent properties is its high vapor pressure, which makes it less efficient. So, this study focused on introducing microencapsulation technology by the technique of complex coacervation to microencapsulate the Palmarosa essential oil to improve its release profile.

1.6 Research question

This study was conducted to address the following research questions

- Does microencapsulation of Cymbopogan *martini* essential oil can improve the release profile of *Cymbopogan martini* essential oil?
- What are the significant factors contributing for *Cymbopogan martini* essential oil microcapsules characteristics?

1.7 Objective of the study

1.7.1 General objective

The objective of the present study was to formulate and optimize microcapsules of *Cymbopogan martini* essential oil and evaluation of its *invitro* release.

1.7.2 Specific objectives

- To screen significant factors for microcapsule characteristics
- To optimize microcapsule formulation of *Cymbopogan martini* essential oils
- To evaluate the *in vitro* release profile of optimal microcapsule formulation.

2. Materials and methods

2.1 Materials

Ammonia solution (28-30%) (Supelco®, Germany), Diethyl ether (Chemical Udyog Pvt. Ltd, India), Ethanol absolute (ROMIL Ltd, UK), Gelatin type B. (gel strength 250, Sino-Ethiop Associate Africa PLC donation), Hydrochloric acid (Sigma-Aldrich®, Germany), n-Hexane 95% HPLC grade (Park Scientific Ltd, UK), sodium alginate (Sisco Research Laboratories Pvt. Ltd, India), Sodium hydroxide (Loba Chemie Pvt Ltd, India), tannic acid (LABPAK LIMITED, UK), Tween 80 sigma (Sigma-Aldrich®, Germany), and deionized water were used as received.

Essential oil

Palmarosa essential oil extracted by steam distillation was obtained from Ethio Agri-CEFT PLC. Addis Ababa, Ethiopia.

Table 2.1 Physical and chemical properties Palmarosa essential oil

Test	Specification	Result
Appearance	Colourless to yellow liquid	Conforms
Odour	Characteristic	Conforms
Specific Gravity	0.875 to 0.900	Conforms
Refractive Index	1.470 to 1.480	Conforms

2.2 Methods

2.2.1 Preliminary study

2.2.1.1 Defining optimal polymeric ratio and pH for microcapsules

The optimal polymeric ratio and pH were determined by a slight modification of a method used elsewhere (Shinde & Nagarsenker, 2009). A mixture of gelatin B and sodium alginate aqueous solution (0.2% W/V total polymeric concentration) was prepared separately by varying the gelatin/alginate polymeric ratio between 10:90 and 90:10 and each polymeric ratio of the mixture was set from 1.5 to 4.5 pH, respectively (by using 1N HCl and 1N NaOH) at 50°C temperature and stirring speed of 800 rpm by using hot plate with stirrer (DAIHAN-brand® -SMHS, Korea). After pH adjustment by using pH meter (Hanna instrument HI-2550, UK), the mixture was kept for 30 minutes for coacervate to be formed completely. Finally, dry coacervate yield (wet coacervate dried by oven (BINDER ED 115, USA) at 40°C in the oven) and turbidity measurement by UV-VIS spectrophotometers (Shimadzu UV-1800, Germany) at 600 nm were used as response factors for the optimal polymeric ratio and pH.

2.2.1.2 Experimental factors screening study

2.2.1.2.1 Design of Experiment

2.2.1.2.1.1 Plackett–Burman screening design

Factors that have a significant impact on microcapsule features were identified by the Plackett–Burman screening design by taking eight factors- twelve experimental run choices (Z. Q. Zhang et al., 2011). The microcapsules feature considered as response from those factors were, $\boldsymbol{y_1}$ Encapsulation Efficiency (EE%, y_1 %), microcapsule surface property y_2, microcapsules size distribution $\boldsymbol{y_3}$ and microcapsules mean size $\boldsymbol{y_4}$. The following Fist-order polynomial model (Equation 2.1) was used to investigate the association between factors x_i (indicted in Table 2.2 and Table 2.3) and responses (y_i).

$$\boldsymbol{y_i = b_0 + \sum_{i=1}^{8} b_i x_i} \quad \textbf{(eq 2.1)}$$

Where: $\mathbf{b_0}$ model intercept and

$\mathbf{b_i}$ linear regression coefficient,

MINITAB™ version 19 software was used for the statistical analysis of data that was generated from the experimental run.

Table 2.2 Factors with coded levels for Plackett–Burman screening design

Symbol	Factor	unit	Coded levels	
			Low level (-1)	High level (+1)
x_1	Total polymeric concentration	% w/w	1	3
x_2	Palmarosa essential oils	% w/w	1	3
x_3	Tween 80	% w/w	0.01	0.1
x_4	Temperature	°c	40	50
x_5	Reaction time	minute	10	30
x_6	String speed	rpm	600	900
x_7	Tannic acid	% w/w	0.3	0.5
x_8	Crosslinking time	hour	4	6

Table 2.3. Design of Experiment by Plackett-Burman screening design

Formulation code	**Coded Factors* Level**							
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8
m_1	+1	-1	-1	-1	+1	+1	+1	-1
m_2	+1	+1	+1	-1	+1	+1	-1	+1
m_3	-1	+1	+1	+1	-1	+1	+1	-1
m_4	-1	+1	-1	-1	-1	+1	+1	+1
m_5	+1	-1	+1	+1	-1	+1	-1	-1
m_6	+1	+1	-1	+1	-1	-1	-1	+1
m_7	+1	+1	-1	+1	+1	-1	+1	-1
m_8	-1	-1	-1	+1	+1	+1	-1	+1
m_9	-1	-1	+1	+1	+1	-1	+1	+1
m_{10}	-1	+1	+1	-1	+1	-1	-1	-1
m_{11}	+1	-1	+1	-1	-1	-1	+1	+1
m_{12}	-1	-1	-1	-1	-1	-1	-1	-1

*** x_i is describe in table 2.1**
(i, 1-8)

2.2.1.2.2 Microcapsules preparation

Gelatin B and sodium alginate aqueous solution mixtures with known total polymeric concentration at optimal polymeric ratio of sodium alginate/gelatin (0.3:1) were was prepared. A defined amount of Palmarosa oil and Tween 80 were added to the prepared mixtures and emulsified by using a homogenizer at 15,000 rpm for 3 minutes. Then, the emulsion pH was adjusted to 3.5 by using 1N HCl and 1N NaOH to induce coacervation at the specified temperature, stirring speed, and reaction time. After coacervate formation, the system temperature was lowered and maintained below 10°C for 15 minutes to harden the formed coacervates by using an ice bath. After chilling, the coacervate was brought to room temperature and treated with a defined amount of tannic acid for a specified time to crosslink the wall material. Finally, the formulated microcapsules were harvested by simple decantation and drying in a vacuum oven at room temperature. After screening the important formulation/process factors that contributed to the response variables, the microcapsules for optimization were also prepared by the same procedure except for factors, that were not significant kept constant in the experimental run. The method was adopted from (Devi et al., 2012) with moderate modification.

2.2.1.2.3.1 Measurement of encapsulation efficiency

Determination of encapsulation efficiency was done by a slight modification of method described elsewhere (Liu et al., 2010). One gram of microcapsules was added to a test tube that contained 30 ml of hexane and was vigorously shaken for 30 seconds by vortex mixer and centrifuged for 20 minutes at 2720 rpm. Then, the supernatant was decanted and the sediment dried in air to remove surface oil. The dried microcapsules were added to 20 ml of warm deionized water (40°C) and stirred for 15 minutes at 40°C. Then, 2 ml of ammonia solution 28–30% (w/w) was added and stirred for another 15 minutes at 40 °c. The mixture was temperature cooled to room temperature. After cooling, the mixture was transferred to a separatory funnel and 10 ml of 96% ethanol was added following the gas release 25 ml of each hexane and diethyl ether were added. Gentle shaking was applied to extract the Palmarosa essential oil and then after the mixture settled and separated the organic phase was collected. The extraction was continued on the aqueous phase for further two consecutive rounds by using 5 ml of 96% ethanol and 15 ml of each hexane and diethyl ether to exhaust the extraction process. Finally, the collected organic phase from the three extraction batches was collected and dried in a vacuum oven at room temperature to recover the Palmarosa essential oil by evaporating the solvent.

The encapsulation efficiency (EE%) $\mathbf{y_1}$ of Palmarosa oil is defined by the following equation (Equation 2.2).

$$y_1 = \frac{Ma}{Mth} \times 100 \qquad \text{(eq 2.2)}$$

Where: Ma the actual mass of Palmarosa oil in encapsulated

M^{th} the theoretical mass of Palmarosa oil encapsulated

2.2.1.2.4.2 Microcapsules surface property

The microcapsules $\boldsymbol{m_i}$ surface property $\boldsymbol{y_2}$ were determined by visual inspection. Oily and dry microcapsules were assigned as 0 and 1, respectively for statistical analysis.

2.2.1.2.4.3 Microcapsule size and distribution analysis

Microcapsules size means $\boldsymbol{y_4}$ that was the mean of the volume diameter in micrometers and microcapsules size distribution $\boldsymbol{y_3}$ of microcapsule (Z. Q. Zhang et al., 2011) were analyzed by a Laser diffraction particle size analyzer (Mastersizer 3000 Malvern Instruments Ltd., UK). The span $\boldsymbol{y_3}$ was calculated by the Equation 2.3 and microcapsules size mean $\boldsymbol{y_4}$ was determined by using Fraunhofer diffraction equation as shown in Equation 2.4.

$$\boldsymbol{y_3 = \frac{d_{v,90} - d_{v,10}}{d_{v,50}}} \qquad (2.3)$$

Where: $d_{v,10}$ is microcapsules diameter below 10% of volume of microcapsule found.

$d_{v,50}$ is microcapsules diameter below 50% of volume of microcapsule found.

$d_{v,90}$ is microcapsules diameter below 90% of volume of microcapsule found.

$$\mathbf{Sin}\ \boldsymbol{\theta_n = \frac{n\lambda}{x}} \qquad (2.4)$$

Where θ is diffraction angle

λ is wave length

n is ordinal number

x is particle size

2.2.2 Formulation optimization study

2.2.2.1.1 Central composite design

Plackett–Burman screening design selected three factors that have a significant impact on response y_1 encapsulation efficiency (EE%) and those factors (Table 2.4) were optimized to get optimal response y_1 by using the central composite design that includes 8 Factorial points, 6 center points in the cube and 6 axial points (Table 2.5). The total of the experiment run was 20 (Z. Q. Zhang et al., 2011).

Nonlinear quadratic equation represents the association of factor x_i and response Y_i (eq 2.5)

$$y_i = b_0 + \sum_{i=1}^{3} b_i x_i + \sum_{i=1}^{3} b_{ij} X^2 + \sum_{i=i}^{2} \sum_{j>i}^{3} b_{ij} x_i x_j$$

Where: b_0 model intercept

b_i is linear regression coefficient

b_{ii} is quadratic regression coefficient

b_{ij} is interactive regression coefficient

Table 2.4 Factors and coded levels for central composite optimization design

Factors*	Coded levels				
	- α	-1	0	+1	α
x_1	0.31821	1	2	3	3.68179
x_2	0.31821	1	2	3	3.68179
x_3	0.006137	0.03	0.065	0.1	0.123863

*** *xi* defined in table 2.1; α=1.68**

Table 2.5 Design of experiment by Central composite design

Formulation code	Point type	Coded Factor* Level		
		x_1	x_2	x_3
m_1	Factorial point	+1	-1	+1
m_2	Axial	0	0	-α
m_3	Factorial point	-1	+1	-1
m_4	Factorial point	+1	+1	-1
m_5	Factorial point	+1.	-1	-1
m_6	Factorial point	-1	+1.	+1
m_7	Center point	0	0	0
m_8	Factorial point	-1	-1	-1
m_9	Center point	0	0	0
m_{10}	Center point	0	0	0
m_{11}	Factorial point	-1	-1	+1
m_{12}	Center point	0	0	0
m_{13}	Center point	0	0	0
m_{14}	Axial	-α	0	0
m_{15}	Factorial point	+1	+1	+1
m_{16}	Axial	0	α	0
m_{17}	Axial	0	-α	0
m_{18}	Center point	0	0	0
m_{19}	Axial	α	0	0
m_{20}	Axial	0	0	α

*** x_i defined in table 2.1; α=1.681**

2.2.3 Validation of the experimental design

Experimental design validation was done by quantitative comparison of the response factors (y_i) with software generated predicted response (Z. Q. Zhang et al., 2011). Percentage relative error was defined by Equation 2.6

$$\%\ \text{Relative error} = \frac{(Predicted\ value - Experimental\ Value)}{Predicted\ value}\ x100 \quad \text{(eq 2.6)}$$

Response factors (y_i): encapsulation efficiency y_1

2.2.4 *Invitro* release kinetics of encapsulated Palmarosa essential oils

A known amount of optimized dried microcapsule (w_1) was placed in the oven at 32 °C and the microcapsule was weighed in a specified time interval to gain (w_2) (Chaib et al., 2021). Then Palmarosa essential oil release was determined by Equation 2.7

$$Release(\%) = \frac{w_1 - w_2}{w_1} \times 100 \qquad \text{(eq 2.7)}$$

2.2.5 Microcapsules of Palmarosa essential oil release kinetics

The *invitro* release study was done on average of triplicate cumulative percentage release of optimized microcapsules. Two mathematical models, zero order and first order (Equation 2.8 and 2.9) were fitted to determine the pattern of *invitro* release of the Palmarosa essential oil from the optimized microcapsules (Paarakh et al., 2019).

2.2.5.1 Zero order release kinetics model

Mathematical equation $\mathbf{Q = Q_0 + K_0\ t}$ (eq 2.8)

Where: Q_0 is the initial amount of oil in microcapsules,

Q is the amount of oil released in time,

k_0 is the zero-order release constant,

t is time.

2.2.5.2 First order release kinetics model

Mathematical equation **$\log Q = \log Q_0 - K_1 t / 2.303$** (eq 2.9)

Where: Q_0 is the initial amount of oil in microcapsules,

Q is the amount of oil released in time,

k is the first-order release constant

t is time.

2.2.6 FTIR analysis

Functional group analysis of polymers and microcapsules was analyzed by FTIR spectroscopy (FTIR-8400S, Shimadzu, Japan) at transmittance mode at room temperature. The optimal microcapsules and the two coating materials (Gelatin B and Sodium alginate) were grounded separately and about 8 grams of each mixed with liquids paraffin and were placed on potassium bromide plate then compressed to have thin film and scanned by IR spectrometer from 4000-400 cm^{-1} with 4 cm^{-1} resolution. Background spectrum was obtained before analyzing each sample. IR software was used for data interpretation.

2.2.7 Statistical analysis

To represent numerical data in graph, origin software (OriginLab Corporation, MA, and USA) was used. For screening significant factors contributing to the formulation of microcapsules and optimization of the significant factors Multi-Way ANOVA (ANOVA) was applied at 95 % confidence interval where the statistically significant value of p was < 0.05 by using MINITAB™ version 19 software.

3. Results and discussions

3.1 Preliminary study

3.1.1 Optimal polymeric ratio and pH

At pH 2.5 and below from 10:90 to 90:10 gelatin/alginate polymeric ratio coacervates were loose and soggy even if the coacervate yield increased from 90:10 down to 10:90 at each pH. This was because of the precipitation of alginate due to excessive protonation at low pH less than its pKa (mannuronic acid pKa = 3.38 and guluronic acid pKa = 3.65) that favor strong hydrogen bonding within the molecule than the repulsion force between molecules (Razzak et al., 2016). At pH 3 and 3.5 (Figure 3.1 a and 3.1 b), particularly at 70:30 gelatin B/sodium alginate polymeric ratio, intact and robust coacervates were formed compared to other polymeric ratios at this pH region and exhibited the highest coacervate yield (82.1 and 82.6, respectively). Turbidity measurement at 600 nm also aligns with coacervate yield results (Tables 3.1 and 3.2).

