Extraction and Encapsulation of Bioactive Compounds Using Ultrasound Approach: Parameter Optimization

A Thesis submitted in the partial fulfilment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

By

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Dedicated to my family

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DECLARATION

This is to certify that the work presented in the thesis entitled **"Extraction and Encapsulation of Bioactive Compounds Using Ultrasound Approach: Parameter Optimization"**, is a bonafied work done by me under the supervision of Dr. Shirish H. Sonawane and Dr. Prakash Saudagar and was not submitted elsewhere for the award of any degree.

I declare that this written submission represents my idea in my own words and where other's ideas or words have not been included. I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misinterpreted or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be a cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not taken when needed.

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CERTIFICATE

This is to certify that the thesis entitled **"Extraction and Encapsulation of Bioactive Compounds Using Ultrasound Approach: Parameter Optimization"** that is being submitted by **Ms. Shital Balasaheb Potdar** in partial fulfilment for the award of Doctor of Philosophy (Ph.D) in the Department of Chemical Engineering, National Institute of Technology, Warangal, is a record of bonafied work carried out by her under my guidance and supervision. The results of embodied in this thesis have not been submitted to any other Universities or Institutes for the award of any degree or diploma.

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(Shital Balasaheb Potdar)

Abstract

In the present time, people have become more cautious about their diet. As they have experienced the side effects caused due to consumption of junk food containing artificial food preservatives and flavour enhancers. Thus, the trend is moving towards the use of bioactive compounds in food processing. Out of several bioactive compounds of health benefit, ginger is being used since dawn of medicine. Use of bioactive compounds such as ginger in the food matrix has two main steps; first is extraction of active ingredients from its source, followed by encapsulation with suitable carrier.

In the present study, ultrasound assisted extraction of ginger oil has been carried out from its root (*Zingiber officinale)*. The effect of different parameters such as different solvents, sonication time, applied sonication power, and ginger powder to solvent ratio on extraction yield of ginger oil was investigated. Further, to preserve the bioactivity of extracted compounds, encapsulation has a great potential. Therefore, ginger oil is encapsulated with gum arabic (GA) shell. In practise, many parameters affect the encapsulation process. To evaluate the effect of all process parameters is a complex, time-consuming and costly process. Hence, the design of experiments (DOE) was carried out with Taguchi approach. In addition, to verify the predicted results using Taguchi approach, Artificial Neural Network (ANN) model was developed. Further the study was extended to evaluate the controlled release study of encapsulated ginger oil in GA shell. More than 90% of ginger oil was observed in acidic pH in 300 min.

Another way of incorporating the bioactive compounds in the food matrix is through emulsion. Hence, in order to incorporate the bioactive compounds in the food matrix, the study was extended to attempt novel, energy efficient, ultrasonic atomization approach for emulsification. Typically, the size of particles is under control of the parameters like oil concentration, surfactant concentration, and viscosity of continuous phase and through energy input to solution.

Overall, the study showed that ultrasound has great potential in extraction and encapsulation of ginger oil. The use of Taguchi for design of experiments along with ANN is a great choice to predict the encapsulation process with more accuracy. The ultrasonic atomization can be exploited as a novel approach for emulsification.

Keywords: Extraction, Encapsulation, Sonochemistry, Ginger oil, Gum arabic, Taguchi, Artificial neural network, Controlled release, Ultrasound atomization.

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List of Abbreviations

Abbreviations	Description
GA	Gum acacia
MD	Maltodextrin
CD	Cyclodextrin
WPI	Whey protein isolate
LPI	Lentil protein isolate
WPC	Whey protein concentrate
MS	Modified starch
CG	Cashew gum
CH	Chitosan
IN	Inulin
BG	Brea gum
OLE	Olive leave extract
SW	Subcritical water
HPH	High pressure homogenization
UAE	Ultrasound-assisted extraction
MAE	Microwave-assisted extraction
HAE	Heat assisted extraction
SFE	Supercritical fluid extraction
PLE	Pressurized liquid extraction
TPC	Total polyphenol content
TFC	Total flavonoid content
ANOVA	Analysis of variance
RSM	Response surface methodology
SEM	Scanning electron microscopy
GAE	Gallic acid equivalents
QE	Quercetin equivalents

Chapter 1

Introduction

The chapter provides a brief overview about the importance of bioactive compounds as a food preservative, nutraceutical and flavouring agent in food processing. The need and methods for extraction of active ingredients from the bioactive source is illustrated. Introduction to encapsulation process in food industries along with different approaches used for encapsulation are described. The next phase of chapter deals with explaining the different approaches used for process optimization in food processing. The role of Taguchi and artificial neural network (ANN) modelling in process parameter optimization and prediction of process outputs is described. This chapter also gives overview about the different controlled release mechanisms of encapsulated active compound from the shell. Further, the potential of ultrasonic atomization approach for emulsification is explained.

1.1 Importance of bioactive compounds in food matrix

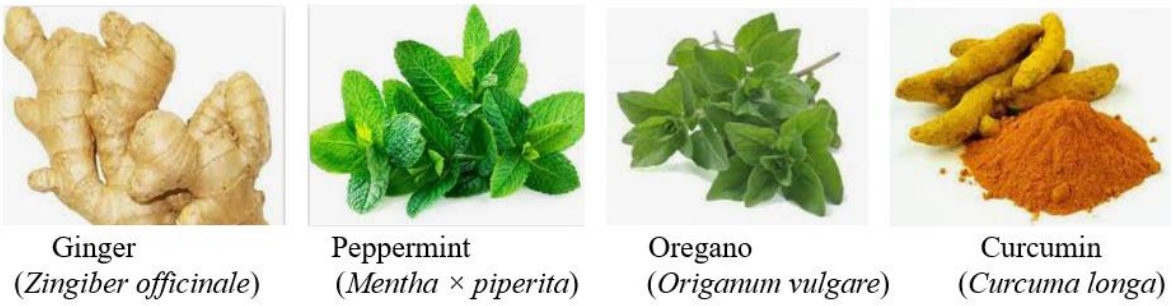

Figure 1.1: Few health beneficial bioactive compounds

In the present time, people have become more cautious about their diet. They have experienced the side effects caused due to the consumption of junk food. The majority of the side effects indulged from junk food are due to the use of artificial food preservatives and flavour enhancers in food processing. Therefore, people prefer to include bioactive compounds such as ginger, curcumin, garlic and many other spices as a preservative and flavour enhancer. Also, from decade's, plant-derived bioactive compounds are being used to cure and get relief from chronic diseases (Martirosyan, D., and Miller 2018; Banwo et al. 2018). Few representative bioactive compounds are shown in figure 1.1. Along with imparting a flavour to food, these are having other health-beneficial properties like antioxidant and antimicrobial activity. They are also used as a treatment of many chronic diseases (Burt 2004).

1.1.1. *Zingiber Officinale* (Ginger) as a bioactive compound

Out of several bioactive compounds having health benefits, ginger is being used since dawn of medicine and food processing. Ginger is scientifically known as *Zingiber Officinale*, it is a member of the *Zingiberaceae* kingdom. It is a rhizomatous plant grown across south-eastern China, India and in parts of Africa, Austria, Japan and Latin America. The ginger has functioned as a spice and medicine in India since ancient past (Ali et al. 2008; Hasan et al. 2012).

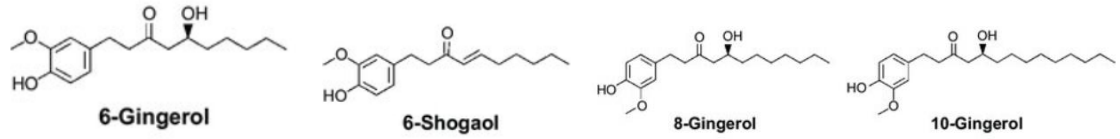

Figure 1.2: Major active components present in the ginger extract

The major active ingredients of ginger extract responsible for health beneficial properties are gingerol, and shogaol (figure 1.2). The biological activities of ginger can be listed as analgesic, anticancer, antiemetic, antidiabetic, anti-inflammatory, antimicrobial, antinausea, antiobesity, antioxidant, antipyretic, anti-tumour, cardiovascular, neuroprotective and respiratory protective (Mao et al. 2019). It can be exploited as a natural drug molecule to prevent various diseases such as cancer, fever, cold, upset stomach, nausea, menstrual cramps, rheumatism, respiratory discomforts and so on (Kumar and Sharma 2014). The food preservative action of ginger is due to its antioxidant property which inhibits the oxidation of oils as well as fat containing food products, giving shelf life to the food. Ginger also has distinctive characteristic flavour and can also be used as flavour enhancer in the various food matrices. Hence the use of ginger is favoured in all the ages of time.

1.2. Extraction of bioactive compounds

1.2.1. Need for extraction of bioactive compounds

The use of bioactive compounds in food processing has certain constraints, as majority of the bioactive compounds are sensitive to environmental conditions. Active ingredients get degraded at environmental conditions also the availability of the bioactive compounds is a function of regional and seasonal availability. Thus, it is needed to extract the bioactive compounds, and preserve them against environmental degradation to preserve their bioavailability. Thus, bioactive compounds can be used throughout the year and at all regions.

1.2.2. Methods of extraction

Several approaches have been reported for the extraction of active ingredients from the source bioactive compounds including hot water extraction, maceration, microwave assisted extraction, Soxhlet extraction and ultrasound assisted extraction (Azmir et al. 2013). The selection of the extracting method is based on the nature of bioactive compound. The detailed mechanism of all extraction technique is explained in the section below.

1.2.2.1. Extraction using hot water bath

Typically, water is exploited as a solvent for the extraction. However, sometimes depending on the nature of bioactive compound (hydrophilic/lipophilic) solvents such as ethanol is also used. In an extraction process, initially dissolved oxygen from the water is removed using ultrasound or inert gas purging in the solution. Ultrasonication is an economical approach for degassing when the choice is available. To maintain the pressure during extraction, water level is sustained by adding water manually or using the pump. The heat required for extraction of bioactive compound is supplied by using heating jacket or oven. Convective heat transfer takes place from water to bioactive material and extraction is obtained (Hassas-Roudsari. et al. 2009)

1.2.2.2. Extraction of bioactive compounds with Soxhlet extractor

The term Soxhlet extraction is coined after 'Franz Ritter von Soxhlet', a German agricultural chemist. Soxhlet extraction is a great choice for the continuous extraction of a bioactive compounds by a hot solvent (Grigonis et al. 2005). It is conventional method for lipid extraction when the bioactive compound of concern is having less solubility in a solvent (Castro and Priego-Capote 2010). The main parts of the Soxhlet extractor are extraction chamber distillation flask, siphon and condenser. In the first step, bioactive compound to be extracted is kept in crushed form in a distillation flask equipped with the facility for simultaneous heating and stirring. The vapour produced in the distillation chamber are carried to condenser, where the solvent is cooled and sent back to extraction chamber. When extraction chamber is full, solvent is transferred to the distillation flask using siphon. The thimble helps to transfer the solvent rapidly. The thimble allows the entry of solvent however impede the mutation of solids inside the still pot. Cycle is repeated multiple times to obtain concentrated extract. Finally, extract gets concentrated in distillation flask. After complete extraction the solvent is recovered using rotary evaporator.

The benefits of Soxhlet extraction can be listed as higher extraction yield, stable operation, feasibility of continuous extraction and lower energy requirement as against other extraction methods. The limitations of this approach are the requirement of hazardous and flammable solvents, need of expensive and pure solvents (Smith and Tschinkel 2009, Abubakar and

Haque2020). Many cycles are required for complete extraction, therefore the time required for extraction is also higher. Also, Soxhlet extractor have limitation in the extraction of temperature sensitive bioactive compounds (Ramluckan et al.2014).

1.2.2.3. Maceration process of extraction

For the extraction with maceration, the bioactive plant material is crushed finely, kept in a sealed container and extracting solvent is supplemented to it. The mixture is kept for 7-8 days, and the solution containing sample is frequently shaken. The maximum possible extraction is determined usually by identifying the change in colour and its intensity to desired level. Extract and biomass are separated after extraction with suitable technique (Plaza and Turner 2015).