Because of the high yield and the robustness of the coacervate, pH 3.5 and 70:30 gelatin B/sodium alginate were chosen as optimal pH and polymeric ratio respectively for the subsequent experimental investigations. Optimal pH at 3.5 and closet optimal polymeric ratio of 70:30 gelatin B/sodium alginate were also confirmed by other investigators (Bouchal et al., 2023; Lemos et al., 2017; Shinde & Nagarsenker, 2009; Wang et al., 2016). This is because the optimal pH 3.5 is less than the isoelectric point of gelatin B (isoelectric point of gelatin B 4.6-5.2) thus making the gelatin more cationic (more free functional amino groups available) and at the same time higher than the pKa of sodium alginate which also makes sodium alginate to become more anionic (more free functional carboxylic groups available) allowing for an intensive interaction between the two polymers (X. Ren et al., 2023). The optimal polymeric ratio also offers a generous amount of functional groups for interaction to happen.

From 60:40 to 10:90 gelatin/ alginate polymeric ratio at pH 3 and 3.5, all coacervates formed were loose and soggy. This was because of the un proportional ratio of the polymers even if it exists at optimal pH. Hence, for a robust and intact coacervate to form, there is a need to find an optimal pH and the polymeric ratio of polymers. At pH 4, from 90:10 to 60:40 gelatin/ alginate polymeric ratio, the formed coacervates were again loose and soggy. Below 60:40 polymeric ratio at pH 4 and all polymeric ratio at pH 4.5, no coacervate were formed. This is due to the closeness of the pH to the isoelectric point of gelatin B (4.6-5.2) which makes the net charge close to zero thus lowering the interaction to form coacervates (Pal et al., 2019).

Table 3.1 Percentage yield of coacervates prepared at different pH and gelatin B/sodium alginate ratios.

GelatinB/sodium alginate polymeric ratio)	**p^H 1.5**	**p^H 2**	**p^H 2.5**	**p^H 3**	**p^H 3.5**	**p^H 4**	**p^H 4.5**
90:10	NP	NP	NP	NP	51.17	72.81	NP
80:10	34.5	46.87	52.32	65.5	78.96	64.51	NP
70:30	57.35	68.7	68.31	82.16	82.6	65.76	NP
60:40	70.12	81.97	84.18	79.21	75.93	37.22	NP
50:50	81.38	86.01	83.72	72.85	74.75	NP	NP
40:60	84.65	86.96	79.52	72.8	52.77	NP	NP
30:70	94	87.27	66.86	66.53	45.72	NP	NP
20:80	93.36	75.56	66.62	61	42.23	NP	NP
10:90	89.96	58.43	NP	NP	NP	NP	NP

Where NP, is No phase separation

Table 3.2 turbidity measurement at 600 nm of coacervates prepared at different pH and gelatin B/sodium alginate ratios.

Gelatin B/sodium alginate polymeric ratio)	**p^H 1.5**	**p^H 2**	**p^H 2.5**	**p^H 3**	**p^H 3.5**	**p^H 4**	**p^H 4.5**
90:10	NP	NP	NP	NP	0.033	0.302	NP
80:10	0.043	0.217	0.314	0.454	0.594	0.205	NP
70:30	0.068	0.486	0.258	0.617	0.523	0.011	NP
60:40	0.089	0.097	0.148	0.522	0.607	0.007	NP
50:50	0.100	0.139	0.151	0.676	0.333	NP	NP
40:60	0.077	0.097	0.159	0.130	0.149	NP	NP
30:70	0.077	0.081	0.060	0.044	0.069	NP	NP
20:80	0.057	0.039	0.034	0.032	0.043	NP	NP
10:90	0.026	0.023	0.011	0.010	0.024	NP	NP

Where NP, is No phase separation

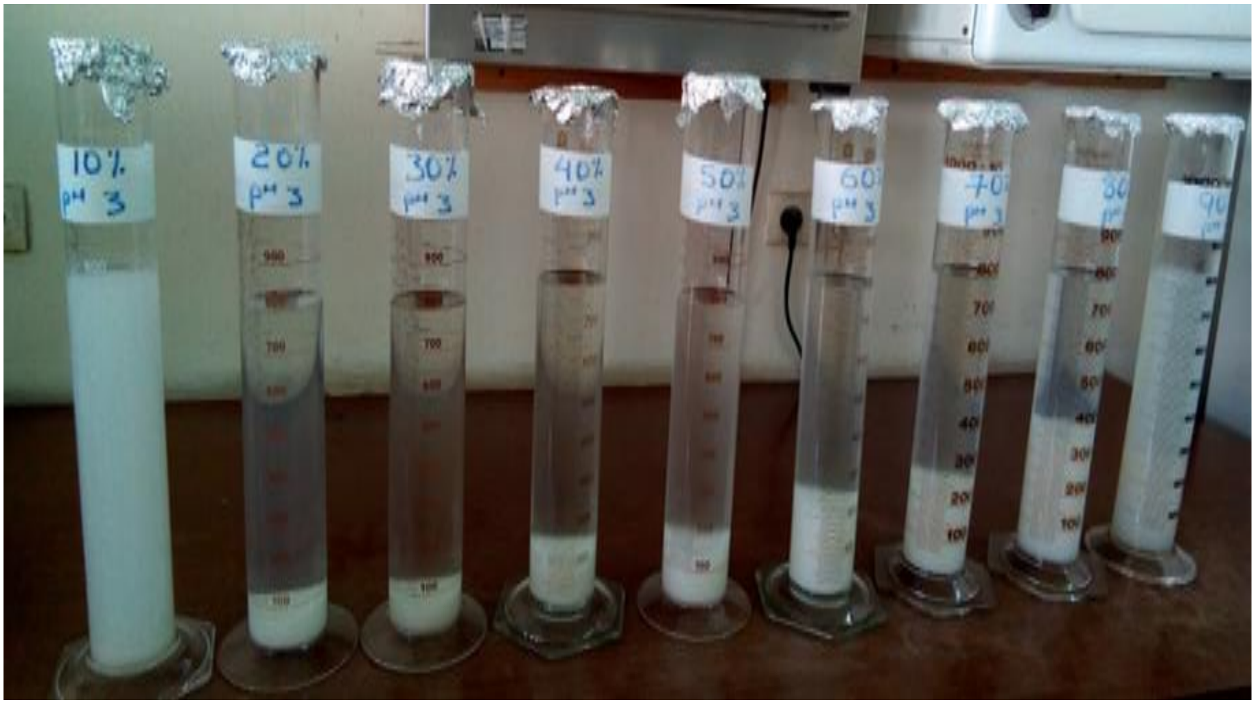


Fig 3.1a. Pictures of coacervates prepared at pH 3 and 10/90 to 90/10 sodium alginate/gelatin B polymeric ratio

Fig 3.1b Pictures of coacervates prepared at pH 3.5 and 10/90 to 90/10 sodium alginate/gelatin B polymeric ratio

3.1.2 Experimental factors screening study

3.1.2.1 Encapsulation efficiency as response for microcapsule characteristics.

Microcapsules formulation $\boldsymbol{m_i}$ proposed by Plackett–Burman screening design and, their encapsulation efficiency (EE%) $\boldsymbol{y_1}$ were determined as shown in table 3.3. Microcapsules m_i encapsulation efficiency ranged from 11.9 % to 86.31%. The analysis of variance of Plackett–Burman screening design showed that the total polymeric concentration.$\boldsymbol{x_1}$, oil load $\boldsymbol{x_2}$ and Tween 80 concentration $\boldsymbol{x_3}$ had a significant effect on microcapsules $\boldsymbol{m_i}$ encapsulation efficiency y_1 (Table 3.3 and Figure 3.4). The P-value for $\boldsymbol{x_1}$, $\boldsymbol{x_2}$ and $\boldsymbol{x_3}$ in relationship with $\boldsymbol{y_1}$ response were 0.003, 0.002 and 0.017, respectively. But, the other factors temperature $\boldsymbol{x_4}$, reaction time $\boldsymbol{x_5}$, stirring speed $\boldsymbol{x_6}$, tannic acid concentration $\boldsymbol{x_7}$ and crosslinking time $\mathbf{x_8}$ were not statistically significant so in the optimization study, they were assigned based on the regression equation slope sign in Equation 3.1 and remain constant throughout the experimental run. Except $\boldsymbol{x_7}$, all factors had negative slope sign so they were kept at lower level (**-1**) and $\mathbf{x_7}$ which had positive slope was kept at higher level (**+1**). The statistical model $\mathbf{R^2}$ and $\mathbf{R^2adj}$ were 98.74% and 95.40%, respectively (Table 3.5). The encapsulation efficiency (EE%) $\boldsymbol{y_1}$ as response variable for microcapsule characteristics by taking $\boldsymbol{x_1}$, $\boldsymbol{x_2}$and $\boldsymbol{x_3}$ as factors were further optimized by using central composite design.

Table 3.3 Encapsulation Efficiency of microcapsules mi prepared according to the Placket-Burman screening design.

Formulation code	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	y_1
m_1	3	1	0.01	40	30	900	0.5	4	57.58
m_2	3	3	0.10	40	30	900	0.3	6	33.22
m_3	1	3	0.10	50	10	900	0.5	4	11.90
m_4	1	3	0.01	40	10	900	0.5	6	12.28
m_5	3	1	0.10	50	10	900	0.3	4	70.03
m_6	3	3	0.01	50	10	600	0.3	6	20.75
m_7	3	3	0.01	50	30	600	0.5	4	26.39
m_8	1	1	0.01	50	30	900	0.3	6	16.41
m_9	1	1	0.10	50	30	600	0.5	6	39.00
m_{10}	1	3	0.10	40	30	600	0.3	4	12.00
m_{11}	3	1	0.10	40	10	600	0.5	6	86.31
m_{12}	1	1	0.01	40	10	600	0.3	4	30.87

*** x_i defined in table 2.2**

Table 3.4 Analysis of Variance for microcapsules mi encapsulation efficiency y_1 as response variable

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Model	**8**	**6523.64**	**815.46**	**29.49**	**0.009**
Linear	**8**	**6523.64**	**815.46**	**29.49**	**0.009**
x_1	**1**	**2460.18**	**2460.18**	**88.96**	**0.003**
x_2	**1**	**2810.92**	**2810.92**	**101.64**	**0.002**
x_3	**1**	**647.98**	**647.98**	**23.43**	**0.017**
x_4	**1**	**190.24**	**190.24**	**6.88**	**0.079**
x_5	**1**	**188.34**	**188.34**	**6.81**	**0.080**
x_6	**1**	**16.10**	**16.10**	**0.58**	**0.501**
x_7	**1**	**209.84**	**209.84**	**7.59**	**0.070**
x_8	**1**	**0.05**	**0.05**	**0.00**	**0.968**
Error	**3**	**82.96**	**27.65**		
Total	**11**	**6606.60**			

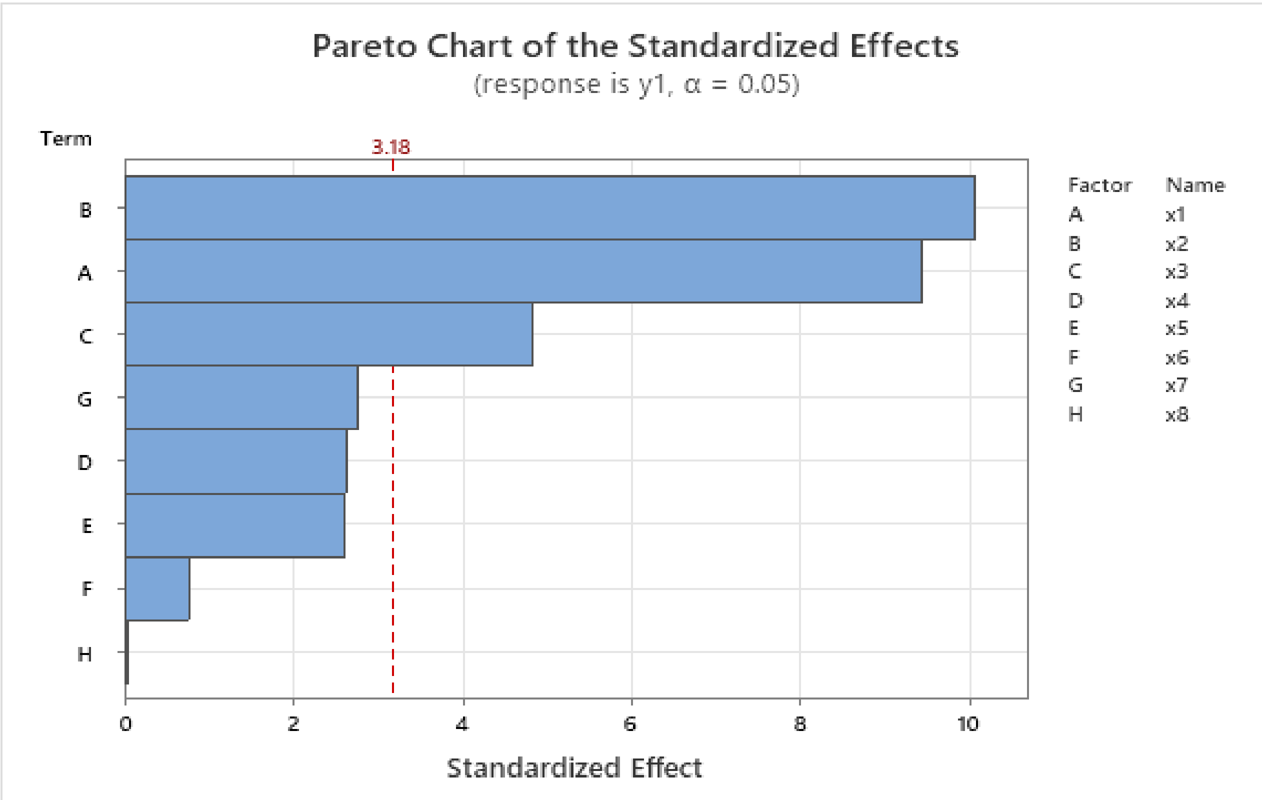
*** x_i defined in table 2.2**

Regression Equation for microcapsules *m_i* Encapsulation efficiency *y_1* as response variable.

$$y_1 = 60.9 + 14.32\,x_1 - 15.30\,x_2 + 163.3\,x_3 - 0.796\,x_4 - 0.396\,x_5 - 0.0077\,x_6 + 41.8x_7 - 0.07\,x_8 \quad \text{(eq 3.1)}$$

Table 3.5 model summery for microcapsules mi Encapsulation efficiency y_1 as response variable

SD	R-sq	R-sq(adj)	R-sq(pred)
5.25877	**98.74%**	**95.40%**	**79.91%**



*** xi defined in table 2.2**

Fig 3.4 pareto chart for microcapsules mi encapsulation efficiency as response variable.

3.1.2.2 Microcapsule surface property as response for microcapsule characteristics

Microcapsules formulation m_i proposed by Plackett–Burman screening design, their surface property $\mathbf{y_2}$ were analyzed by visual inspection where oily and dry microcapsules were represented by $\mathbf{y_2} = 0$ and $\mathbf{y_2} = 1$, respectively as shown in Table 3.6. The analysis of variance of the Plackett–Burman screening design (Table 3.7 and Figure 3.5) showed that the only factor which had a significant effect at p value = 0.015 was oil load $\mathbf{x_2}$ and also the Regression Equation of the screening design in Equation. 3.2 showed that the slope of $\mathbf{x_2}$ was negative which confirmed $\mathbf{x_2}$ affects $\mathbf{y_2}$ negatively which means when the oil load increases the surface nature of microcapsules becomes oily since the oily nature represented by 0 in Statistical analysis. The statistical model $\mathbf{R^2}$ was 91.43% which seems good however $\mathbf{R^2adj}$ was only 68.57% (Table 3.8) this was not a surprise since the screening design only use Fist-order polynomial model and only for screening purpose on other hand the response variable were categorical by nature which is a limitation of the model. However, the model accurately identified the oily nature of some of the microcapsule formulation was due to the oil load $\mathbf{x_2}$ burden.