1.2.2.4. Bioactive compounds extraction using supercritical fluid

To carry out supercritical fluid extraction (SCF) of bioactive compounds, initially the fluid is exposed to the critical pressure and temperature to achieve SCF properties suitable for extraction. After completion of extraction, extract and SCF are isolated by reducing the solution pressure. The merit of SCF extraction method over solvent extraction and mechanical-pressing method is that the aroma is preserved to large extent (Ozkal et al. 2005).

1.2.2.5. Microwave-assisted extraction of bioactive compounds

Microwave-assisted extraction (MAE) is a recently emerged, rapid, safe and cost-effective technique of extraction. The bioactive compound is extracted, crushed/grounded to which the solvent like methanol is added and the mixture is brought in contact with microwave irradiation for time of nearly 30-50 sec. The caution should be exercised that sample is not boiling in the microwave. Subsequently, extract is separated and cooled. The repetition of cycle is required several times to ensure complete extraction (Chemat and Cravotto 2012). The solvent containing extract and biomass residue are separated using centrifuge. The salient feature of this approach is that process does not require the sample to be free from water. It is a suitable technique for segregating pesticides and lipids from seeds, feeds, foods, and soil (Asghari et al. 2011).

1.2.2.6. Ultrasound-assisted extraction of bioactive compounds

The above mentioned conventional extraction techniques are inflicted with some pros and cons which restricts in scale up. The main challenges of the above techniques are reduced mass transfer rate, active component degradation at higher temperature and prolonged extraction time. To overcome such difficulties and to obtain easy and simple approach, acoustic cavitation can be exploited for extraction of bioactive compounds. In the ultrasound-assisted extraction

(UAE) technique, the waves above 20 kHz are passed in the solution of bioactive compounds which needs to be extracted. Ultrasonic waves passed through this medium leave a number of physical and chemical effects. Microstreaming, turbulence, rupture of cell wall are the physical effects and free radical formation is a chemical effect. These physical and chemical effects are attributed to cavitation phenomenon (Zhou et al. 2010).

High quality extract is obtained with this technique due to shear mechanism which is responsible for the rupture of cell wall and allowing the easy penetration of solvent and thus boosting the mass transfer. Short extraction time, low energy requirement, high product yield and preservation of active compounds are the striking advantages of ultrasound assisted extraction (UAE). Another advantage is low solvent usage which is the major concern in extraction process with other conventional techniques (Jadhav et al. 2021). Among all the extraction techniques UAE was found to be most appropriate with higher extraction efficiency for thermally sensitive food giving higher extraction efficiency and retaining the activity of valuable bioactive compounds. The extraction time required found to be is less than 1 h which is very high for conventional processes and also it is suitable for thermally sensitive materials (Sharayei et al. 2019).

1.3. Preservation of bioactive compounds through encapsulation

1.3.1. Introduction to encapsulation

Once the active ingredients are extracted from the source, the preservation of active ingredients from the environmental conditions is very essential. Encapsulation has a great potential in preserving these bioactive compounds.

1.3.1.1. Basics of encapsulation

Encapsulation refers to stabilization of active compound in a core-shell system, efficient for preserving the physical, chemical, and biological properties, along with its controlled release at desired conditions (Silva et al. 2012). During encapsulation, active ingredient is wrapped within one or blend of shell materials. There are two important terms commonly used in encapsulation. The first term refers to the material which is being coated is also referred as active/core material and another term is the shell/carrier material or encapsulating agent which is referred to the material used as a coating to the core compound. The classification of an encapsulation system is on the basis of the arrangement of the core substance and deposition process of the shell material. These structures are broadly classified into reservoir and matrix type as shown in figure 1.3 below.

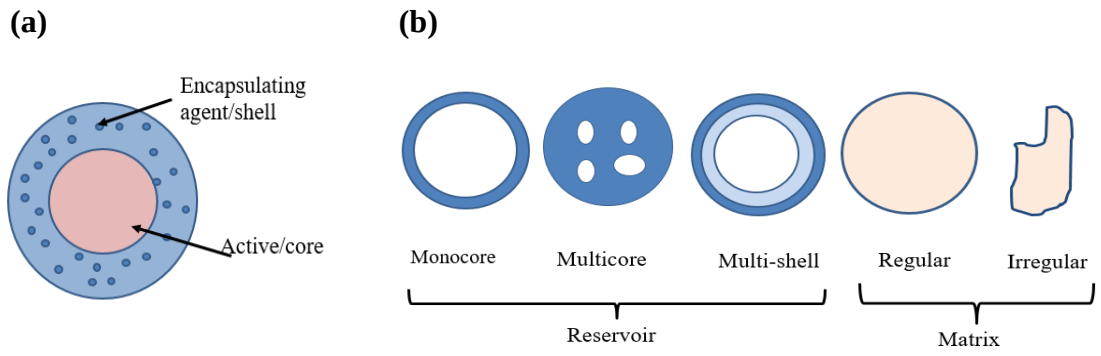

Figure 1.3: Encapsulation (a) core-shell morphology (b) different arrangements in encapsulation

In reservoir type, the active compound is surrounded with shell around. It is further divided as mono-core, multi-core and multi-shell based on the number of shell layers and active molecules present in it, whereas in case of matrix type, encapsulated material is having structure in which the core material is uniformly dispersed or sprayed over the outside coating layer.

1.3.1.2. Need of encapsulation

In the present time, consumers have become more cautious about the food. The trend is moving towards the food having natural ingredients (preservatives and flavour enhancers) as against synthetic food preservatives. The major reason behind this trend is due to the side effects experienced in last few decades on consumption of food with synthetic food ingredients (Teixeira et al. 2004). This issue motivates to search a technique for the use of bioactive compounds in food processing while taking into account the limitation of bioactive compounds as they get degraded at harsh environmental conditions. The encapsulation technique helps to fulfil this need. For instance, many times it is observed that flavour has reduced to large extent in various food-processing steps, therefore it is desired to add flavour externally. Here, the encapsulated flavour has high potential to maintain the lost flavour. Among the several motives to encapsulate flavour, preservation of functionality and stability are of main interest. The food with the use of encapsulated flavour compounds catches the attraction of consumers due to its smell and taste and is also easily digestible. The encapsulated flavour powder is superior to handle and dust free (Berger 2012).

The contamination of bioactive compounds in liquid form (extract) is reduced due to liquid bioactive compounds are converted to solid powder with the help of an encapsulating agent. Encapsulated food ingredients are stable with less chance of bacterial growth. They are less volatile in nature compared to natural flavour as well as other bioactive compound. Another key feature is due to ability of controlled release. Increased safety due to decreased

flammability is also an additional advantage of encapsulation. Bioactive compounds are encapsulated using various methods that provide the material with controlled particle size distribution and morphology. The above-mentioned benefits are assists to minimize some limitations of encapsulation. Beside to this encapsulation will assist to preserve bioactive compounds, the cost of encapsulation synthesis is increased. The major thrust of research is to make this process economical. Sometimes, externally added flavour may tend to change the taste of food. Hence, retaining its natural taste is also a significant factor.

1.3.2. Chemical and physical methods of encapsulation

The methods of encapsulation are primarily classified in two broad categories as chemical and physical encapsulations as shown in figure 1.4. Chemical methods of encapsulation can be further classified as coacervation, molecular inclusion, and co-crystallization. Physical method of encapsulation is also called as mechanical encapsulation. Spray-drying, freeze-drying, extrusion and vacuum drying, spray-cooling or chilling as well as fluidized bed coating are the approaches in physical encapsulation. The choice of the encapsulation technique is based on the physical and chemical properties of active and shell materials and the targeted application of synthesized food matrix. A combination of two or more of techniques is employed to achieve the successful encapsulation.

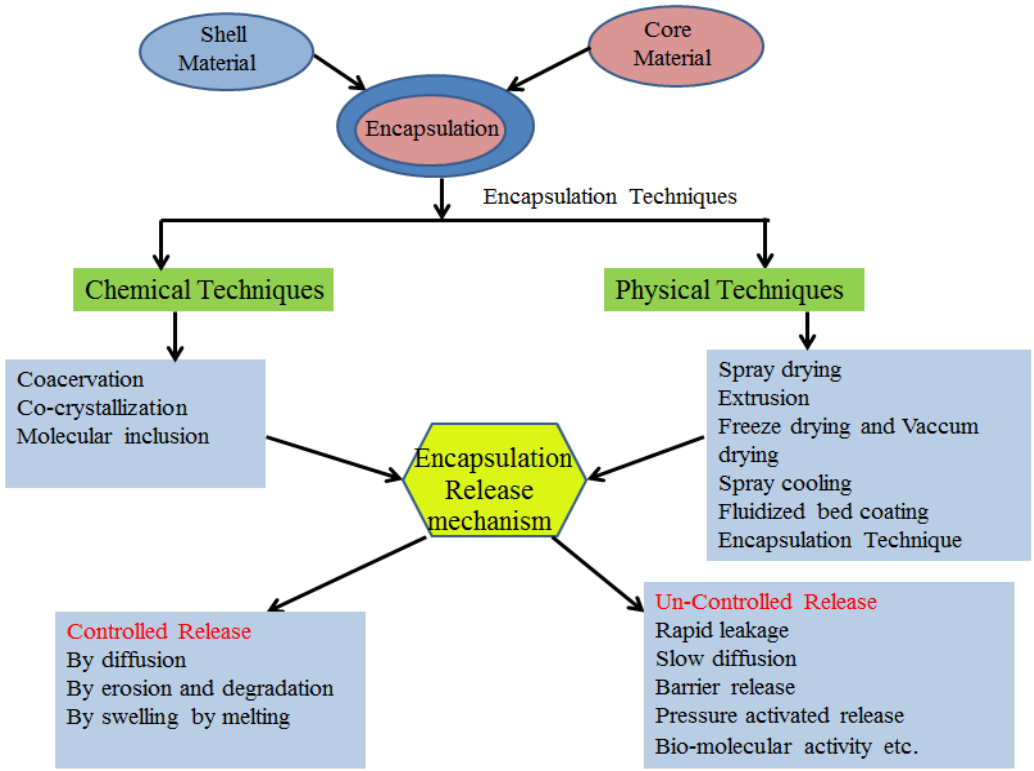

Figure 1.4: Encapsulation techniques and release mechanisms

1.3.2.1. Chemical methods of encapsulation

Coacervation: Coacervation is a conventional method of encapsulation. This approach permits addition of polymer droplets in a suspension of core compound. The concept of coacervation involves the separation of the coacervate phase and the very dilute colloidal phase in a single concentrated colloidal phase. Coacervation has three phases: the solvent phase, core phase, and polymer-rich phase. The liquid phase in which polymer (shell material) is dissolved is termed coacervate. During the coacervation, the coating of shell material is formed around the core phase, further the shell formed is allowed to make rigid structure surrounding the core and thus, microcapsules are synthesized (Kaushik et al. 2015, Yan and Zhang 2014).

Simple coacervation: Here, a weak solvent is mixed with a hydrophilic colloidal solution. This gives rise to the colloid molecules (coacervate) rich phase, and the second phase is coacervate free (Asbahani et al. 2015).

Complex coacervation: It takes place between two oppositely charged polymers. Initially, dispersion of lipid/core in an aqueous phase containing shell material is carried out by either change in temperature or by change in pH. In the next step, these phases are separated into insoluble polymer-rich phase and an aqueous phase as a consequence of attractive electrostatic force (Piacentini et al. 2015, Eghbal and Choudhary 2018). The schematic of encapsulation by coacervation is depicted in figure 1.5 below.

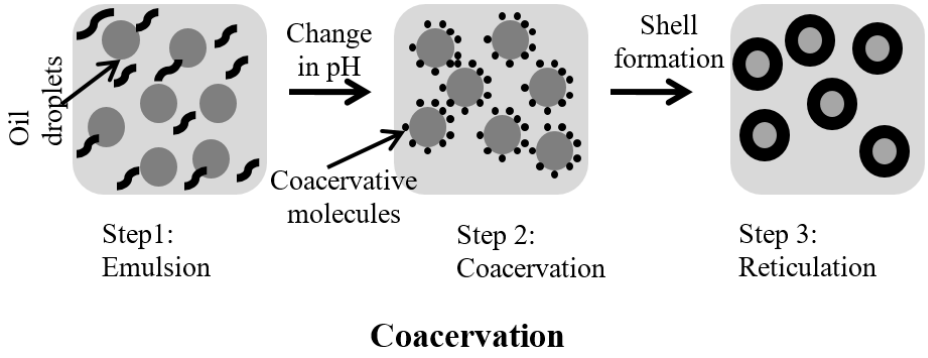

Figure 1.5: Schematic of encapsulation by coacervation

Molecular inclusion: In the molecular inclusion approach for encapsulation, the shell cavity (generally hydrophilic) acts as a host in which a hydrophobic core is accommodated (host-guest-like structure) (figure 1.6). Enzymatically modified starch molecules like cyclodextrin are used as a shell. Fragrances/flavours are commonly encapsulated in the core. Retention of aroma is a function of the molecular weight, polarity, shape, volatility and chemical functionality. The weight of the encapsulated structure formed is in micron range because of

the small size of the ring. The limitation of this approach is usage of large quantity of water in the process which often requires additional steps of separation and drying.

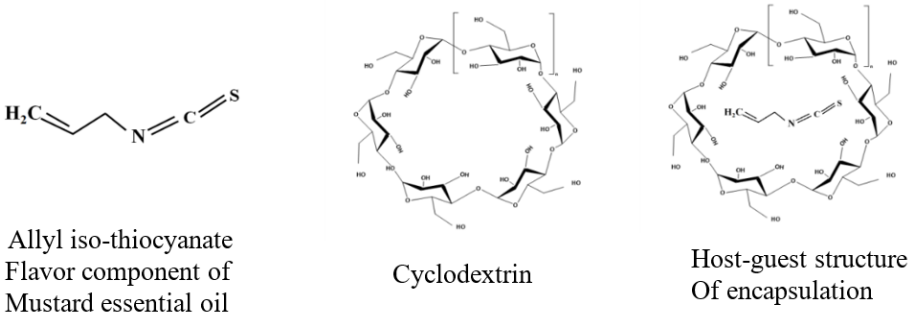

Figure 1.6: Schematic of encapsulation by molecular inclusion

Co-crystallization: In the co-crystallization approach, supersaturated sucrose solution is prepared. In the next step, the crystalline sucrose is rearranged from an ideal to an uneven agglomerated crystal, to give a porous matrix. Then core material is included in the supersaturated solution. In the last step, substantial heat is removed after the solution reaches the sucrose crystallization temperature. This approach allows the retention of volatiles to a great extent and the product obtained is a dry powder. The shortcoming the process is that, it regulates the addition of external anti-oxidant to denigrate the oxidation in the storage period.

1.3.2.2. Physical methods of encapsulation

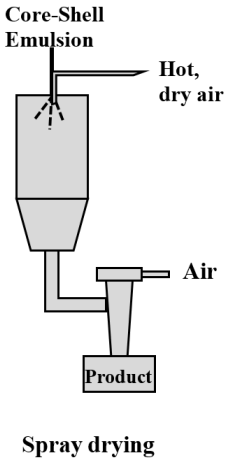

Figure 1.7: Encapsulation using spray drying

Spray drying: Encapsulation by spray drying is a developed technique at the industrial level. It is in easy to perform and permits continuous production. In the spray drying, emulsion containing core, shell is passed through the spray drying chamber equipped with an arrangement for hot gas. This method allows the conversion of liquid or a semisolid mixture of core-shell

into a free-flowing powder and spherical particles can be obtained, with a typical particle size below 10 mm of a multiple core structure. Air and nitrogen are the commonly used gases in drying. The co-current gas flow with temperature above 150 ºC is employed in the spray drying chamber for food components such as flavours and oils. However, rapid water evaporation from emulsion assists in maintaining the temperature of the core below 100 ºC. The emulsion is exposed to heat for a very few seconds to retain the integrity of the bioactive compounds (Kanakdande et al. 2007).

Freeze drying: This method is employed for the encapsulation of heat sensitive bio-ingredients. The process is accomplished with three major steps, the first step is about freezing the mobile phase, while the second, third step is primary and secondary drying respectively. Primary drying is accomplished by decreasing the pressure in the chamber. Secondary drying is employed to assure the complete elimination of water. Then the freeze dried bioactive compounds are plugged to prevent oxidation and water absorption. The only limitation of freeze drying lies in the higher time required for the process against spray drying (almost fifty times higher) (Ballesteros et al. 2017).

Fluidized bed coating:

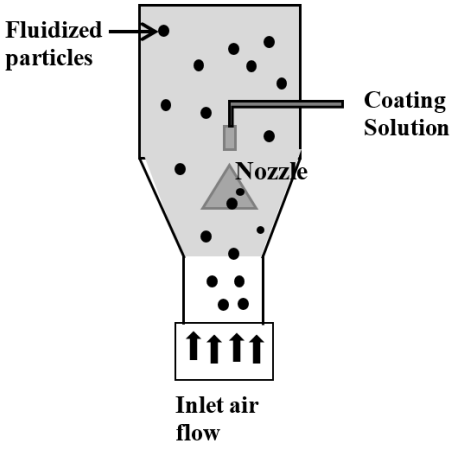

Figure 1.8: Encapsulation by fluidized bed coating

It is the only encapsulation approach that applies to core and shell materials in powder form. This approach allows core material to be evenly coated with shell material. In an encapsulation process, the bioactive core material is fluidized in the bed, wherein shell material is sprayed. Core and shell materials must be viscous enough to get fluidized in the reactor. Because high viscous materials have limitations to move freely inside the chamber. In contrast, less viscous

materials escape without coating the bioactive material. Encapsulation efficiency of the fluidized bed is decided by its retention time, which is a function of the flow rate of the shell material. The fluidized bed reactor has superior temperature control and it is advantageous for processing nutritional products (Coronel-Aguilera and San Martin-Gonzalez. 2015). However, at higher speed, both active material and shell materials get carried away from the reactor. The pressure drop within the reactor is a limiting factor associated with fluidized bed reactor. This approach is employed to process hydrogenated vegetable oil of fatty acids.

Extrusion method of encapsulation

Extrusion is the second widely used encapsulation approach after spray drying. The major step in encapsulation by extrusion is the preparation of the core-shell solution and passing it from an aperture. The laboratory needle of syringe beads is used as an aperture and droplet formation towards the nozzle discharge section. The extrusion approach is further categorized as dripping extrusion, coaxial air flow, jet cutting and spinning disk, dripping extrusion atomization, co-axial double capillary, centrifugal extrusion and vibrating jet. After passing the core-shell solution from the aperture it is solidified by applying a chemical (gelation) and physical (cooling/ heating) approach to form an encapsulating structure. The particles constituted with extrusion are bigger in volume as against formed by spray drying. The less porous encapsulation can be achieved than spray drying. The oxidation of bioactive compounds in extrusion is prevented as the entry of oxygen is restricted. Hence shelf life of the food improves to 4-5 years from few months for unencapsulated food (Belscak-Cvitanovic et al. 2011).

1.4.Taguchi and ANN approaches for process design, optimization and prediction

1.4.1. Different approaches used for process optimization

As discussed in above sections the preservation of bioactive compounds needs extraction of active compounds from the source and its encapsulation with suitable approach. In practice, in numerous cases, carrying out the experiments and collecting the output information is much time consuming, costly and complex process which requires several steps to obtain the result. In such cases it is not feasible to perform more number of experiments due to all above reasons. Such situations may result into the common error of overfitting. Encapsulation is amongst such complex process and many factors affect in encapsulation such as core and shell concentration, their ratios and other parameters based on the encapsulation method chosen.

The conventional one factor at a time experiment is a very time-consuming technique and possesses the limitation that it fails to provide interactive information. In this regard many machine learning and statistical learning approaches have been emerged.

Taguchi DOE approach is very effective and popular statistical tool to identify the significant factors as well as to reduce the total number of runs (Ashengroph et al. 2010). Response surface methodology (RSM) is an efficient statistical and mathematical tool which is generally employed to identify the probable interactions between the parameters. RSM considers the relationships among the explanatory variables and one or more response variables. RSM was adopted in optimization of extraction conditions (Ayim et al. 2018). The combination of Taguchi and RSM approach has been also employed for the process optimization (Dash et al. 2016; Thakur et al. 2016). However, any form of a non-linearity between the variables may affect the prediction accuracy of the RSM (Desai et al. 2008). Hence, artificial neural network (ANN) has been evolved as an alternative to the RSM for complex non-linear multivariate modelling.

1.4.2. Design of experiments: Taguchi approach

The design of experiments (DOE) with Taguchi orthogonal array is a best choice to observe the effect of all parameters with a minimum number of experiments. Hence, in the present study, DOE is performed with Taguchi approach to understand the significant factors affecting on encapsulation efficiency as well as to provide the interaction effects between the input process parameters. Analysis of variance (ANOVA) analysis in Taguchi is useful to better understand the contribution of each fact affect in deciding the output value. Analysis of variance (ANOVA) is an analysis tool applied in statistics that divides an observed aggregate variability observed in a sets of data in systematic and random factors. Further Taguchi approach gives regression equation to predict the output.

1.4.3. Artificial neural network

ANN is found to be an effective tool for better prediction of the output responses. However, the data required for teaching the ANN need to be properly selected, and a wide range of data needs to be trained. Hence in the present study, the data required for teaching the ANN is selected from the designed data set that has been used in the Taguchi approach.

The ANN has been popularly used to discover and find multivariate dependencies amongst the process parameters (Olden et al. 2002; Bhagya and Dash 2020; Dash and Das 2021). The experimental outputs help to find out the fundamental phenomenon of encapsulation. To investigate the effect of a broad spectrum of input parameters, a data-driven approach like ANN is helpful. The current computational system of ANN is invented with the aim to imitate the functioning of biological neurons. The ANN network has three layers of neurons namely the input layer, hidden layer and output layer. These three layers are connected by synaptic connections. ANN allows useful and realistic method of describing the complex process of

interest and proposes a nonlinear correlation between dependent and independent parameters of existing experimental data. In the model building, an algorithm is set based on the known input and output parameters. ANN model have excellent ability to train the available experimental data precisely that it captures non-existing correlation. Another striking advantage of ANN lies in its ability of parallel processing instead of sequential programming as adopted in other approaches.

1.5. Controlled release of encapsulated bioactive compounds

The most fascinating fact about encapsulation lies in its ability of controlled release. Controlled release is the process in which active ingredient breaks the core-shell structure and moves from one environment to particular another molecular environment in the surrounding saliva (Silva et al. 2012).

1.5.1. Different mechanisms of controlled release

Different mechanisms are proposed in the literature for the release of active compound namely diffusion, degradation/erosion, melting, swelling and fragmentation (Siegel and Rathbone 2012).