Table 3.6 Surface property of microcapsules m_i prepared according to the Placket- Burman screening design

Formulation Code	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	y_2
m_1	3	1	0.01	40	30	900	0.5	4	1
m_2	3	3	0.10	40	30	900	0.3	6	0
m_3	1	3	0.10	50	10	900	0.5	4	0
m_4	1	3	0.01	40	10	900	0.5	6	0
m_5	3	1	0.10	50	10	900	0.3	4	1
m_6	3	3	0.01	50	10	600	0.3	6	0
m_7	3	3	0.01	50	30	600	0.5	4	1
m_8	1	1	0.01	50	30	900	0.3	6	1
m_9	1	1	0.10	50	30	600	0.5	6	1
m_{10}	1	3	0.10	40	30	600	0.3	4	0
m_{11}	3	1	0.10	40	10	600	0.5	6	1
m_{12}	1	1	0.01	40	10	600	0.3	4	1

* x_i defined in table 2.2

Table 3.7 Analysis of Variance for microcapsules surface property (y_2) of microcapsules mi as response variable

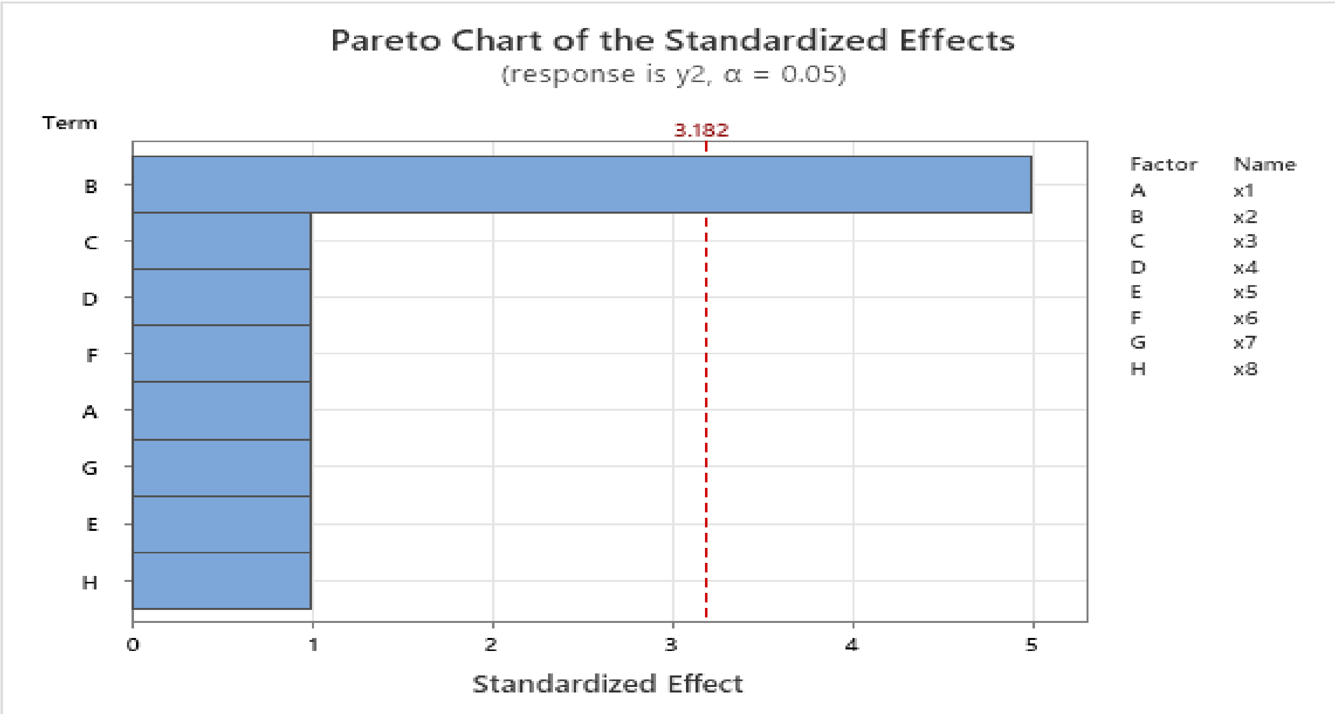
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	2.66667	0.33333	4.00	0.141
Linear	8	2.66667	0.33333	4.00	0.141
x_1	1	0.08333	0.08333	1.00	0.391
x_2	1	2.08333	2.08333	25.00	0.015
x_3	1	0.08333	0.08333	1.00	0.391
x_4	1	0.08333	0.08333	1.00	0.391
x_5	1	0.08333	0.08333	1.00	0.391
x_6	1	0.08333	0.08333	1.00	0.391
x_7	1	0.08333	0.08333	1.00	0.391
x_8	1	0.08333	0.08333	1.00	0.391
Error	3	0.25000	0.08333		
Total	11	2.91667			

Table 3.8 Model summery for microcapsules surface property (y_2) of microcapsules m_i as response variable

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
0.288675	91.43%	68.57%	0.00%

Regression Equation in Uncoded Units for microcapsules surface property (y_2) of microcapsules m_i as response variable **(eq 3.2)**

$$y_2 = 0.94 + 0.0833\, x_1 - \mathbf{0.4167}\, x_2 - 1.85\, x_3 + 0.0167\, x_4 + 0.00833\, x_5 - 0.000556\, x_6 + 0.833\, x_7 - 0.0833\, x_8$$



*** x_i defined in table 2.2**

Fig 3.5 pareto chart for microcapsules surface property (y_2) of microcapsules mi as response variable

3.1.2.3 Microcapsules size distribution (span) as response for microcapsule characteristics

Microcapsules formulation m_i proposed by Plackett–Burman screening design, their size distribution (span) y_3 as response for microcapsule characteristics were determined by laser diffraction particle size analyzer. The microcapsules formulation m_i span ranged from 1.588 to 2.838 (Table 3.9). Analysis of variance showed both total polymeric concentration x_1 and oil load x_2 had significant effects at p value = 0.020 on microcapsules size distribution y_2 (Table 3.10 and Figure 3.6) and Regression Equation slope of both factors x_1 and x_2 in Equation 3.3 were negative which tells the relationship between both factors and the response y_3 (span) were inversely related. When total polymeric concentration become less and the oil load became high the produced microcapsules become oilier, this is because of polymer concentration became insufficient to coat the oil, so this led to excessive oil accumulation on surface of microcapsules. But this was an interesting property which helps wetting microcapsules so in the gridding process, the oilier microcapsules became finer whereas the dry microcapsules became coarser. This phenomenon was observed in laser diffraction particle size distribution analysis (Figure 3.7) the oily microcapsules were fine compared to the dry microcapsules. The statistical model $\mathbf{R^2}$ and $\mathbf{R^2adj}$ was **96.22%** and **86.15%,** respectively (Table 3.11).

Table 3.9 Microcapsules size distribution (span) of microcapsules mi prepared according to the Placket- Burman screening design

Formulation Code	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	y_3
m_1	3	1	0.01	40	30	900	0.5	4	2.024
m_2	3	3	0.10	40	30	900	0.3	6	2.038
m_3	1	3	0.10	50	10	900	0.5	4	1.630
m_4	1	3	0.01	40	10	900	0.5	6	1.926
m_5	3	1	0.10	50	10	900	0.3	4	1.588
m_6	3	3	0.01	50	10	600	0.3	6	1.955
m_7	3	3	0.01	50	30	600	0.5	4	1.771
m_8	1	1	0.01	50	30	900	0.3	6	2.838
m_9	1	1	0.10	50	30	600	0.5	6	2.660
m_{10}	1	3	0.10	40	30	600	0.3	4	2.216
m_{11}	3	1	0.10	40	10	600	0.5	6	2.164
m_{12}	1	1	0.01	40	10	600	0.3	4	2.689

*** xi defined in table 2.2**

Table 3.10 Analysis of Variance for microcapsules size distribution (span) y_3 of microcapsules m_i as response variable

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	1.79696	0.22462	9.56	0.045
Linear	8	1.79696	0.22462	9.56	0.045
x_1	1	0.48763	0.48763	20.75	0.020
x_2	1	0.49086	0.49086	20.88	0.020
x_3	1	0.06855	0.06855	2.92	0.186
x_4	1	0.03152	0.03152	1.34	0.331
x_5	1	0.21200	0.21200	9.02	0.058
x_6	1	0.16591	0.16591	7.06	0.077
x_7	1	0.11002	0.11002	4.68	0.119
x_8	1	0.23046	0.23046	9.80	0.052
Error	3	0.07052	0.02351		
Total	**11**	**1.86747**			

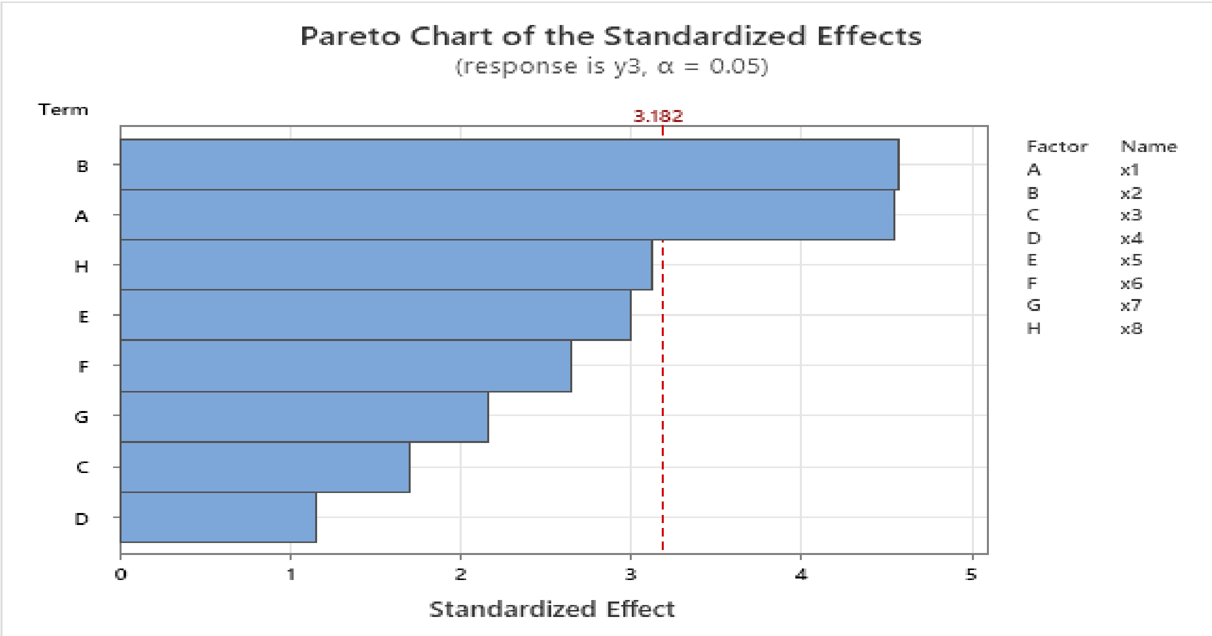
*** x_i defined in table 2.2**

Table 3.11 statistical model Summary for microcapsules size distribution (span) of microcapsules mi as response variable

S	R-sq	R-sq(adj)	R-sq(pred)
0.153315	96.22%	86.15%	39.58%

Regression Equation for microcapsules size distribution (span) of microcapsules m_i as response variable

$$y_3 = 3.498 - 0.2016\, x_1 - 0.2023\, x_2 - 1.680\, x_3 - 0.01025\, x_4 + 0.01329\, x_5 - 0.000784\, x_6 - 0.958\, x_7 + 0.1386\, x_8 \quad \textbf{(eq 3.3)}$$



*** x_i defined in table 2.2**

Fig 3.6 pareto chart for microcapsules size distribution (span) of microcapsules mi as response variable

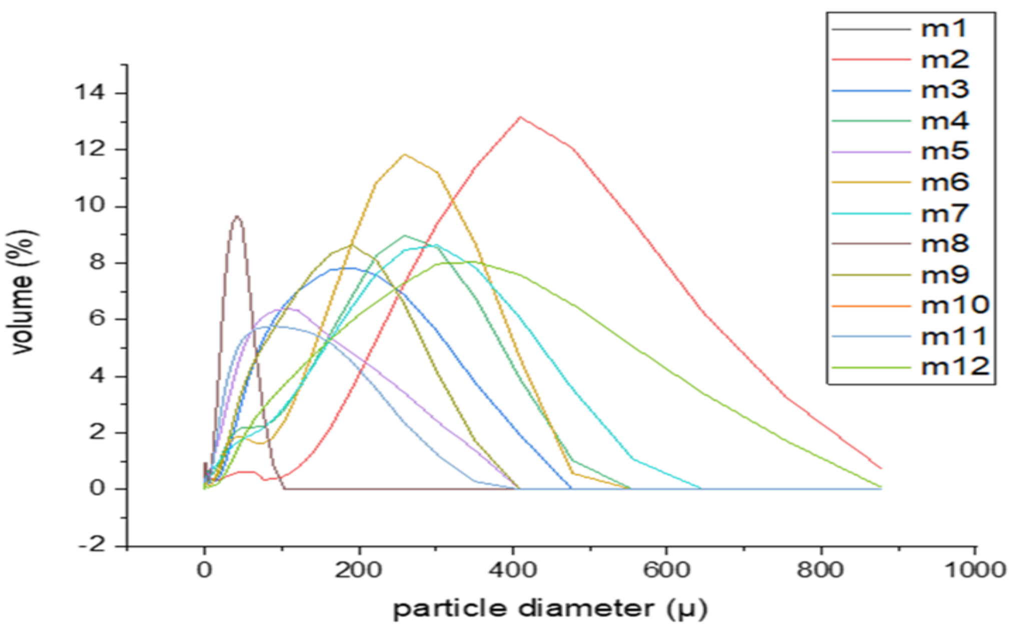


Fig 3.7 Microcapsules size distribution (span) of microcapsules mi prepared according to the Placket- Burman screening design

3.1.2.4 Microcapsules mean size as response for microcapsule characteristic

Microcapsules formulation m_i proposed by Plackett–Burman screening design and, their microcapsules size means y_4 that was the mean of the volume diameter in micrometers analyzed by laser diffraction particle size analyzer are shown in Table 3.12. The microcapsules m_i size mean ranged from 28.4 to 325.58 µm. The analysis of variance showed all the eight factors didn't have any statistically significant effect on response y_4 (Table 3.13 and Figure 3.8). Even if the microcapsule surface property y_2 was affected by oil load x_2 and thus the oily nature of the microcapsules also affects the microcapsule distribution (span) y_3, overall, the mean size of the microcapsules was not affected by any of the eight factors. The mean size value only focusses on the mean volume diameter of the microcapsules which is not dealing with the size distribution. Hence, microcapsules mean size was not affected by any of the eight factors. Accordingly, microcapsules surface property, size and distribution were not used as response for microcapsules characteristic in the optimization study.