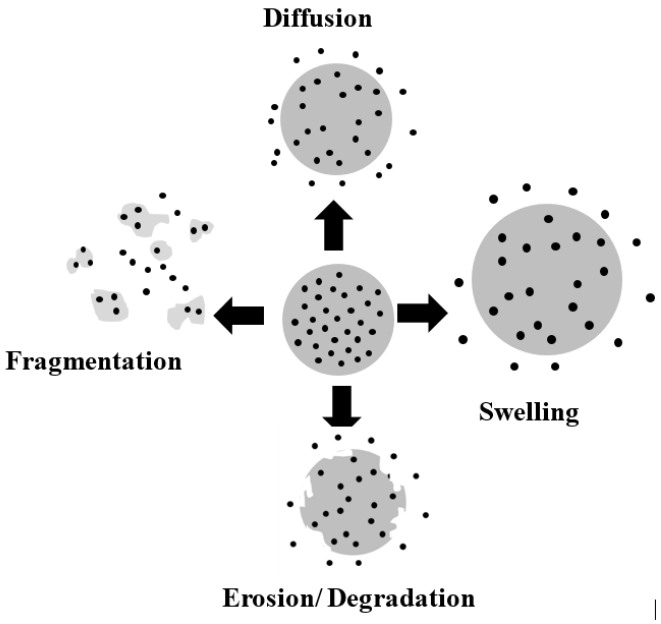

Figure 1.9: Different mechanisms of controlled release of encapsulated bioactive compound

1.5.1.1.Controlled release by the way of diffusion

Diffusion is commonly observed and effective mechanisms for releasing the core compound in a controlled way in a food matrix for their bioavailability. Solubility of core compound in the food matrix and diffusion of the core compound through the matrix are the controlling factors

in the release by diffusion. The thrust for the release by diffusion can be attributed to vapour pressure on the respective side of the matrix (Singh et al. 2002). Release of core from the shell is accomplished in two steps; in the first step, the active core material diffuses and comes to the surface. In the second step, the active component at the surface completely disintegrates and is then transported away from the shell to a food matrix. Static and convective are the two mechanisms for the release by diffusion. Random motion of the molecules in a stationary fluid causes static diffusion (also known as molecular diffusion). During convective diffusion, the core compound of the encapsulates is transported from the core to surrounding food matrix. The kinetics of eddy diffusion is significantly dominant over the kinetics of molecular diffusion. Moreover, it is invariant with the nature of bioactive compound (de Roos et al. 2003).

1.5.1.2.Controlled release by degradation

The core material may get released because of the degradation of shell material in the food matrix or with a combined approach of diffusion and degradation. During erosion, the disintegration of biopolymers forming the shell takes place. It is characterized by the loss of material from the encapsulation system. Erosion of encapsulates may occur in homogeneous (bulk erosion) or heterogeneous way (surface erosion). When degradation is restricted by a weak film approaching the boundary of the feed matrix, heterogeneous degradation takes place. Whereas, erosion at a uniform rate across the polymer matrix results in homogenous degradation. Controlled release by degradation is facilitated by adopting external stimuli such as enzymatic activity, pH change, or by applying osmotic pressure.

1.5.1.3. Controlled release by swelling

In the encapsulation system, controlled release by swelling occurs because core which is dissolved/ dispersed with a biopolymer cannot diffuse to any substantial degree through the encapsulation matrix. However, controlled release is possible if the system is kept in a thermodynamically compatible system. The shell formed of biopolymer swells because of the immersion of solvent from the solution. The active compounds from the swollen section of the shell come out in the food matrix. The extent of swelling is dependent on its water absorption capacity.

1.5.1.4. Controlled release of encapsulation through fragmentation

In this particular approach for release, the encapsulation structure initially breaks down into several daughter encapsulates. Further, the release of active ingredients takes place by one of the techniques mentioned above.

1.6. Application of ultrasound atomization in emulsification

1.6.1. Emulsions in food industry

Another way of incorporating bioactive compounds in food matrix is through emulsification. Basically, emulsions are thermally unstable systems, where droplets of one phase are uniformly dispersed in another continuous phase. Emulsions are broadly categorized into two types based on continuous phase and dispersion phase. When oil particles are distributed in continuous aqueous phase, it is called as oil in water emulsion. Whereas when continuous phase is lipid in nature and the aqueous phase is dispersed phase, it is called water in oil emulsion (Tadros 2013). Emulsions have significant contributions in many industries like pharmaceutical, cosmetic, home care products, paint and food. Among such a wide application sector, an emulsion in food industries is fascinating and necessarily used to deliver bioactive compounds. In food industries, emulsions used not only contributes in imparting a mouthfeel and taste to food products but also helps to improve the physical form (appearance, texture, colour) of food and thus takes the attention of consumers (Torrico 2015). The application products of emulsion in the food sector are not limited to mayonnaise, milk, beverages, sauces salad dressings, gravies, peanut butter, ice cream and other whipped dessert toppings.

1.6.2 Basics of ultrasound atomization

In brief, the phenomenon of atomization can be explained as below: when liquid passes through a piezoelectric element, a thin film of liquid is formed. During the vibrating motion of sonication waves perpendicular to liquid film, liquid film absorbs the vibrational energy. With the absorption of more amount of energy, the crest of these waves becomes taller and trough becomes deeper. Critical amplitude reaches whereat the amplitude of the capillary waves is above that requires sustaining the stability. As a result, the waves collapse, and tiny liquid drops are ejected from the top of the degenerating waves normal to the atomizing surface (Deepu et al. 2018).

1.6.3 Capillary wave and cavitation hypothesis in ultrasonic atomization

Atomization using ultrasound is based on two principal hypotheses, namely capillary wave hypothesis and the cavitation hypothesis.

Capillary wave hypothesis: The capillary wave hypothesis assumes that capillary waves consist of crests and troughs formed on the vibrating surface. When more amount of vibrational energy is absorbed, the crest of these waves becomes taller and trough becomes deeper. Critical amplitude reaches whereat the amplitude of the capillary waves is above that requires

sustaining the stability. As a result, the waves collapse; as a result, small liquid drops are emitted from the top of the degenerating waves normal to the atomizing surface.

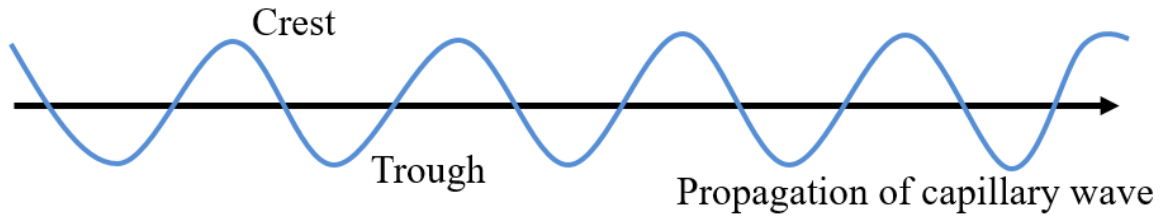

Figure 1.10: Representation of capillary wave hypothesis

Cavitation hypothesis: The liquid coming in contact with ultrasound transducer forms a liquid film on the vibrating surface, cavitation bubbles are developed on the vibrating surface. These bubbles are responsible for nuclei formatting, growth and intense collapse phenomenon of cavitation. In the course of the implosive collapse of these cavities, high intensity hydraulic shocks are produced. These hydraulic shock waves cause the breakdown of the liquid film and cause direct ejection of the droplets.

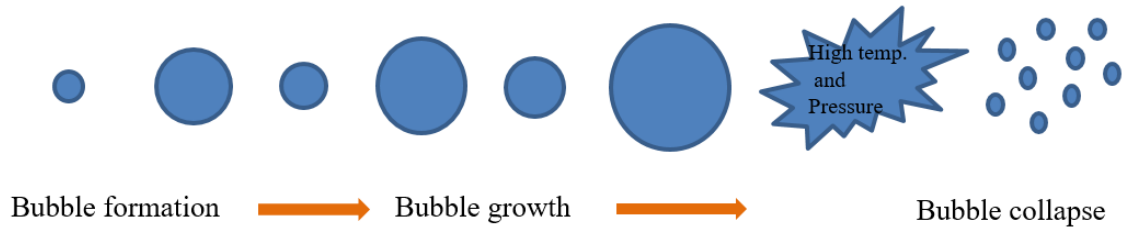

Figure 1.11: Representation of cavitation hypothesis

The energy consumption with the ultrasonic atomization approach is one order lower than that of other high energy techniques. The ultrasonic atomizer is superior to other types of ultrasonic equipment's like welders, ultrasound bath and probe sonication in terms of fine control of energy. While other devices depend on control of energy input, ultrasonic atomization typically requires power levels below 15 W (Dalmoro et al. 2012).

1.7 Motivation and scope of the current research

In the present, the application of bioactive compounds in food processing has become an essential in food processing. Because, last few decades have experienced the side effects of artificial preservatives and flavouring agent in the food processing. In this regard, it is very much important to have efficient processes for extraction and encapsulation of bioactive compounds especially lipid compounds. Hence, we have attempted extraction and encapsulation of ginger oil. However, understanding the effect of all input parameters on

process output is complex. Therefore, it is necessary to study the process of concern with a technique having ability of predicting the process with more accuracy and minimum number of experiments. This fact has motivated the present research to extract the ginger oil from the root and encapsulation of active ingredients using ultrasound approach. The effectiveness of integrated Taguchi and ANN approaches observed from literature has motivated its use in process parameter optimization.

1.8 Organization of the dissertation

The dissertation has been organized under eight chapters with the contents summarized as follows:

Chapter 1 explains the importance of bioactive compounds in food processing steps. The need of extraction and encapsulation of bioactive compounds for their preservation are reported. Different techniques for extraction of bioactive compounds are illustrated. Further the potential of encapsulation in preservation of extracted bioactive compounds and different approaches currently practised are illustrated with their mechanism. The usefulness and role of Taguchi and ANN approaches for process design, prediction and optimization are summarized. This chapter also explains different mechanisms of controlled release of active component from encapsulation. The insight into ultrasonic atomization approach as an alternative to high energy emulsification techniques is provided.

Chapter 2 consists of an extensive literature survey in the area of extraction of bioactive compounds with different extraction techniques, different extraction approaches used, core and shell material used for encapsulation process. The practical examples of controlled release of encapsulated compounds are elaborated. The purpose of this chapter is to understand the selection criteria for choice of extraction, encapsulation technique, controlled release mechanism and finding best suitable method for process optimization. Use of ultrasound atomization in food sector is reported. Eventually, the research gaps were identified and research objectives are established to fulfil the identified gaps.

Chapter 3 presents the extraction of ginger oil from *Zingiber Officinale Roscoe* herb using cavitation technique. The extraction efficiency of different polar and nonpolar solvents is evaluated. The effect of different process parameters namely sonication time, applied sonication power and feed to solvent ratio are also evaluated in terms of extraction yield. The obtained extracts are characterised using UV-visible spectrophotometry and Fourier transformation infrared spectroscopy analysis.

Chapter 4 deals with encapsulation of ginger oil with gum arabic (GA) shell using an ultrasound approach. Taguchi approach with standard L^{27} orthogonal array is applied to understand the effect of various process parameters on the process of an encapsulation. The model validation using the analysis of variance (ANOVA) technique is explained. Parameter optimization study by considering the various input parameters such as ginger oil and GA concentrations, sonication time, and drying parameters like temperature and flowrate using integrated Taguchi and ANN are described.

Chapter 5 demonstrates parametric optimization for the encapsulation process of the Peppermint flavour in GA shell studied by Sangolkar et al (2019). Based on this literature data, different ANN networks were developed and trained by varying the combination of transfer functions, number of neurons in the hidden layer and learning rate. The best performing model was optimized. To further investigate the consequence of a wide range of parameters, the developed network was used to simulate the outputs for non-existing combinations of input parameters (interpolation and extrapolation).

Chapter 6 demonstrates the controlled release study of encapsulated ginger oil in GA shell material. The effect of pH of phosphate buffer solution, the concentration of GA, emulsification time on % cumulative release over a period of time was studied.

Chapter 7 ultrasonic atomization as a novel approach for the synthesis of sunflower oil-in-water emulsification is exploited. The effects of oil concentration, surfactant concentration atomization frequency and flowrate to atomizer on particle size were investigated using particle size analysis and optical microscopy analysis.

Chapter 8 presents the overall conclusions drawn from the investigations carried out and it also suggests further recommendations for the future work.

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Chapter 2

Literature Review

The current chapter deals with an extensive literature review on extraction of various bioactive compounds. The section 2.1 focuses on highlighting the literature of extraction of bioactive compounds with different extraction techniques. Further, the literature study for encapsulation of various bioactive compounds with different shell materials and suitable encapsulation technique is presented in section 2.2. An attempt is made to focus on encapsulation process with the use of different process optimization and prediction techniques applied in food processing. The observation and factors affecting on controlled release of encapsulated core compounds are described in section 2.3. Eventually, the potential of ultrasound atomization in food processing is studied from the literature and detailed in section 2.4. This chapter also provides a summary of the literature review, gaps identified in the existing research and objectives of the present work.