Table 3.12 Microcapsules mean size of microcapsules m_i prepared according to the Placket-Burman screening design

Formulation code	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	y_4 (µm)
m_1	3	1	0.01	40	30	900	0.5	4	134.31
m_2	3	3	0.10	40	30	900	0.3	6	282.63
m_3	1	3	0.10	50	10	900	0.5	4	176.8
m_4	1	3	0.01	40	10	900	0.5	6	28.4
m_5	3	1	0.10	50	10	900	0.3	4	325.58
m_6	3	3	0.01	50	10	600	0.3	6	119.43
m_7	3	3	0.01	50	30	600	0.5	4	268.61
m_8	1	1	0.01	50	30	900	0.3	6	77.15
m_9	1	1	0.10	50	30	600	0.5	6	239.2
m_{10}	1	3	0.10	40	30	600	0.3	4	147.17
m_{11}	3	1	0.10	40	10	600	0.5	6	171.54
m_{12}	1	1	0.01	40	10	600	0.3	4	89.93

Table 3.13. Analysis of Variance for microcapsules mean size y_4 of microcapsules m_i as response variable

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	76496.3	9562.0	2.00	0.308
Linear	8	76496.3	9562.0	2.00	0.308
x_1	1	24611.5	24611.5	5.14	0.108
x_2	1	17.9	17.9	0.00	0.955
x_3	1	32561.5	32561.5	6.80	0.080
x_4	1	10371.7	10371.7	2.17	0.237
x_5	1	4696.2	4696.2	0.98	0.395
x_6	1	10.1	10.1	0.00	0.966
x_7	1	44.2	44.2	0.01	0.929
x_8	1	4183.2	4183.2	0.87	0.419
Error	3	14354.9	4785.0		
Total	11	90851.2			

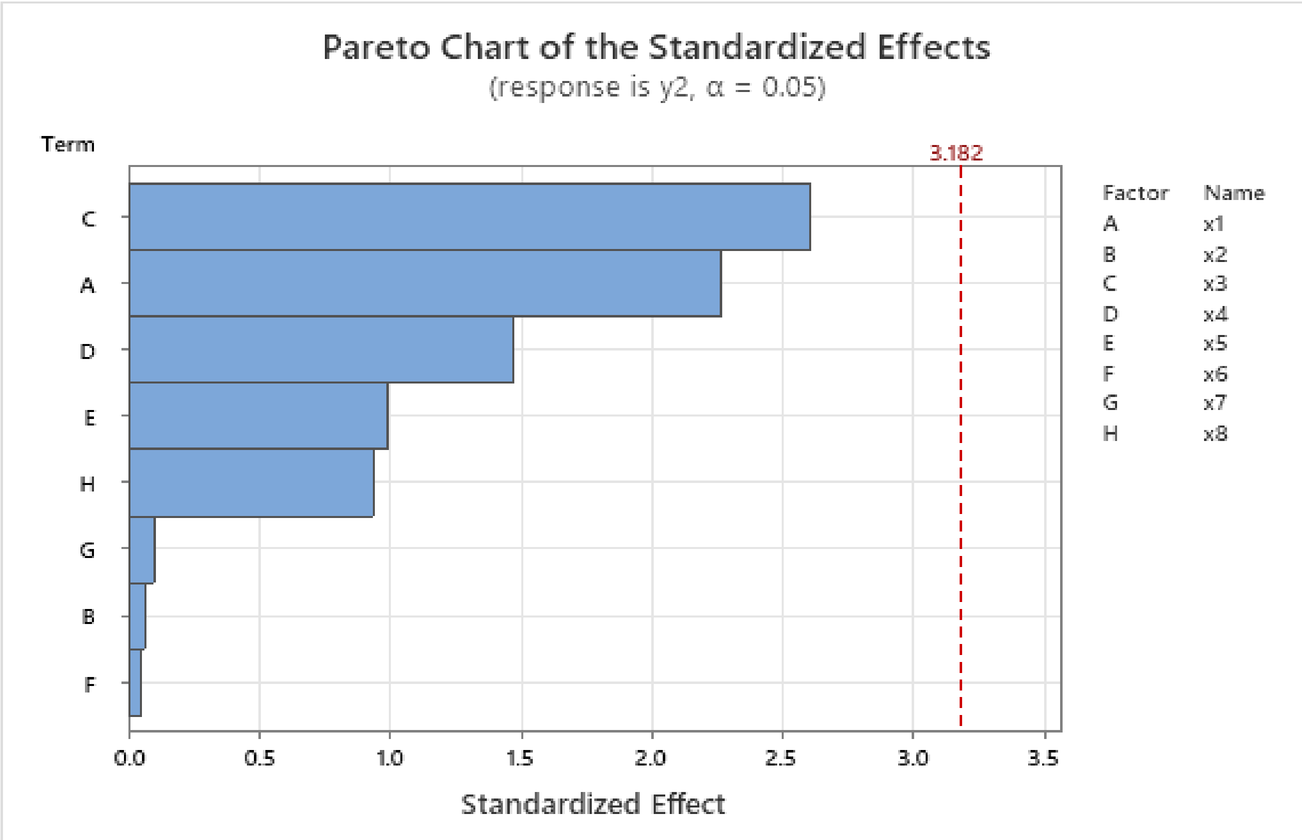
*** xi defined in table 2.2**

Table 3.14 Model Summary for microcapsules mean size y_4 of microcapsules m_i as response variable

S	R-sq	R-sq(adj)	R-sq(pred)
69.1734	**84.20%**	**42.07%**	**0.00%**

Regression Equation for microcapsules mean size y_4 of microcapsules mi as response variable

$$y_4 = -179 + 45.3\,x_1 - 1.2\,x_2 + 1158\,x_3 + 5.88\,x_4 + 1.98\,x_5 - 0.006\,x_6 - 19\,x_7 - 18.7\,x_8 \quad \text{eq 3.4}$$



* x_i defined in table 2.2

Fig 3.8 pareto chart for microcapsules mean size y4 of microcapsules mi as response

3.2 Optimization study

3.2.1 Factors and response variables selection

As per the Plackett–Burman screening design suggestion, statistically significant factors were total polymeric concentration x_1, oil load x_2 and tween 80 concentration x_3 and response for microcapsules m_i characteristic was encapsulation efficiency (EE%) $\mathbf{y_1}$. So, these factors and response variable were further optimized by central composite design as shown in Table 3.15.

Table 3.15 Encapsulation efficiency of microcapsules Mi proposed by central composite design.

Formulation Code	x_1	x_2	x_3	y_1
M_1	3.00000	1.00000	0.100000	86.31
M_2	2.00000	2.00000	0.006137	18.56
M_3	1.00000	3.00000	0.030000	10.35
M_4	3.00000	3.00000	0.030000	24.19
M_5	3.00000	1.00000	0.030000	76.36
M_6	1.00000	3.00000	0.100000	10.44
M_7	2.00000	2.00000	0.065000	26.08
M_8	1.00000	1.00000	0.030000	28.26
M_9	2.00000	2.00000	0.065000	18.56
M_{10}	2.00000	2.00000	0.065000	26.08
M_{11}	1.00000	1.00000	0.100000	38.77
M_{12}	2.00000	2.00000	0.065000	27.99
M_{13}	2.00000	2.00000	0.065000	26.08
M_{14}	0.31821	2.00000	0.065000	11.21
M_{15}	3.00000	3.00000	0.100000	28.15
M_{16}	2.00000	3.68179	0.065000	15.01
M_{17}	2.00000	0.31821	0.065000	96.70
M_{18}	2.00000	2.00000	0.065000	26.08
M_{19}	3.68179	2.00000	0.065000	57.62
M_{20}	2.00000	2.00000	0.123863	17.46

* x_i defined in table 2.2

3.2.2 Mathematical model section

Under Response surface methodology central composite design was used to analyses the relationship between three factors, total polymer concentration x_1, oil load x_2 and Tween 80 concentration x_3, and response for microcapsule characteristic encapsulation efficiency (EE%) y_1. Linear, linear + square, linear +interaction and full quadratic statistical models (Table 3.16) were generated by Minitab software for a response variable y_1 and statistically sound mathematical model was selected based on multiple correlated coefficient R^2, adjusted multiple correlated coefficients R^2adj and predicted R^2pred. Quadratic model compare to other had less standard deviation and all its multiple correlation coefficients were > 90% (R^2 =98.47%, R^2adj=97.09% and R^2 pred =91.08%) which shows quadratic mathematical model was an excellent fit to analyze the relationship between factors and response.

Table 3.16 Mathematical models generated for encapsulation efficiency of microcapsules Mi proposed by central composite design.

Response	Source	SD	R^2	R^2 adj	R^2pred
y_1	Linear	13.1904	77.24%	72.98%	61.11%
	Liner + square	7.52494	93.98%	91.21%	85.37%
	Liner + interaction	13.1129	81.73%	73.29%	59.36%
	Quadratic	**4.33185**	**98.47%**	**97.09%**	**91.08%**

3.2.3 Mathematical model fitness analysis

Analysis of variance (ANOVA) was used as fitness verifying tool for full quadratic mathematical model generated by Minitab. ANOVA at 95% confidence interval, analyzed the relationship between factors and response as shown in Table 3.17. The analysis of variance set out Linear (p value < 0.0001), square (p value <0.0001) and 2-way interaction (p value < 0.001) were significant. The quadratic model itself was strongly significant based on the analysis of ANOVA where p <

0.0001. The normal probability plot showed that the point where symmetrical to the line and versus fits plot also showed that the points were random which shows model fitness (Figure 3.9). And of course, the Lack-of-Fit was insignificant (p value = 0.178) which also indicted the fitness of the model.

Table 3.17 Analysis of variance for encapsulation efficiency of microcapsules Mi proposed by central composite design.

Source	DF	Adj SS	Adj MS	F-Value	P-Value	Level of significance α=0.05
Model	9	12045.5	1338.38	71.32	< 0.0001	***Significant***
Linear	3	9449.3	3149.77	167.85	< 0.0001	***Significant***
X_1	1	3084.5	3084.48	164.38	< 0.0001	***Significant***
X_2	1	6327.2	6327.22	337.18	< 0.0001	***Significant***
X_3	1	37.6	37.60	2.00	0.187	***Not significant***
Square	3	2047.7	682.56	36.37	< 0.0001	***Significant***
x_1*x_1	1	177.4	177.43	9.46	0.012	***Significant***
x_2*x_2	1	1772.1	1772.09	94.44	< 0.0001	***Significant***
x_3*x_3	1	75.7	75.66	4.03	0.072	***Significant***
2-Way Interaction	3	548.5	182.82	9.74	0.003	***Significant***
x_1*x_2	1	513.4	513.44	27.36	< 0.0001	***Significant***
x_1*x_3	1	1.4	1.37	0.07	0.793	***Not significant***
x_2*x_3	1	33.7	33.66	1.79	0.210	***Not significant***
Residual	10	187.6	18.76			
Lack-of-Fit	5	132.7	26.54	2.41	0.178	***Not significant***
Pure Error	5	55.0	10.99			
Total	19	12233.1				

* x_i defined in table 2.2

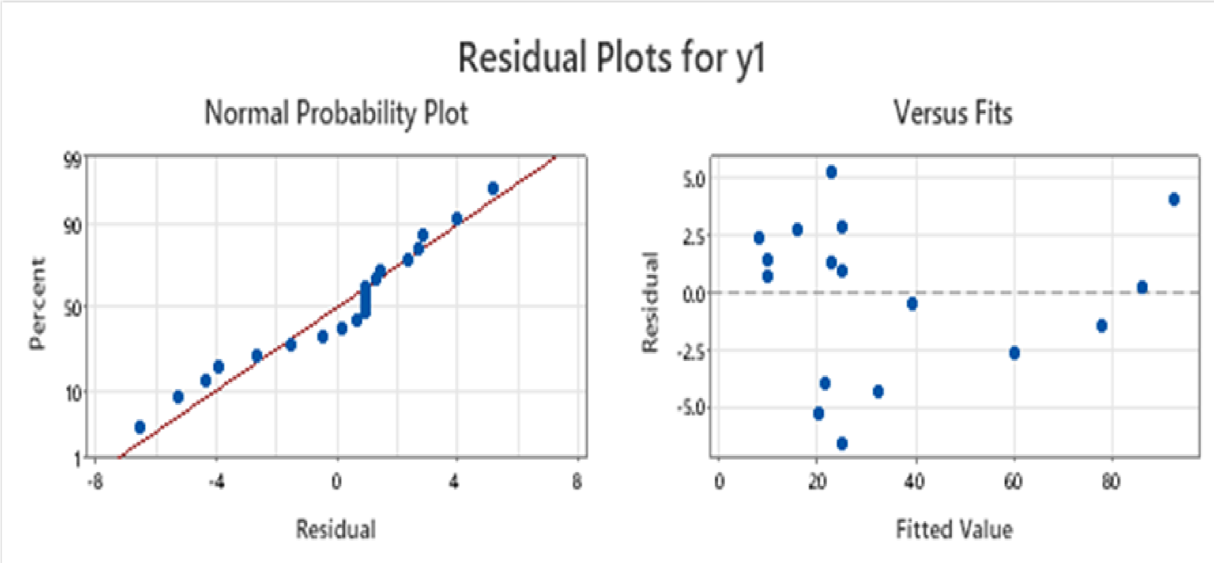


Fig 3.9. Residual plots for y_1 for encapsulation efficiency of 20 microcapsules formulations M_i proposed by central composite design.

To define the relation between factors and optimal response regression equation in **Equation 5.5.** was generated by Minitab software with their significance level. Linear relationship between factors and response showed that x_1 and x_2 had significant effect, where p value <0.0001, on response y_1 and the slope for both factors were positive. This indicates both factors and response had direct relation. Unlike other factors, x_3 had no impact on the response where p value was 0.124 which was not significant. The square relationship of factors and response showed that all $x_1(-1)*x_1(+1)$, $x_2(-1)*x_2(+1)$ and $x_3(-1)*x_3(+1)$ had significant effects on response y_1 and the p value was 0.008, < 0.0001 and 0.022, respectively. But, only $x_3(-1)*x_3(+1)$ effect had negative slope which showed the relationship of factors with response y_1 was inversely related. The 2 -way effects on response were also analyzed and the only factors interaction that had an impact on response y_1 was x_1*x_2 where the p value was < 0.0001 and the slope was negative which indicated that the relationship between the factors interaction and response was inversely related. Both interaction of factors x_1*x_3 and x_2*x_3 were not significant for response y_1 with the p value of 0.755 and 0.143, respectively. The graphical presentation of 2-way interaction were also described as contour and surface plots as shown on Figure 3.10 and 3.11, respectively and the main effect (linear) and interaction plot are also shown in Fig 3.16 and 6.17 respectively.

Regression Equation for y_1 as response

$$y1 = 47.4 + 16.25\, x_1 - 46.05\, x_2 + 384\, x_3 + 3.51\, x_1{*}x_1 + 11.09\, x_2{*}x_2 - 1870\, x_3{*}x_3 - 8.01\, x_1{*}x_2 + 11.8\, x_1{*}x_3 - 58.6\, x_2{*}x_3 \qquad \text{(eq3.5)}$$

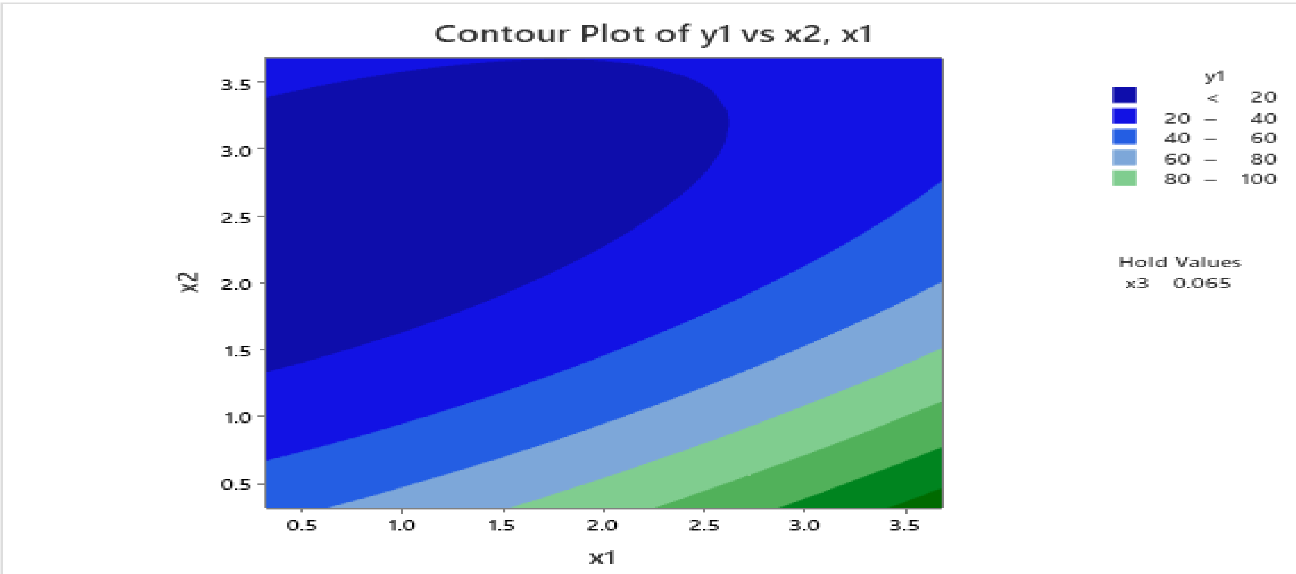
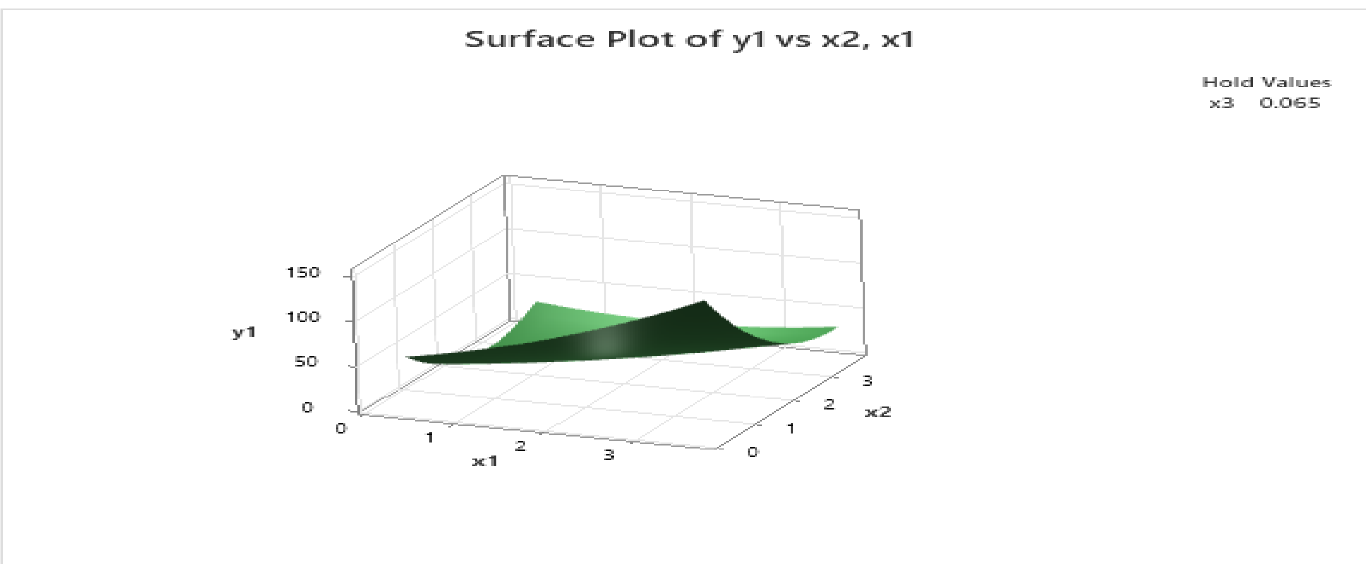


Fig 3.10 Contour plot for 2-Way Interaction for encapsulation efficiency (*y_1*) of microcapsules Mi proposed by central composite design.



* x_i defined in table 2.2

Fig 3. 11 Surface plot for 2-Way Interaction for encapsulation efficiency *y_1* of microcapsules Mi proposed by central composite design.

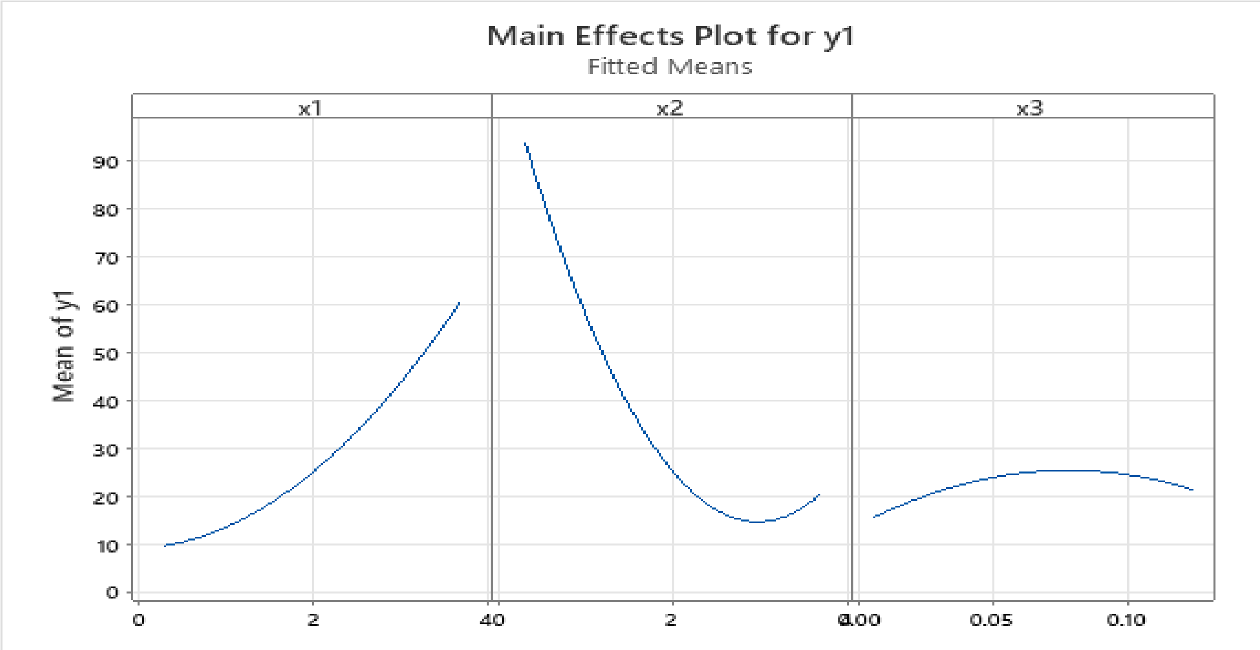
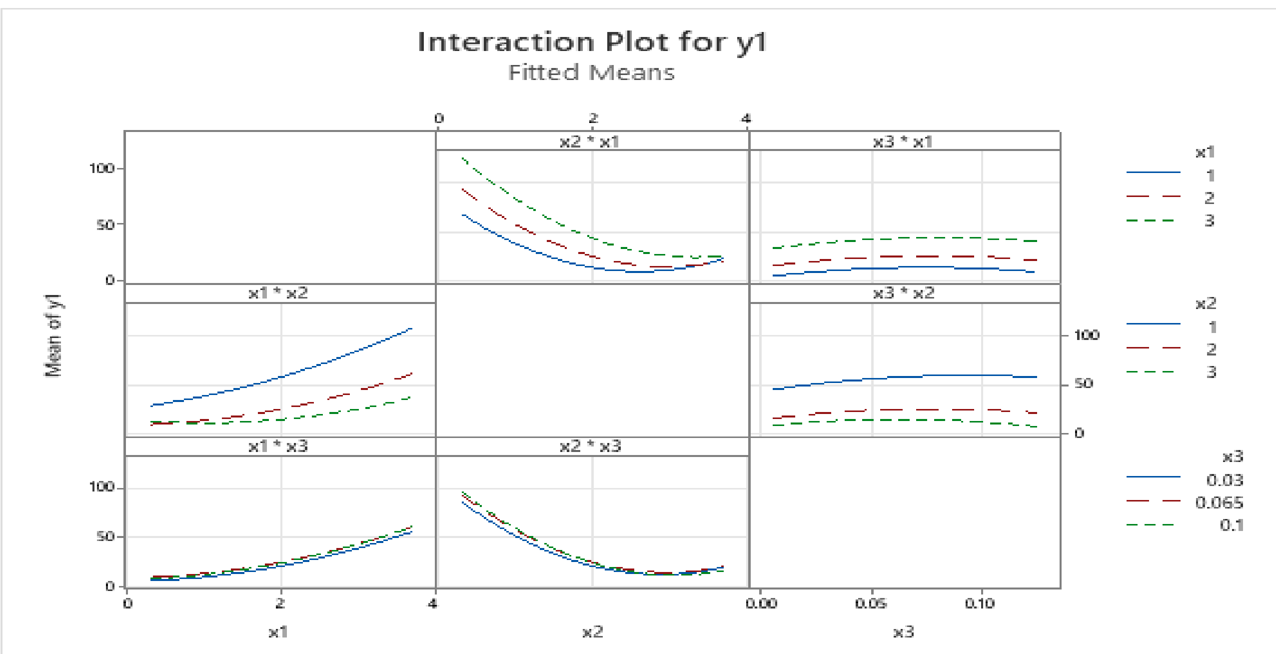


Fig 3.12 Main effect plot for encapsulation efficiency y_1 of microcapsules Mi proposed by central composite design.



* x_i defined in table 2.2

Fig 3.13 Interaction plot for encapsulation efficiency y_1 of microcapsules Mi proposed by central composite design.

3.2.4 Response optimization

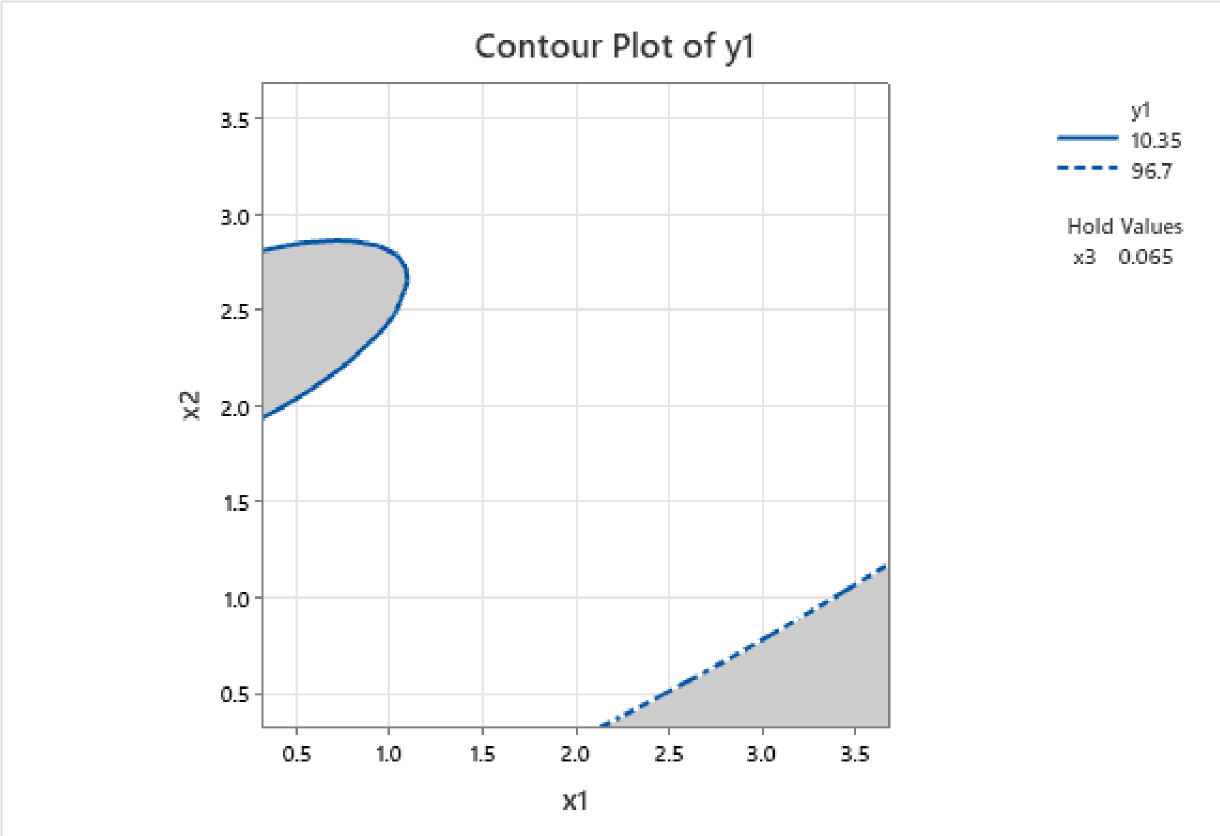
The optimal encapsulation efficiency y_1 obtained from experimental run, proposed by central composite design, was 96.7%. Response optimizer of, Minitab software, proposed further experimental run to optimize the response as shown Table 3.18 and 3.19. However, the proposed maximum response was not realistic 152.890% which was greater than 100%. This was because the response, encapsulation efficiency (EE%) y_1, gained by experimental run was maximum that was 96.7% so the microcapsules M_i formulation that yield 96.7% encapsulation efficiency was taken as optimized formulation.

Table 3.18 Parameters for response optimizer for encapsulation efficiency y_1 of microcapsules Mi proposed by central composite design.

Response	Goal	Lower	Target	Upper	Weight	Importance
y_1	Maximum	10.35	96.7		1	1

Table 3.19 Solution proposed by Response Optimizer Minitab software for encapsulation efficiency y_1 of microcapsules Mi proposed by central composite design.

Solution	x_1	x_2	x_3	y_1 Fit	Composite Desirability
1	3.68179	0.318207	0.109593	154.208	1



Where below simi oval curve y1 is low and above the broken line y_1 is high
* xi defined in table 2.2

Fig 3.14 Overlay plot for encapsulation efficiency of microcapsules Mi proposed by central composite design.

3.2.5 Validation of the optimized microcapsule formulation

The optimal microcapsules M_i formulation, where total polymeric concentration x_1 2 % (w/v), oil load x_2 0.318207% (w/v) and Tween 80 concentration x_3 0.065000% (w/v), were validated by comparison with predicted value by Minitab software at 95% confidence interval as shown in Table 3.20. The percentage error was calculated by compering predicted and experimental values. (Table 3.21) The percentage error was 4.31% which was less than 5 so this confirmed that the optimal microcapsules formulation aligns with predicted value.

Table 3.20 Prediction of optimal formulation by Minitab software for encapsulation efficiency of microcapsules Mi proposed by central composite design

Fit	SE Fit	95% CI	95% PI
92.7	3.38	(85.1517, 100.195)	(80.4367, 104.910)

Table 3.21 Percentage error for predicted and experimental values for encapsulation efficiency of microcapsules Mi proposed by central composite design.

Response	Predicted value	Experimental value	% Error
Encapsulation Efficiency (EE%)	92.7	96.7 %	4.31 %

3.3 Evaluation of optimized microcapsule formulation

3.3.1 *In vitro* release study

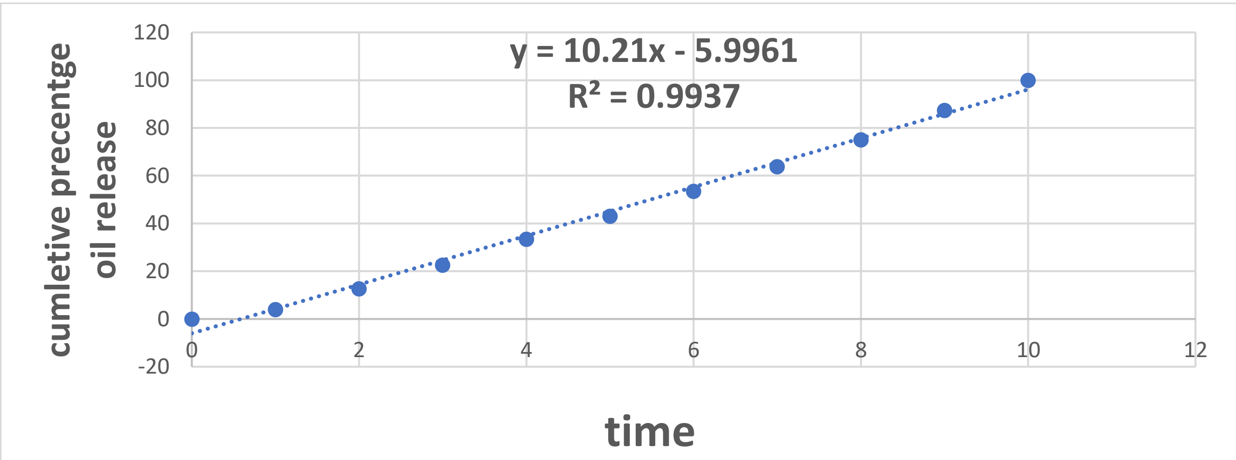
Optimized microcapsules proposed by central composite design, were prepared as shown on Table 3.22. The invitro release study were done on average of triplicate cumulative percentage release of optimized microcapsules for 10 hr. (Table 3.23). Two mathematical models, zero order and first order, were fitted to determine the pattern of invitro release of the Palmarosa essential oil from optimized microcapsules as shown Figure. 3.16 and 3.17, The best model fit was zero order kinetics model (where R^2 = 0.9937). The model explain that the Palmarosa essential oil release from microcapsule at 32 °C (assuming the skin temperature of human being at average is 32 (Solomon et al., 2012)) was a controlled release which means it was independent of concentration of the essential oil (Paarakh et al., 2019) so microencapsulation of Palmarosa oil by complex coacervation method improve the release profile of the essential oil.