2.1. Extraction of bioactive compounds

The plant based bioactive compounds have proved their importance in food processing and in nutraceuticals. The primary limitation for the exploitation of bioactive compounds in food processing is due to its degradation under environmental conditions (Cikos et al. 2018). Therefore, it is very much essential to extract active ingredients from the bioactive source. This section explains extraction of various bioactive compounds using the different extraction techniques mentioned below. This section aims to provide a knowledge in the selection of extraction method for the extraction of desired bioactive compound.

Azian et al. (2014) proposed mechanism of solute extract from plant matrix as shown in figure 2.1 below. Initially, solvent penetrates from bulk solvent (liquid) into the matrix through stagnant film layer takes place. Then, the transportation of solutes within plant matrix to bulk solvent occurs because of the concentration gradient of the solutes. The transportation phenomenon is explained mathematically using Fick's law as given below.

$$\frac{\partial C_s}{\partial t} = D\,\frac{\partial^2 C_s}{\partial r^2} \qquad (2.1)$$

In the equation, D refers to diffusion coefficient, Cs is the concentration of solute in (mg/g) at that time instant and r is the radial distance (m) of the solid matrix. The external diffusion takes

place due to the transport of solute through the stationary film enclosing the solid matrix. The external mass transfer is a convection process, where bulk solvent plays an important role.

$$\frac{dC_f}{dt} = 3\frac{k_f}{R}\frac{m}{\rho^V}(C_{r=R} - C_s) \quad (2.2)$$

Also, it is commonly observed that prolonged extraction of bioactive compounds leads to degradation of active compounds. The rate of degradation of active components is given by k_{deg}.

$$\frac{dC_{deg}}{dt} = -k_{deg}t \quad (2.3)$$

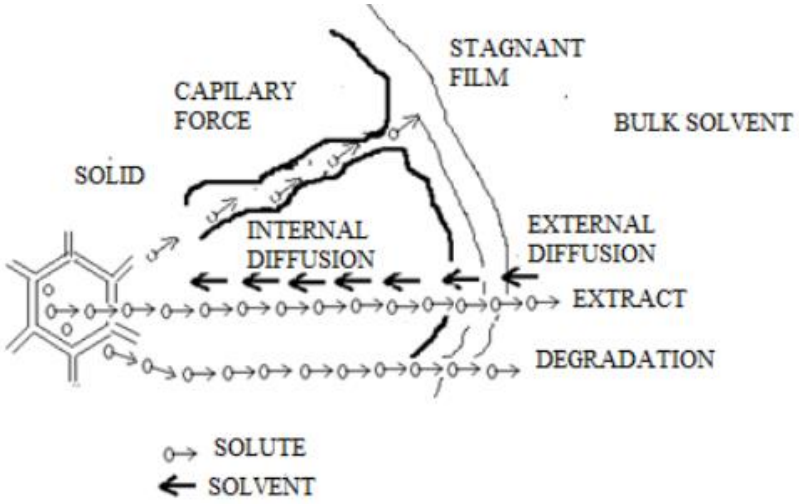

Figure 2.1: Mechanisms of solute extract for typical solvent (Azian et al. (2014)).

The reported extraction of various bioactive compounds with different extraction technique is illustrated in the section below.

2.1.1. Hot water bath extraction

It is an old age technique; it is used from ancient time. However, with time, technology has experienced a revolution. Lee et al. (2014) used this technique for the extraction of onion peels (Allium cepa L.) using hot water, ethanol and subcritical water (SCW) as a solvent. The study reported that, extraction with ethanol as a solvent is favourable, the total phenolic and flavonoids contents increased to 327.5 mg gallic acid equivalents (GAE)/g extract and 183.95 quercetin equivalents in mg per g (mg QE/g) extract respectively. The study also highlighted the importance of onion peel extracts produced by SCW extraction as a source for valuable antioxidant. Similarly, Hassas-Roudsari et al. (2009) extracted antioxidant compounds from canola meal with three different techniques namely; SCW at 110 ºC, ethanol (95 %, v/v) and hot water at 80 ºC. The similar yield was obtained with subcritical water extraction and hot

water extraction (0.19– 0.21 g/g meal). The lower yield with ethanol in the range of (0.14–0.15 g/g meal) was attributed to lower solubility of protein in ethanol. The important note from their study is on the effect of temperature on polarity of water. It is well known that water is highly polar solvent with an ability to dissolve polar compounds. However, it is interesting to note that the polarity of water lowers with increase in temperature. In particular, when water temperature increases from 25 to 200 ºC, its dielectric constant reduced from 79 to 35, a value equivalent to that of methanol =35 and same as that ethanol=24 (Cacace & Mazza, 2006). This shows that, beside the polar nature, water has an ability to dissolve non-polar compounds at higher temperature (Lide 2000). Another reason for solubility of non-polar compounds is due to increase in temperature of water contributes in lowering the surface tension and viscosity. Thus, diffusion rate and mass transfer rate also enhance promoting the extraction (Ramos et al., 2002).

Tunchaiyaphum et al. 2013 compared the performance of supercritical water extraction and Soxhlet extraction techniques with practical example of extraction of antioxidant compounds from mango peel. They concluded that SCW extraction as a green technique for extraction of phenolic compounds from agricultural wastes.

2.1.2. Soxhlet extraction

The quantity of solvent required for extraction is a function of weight of the plant material. For instance, root and stem of the tree are heavier than leaf. Hence, the solvent requirement is higher for root and stem than the leaf. The selection of solvent is carried out carefully in particular, hexane is the more suitable for oily and fatty materials (polar), while methanol and chloroform are best solvents in the extraction of chlorophyll (slightly polar), ethyl acetate is typically used for extraction of phenolic compounds. Methanol and acetone are beneficial to extract all components (polar).

Alara et al. (2018) performed the extraction of phenolic compounds with Soxhlet extractor from Vernonia cinerea leaves. The range of parameters considered for extraction is 1–4 h extraction time, 1:10-1:25 g/mL powder-to-solvent and 20–80 % v/v ethanol concentration. The maximum extraction yields, total flavonoid content (TFC) and total polyphenol content (TPC) were attained at 2 h of extraction time. With continued extraction beyond 2 h, led to degradation of phenolic compounds. All three output parameters improved with increase in ethanol concentration from 20 to 60 % v/v of, beyond this range linear decrease in yield was observed. The outputs at optimized conditions are- extraction yield (10.01 ± 0.85 % w/w), TPC (53.96 ± 1.45 mg GAE/g d.w.) and TFC (30.09 ± 0.44 mg QE/g d.w.). Similar study was carried out by Lama-Muñoz et al. (2020). They attempted extraction of phenolic compounds and

mannitol from olive leaves employing Soxhlet technique. The same range of parameters as given by Alara et al. (2018) was used for extraction. The process was performed for 4 h using dry olive leaves (10 g) with a mixture of ethanol–water (60:40, v/v). They concluded that Soxhlet technique gives a greater recovery of apigenin-7-Oglucoside, luteolin-7-O-glucoside, oleuropein, and verbascoside. The performance of Soxhlet extraction was compared with pressurized liquid extraction. The yield of mannitol and cultivar extraction was found to be 63.72 g/kg and 82.21 g/kg by PLE and Soxhlet approach respectively. The lower yield with PLE as compared to Soxhlet was attributed to degradation of active compounds owing to the hydrolysis reactions and oxidation taking place at higher temperatures (190 °C) used in PLE, as compared to Soxhlet extraction where mild temperatures used ranging between 81.63 °C and 91.58 °C. The only limitation reported with Soxhlet extraction is due to higher time (4 h) Vs. 5 min with PLE. This comparison indicated the potential of Soxhlet extraction if time constraints are not applied.

The selection of suitable solvent is a key challenge in extraction process. The selection largely depends on the physicochemical properties of solvent which includes polarity, boiling point and viscosity of the solvent. To give the brief overview and guidance for the selection of solvent Ramluckan et al. (2014) performed the Soxhlet extraction of oil from algal biomass. The table 2.1 below gives the physiochemical properties and the quantification of extracted compounds

Table 2.1: Physiochemical properties and the quantification of extracted compounds in Soxhlet extraction

Sr. No.	Solvent	Polarity index Units	Boiling point °C	Density @ 25 °C g/m	Chlorophyll conc. in Lipid extract % m/m	Chlorophyll conc. in Lipid extract % m/m
1	Petroleum ether	0.1	35.0–60.0	0.640	0.10	4.82
2	Hexane	0.1	69.0	0.659	0.78	10.02
3	Cyclohexane	0.2	80.7	0.779	0.14	6.87
4	Isooctane	0.4	99.2	0.690	0.51	8.69
5	Toluene	2.4	110.0	0.865	0.41	9.83
6	Benzene	2.7	80.0	0.874	0.67	7.03
7	Diethyl ether	2.8	34.6	0.706	0.63	6.96
8	Dichloromethane	3.1	39.8-40.0	1.325	0.22	5.98
9	Isopropanol	3.9	82.0	0.785	0.43	5.22
10	Chloroform	4.1	60.5-61.5	1.492	0.26	10.73

11	Acetone	5.1	56.0	0.791	0.16	2.15
12	Methanol	5.1	64.7	0.792	1.55	6.68
13	Ethanol	5.2	78.0	0.789	0.57	10.21
14	Water	10.2	100.0	1	0.10	4.82

2.1.3. Maceration process of extraction

Maceration is an ancient technique of extraction, where the leaves of Basil and so many other plants were used as a remedy in many diseases not limited to cold and cough. Jovanović et al. (2017) carried out extraction of polyphenols from Thymus serpyllum L. herb by three different methods namely maceration, HAE and UAE. Effect of extraction parameters like solid-to-solvent ratio, particle size, extraction time and solvent type was investigated employing statistical analysis. Extraction efficiency was evaluated in terms of TPC and TFC. They reported that best polyphenols yield was obtained with powder size of 0.3 mm, 1:30 feed-to-solvent ratio and 50 % ethanol. With the above said conditions, the measured TPC was 26.6, 29.8 and 32.7 mg GAE/L in maceration, HAE and UAE respectively. Beside the lower yield, less energy and equipment requirement are the striking advantages on maceration process.

Maceration approach is typically employed to extract phenolic compounds from the grape in wine preparation. Setford et al. (2017) reviewed the various factors affect extraction and evolution of phenolic compounds in the red wine maceration. Temperature, solvent composition and sold-liquid contact area the important parameters affecting the extraction with maceration.

Temperature: It is a significant parameter in the extraction of phenolic compounds in the fermentative maceration because it affects the penetrability of the cell membranes in the grape solids (Boulton 2001).

Solvent composition: At higher concentrations of ethanol and sulphur dioxide in water, the dielectric constant of the solvent is decreased, that reduces the solvation of molecule. Therefore, it is recommended to increase the concentration of these molecules in the solvent which helps to reduce their interactions with solute molecules and enhances the rate of internal diffusion (Karacabey and Mazza 2008).

Sold-liquid contact area: It is required to improve the solid-liquid contact area to keep constant temperature as well as to limit the potential contamination by aerobic spoilage microorganisms (Sparrow et al. 2015).

Fick's second law was applied to elaborate the extraction phenomenon, which says that the rate of diffusion is a dependent on the internal diffusion coefficient and the solute concentration gradient. Temperature is a major contributing factor affecting the extraction process in fermentative maceration (Koyama et al. 2007).

2.1.4. Supercritical fluid (SCF) extraction

It is a green extraction technique because organic solvents are not required in the process. The extract obtained is pure in nature hence it can be readily used. Gopalan et al. (2000) performed turmeric oil extraction from turmeric (Curcuma longa) with supercritical carbon dioxide in a semicontinuous-flow extractor. They observed that the use of high pressure (20-40 MPa) and low temperature (313-333 K) is more suitable for extraction. Because in such conditions solvent density is high and therefore the solubility of the oil in the solvent is improved. Another observation reported by their group is based on the particle size of component to be extracted. Typically, grinding the bioactive compounds prior to extraction is useful to obtain particles with higher surface area that assists in mass transfer dissolving the essential oil in the solvent an as a result, extraction rate is improved.