Table 3.22 Optimized microcapsules proposed by central composite design

Symbol	Factor	unit	Uncoded factor
x_1	**Total polymeric concentration**	% w/w	2
x_2	**Palmarosa essential oils**	% w/w	0.318207
x_3	**Tween 80 concentration**	% w/w	0.065000
x_4	**Temperature**	°c	40
x_5	**Reaction time**	minute	10
x_6	**String speed**	rpm	600
x_7	**Tannic acid**	% w/w	0.5
x_8	**Crosslinking time**	Hour	4

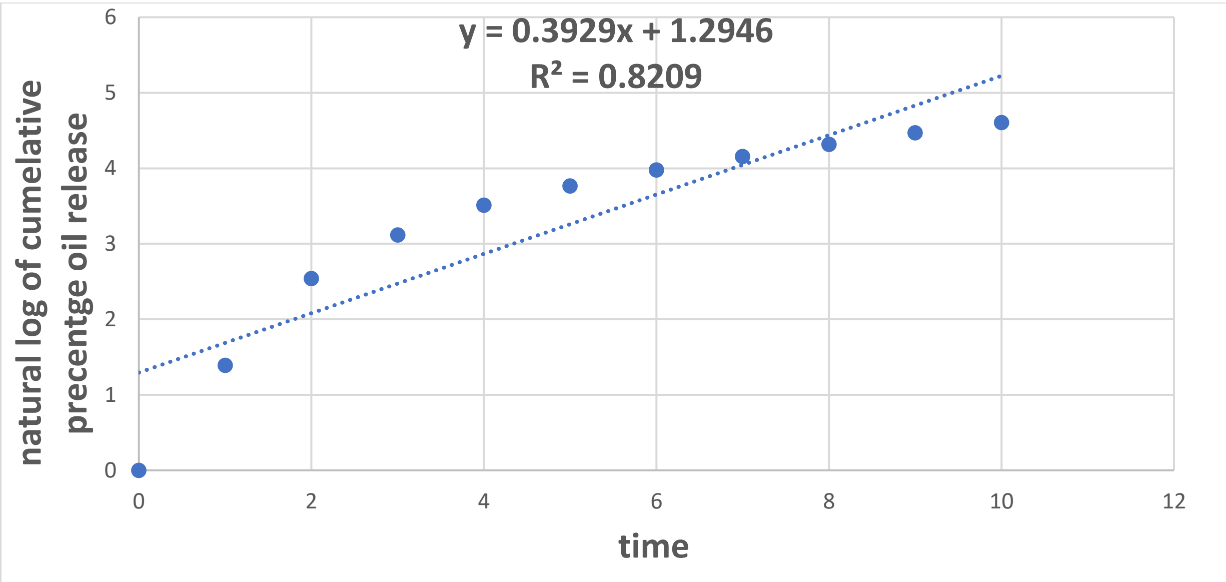
Table 3.23 cumulative percentage release of Palmarosa essential oil from optimal microcapsules (triplicate)

Time (hr)	Cumulative percentage oil release
1	4.02 ± 0.42
2	12.66 ± 0.38
3	22.61 ± 0.65
4	33.45 ±0.18
5	43.1± 0.76
6	53.47 ± 0.64
7	63.87± 0.47
8	74.99± 0.25
9	87.41± 0.22
10	100



Mathematical equation $Q = Q_0 + K_0 t$, where: Q_0 is the initial amount of oil in microcapsules, Q is the amount of oil released in time, k_0 is the zero-order release constant, t is time.

Fig 3.16 zero order release kinetics plot for optimized microcapsules proposed by central composite design.



Mathematical equation $\log Q = \log Q_0 - K_1 t / 2.303$ Where: Q_0 is the initial amount of oil in microcapsules, Q is the amount of oil released in time, k is the first-order release constant and t is time

Fig 3.17 first order release kinetics graph for optimized microcapsules proposed by central composite design.

3.3.2. FTIR analysis

Gelatin below its isoelectric point, becomes cationic polymer and its free amino group interact with carboxylic functional groups of polysaccharide polymer such as sodium alginate and the interaction of the two functional groups form amide (Thies,2007). Fourier-transform infrared spectroscopy (FTIR) analysis was caried out (4000-400 cm^{-1}) to analyze the functional groups of gelatin B, sodium alginate and optimized microcapsules formulation (Figure 3.18). The amide band I (1625-1750 cm ^{1}) due to CO and CN stretch, the amide band II (1475-1575 cm^{1}) due to N–H stretch and amide band III (1225 - 1425 cm^{-1}) due to C–N and N–H stretch was identified on optimized microcapsules formulation FTIR spectrum at 1621,1713,1445 and 1315 cm^{-1} respectively. And also, at 1318 cm^{-1} C-N stretch of amine (1335-1250cm^{-1}) groups were observed in gelatin B spectrum. The presence of the COO^{-} of carboxylic acid (1650–1550 cm^{-1}) of sodium alginate were identified at 1594cm^{-1} and the band at 1402 also indicts the C–O bond (1440–1395 cm^{-1}) of carboxylic acid. These findings also align with other studies (Heckert Bastos et al., 2020; Wang et al., 2016).

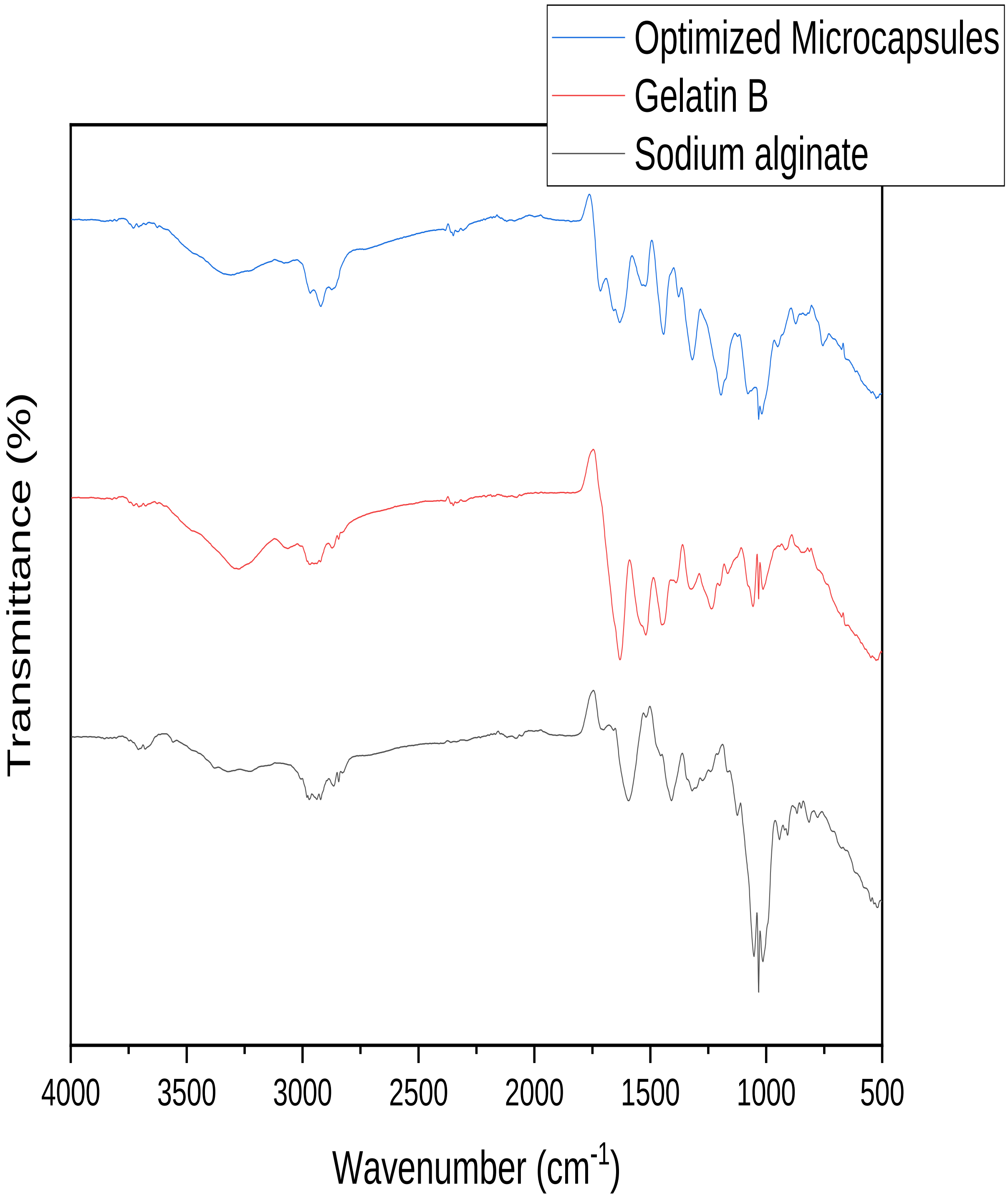


Fig 3. 18 FTIR spectra of optimized microcapsules, Gelatin B and Sodium alginate

4 Conclusions

Microcapsules formulation of Palmarosa essential oil was prepared by complex coacervation technique. Three factors, total polymeric concentration, Palmarosa oil concentration and Tween 80 concentration, had significant effect on encapsulation efficiency and further optimized by central composite design. The optimal microcapsule proposed by central composite design had 96.7 % encapsulation efficiency Two mathematical models, zero order and first order were fitted to determine the pattern of invitro release of the Palmarosa essential oil from optimized microcapsules formulation. The best models fit was zero order kinetics model kinetics model. Models explained that the Palmarosa essential oil release from optimized microcapsule formulation at 32 °C, assuming the skin temperature of human being at average is 32, was a controlled release which means it was independent of concentration of Palmarosa the essential oil. FTIR spectra of gelatin B, sodium alginate and optimized microcapsules showed the amide formation due to interaction of amino group of gelatin and carboxylic group of sodium alginate during complex coacervation process.

5. Suggestions for further work

The scope of this study was to formulate, optimize microcapsules and understanding of the Palmarosa essential oil release pattern from microcapsules. In addition, it was done under limited time and resource so farther research work is need. Conducting, Characterization and stability study on optimized microcapsules is recommended. Incorporating optimal microcapsules in topical formulation and assessing the compatibility, release pattern, activity test (repellent efficacy test) and shelf-life determination for final product also needed.

References

Achee, N. L., Grieco, J. P., Vatandoost, H., Seixas, G., Pinto, J., Ching-Ng, L., Martins, A. J., Juntarajumnong, W., Corbel, V., Gouagna, C., David, J. P., Logan, J. G., Orsborne, J., Marois, E., Devine, G. J., & Vontas, J. (2019). Alternative strategies for mosquito-borne arbovirus control. *PLoS Neglected Tropical Diseases*, *13*(1), 1–22. https://doi.org/10.1371/journal.pntd.0006822

Ansari, M. A., & Razdan, R. K. (1994). Repellent action of Cymbopogan martinii martinii Stapf var. sofia oil against mosquitoes. *Indian Journal of Malariology*, *31*(3), 95–102. http://www.ncbi.nlm.nih.gov/pubmed/7713271

Aryee, F. N. A., & Nickerson, M. T. (2012). Formation of electrostatic complexes involving mixtures of lentil protein isolates and gum Arabic polysaccharides. *Food Research International*, *48*(2), 520–527. https://doi.org/10.1016/j.foodres.2012.05.012

Asebe, G., Mamo, G., Wieland, B., Medhin, G., Tilahun, G., Abegaz, W. E., & Legesse, M. (2021). Community awareness and experiences of health workers concerning mosquito-borne viral diseases in selected districts of Gambella Region , Southwestern Ethiopia. *Infection Ecology & Epidemiology*, *11*(1). https://doi.org/10.1080/20008686.2021.1988453

Baldini, F., Segata, N., Pompon, J., Marcenac, P., Robert Shaw, W., Dabiré, R. K., Diabaté, A., Levashina, E. A., & Catteruccia, F. (2014). Evidence of natural Wolbachia infections in field populations of Anopheles gambiae. *Nature Communications*, *5*, 1–7. https://doi.org/10.1038/ncomms4985

Barbosa, H., Barthelemy, M., Ghoshal, G., James, C. R., Lenormand, M., Louail, T., Menezes, R., Ramasco, J. J., Simini, F., & Tomasini, M. (2018). Human mobility: Models and applications. *Physics Reports*, *734*, 1–74. https://doi.org/10.1016/j.physrep.2018.01.001

Baumert, B. O., Carnes, M. U., Hoppin, J. A., Jackson, C. L., Sandler, D. P., Freeman, L. B., Henneberger, P. K., Umbach, D. M., Shrestha, S., Long, S., & London, S. J. (2018). Sleep apnea and pesticide exposure in a study of US farmers. *Sleep Health*, *4*(1), 20–26. https://doi.org/10.1016/j.sleh.2017.08.006

Benelli, G., & Beier, J. C. (2017). Current vector control challenges in the fight against malaria. *Acta Tropica*, *174*, 91–96. https://doi.org/10.1016/j.actatropica.2017.06.028

Besansky, N. J., & Collins, H. (1992). *The Mosquito Genome : Organization , Evolution and Manipulation*. *8*(6), 186–192.

Bhatt, S., Weiss, D. J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K. E., Moyes, C. L., Henry, A., Penny, M. A., Smith, T. A., Bennett, A., Yukich, J., Eisele, T. P., Eckhoff, P. A., Wenger, E. A., Brie, O., Griffin, J. T., Fergus, C. A., … Gething, P. W. (2015). *The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015*. https://doi.org/10.1038/nature15535

Bouchal, F., Ayachi, N., Benmeziane, R., & Lahlou, S. (2023). *Effect of Sodium Alginate-Gelatin System on the Dissolution Behavior of Diclofenac Sodium Microparticles*. 5–10.

Bui, L. (2012). *Fluidised Bed Microencapsulation of Ascorbic Acid: Effectiveness of Protection under Simulated Tropical Storage Conditions*.

Burgess, D. J., & Carless, J. E. (1985). Manufacture of gelatin/gelatin coacervate microcapsules. *International Journal of Pharmaceutics*, *27*(1), 61–70. https://doi.org/10.1016/0378-5173(85)90185-1

Campbell, G. L., Hills, S. L., Fischer, M., Jacobson, J. A., Hoke, C. H., Hombach, J. M., Marfin, A. A., Solomon, T., Tsai, T. F., Tsu, V. D., & Ginsburg, A. S. (2011). Estimated global incidence of Japanese encephalitis: A systematic review. *Bulletin of the World Health Organization*, *89*(10), 766–774. https://doi.org/10.2471/BLT.10.085233

Casida, J. E. (2010). Pest Toxicology: The Primary Mechanisms of Pesticide Action. *Hayes' Handbook of Pesticide Toxicology*, 103–117. https://doi.org/10.1016/B978-0-12-374367-1.00002-1

Chaib, S., Benali, N., Arhab, R., Sadraoui Ajmi, I., Bendaoued, H., & Romdhane, M. (2021). Preparation of Thymus vulgaris Essential Oil Microcapsules by Complex Coacervation and Direct Emulsion: Synthesis, Characterization and Controlled Release Properties. *Arabian Journal for Science and Engineering*, *46*(6), 5429–5446. https://doi.org/10.1007/s13369-020-05223-w

Chen, C., Yang, H., Yang, X., & Ma, Q. (2022). Tannic acid: A crosslinker leading to versatile functional polymeric networks: A review. *RSC Advances*, *12*(13), 7689–7711.

https://doi.org/10.1039/d1ra07657d

Cho, J. S., Kwon, A., & Cho, C. G. (2002). Microencapsulation of octadecane as a phase-change material by interfacial polymerization in an emulsion system. *Colloid and Polymer Science*, *280*(3), 260–266. https://doi.org/10.1007/s00396-001-0603-x

Chung, S. K., Seo, J. Y., Lim, J. H., Park, H. H., Yea, M. J., & Park, H. J. (2013). Microencapsulation of essential oil for insect repellent in food packaging system. *Journal of Food Science*, *78*(5), 1–6. https://doi.org/10.1111/1750-3841.12111

COSTA, L. G. (2013). *Toxic effects of pesticides* (C. Klaassen (ed.); 8th Editio). Casarett and Doull's Toxicology: The Basic Science of Poisons.