Czaikoski et al. (2015) performed SFE with CO_2 and propane as a fluid for the extraction of Eupatorium intermedium. The study revealed that, with CO_2 as a fluid, only pressure is a major factor affecting the extraction yield. Whereas, in case of propane as a fluid, both pressure and temperature contribute in deciding the extraction yield. They found that obtained extract possess antibacterial activity.

2.1.5. Microwave assisted extraction

It is a low cost, rapid and intensified extraction process. Hence, in the present time, the research is moving towards carrying out extraction of many plant materials. Also, the trend is moving towards the use of this technology in integration with other extraction technologies such as Soxhlet extraction. Ganzler et al. (1986) studied this approach to extract different polar (vicine, convicine, gossypol and pesticides) and non-polar (crude fat) compounds. They observed that MAE was more effective than the conventional Soxhlet or shake flask extraction methods. Higher extraction yield with MAE in comparison with conventional technique was attributed to sensitivity of selected bioactive compounds to high temperatures, which might have degraded at higher time in Soxhlet extraction procedure. Because of the substantial reduction in time and energy and projected the potential of microwave assisted extraction for rapid extractions of large sample series.

Dahmoune et al. (2015) investigated the MAE of phenolic compounds from Myrtus communis leaves. They studied the effect of various parameters including ethanol concentration, microwave power (400–600 Watt), irradiation time (30-90 sec) and solvent-to-solid ratio (20–40 mL/g). The optimal MAE conditions were determined using RSM. A second-order regression equation and ANOVA analysis was performed to determine the significant parameters affecting the extraction yield. They found that ethanol concentration and liquid-to-solid ratio are the major factors affecting the extraction process ($p < 0.01$). Optimum parameters of extraction were ethanol concentration- 42 %, microwave power- 500 W, irradiation time- 62 sec and solvent to powder ratio- 32 mL/g, TPC- 162.49 ± 16.95 mg g GAE/g dry weight. Antioxidant activity of the obtained extract was estimated using ABTS assay. More than 50 % of oxidation inhibition was achieved with concentration of 70 µg GAE/mL.

2.1.6. Ultrasound-assisted extraction

Ultrasound assisted extraction (UAE) is a rapid, intensive and green approach for the extraction of bioactive components. Nawaz et al. (2019) reported the influence of polarity of the solvent and extraction technique on phytochemical composition and antioxidant activity of corn silk. Extraction was carried out in two different ways. In the first approach extraction was performed with each solvent separately. Whereas in the second approach, sequential extraction with solvents of increasing polarity were used, here five different solvents with higher polarity were employed for extraction of phytochemicals. Corn silk was analysed to confirm various bioactive phytochemical compounds present in it namely flavonoids, phenolic acids, tannins, ascorbic acid and cardiac glycosides. Polar solvents were found to be more effective in the extraction of phytochemicals from corn silk.

Jadhav et al. (2022) carried out an extraction of oil from waste custard apple seed (CAS) with ultrasound cavitation. The effect of different extracting solvents namely methanol, hexane, toluene, ethanol and tetrahydrofuran (THF) on extraction yield was observed. The effect of sonication time (5, 10, 15, 20, 25 and 30 min). The study reported that 10 min time is enough to accelerate the collision between the powder and ultrasonic waves which is responsible for the breakage of the cell wall of the powder particles, reduced particle size and thus promote the mass transfer. At high temperature, the vapor pressure of the solvent increases and the cavitation collapse is less intense. Hence, with increase in temperature from 20-50 ºC decreases in yield from 33.60 to 32.1 g of oil/ 100 g of CAS was observed. With applied sonication power (20, 25, 30, 35, and 40 % amplitude of 750 W power). The reason behind increase in extraction

yield with sonication power was attributed to an increase in the rate of formation of cavities and the number of cavitation events with power. The comparison of UAE against Soxhlet showed that the time of extraction with ultrasound is only 10 mins as against 4.5 h with Soxhlet method. Also, ultrasound was found to be superior over Soxhlet in terms of energy consumption (84.51 % less).

Liu et al. (2014) developed and optimized high-performance liquid chromatography with diode-array detection (HPLC-DAD) approach for the estimation of 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol in ginger extracts. Twenty-one extracts were obtained by variation in type of ginger used (dry vs. wet) different extracting methods and using various solvents. The ANOVA analysis helped them to understand the effect of extraction methods on extraction yields. They observed that of gingerol content increased in following order: high pressure > high temperature > blender > low pressure. It was also observed that dry ginger possesses higher extraction ability as compared to wet ginger. The most interesting aspect is that heat drying of fresh ginger root before extractions did not degrade gingerol. The extraction yield of ethanol solvent obtained was much higher than aqueous extract. Aqueous ginger root extracts had considerably less gingerol yield as compared to aqueous ethanol extracts. At high pressure and temperature higher gingerol yield was obtained over other techniques.

Sasikala et al. (2018) carried out UAE of polyphenols from ginger rhizome (*Zingiber officinale*) followed by adsorption and desorption. The maximum degree of extraction was obtained with methanol solvent (0.55 mg/ml) followed by acetone (0.52 mg/ml) and ethanol (0.37 mg/ml). Different solid-solvent ratios (1:5, 1:10, and 1:15 (w/v)) were studied. Among, 1:10 (w/v) solid solvent ratio was effective in extracting maximum polyphenols.

2.1.7. Comparison of different extraction techniques

From above mentioned techniques of extraction, it is clear that certain bioactive compound can be extracted with more than 2 extraction approaches. However, the choice of extraction technique not only depends on the higher recovery of bioactive compounds but other parameters like cost, extraction time and solvent requirement also need to be considered in order to make the extraction process more efficient. Leach 2004 performed such comparison as shown in table 2.2 below.

Table 2.2: Comparison of different extraction techniques

Extraction	**Soxhlet**	**USE**	**ASE**	**MAE**	**SFE**
Cost of process	Low	Low	High	Medium	High
Extraction time	6-48 h	< 30 min	< 30 min	< 30 min	< 630 min
Solvent required (mL)	200-600	< 50	< 100	< 40	< 10

Ong and Len 2003 compared the baicalein extraction performance of Soxhlet extraction with PLE. They found that parameters of extraction are 100-120 mL methanol and water mixture in the ration of 70:30 in 3–4 h time with Soxhlet as against 20–25 ml methanol at a pressure 10–30 atm and at 100 °C over a period of 20 min with PLE.

Grigonis et al. (2005) investigated the comparative performance of Soxhlet extraction, MAE, and SFE in 6 h, 0.25 h, and 2/h respectively. MAE and SFE were observed to be efficient in the extraction of antioxidants from sweet grass. Two step extraction process helped to improve extraction yield from 0.46 % to 0.058 %. Similar study was performed by Pan et al. (2003). They compared extraction performance of three different techniques namely, MAE liquid/liquid extraction, UAE and HAE for the extraction of caffeine and polyphenols from green tea leaves. They found that extraction yield with MAE in 4 min was greater as compared to 20 h extraction in atmospheric conditions and UAE in 90 min. To obtain the same yield, 45 min time was required with HAE. Teixeira et al. (2006) investigated the comparative performance of disruption technique and solid-liquid extraction technique for the extraction of extract phenolic compounds from Ficus carica levels. From ANOVA analysis and performance comparison it was observed that higher extraction yields can be achieved with disruption technique.

2.1.8. Extraction of ginger

Out of several bioactive compounds of interest, ginger is being employed from ancient time as a spice, aroma and natural drug molecule. Interestingly, India is the second most ginger producing country in the world with annual production of 1.89 million metric tons of the spice from an area of 157839 hectares in 2021. Thus, it is of great interest to use ginger in food processing.

Many investigations have been performed in recent past to evaluate the composition of active compounds in the ginger extract. Also, authors have investigated the extraction of ginger oil from the source employing different methods and different solvents. Out of the reported solvents methanol and hexane are found to be effective and commonly used solvents in

extraction (Hasan et al., 2012). It is observed that HPLC-UV is a good approach to determine the chemical composition of ginger extract.

Supardan et al. (2012) investigated the extraction of ginger oleoresin with ethanol as a solvent, at 42 kHz frequency and at 60 ºC and mentioned that UAE was nearly 1.75 times accelerated as compared to traditional methods. Combined UAE and supercritical carbon dioxide approach was used to extract oleoresin and study reported 8.14 % of oleoresin yield at 35 °C and 25 MPa temperature and pressure respectively. Kadam et al. (2019) performed the extraction the ginger oil from raw ginger by hydro distillation method and observed changes in the physical and chemical properties namely colour pale (yellow to brown), odour (warm, citrus and woody) appearance (mobile liquid), flash Point (56 °C), specific Gravity (at 20 °C, 0.8765 - 0.8892), refractive index (at 20 °C 1.48 - 1.49), non-volatile matter content (4.17 %), optical rotation (-29 to -48 °). They found that the extract has 95 % solubility in alcohol. Acid value obtained was 5.28 and moisture content was 8.76 %.

Malu et al. (2009) carried out extraction of *zingiber officinale* with different solvents namely, n-hexane, ethanol, ethyl acetate and water. Obtained extracts were evaluated for antibacterial activity and bacterial growth inhibition activity. Except the water, all other extracts exhibited antibacterial activity and that the inhibition of bacterial growth.

Azian et al. (2014) performed extraction of ginger with two different methods namely Soxhlet extraction and an accelerated water extraction. The highest yields of 6-, 8-, 10-gingerols and 6-shogaol with Soxhlet extraction was observed to be 13.948, 7.12, 10.312 and 2.306 mg/g, respectively. Whereas, for the same compounds of ginger, extraction yield with accelerated water extraction was 68.97 ± 3.95 mg/g at 3min, 18.98 ± 3.04 mg/g at 5min, 5.167 ± 2.35 mg/g at 3 min and 14.57 ± 6.27 mg/g at 3min, respectively. They observed that at 3 mins temperature affects and indicated rapid increase in the concentration of 6-shogaol and decrease in the yield of 6-gingerol. Extended extraction beyond 8 h led to degradation of all active compounds.

2.2. Encapsulation approach for preservation of bioactive compounds

The effectiveness of polyphenols is influenced by the preservation of their stability, bio-chemical-activity, and also bioavailability. It was observed that encapsulated polyphenols, in place of free compounds, can effectually mitigate some deficiencies Also, the hygroscopic property of some compounds can be preserved from moisture attack (Fang and Bhandari 2010).

2.2.1. Potential core materials of interest for encapsulation

Table 2.3 gives the classification of bioactive compounds encapsulated in the food sector.

Different fatty acids for instance ω-3 fatty acids, flaxseed oil, clove extract, soybean oil, canola oil is encapsulated as they have health benefits in the treatment of coronary heart disease,

immune response disorders, bone health, stroke and weight gain (Shaw et al. 2007, Chatterjee et al., 2013). Similarly, different Carotenoids which are the rich source of Lycopene, the rubixanthin, and β-cryptoxanthin, vitamin-A are encapsulated to decrease the risk of cancers and eye disease (de Campo et al. 2018). The bioactive compounds like ginger and red cabbage which are the prime source of antioxidants and polyphenols are encapsulated to maintain their bioactive properties (de Barros Fernandes et al. 2016).

Table 2.3: Classification of bioactive compounds encapsulated in the food sector

Name	Shell material	Encapsulation method	Findings	References
Fatty acids	**Coronary heart disease, immune response disorders bone health, stroke, weight gain**			
ω-3 fatty acids	Chitosan, spray-dried	Lecithin	Encapsulation offered the highest oxidative stability to compounds in powder and emulsion form.	Shaw et al. (2007)
Flaxseed oil	Gelatin-GA	Complex coacervation	Primary and secondary oxidation was inhibited with the formation of capsules when compared with non-encapsulated oil	Liu et al. (2010)
Clove extract, soybean oil	MD and GA matrices	Spray dryer	Clove extract proved as an optimistic natural antioxidant in the same.	Chatterjee and Bhattacharjee (2013)
Canola oil	Alginate	Co-extrusion	Encapsulates characteristics were affected by the core and shell flowrates as well as their formulations.	Wang et al. (2013)
Flaxseed oil	Blend of MD, GA, WPC and MS (Hi-Cap 100 TM and capsule TA)	Spray drying	EE have following order MD: Hi-Cap>MD: Capsul> MD: WPC Oxygen diffusion from the glassy wall affected the reaction rate	Carneiro et al. (2013)
Flaxseed oil	Chickpea CPI) or LPI WPI and MD	Emulsion and freeze drying	CPI and LPI proprtected flaxseed oil and its release GI tract	Karaca et al. (2013)
ω-3 fatty acids	Nanoemulsionbased	Fish oil	Sunflower phospholipids served as a natural emulsifier to deliver x -3	Komaiko et al. (2016)

	delivery systems		fatty acids in the food matrix and beverages.	
OLE in Soybean oil	WPC, and mixture of WPC–pectin	Stirring and homogenizer	Higher antioxidant of emulsion revealed its increased solubility and controlled release of olive leaf phenolic compounds	Mohammadi et al. (2016)
Carotenoids	**Lycopene, the rubixanthin, and β-cryptoxanthin, vitamin A decreases the risk of cancers and eye disease**			
Lycopene	β-cyclodextrin freeze-drying.	Spray-drying or molecular inclusion	Purity of lycopene enhances from 96.4 to 98.1% with spray-drying, decreased from 97.7 to 91.3% with complex formation and freeze-drying	Nunes and Mercadanteet al. (2007)
β-carotene	Oil-in-water nanoemulsions	homogenization	β-carotene bio-accessibility decreased in the order LCT ≫ MCT > orange oil	Qian et al. (2012)
Lutein	porous starch and gelatin	Spray-drying	Encapsulation provided lutein stability from heat, pH, light and oxygen and their retention rates by 15–50% in comparison with free lutein.	Wang et al. (2012)
Zeaxanthin	Cactus cladode mucilage (CM)	HPH	CM offered a protection to Zeaxanthin indicating that nanoencapsulation can improve zeaxanthin stability	De Campo et al. (2018)
Antioxidants and polyphenols	**Tocopherols, flavonoids, polyphenols (Belscak et al. 2011) (Busic et al., 2018)** **Coronary heart disease, urinary tract disease and cancer**			
Ginger	CG and IN	Spray drying	EE was affected by the blend of the shell, Highest EE 89.80% obtained with higher IN concentrations were applied	de Barros et al. (2016)
Red cabbage	MS	Spray-drying	Encapsulation helped to improve the retention of polyphenols and antioxidant capacity upon thermal treatment without altering colour properties	Zanoni et al. (2020)
Phytosterols	Stigmasterol, β-sitosterol coronary heart disease			

Wheat germ oil	WPC, MD	Ultrasonication and freeze drying	Increase in WPC and ultrasonication time have positive impact on EE	Yazicioglu et al. (2015)
Rice bran oil	Jackfruit Seed Starch – WPI	Spray drying	Optimum conditions obtained were 20 % oil, 3:1 ratio of starch to protein ratio and inlet air temperature of 140 °C in spray drying	Murali et al. (2017)
Corn oil	Brea gum (BG), IN, GA	Homogenizer, Ultrasonication spray drying	BG and IN was good choice as an alternative to GA for hydrophobic compounds encapsulation	Castel et al. (2018)

2.2.2. Shell materials used in food encapsulation

The basic step in the encapsulation is to find a suitable shell material. Classification and properties of the food grade materials utilized as a shell for the encapsulation is illustrated with table 2.4.

Starch: Starch acts as a shell and as emulsion stabilizers. Due to their ability to retain the flavour they are widely used for flavour encapsulation. Newly developed microporous starch materials possess improved aroma retention (Murua-Pagola et al., 2009, Cortes et al., 2014). In practise starch granules are employed in combination with proteins/ polysaccharides for protecting the bioactive substances. Hydrolysed starches formed strong oxygen-barrier, whereas starch in the modified form is an excellent emulsion stabilizer.

Maltodextrin (MD): is polysaccharide, product of partial acids/enzymes hydrolysis of cornflour/rice/ potato. It helps to improvise the thickness and shelf life of food to which it is being added. MDs are available in different dextrose equivalents. They possess good film forming properties (Yoshii et al., 2001). They are commonly blended with other shell materials such as GA, starch, WPI to decrease the overall cost of product as they are easily available and cheaper as compared to other shell material.

Gums: It is hardened sap of acacia Senegal and Vachellia (Acacia) seyal. GA is a mixture of polysaccharides and glycoproteins. GA is most significantly used for the encapsulation of lipid based bioactive compounds. During emulsification of core-shell mixture, it serves as a surfactant and drying matrix. GA is expensive, and its availability is also another issue hence often it is blended with MD. GA in blend with MD allows loading of higher solids in encapsulation by controlling the viscosity (Agnihotri et al., 2012). Encapsulates formed by

spray drying approach and GA-MD blend gives particle size distribution in the range of 10-200 nm and retains volatile compounds more than 80 %. It is reported that 70:30 mixtures of GA and MD offers superior quality microcapsules (Yoshii et al., 2001).

Soya bean essential oil was encapsulated employing GA shell material. The study demonstrated the impact of the core to shell ratio on encapsulation efficiency, increase in soya bean oil to GA ratio was from 0.25 to 5.0 produced a negative impact on encapsulation efficiency (Hogan et al. 2001).

Proteins: are polymers constituted of molecular structure called amino acids. Different types of proteins when used in encapsulation, they get self-associated at oil and water interface so as to form the protective coating to inner compound. During emulsification, the protein molecules get readily adsorbed at oil–water interface. Resultant steric-stabilizing film promptly protects the oil globules from recoalescence and thus imparts physical stability to the emulsion in the period of food processing and preservation. (Dickinson, 2001).

Table 2.4: Food grade materials utilized as a shell for the encapsulation

Shell Material	**Examples**	**Characteristics**	**Constraints in employing as a shell**	**References**
Carbohydrate	Starch, Pectin Cellulose, Gums MD, CD	Serve as flavour binder Inexpensive, good solubility, low viscosity	Alginate has low encapsulation efficiency and gives burst release	Robert et al. (2012), Takei et al (2010) Jin et al. (2012),
Proteins	Sodium caseinate, SPI, WPI, gelatin	Serves as an emulsifier, less viscous, solubile in the aqueous phase, forms a coating on the core	Some of the Proteins are costly	Hogan et al. (2001) Charve and Reineccius (2009), Gunasekaran et al. (2007), Kaushik et al. (2007)
Starch	MDs, MS β-CD	Preserves aroma and flavour, substitute to fat, stabilizer in emulsion preparation	Sensitivity to acid attack and amylase hydrolysis	Murúa-Pagola et al. (2009), Gandia-Herrero et al. (2010)

MD	Cornflour	Cost effective, can be used in blend with costly shell material, less viscous at a higher shell concentration	Less emulsifying properties, Flavour retention capacity is low	Dorner et al. (2003)
Gums	GA, CG	Taste and flavour retention capacity is good, emulsifier	Often form highly viscous solutions, More expensive, supply and cost are subject to fluctuations	Sangolkar et al. (2019)

2.2.3. Effect of core-shell concentration

Core concentration: It is always a goal in encapsulation to use the maximum practicable active material concentration that offers higher core preservation within the capsules. This is beneficial because with a high core concentration, amount of shell material required for encapsulation will be less with high product yield. However practically, we need to choose an optimal active material concentration that can be encapsulated efficiently. Hogan et al. (2001) in their investigation observed that loading of higher core (oil) concentration leads to impoverished retention or decreased encapsulation efficiency, and much of the loaded oil remains at the surface of the powder. In particular, when active material concentration was decreased by 10 %, active volatile material retention was increased to 150 %. Encapsulation using sodium caseinate shell and spray drying approach demonstrated that with increasing core to shell ratio from 0.25 to 3.0 drastically decreases encapsulation efficiency from 89.2 % to 18.8 %. The trend of reduction in encapsulation efficiency with higher core concentration might be due to the volatility of active compounds near to the drying temperature, consequently reduction in the diffusion path length to the air-particle interface (Mehran et al., 2020). Despite this trend, in certain applications, the loading of higher volatility resulted in higher retention. The literature available to date describing core, shell concentration, and their ratio indicates that a 1:4 ratios (20 % core at the final encapsulated powder) of the core to shell results in good preservation ability with the highest possible encapsulation efficiency for frequently employed shell materials such as GA and modified starches.

Shell concentration: Higher shell concentration to prepare emulsion has positive impact on volatile matter retention, here higher solid content shortens the time needed to develop a coating surrounding the core also shell gets formed around the core material easily. The

reduction in encapsulation efficiency with an increase in shell concentration beyond certain limits might be due to two reasons. First is, the insolubility of solids beyond certain limits, thus shell material does not become available for encapsulation. Second reason they can be due to increase in viscosity of emulsion at higher solid content which restricts the circulation movement within the droplets consequently. (Satpathy and Rosenberg, 2003). However, the determination of shell concentration is also decided by the type and characteristic of the active compound.

2.2.4. Methods of encapsulation

2.2.4.1 Chemical Methods:

Coacervation: Xiao Jun-xia et al. performed coacervation between soybean protein isolate (SPI) and GA for sweet orange oil micro-encapsulation. The effect of pH, ionic strength, different SPI:GA ratios and loading of core material was investigated. The maximum encapsulation efficiency was observed at pH 4.0. They observed that, high ionic strength decreases the coacervation between the two biopolymers. Further, 1:1 ratio of SPI/GA ratio provided highest coacervate yield microencapsulation efficiency (MEE). In particular, with increase of 10 % sucrose in sucrose/soy protein isolate ratio 1:1 improved the yield by 20 %, achieving 78 % compared to 65 % of control. No cavities were observed on the surface of microcapsules. The reason for this observation was attributed to addition of sucrose, that might have filled in the cavities formed during spray-drying.

Similarly, Wu and Xio (2005) performed encapsulation of fish oil with combined coacervation and spray drying approach. The aim behind encapsulation of fish oil was to preserve its oxidative stability. The oxidative stability of fish oil was boosted due to microencapsulation. The oxidative stability was more when MD was partially (40 %) substituted with GA. SEM analysis depicted that the microcapsules were spherical, smooth and hollow with its shell made up of innumerable small and solid submicrocapsules having fish oil in the core.

Co-crystallization: Deladino et al. (2007) were the pioneer to investigate encapsulation of yerba mate extract (phenolic compound) in a sucrose matrix with a co-crystallization approach. They investigated the effect of core concentration on the physicochemical properties of the co-crystallized product. Free flowing, stable powder was obtained with technique even at high humidity conditions, and encapsulation helped to reduce the hygroscopic characteristics without affecting their high solubility. 72 ± 7.8 % extraction efficiency was obtained at

operating conditions of 0.7 g extract/100 g sucrose and maximum temperature conditions of 140 °C, total phenolic content: 0.58–1.75 mg GAE/g powder.

Sarabandi et al. (2019) also carried out encapsulation of 10 mL marjoram extract in 50 g sucrose solution. Maximum operating temperature was 132 °C. At these conditions TPC obtained was 0.317–1.162 mg GAE/g powder, antioxidant activity 14.67– 43.89 and 83 % encapsulation efficiency were achieved. DSC and FTIR graphs helped them to prove that encapsulation helps to avoid interaction among core and matrix components and shelf life increases to 120 days in different conditions.

To overcome the insolubility problem of curcumin in water, Katherine et al. (2021) synthesized curcumin-dextrose cocrystal, a remarkable increase in solubility of curcumin up to 25 mg/mL water was in comparison with pure curcumin. Their study provided the guidelines in selection of various parameters of crystallization. They observed that absence of water requires high heating temperature to completely melt dextrose. Also, at high temperature dextrose begins to caramelize. Hence, the optimum temperature was set to 100°C with water to dextrose ratio of 1:15. The crystals were obtained in 7 minutes.