Da Silva Junior, G. B., Pinto, J. R., Mota, R. M. S., Da Justa Pires Neto, R., & De Francesco Daher, E. (2019). Risk factors for death among patients with Chikungunya virus infection during the outbreak in northeast Brazil, 2016-2017. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *113*(4), 221–226. https://doi.org/10.1093/trstmh/try127

Dangol, S., Poudel, D. K., Ojha, P. K., Maharjan, S., Poudel, A., Satyal, R., Rokaya, A., Timsina, S., Dosoky, N. S., Satyal, P., & Setzer, W. N. (2023). Essential Oil Composition Analysis of Cymbopogon Species from Eastern Nepal by GC-MS and Chiral GC-MS, and Antimicrobial Activity of Some Major Compounds. *Molecules*, *28*(2). https://doi.org/10.3390/molecules28020543

Davis, K. J., & Fitzhugh, O. G. (1962). Tumorigenic potential of Aldrin and Dieldrin for mice. *Toxicology and Applied Pharmacology*, *4*(2), 187–189. https://doi.org/10.1016/0041-008X(62)90056-X

Devi, N., Hazarika, D., Deka, C., & Kakati, D. K. (2012). Study of complex coacervation of gelatin a and sodium alginate for microencapsulation of olive oil. *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, *49*(11), 936–945. https://doi.org/10.1080/10601325.2012.722854

Diallo, D., Sall, A. A., Diagne, C. T., Faye, O., Faye, O., Ba, Y., Hanley, K. A., Buenemann, M., Weaver, S. C., & Diallo, M. (2014). Zika virus emergence in mosquitoes in Southeastern Senegal, 2011. *PLoS ONE*, *9*(10), 4–11. https://doi.org/10.1371/journal.pone.0109442

Dieme, C., Bechah, Y., Socolovschi, C., Audoly, G., Berenger, J. M., Faye, O., Raoult, D., & Parola, P. (2015). Transmission potential of rickettsia felis infection by Anopheles gambiae mosquitoes. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(26), 8088–8093. https://doi.org/10.1073/pnas.1413835112

Eliasson, H., Broman, T., Forsman, M., & Bäck, E. (2006). Tularemia: Current Epidemiology and Disease Management. *Infectious Disease Clinics of North America*, *20*(2), 289–311. https://doi.org/10.1016/j.idc.2006.03.002

Esvelt, K. (2016). Gene editing can drive science to openness. *Nature*, *534*(7606), 153. https://doi.org/10.1038/534153a

European Centre for Disease Prevention and Control. (2019). *Dengue worldwide overview*. Surveilance and Disease Data. https://www.ecdc.europa.eu/en/dengue-monthly

Franco, R., Cecchi, G., Priotto, G., Paone, M., Diarra, A., Grout, L., Mattioli, R. C., & Argaw, D. (2020). *Monitoring the elimination of human African trypanosomiasis : Update to 2014*. 1–26.

Ghosh, S. K. (2006). Functional Coatings and Microencapsulation: A General Perspective. *Functional Coatings: By Polymer Microencapsulation*, 1–28. https://doi.org/10.1002/3527608478.ch1

Giraldo, M. I., Gonzalez-Orozco, M., & Rajsbaum, R. (2023). Pathogenesis of Zika Virus Infection. *Annual Review of Pathology: Mechanisms of Disease*, *18*, 181–203. https://doi.org/10.1146/annurev-pathmechdis-031521-034739

Giron, S., Franke, F., Decoppet, A., Cadiou, B., Travaglini, T., Thirion, L., Durand, G., Jeannin, C., L'Ambert, G., Grard, G., Noël, H., Fournet, N., Auzet-Caillaud, M., Zandotti, C., Aboukaïs, S., Chaud, P., Guedj, S., Hamouda, L., Naudot, X., … Leparc-Goffart, I. (2019). Vector-borne transmission of Zika virus in Europe, southern France, August 2019. *Eurosurveillance*, *24*(45), 1–4. https://doi.org/10.2807/1560-7917.ES.2019.24.45.1900655

Glomm, W. R., Molesworth, P. P., Yesiltas, B., Jacobsen, C., & Johnsen, H. (2023). Encapsulation of salmon oil using complex coacervation: Probing the effect of gum acacia on interfacial tension, coacervation and oxidative stability. *Food Hydrocolloids*, *140*(January), 108598. https://doi.org/10.1016/j.foodhyd.2023.108598

Golding, N., Wilson, A. L., Moyes, C. L., Cano, J., Pigott, D. M., Velayudhan, R., Brooker, S. J., Smith, D. L., Hay, S. I., & Lindsay, S. W. (2015). Integrating vector control across diseases. *BMC Medicine*, *13*(1), 1–6. https://doi.org/10.1186/s12916-015-0491-4

Govere, J., Durrheim, D. N., Du Toit, N., Hunt, R. H., & Coetzee, M. (2000). Local plants as repellents against Anopheles arabiensis, in Mpumalanga Province, South Africa. *The Central African Journal of Medicine*, *46*(8), 213–216. http://www.ncbi.nlm.nih.gov/pubmed/11317593

Gubler, D. J. (1998). Resurgent vector-borne diseases as a global health problem. *Emerging Infectious Diseases*. https://doi.org/10.3201/eid0403.980326

Guo, W. P., Tian, J. H., Lin, X. D., Ni, X. B., Chen, X. P., Liao, Y., Yang, S. Y., Dumler, J. S., Holmes, E. C., & Zhang, Y. Z. (2016). Extensive genetic diversity of Rickettsiales bacteria in multiple mosquito species. *Scientific Reports*, *6*(August), 1–11. https://doi.org/10.1038/srep38770

Heckert Bastos, L. P., Vicente, J., Corrêa dos Santos, C. H., Geraldo de Carvalho, M., & Garcia-Rojas, E. E. (2020). Encapsulation of black pepper (Piper nigrum L.) essential oil with gelatin and sodium alginate by complex coacervation. *Food Hydrocolloids*, *102*(September 2019). https://doi.org/10.1016/j.foodhyd.2019.105605

Hemingway, J., Field, L., & Vontas, J. (2002). An overview of insecticide resistance. *Science*, *298*(5591), 96–97. https://doi.org/10.1126/science.1078052

Holt, R. A., Mani Subramanian, G., Halpern, A., Sutton, G. G., Charlab, R., Nusskern, D. R., Wincker, P., Clark, A. G., Ribeiro, J. M. C., Wides, R., Salzberg, S. L., Loftus, B., Yandell, M., Majoros, W. H., Rusch, D. B., Lai, Z., Kraft, C. L., Abril, J. F., Anthouard, V., … Hoffman, S. L. (2002). The genome sequence of the malaria mosquito Anopheles gambiae. *Science*, *298*(5591), 129–149. https://doi.org/10.1126/science.1076181

Huang, G. Q., Sun, Y. T., Xiao, J. X., & Yang, J. (2012). Complex coacervation of soybean protein isolate and chitosan. *Food Chemistry*, *135*(2), 534–539. https://doi.org/10.1016/j.foodchem.2012.04.140

Huang, Y. Y., & Chung, T. W. (2001). Microencapsulation of gentamicin in biodegradable PLA and/or PLA/PEG copolymer. *Journal of Microencapsulation*, *18*(4), 457–465. https://doi.org/10.1080/02652040010019479

Jennifer Jung, M. P. (2001). Particle design using supercritical fluids: Literature and patent survey. *Journal of Supercritical Fluids*, *20*, 1–18. http://linkinghub.elsevier.com/retrieve/pii/S089684460100064X

Jun-Seok Hwang, Jin-Nam Kim, Young-Jung Wee, Jong-Sun, Hong-GI Jang, Sun-Ho Kim, and H.-W. R. (2006). Prepartion and Characterization of Melamine-Formaldehyde Resin Microcapsules Containing Fragrant oil. *Biotechnology and Bioprocess Engineering*, *11*, 332–336. https://doi.org/:10.1007/BF03026249

Jun-xia, X., Hai-yan, Y., & Jian, Y. (2011). Microencapsulation of sweet orange oil by complex coacervation with soybean protein isolate/gum Arabic. *Food Chemistry*, *125*(4), 1267–1272. https://doi.org/10.1016/j.foodchem.2010.10.063

Jyothi, N. V. N., Prasanna, P. M., Sakarkar, S. N., Prabha, K. S., Ramaiah, P. S., & Srawan, G. Y. (2010). Microencapsulation techniques, factors influencing encapsulation efficiency. *Journal of Microencapsulation*, *27*(3), 187–197. https://doi.org/10.3109/02652040903131301

Katz, T. M., Miller, J. H., & Hebert, A. A. (2008). Insect repellents: Historical perspectives and new developments. *Journal of the American Academy of Dermatology*, *58*(5), 865–871. https://doi.org/10.1016/j.jaad.2007.10.005

Katzelnick, L. C., Coloma, J., & Harris, E. (2017). Dengue: knowledge gaps, unmet needs, and research priorities. *The Lancet Infectious Diseases*, *17*(3), e88–e100. https://doi.org/10.1016/S1473-3099(16)30473-X

Klassen, D. (2010). Associative Phase Separation in Admixtures of Pea Protein Isolates with Gum Arabic and a Canola Protein Isolate with iota-Carrageenan and Alginate. *University of Saskatchewan Saskatoon, Saskatchewan, Canada, May*.

Knipling, E. F., & Serv, A. R. (1955). *Possibilities of Insect Control or Eradication Through the Use of Sexually Sterile Males 1*. *August*, 459–462.

Kumar, A., Gautam, R. D., Kumar, R., Chauhan, R., Kumar, M., Singh, S., Kumar, D., Singh, S., & Kumar, A. (2021). Floral studies of palmarosa [Cymbopogon martinii (Roxb.) W. Watson] and chemical insights during inflorescence development. *Industrial Crops and Products*, *171*(May), 113960. https://doi.org/10.1016/j.indcrop.2021.113960

Lebov JF, Engel LS, Richardson D, Hogan SL, S. D. and H. J. (2015). Pesticide exposure and end-stage renal disease risk among wives of pesticide applicators in the Agricultural Health Study. *Physiology & Behavior*, *143*, 198-210. https://doi.org/10.1016/j.envres.2015.10.002

Lee, B. Y., Bacon, K. M., Bottazzi, M. E., & Hotez, P. J. (2013). Global economic burden of Chagas disease: A computational simulation model. *The Lancet Infectious Diseases*, *13*(4), 342–348. https://doi.org/10.1016/S1473-3099(13)70002-1

Lemetter, C., F. Meeuse, and N. Z. (2009). Control of the morphology and the size of complex coacervate microcapsules during scale-up. *AIChE Journal*, *55*(6), 1487-1496. https://doi.org/10.1002/aic

Lemos, Y. P., Mariano Marfil, P. H., & Nicoletti, V. R. (2017). Particle size characteristics of buriti oil microcapsules produced by gelatin-sodium alginate complex coacervation: Effect of stirring speed. *International Journal of Food Properties*, *20*(July), 1438–1447. https://doi.org/10.1080/10942912.2017.1349139

Li, T., & Liu, N. (2010). Inheritance of permethrin resistance in Culex quinquefasciatus. *Journal of Medical Entomology*, *47*(6), 1127–1134. https://doi.org/10.1603/ME10142

Lindsay, S. W., & Birley, M. H. (1996). Climate change and malaria transmission. *Annals of Tropical Medicine and Parasitology*, *90*(6), 573–588. https://doi.org/10.1080/00034983.1996.11813087

Liu, S., Low, N. H., & Nickerson, M. T. (2010). Entrapment of flaxseed oil within gelatin-gum Arabic capsules. *JAOCS, Journal of the American Oil Chemists' Society*, *87*(7), 809–815. https://doi.org/10.1007/s11746-010-1560-7

Lucio G. Costa, Gennaro Giordano , Marina Guizzetti, A. V. (2008). Neurotoxicity of pesticides: a brief review. *Frontiers in Bioscience*, *13*, 525–538. https://doi.org/10.1111/j.1471-4159.2009.05969.x

M.J.B. Vreysen, A. S. R. and J. H. (2007). *Area-Wide Control of Insect Pests From Research to Field Implementation*. Springer.

Mackenzie, J. S., Gubler, D. J., & Petersen, L. R. (2004). *Emerging flaviviruses : the spread and resurgence of Japanese encephalitis , West Nile and dengue viruses*. *10*(12), 98–109. https://doi.org/10.1038/nm1144

Mahajan, R., Blair, A., Coble, J., Lynch, C. F., Hoppin, J. A., Sandler, D. P., & Alavanja, M. C. R. (2007). Carbaryl exposure and incident cancer in the Agricultural Health Study. *International Journal of Cancer, 121*(8), 1799–1805. https://doi.org/10.1002/ijc.22836

Manthripragada AD, Costello S, Cockburn MG, Bronstein JM, R. B. (2010). agricultural organophosphate exposure, and Parkinson disease. *Epidemiology (Cambridge, Mass.)*, *21*, 87–94. https://doi.org/10.1097/EDE.0b013e3181c15ec6.Paraoxonase

Mathur, V., Bhatnagar, P., Sharma, R. G., Acharya, V., & Sexana, R. (2002). Breast cancer incidence and exposure to pesticides among women originating from Jaipur. *Environment International*, *28*(5), 331–336. https://doi.org/10.1016/S0160-4120(02)00031-4

McMichael, A. J. (2000). The urban environment and health in a world of increasing globalization: Issues for developing countries. *Bulletin of the World Health Organization*, *78*(9), 1117–1126. https://doi.org/10.1590/S0042-96862000000900007

Montgomery, M. P., Kamel, F., Saldana, T. M., Alavanja, M. C. R., & Sandler, D. P. (2008). Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural Health Study, 1993-2003. *American Journal of Epidemiology*, *167*(10), 1235–1246. https://doi.org/10.1093/aje/kwn028

Mooranian, A., Negrulj, R., Al-Salami, H., Morahan, G., & Jamieson, E. (2016). Designing anti-diabetic β-cells microcapsules using polystyrenic sulfonate, polyallylamine, and a tertiary bile acid: Morphology, bioenergetics, and cytokine analysis. *Biotechnology Progress*, *32*(2), 501–509. https://doi.org/10.1002/btpr.2223

Moran, P. W., Nowell, L. H., Kemble, N. E., Mahler, B. J., Waite, I. R., & Van Metre, P. C. (2017). Influence of sediment chemistry and sediment toxicity on macroinvertebrate communities across 99 wadable streams of the Midwestern USA. *Science of the Total*

Environment, *599–600*, 1469–1478. https://doi.org/10.1016/j.scitotenv.2017.05.035

Ndiath, M. O. (2019). Insecticides and insecticide resistance. *Methods in Molecular Biology*, *2013*, 287–304. https://doi.org/10.1007/978-1-4939-9550-9_18

Nerio, L. S., Olivero-Verbel, J., & Stashenko, E. (2010). Repellent activity of essential oils: A review. *Bioresource Technology*, *101*(1), 372–378. https://doi.org/10.1016/j.biortech.2009.07.048

Nguon, O., Lagugné-Labarthet, F., Brandys, F. A., Li, J., & Gillies, E. R. (2018). Microencapsulation by in situ Polymerization of Amino Resins. *Polymer Reviews*, *58*(2), 326–375. https://doi.org/10.1080/15583724.2017.1364765

Nwachukwu, W. E., Yusuff, H., Nwangwu, U., Okon, A., Ogunniyi, A., Imuetinyan-Clement, J., Besong, M., Ayo-Ajayi, P., Nikau, J., Baba, A., Dogunro, F., Akintunde, B., Oguntoye, M., Kamaldeen, K., Fakayode, O., Oyebanji, O., Emelife, O., Oteri, J., Aruna, O., … Ihekweazu, C. (2020). The response to re-emergence of yellow fever in Nigeria, 2017. *International Journal of Infectious Diseases*, *92*, 189–196. https://doi.org/10.1016/j.ijid.2019.12.034

OMORI, N. (1962). A review of the role of mosquitoes in the transmission of Malayan and bancroftian filariasis in Japan. *Bulletin of the World Health Organization*.

Paarakh, M. P., Jose, P. A. N. I., Setty, C. M., & Peter, G. V. (2019). Release Kinetics – Concepts and Applications. *International Journal of Pharmacy Research & Technology*, *8*(1), 12–20. https://doi.org/10.31838/ijprt/08.01.02

Pal, A., Kumar Bajpai, A., & Bajpai, J. (2019). Study on facile designing, swelling properties and structural relationship of gelatin nanoparticles. *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, *56*(3), 206–214. https://doi.org/10.1080/10601325.2019.1565542

Park, S. J., & Kim, S. H. (2004). Preparation and characterization of biodegradable poly(l-lactide)/ poly(ethylene glycol) microcapsules containing erythromycin by emulsion solvent evaporation technique. *Journal of Colloid and Interface Science*, *271*(2), 336–341. https://doi.org/10.1016/j.jcis.2003.08.067

Park, U., Lee, M. S., Jeon, J., Lee, S., Hwang, M. P., Wang, Y., Yang, H. S., & Kim, K. (2019). Coacervate-mediated exogenous growth factor delivery for scarless skin regeneration. *Acta Biomaterialia*, *90*, 179–191. https://doi.org/10.1016/j.actbio.2019.03.052

Patel, O., Syamlal, G., Henneberger, P. K., Alarcon, W. A., & Mazurek, J. M. (2018). Pesticide use, allergic rhinitis, and asthma among US farm operators. *Journal of Agromedicine*, *23*(4), 327–335. https://doi.org/10.1080/1059924X.2018.1501451

Patel, S., & Sangeeta, S. (2019). Pesticides as the drivers of neuropsychotic diseases, cancers, and teratogenicity among agro-workers as well as general public. *Environmental Science and Pollution Research*, *26*(1), 91–100. https://doi.org/10.1007/s11356-018-3642-2

Pates, H., & Curtis, C. (2005). *MOSQUITO BEHAVIOR AND VECTOR CONTROL*. 53–70. https://doi.org/10.1146/annurev.ento.50.071803.130439

Pavela, R., & Benelli, G. (2016). Essential Oils as Ecofriendly Biopesticides? Challenges and Constraints. *Trends in Plant Science*, *21*(12), 1000–1007. https://doi.org/10.1016/j.tplants.2016.10.005

Possas, C., Lourenço-De-oliveira, R., Tauil, P. L., Pinheiro, F. de P., Pissinatti, A., da Cunha, R. V., Freire, M., Martins, R. M., & Homma, A. (2018). Yellow fever outbreak in Brazil: The puzzle of rapid viral spread and challenges for immunisation. *Memorias Do Instituto Oswaldo Cruz*, *113*(10), 1–12. https://doi.org/10.1590/0074-02760180278

Rahman, M., Sayed, S. K. J. B., Moniruzzaman, M., Kabir, A. K. M. H., Mallik, U., Hasan, R., Siddique, A. B., Hossain, A., Uddin, N., Hassan, M., & Chowdhury, F. R. (2019). Clinical and laboratory characteristics of an acute chikungunya outbreak in Bangladesh in 2017. *American Journal of Tropical Medicine and Hygiene*, *100*(2), 405–410. https://doi.org/10.4269/ajtmh.18-0636

Ramos-Castañeda, J., Barreto dos Santos, F., Martínez-Vega, R., Galvão de Araujo, J. M., Joint, G., & Sarti, E. (2017). Dengue in Latin America: Systematic Review of Molecular Epidemiological Trends. *PLoS Neglected Tropical Diseases*, *11*(1), 1–24. https://doi.org/10.1371/journal.pntd.0005224

Ranade, V. V., & Hollinger, M. A. (2003). Drug delivery systems. In *Drug Delivery Systems,* *Second Edition*. https://doi.org/10.9774/gleaf.9781315302317-14

Razzak, M. A., Kim, M., & Chung, D. (2016). Elucidation of aqueous interactions between fish gelatin and sodium alginate. *Carbohydrate Polymers*, *148*, 181–188. https://doi.org/10.1016/j.carbpol.2016.04.035

Redekop, W. K., Lenk, E. J., Luyendijk, M., Fitzpatrick, C., Niessen, L., Stolk, W. A., Tediosi, F., Rijnsburger, A. J., Bakker, R., Hontelez, J. A. C., Richardus, J. H., Jacobson, J., de Vlas, S. J., & Severens, J. L. (2017). The Socioeconomic Benefit to Individuals of Achieving the 2020 Targets for Five Preventive Chemotherapy Neglected Tropical Diseases. *PLoS Neglected Tropical Diseases*, *11*(1), 1–27. https://doi.org/10.1371/journal.pntd.0005289

Ren, P. W., Ju, X. J., Xie, R., & Chu, L. Y. (2010). Monodisperse alginate microcapsules with oil core generated from a microfluidic device. *Journal of Colloid and Interface Science*, *343*(1), 392–395. https://doi.org/10.1016/j.jcis.2009.11.007

Ren, X., Liu, Y., Wu, W., & Zhang, W. (2023). Microencapsulation by complex coacervation processes. In *Microencapsulation in the Food Industry*. INC. https://doi.org/10.1016/b978-0-12-821683-5.00004-2

Rimando, A. M., & Duke, S. O. (2006). Natural products for pest management. *ACS Symposium Series*, *927*, 2–21. https://doi.org/10.1021/bk-2006-0927.ch001

Ruan, G., & Feng, S. S. (2003). Preparation and characterization of poly(lactic acid)-poly(ethylene glycol)-poly(lactic acid) (PLA-PEG-PLA) microspheres for controlled release of paclitaxel. *Biomaterials*, *24*(27), 5037–5044. https://doi.org/10.1016/S0142-9612(03)00419-8

Sajeev, C., Vinay, G., Archna, R., & Saha, R. N. (2002). Oral controlled release formulation of diclofenac sodium by microencapsulation with ethyl cellulose. *Journal of Microencapsulation*, *19*(6), 753–760. https://doi.org/10.1080/02652040210000022734

Saldana, T. M., Basso, O., Hoppin, J. A., Baird, D. D., Knott, C., Blair, A., Alavanja, M. C. R., & Sandler, D. P. (2007). Pesticide exposure and self-reported gestational diabetes mellitus in the agricultural health study. *Diabetes Care*, *30*(3), 529–534. https://doi.org/10.2337/dc06-1832

Schmitt, C., & Turgeon, S. L. (2011). Protein/polysaccharide complexes and coacervates in food systems. *Advances in Colloid and Interface Science*, *167*(1–2), 63–70. https://doi.org/10.1016/j.cis.2010.10.001

Selemane, I. (2019). Epidemiological monitoring of the last outbreak of yellow fever in Brazil – An outlook from Portugal. *Travel Medicine and Infectious Disease*, *28*, 46–51. https://doi.org/10.1016/j.tmaid.2018.12.008

Shahidi, F., & Han, X. Q. (1993). Encapsulation of Food Ingredients. *Critical Reviews in Food Science and Nutrition*, *33*(6), 501–547. https://doi.org/10.1080/10408399309527645

Sharma, H., Zhang, P., Barber, D. S., & Liu, B. (2010). Organochlorine pesticides dieldrin and lindane induce cooperative toxicity in dopaminergic neurons: Role of oxidative stress. *NeuroToxicology*, *31*(2), 215–222. https://doi.org/10.1016/j.neuro.2009.12.007

Shashikala, P., Lavanya, A., & Bhagavanth Rao, M. (2012). Microencapsulation for preparing sustained release drugs. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*(SUPPL.1), 69–72.

Shepard, D. S., Undurraga, E. A., Halasa, Y. A., & Stanaway, J. D. (2016). The global economic burden of dengue: a systematic analysis. *The Lancet Infectious Diseases*, *16*(8), 935–941. https://doi.org/10.1016/S1473-3099(16)00146-8

Shinde, U. A., & Nagarsenker, M. S. (2009). Characterization of gelatin-sodium alginate complex coacervation system. *Indian Journal of Pharmaceutical Sciences*, *71*(3), 313–317. https://doi.org/10.4103/0250-474X.56033

Silva, N. I. O., Sacchetto, L., De Rezende, I. M., Trindade, G. D. S., Labeaud, A. D., De Thoisy, B., & Drumond, B. P. (2020). Recent sylvatic yellow fever virus transmission in Brazil: The news from an old disease. *Virology Journal*, *17*(1), 1–12. https://doi.org/10.1186/s12985-019-1277-7

Simon LV, Hashmi MF, T. K. (2018). Yillow Fever. *StatPearls: Tampa/St. Petersburg, FL, USA.*

Slager, R. E., Simpson, S. L., Levan, T. D., Poole, J. a, Sandler, D. P., & Hoppin, J. a. (2010). Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study. *J Toxicol Environ Health A.*, *73*(20), 1382–1393. https://doi.org/10.1080/15287394.2010.497443.Rhinitis

Socolovschi, C., Pagés, F., & Raoult, D. (2012). Rickettsia felis in aedes albopictus mosquitoes, libreville, gabon. *Emerging Infectious Diseases*, *18*(10), 1688–1689. https://doi.org/10.3201/eid1810.120178

Solomon, B., Sahle, F. F., Gebre-Mariam, T., Asres, K., & Neubert, R. H. H. (2012). Microencapsulation of citronella oil for mosquito-repellent application: Formulation and in vitro permeation studies. *European Journal of Pharmaceutics and Biopharmaceutics*, *80*(1), 61–66. https://doi.org/10.1016/j.ejpb.2011.08.003

Sousa, V. I., Parente, J. F., Marques, J. F., Forte, M. A., & Tavares, C. J. (2022). Microencapsulation of Essential Oils: A Review. *Polymers*, *14*(9). https://doi.org/10.3390/polym14091730

Spoto, S., Riva, E., Fogolari, M., Cella, E., Costantino, S., Angeletti, S., & Ciccozzi, M. (2018). Diffuse maculopapular rash: A family cluster during the last Chikungunya virus epidemic in Italy. *Clinical Case Reports*, *6*(12), 2322–2325. https://doi.org/10.1002/ccr3.1831

Sukri, N., Putri, T. T. M., Mahani, & Nurhadi, B. (2023). Characteristics of propolis encapsulated with gelatin and sodium alginate by complex coacervation method. *International Journal of Food Properties*, *26*(1), 696–707. https://doi.org/10.1080/10942912.2023.2179635

Swarbrick, J., & Boylan, J. C. (2003). Encyclopedia of pharmaceutical technology. *Choice Reviews Online*, *40*(11), 40-6157-40–6157. https://doi.org/10.5860/choice.40-6157

Tadei, W. P., Thatcher, B. D., Santos, J. M. M., Scarpassa, V. M., Rodrigues, I. B., & Rafael, M. S. (1998). Ecologic observations on anopheline vectors of malaria in the Brazilian amazon. *American Journal of Tropical Medicine and Hygiene*. https://doi.org/10.4269/ajtmh.1998.59.325

Tawatsin, A., Wratten, S. D., Scott, R. R., Thavara, U., & Techadamrongsin, Y. (2001). Repellency of volatile oils from plants against three mosquito vectors. *Journal of Vector Ecology : Journal of the Society for Vector Ecology*, *26*(1), 76–82.

http://www.ncbi.nlm.nih.gov/pubmed/11469188

Thies, C. (2007). *Microencapsulation of Flavors by Complex Coacervation*. 149–170.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W. H., Simberloff, D., & Swackhamer, D. (2001). Forecasting agriculturally driven global environmental change. *Science*. https://doi.org/10.1126/science.1057544

United Nations Population Devision. (2002). World Urbanization Prospects. The 2001 Revision Data Tables and Highlights. *World Urbanization Prospects: The 2001 Revision*, *March*. https://doi.org/ESA/P/ WP/173

Uversky, V. N., Li, J., & Fink, A. L. (2001). Pesticides directly accelerate the rate of α-synuclein fibril formation: A possible factor in Parkinson's disease. *FEBS Letters*, *500*(3), 105–108. https://doi.org/10.1016/S0014-5793(01)02597-2

Varona, S., Martín, Á., Cocero, M. J., & Duarte, C. M. M. (2013). Encapsulation of Lavandin Essential Oil in Poly-(ε{lunate}-caprolactones) by PGSS Process. *Chemical Engineering and Technology*, *36*(7), 1187–1192. https://doi.org/10.1002/ceat.201200592

Velasco, J., Dobarganes, C., & Márquez-Ruiz, G. (2003). Variables affecting lipid oxidation in dried microencapsulated oils. *Grasas y Aceites*, *54*(3), 304–314. https://doi.org/10.3989/gya.2003.v54.i3.246

Verma, S. K., Kumar, B., Ram, G., Singh, H. P., & Lal, R. K. (2010). Varietal effect on germination parameter at controlled and uncontrolled temperature in Palmarosa (Cymbopogon martinii). *Industrial Crops and Products*, *32*(3), 696–699. https://doi.org/10.1016/j.indcrop.2010.07.015

Villamil-Gómez, W. E., Rodríguez-Morales, A. J., Uribe-García, A. M., González-Arismendy, E., Castellanos, J. E., Calvo, E. P., Álvarez-Mon, M., & Musso, D. (2016). Zika, dengue, and chikungunya co-infection in a pregnant woman from Colombia. *International Journal of Infectious Diseases*, *51*, 135–138. https://doi.org/10.1016/j.ijid.2016.07.017

Wang, L., Yang, S., Cao, J., Zhao, S., & Wang, W. (2016). Microencapsulation of ginger volatile oil based on gelatin/sodium alginate polyelectrolyte complex. *Chemical and Pharmaceutical Bulletin*, *64*(1), 21–26. https://doi.org/10.1248/cpb.c15-00571

Watson;, R. T., Zinyowera;, M. C., & Moss, R. H. (1996). Climate Change 1995: The IPCC Second Assessment Report. Scientific-Technical Analysis of Impacts, Adaptations, and Mitigatio of Climate Change. *Ipcc*, 399–426.

Watts, D. M., Burke, D. S., Harrison, B. A., Whitmire, R. E., & Nisalak, A. (1987). Effect of temperature on the vector efficiency of Aedes aegypti for dengue 2 virus. *American Journal of Tropical Medicine and Hygiene*. https://doi.org/10.4269/ajtmh.1987.36.143

WHO. (2016). World Malaria Report 2016. In *World Health Organization*. https://doi.org/10.1071/EC12504

WHO. (2020). *Multisectoral Approach to thenPrevention and Control of Vector-Borne Diseases*. https://apps.who.int/iris/bitstream/handle/10665/331861/9789240004788-eng.pdf?ua=1

WHO. (2021). *Lymphatic filariasis*. Prescrire International. https://doi.org/10.1201/9781420004946.ch8

Wilke, A. B. B., & Marrelli, M. T. (2015). Paratransgenesis: A promising new strategy for mosquito vector control. *Parasites and Vectors*, *8*(1), 1–9. https://doi.org/10.1186/s13071-015-0959-2

World Health Organization. (2011). *Malaria fact sheet #94, 12 2011*.

World Health Organization. (2015). *Investing to overcome the global impact of neglected tropical diseases*.

World Health Organization. (2017a). Global vector control response 2017–2030. *World Health Organization*.

World Health Organization. (2017b). *Global vector control response 2017–2030*. https://apps.who.int/iris/handle/10665/259205

Xiao, Z., Liu, W., Zhu, G., Zhou, R., & Niu, Y. (2014). A review of the preparation and application of flavour and essential oils microcapsules based on complex coacervation technology. *Journal of the Science of Food and Agriculture*, *94*(8), 1482–1494. https://doi.org/10.1002/jsfa.6491

Yang, T., & Liu, N. (2014). Permethrin resistance variation and susceptible reference line isolation in a field population of the mosquito, Culex quinquefasciatus (Diptera: Culicidae). *Insect Science*, *21*(5), 659–666. https://doi.org/10.1111/1744-7917.12071

Zhang, J., Lu, G., Li, J., Kelly, P., Li, M., Wang, J., Huang, K., Qiu, H., You, J., Zhang, R., Wang, Y., Zhang, Y., Wu, H., & Wang, C. (2019). Molecular Detection of Rickettsia felis and Rickettsia bellii in Mosquitoes. *Vector-Borne and Zoonotic Diseases*, *19*(11), 802–809. https://doi.org/10.1089/vbz.2019.2456

Zhang, W., Jiang, F., & Ou, J. (2011). Global pesticide consumption and pollution: with China as a focus. *Proceedings of the International Academy of Ecology and Environmental Sciences*, *1*(2), 125–144.

Zhang, Z. Q., Pan, C. H., & Chung, D. (2011). Tannic acid cross-linked gelatin-gum arabic coacervate microspheres for sustained release of allyl isothiocyanate: Characterization and in vitro release study. *Food Research International*, *44*(4), 1000–1007. https://doi.org/10.1016/j.foodres.2011.02.044

Zheng, T., Zahm, S. H., Cantor, K. P., Weisenburger, D. D., Zhang, Y., & Blair, A. (2001). Agricultural exposure to carbamate pesticides and risk of non-Hodgkin lymphoma. *Journal of Occupational and Environmental Medicine*, *43*(7), 641–649. https://doi.org/10.1097/00043764-200107000-00012

Zygadlo, J.A., Juliani, H. R. (2003). *Recent progress in medicinal plants.* (Majundar & S. V. D.K., Govil, J.N., Singh, V.K., Shailaja, M.S., Gangal (eds.)). Studium Press LLC.