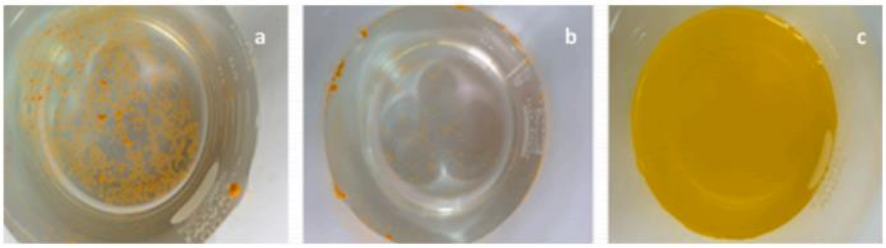

Figure 2.2. Curcumin dispersion in (a) in water, (b) dextrose solution, (c) curcumin – dextrose cocrystal dispersed in water (Katherine et al. 2021).

Pure curcumin (2.2(a)) was not observed by spectrophotometer because it was insoluble. Further, curcumin dispersed in dextrose solution was also insoluble (2.2(b)). The crystal prepared with dextrose dissolved in water depicted high solubility (2.2(c)). This observation assures that solubility of curcumin is not due to presence of dextrose.

Molecular inclusion: With the aim to improve the solubility and characteristics of poorly water-soluble drugs Rahman et al. (2012) performed molecular inclusion of curcumin in the cavity of β-cyclodextrin. Poorly water-soluble compounds like curcumin can be delivered. From UV-absorbance analysis colour characteristics were evaluated. They observed that when curcumin is enclosed in the β- cyclodextrin, preservation of curcumin's orange colour can be achieved. In addition, encapsulated curcumin absorbed less lightly than native curcumin. The

study reported that more than 80% encapsulation efficiency can be achieved with molecular inclusion for the three ratios of curcumin: βCD (1:1, 1:2 and 1:5) However at 1: 0.5 ratio only 35% of encapsulation efficiency was achieved. The retention of aroma compounds inside the cavity was highly affected due to factors such as molecular weight, shape, chemical functionality, polarity and volatility of the core material. Provides protection against heat and evaporation.

The same method was used by Yadav et al. for inclusion of curcumin. They studied the effect of different cyclodextrins namely βCD, γCD (gamma cyclodextrin), HPβCD (hydroxypropyl-β-CD), and MβCD (methyl β-CD). The stability constants of the CD are in the order of HPβCD (424 M^{-1}) > MβCD (401 M^{-1}) > γCD (154 M^{-1}) > βCD (134 M^{-1}). Stability constant (Ks) value between 200 and 5,000 M^{-1} is referred as most suitable for the enhancement of solubility and stability of poorly soluble drug. In another study, Yallapu et al. 2010 also reported inclusion of curcumin in βCD. The synthesized self-assembly was used to deliver curcumin in prostate cancer cells. Encapsulation efficiency of curcumin was enhanced by increasing the ratio of curcumin to CD. Curcumin was efficiently encapsulated in β-CD cavities and formed different types of self-assemblies. Anti-cancer efficacy of the Curcumin (CUR) and CD30 (CD-CUR) inclusion complex cell proliferation assay was performed in two prostate cancer cell lines. They observed that CD30 (30% complex) formulation has a considerably greater anti-proliferative effect as against free CUR which is evident from their IC50 values. The IC50 of CD30 is 16.8 µM and 17.6 µM in C4-2 and DU145 Prostate cancer cell lines, respectively. This shows that encapsulated curcumin in CD is more efficient as compared to bare curcumin.

2.2.4.2 Physical Methods of Encapsulation

Spray drying: Navidad-Murrieta et al. (2020) carried out the encapsulation of extract from Hibiscus sabdariffa with spray drying approach. The interactions between core and shell material were confirmed with UV-absorbance at 211 and 282 nm respectively. Taguchi design with L8 orthogonal array was employed to optimize the multiple variables of extraction and spray drying process with a minimum number of runs. From SEM analysis they observed with spray drying particles formed are spherical in shape with different diameters. The outer smooth surface observed was attributed to shell materials (MD and GA). The particles were observed to have of some concavities with a wrinkled surface. They reported that encapsulation is a potential technique to preserve the phenolic compounds and antioxidant activity of plant material.

To fortify the vitamin D deficiency yogurt powders fortified with nano-liposomal encapsulated vitamin D was incorporated. Jafari et al. (2019) performed the study to observe the effect spray drying conditions temperature (160–190 °C), and blends of shell material (milk protein concentrate (0– 3 % w/w), MS starch (0–3 % w/w), GA (0–3 % w/w) and MD (10–25 % w/w)) toward the physicochemical characteristics of the spray-dried whey powder enriched with vitamin D3. For the encapsulation of vitamin D3 nanoliposome was synthesized using egg yolk lecithin, sesame oil, and glycerol applying thin film dispersion technique. The optimal conditions of shell material and spray drying were chosen from Taguchi design. From SEM analysis, they observed that addition of MD leads to spherical particles with an uniform distribution in the matrix.

Freeze drying process: Horszwald et al. (2013) and Ballesteros et al. (2017) carried out the extraction of antioxidant phenolic compounds from spent coffee grounds and encapsulated them with both freeze drying and spray drying approaches separately. The study revealed that polyphenols are preserved with better quality using freeze drying. They observed that colour retention for the freeze dried samples was higher as against to spray drying. On the other side TPC was 20 % lower in case of freeze drying as against spray drying.

This was due to low temperatures and higher drying time of 140 h in freeze drying which might have led to the degradation of polyphenols.

Fluidized bed coating: Coronel-Aguilera et al. (2015) investigated encapsulation of an emulsion containing 0.06 g β-carotene/100 g. Initially, an emulsion was prepared using spray drying. This mixture was further fluidized to obtain an encapsulation. The effect of fluidization parameters namely temperature and feed rate of coating solution on the stability of colour of β-carotene was evaluated at different storage temperature. It was reported that coating solution does not have significant impact on retention of properties of β-carotene including moisture content, particle size, melting point, water activity and colour. Coated powders showed colour stability of 4 weeks, when loaded in yogurt.

Extrusion method of encapsulation: Belscak- Cvitanovic et al. (2011) investigated encapsulation of raspberry leaf, hawthorn, and olive leaf extracts in alginate–chitosan. The shell was dissolved in the ascorbic acid by electrostatic extrusion. The study reported that Nettle extract with microparticles have large sized particles with irregular shape. This was attributed to gelling process of alginate due to presence of microelements (strontium, copper and zinc). The shelf life of antioxidants of hydrogel microcapsules lowered due to instability of ascorbic acid. Overall, the synthesized microbeads depicted substantial biological activity and antioxidant activity that helped to enhance the daily dosage of antioxidants when supplemented

in a food product. The oxidation of bioactive compounds in extrusion is prevented as the entry of oxygen is restricted. Hence shelf life of the food improves to 4-5 years from few months for un-encapsulated food. The table 2.5 given below tabulates and give comparison on encapsulation method, core, shell and their scientific findings.

Table 2.5: Encapsulation of different active materials with different shell and encapsulation approaches

Encapsulation method	Core	Shell	Scientific findings	Reference
Coacervation	Sunflower oil, orange oil, lemon oil	WPI /GA	At pH 4.0 controlled-release occurs	(De Kruif et al. 2004)
Compound coacervation	Camphor oil with added polystyrene	Gelatin blended with GA	Encapsulation efficiency of 99.6 wt. %. The release rate depends on the quantity of PS	(Chang et al. 2006)
Emulsification and spray drying	3-methyl-butyraldehyde	WPC, sodium octenyl, MD, GA and GA	Investigated the effect of solid content on flavour retention and intensity. GA allowed exhibited highest (45 %) solid loading	(Bruckner et al. 2007)
Spray drying	Cumin oleoresin	GA, MD, and MS	GA proved to be superior for the core protection in comparison with MD and MS	(Kanakdande et al. 2007)
Complex coacervation	Sunflower oil	Gelatin and GA mixture	Particle size of capsules depends on initial oil size and it is a function of emulsification step chosen	(Lemetter et al. 2009)
Ultrasound	lysozyme	tetradecane, dodecane, perfluorohexane	Highly stable of perfluorohexane microspheres, encapsulated fluroscent dye gives controlled release	(Zhou et al. 2010)
Electrostatic extrusion	raspberry leaf	alginate–chitosan	Microbeads supply necessary biological activity and antioxidant	(Belscak-Cvitanovic et al. 2011)
HPH	Curcumin, resveratol	Soy lecithin, sugar ester, MS	Improvement in degradation and food stability	(Donsì et al. 2011)

Spray drying	Colistin sodium methane sulfonate	GA and MD	Microcapsules prepared using spray drying protects antimicrobial activity and also fulfils uniform particle size critrerion	(Lolodi et al. 2011)
Molecular inclusion	Rosmarinic acid	β-CD	Antioxidant activity of Rosmarinic acid improved slightly upon molecular inclusion with CDs	(Celik et al. 2011)
Molecular inclusion	Curcumin	CD	Poorly water-soluble compounds like curcumin can be delivered. Bioactive compounds were preserved within the aqueous compartment	(Rahman et al. 2012)
Freeze drying	Red wine Polyphenols	MD	Polyphenols were preserved to great extent found; Capsules with MD shell yielded a free-flowing powder	(Sanchez et al. 2013)
Extrusion	Ascorbic acid	MD	Ascorbic acid content in encapsulates increased to 18.59 g per 100 g extrudates (yield = above 96 %)	(Chang et al. 2010)
Fluidized bed coating	Spray-dried β-carotene	Hydroxypropyl cellulose	Coated powders showed colour stability of 4 weeks, when loaded in yogurt	(Coronel-Aguilera et al. 2015)
Spray drying	Menhaden oil (Fish-oil)	MD fish, κ-carrageenan gelatine	MD has positive effect on encapsulation efficiency and controlled release	(Mehrad et al. 2015)
Freeze-drying and spray-drying	Spent coffee grounds extract	MD, GA and their blend	62 % phenolic compounds were preserved in encapsulated product	(Ballesteros et al. 2017)
Complex coacervation	Ginger essential oil	WPI/ GA, CH	The thermo-gravimetric analysis demonstrated that shell materials have significant contribution to increase thermal stability	(Tavares et al. 2020)

2.3 Controlled release of encapsulated bioactive compounds

Controlled release is very attracting phenomenon concerning encapsulation. This section gives practical examples of controlled release and different factors affecting the controlled release are illustrated.

2.3.1 Factors affecting retention of active compound and its controlled release

The rate of release of active component out of the core-shell structure is function of many factors including- physicochemical properties of core and shell material used in preservation, method of encapsulation, physical properties of the encapsulate formed like size, molecular weight, pH sensitivity and dissolution ability of encapsulate in the surrounding solution. Two other factors of consideration in the controlled release are-thermodynamic factor (volatility of the active compound) and kinetic factor (resistance to mass transfer from core to emulsion and from emulsion to surrounding environment). The nature of active ingredient also has high impact in deciding the controlled release rate (Dadsetan et al. 2013; Naknean and Meenune 2010).

Molecular weight: In practise, high molecular weight compounds have hight retention ability as compared to low molecular weight compound. This behaviour was discovered by Rosenberg et al. 1990 and Goubet et al. 1998 for esters spray dried with GA. They observed that ethyl hexanoate (MW = 144) was retained to efficiently than ethyl butyrate (MW = 116). Moreover, when these compounds are stored at different relative humidities (range from 11 to 97%), ethyl hexanoate have encapsulation efficiency than ethyl butyrate. This occurs because, with increase in molecular weight its molecular size increases. Thus, the diffusion of active compound to the surface is retarded giving high retention of bioactive compounds.

Chain length: Chain length of bioactive compounds is another characteristic that influence on retention and release of flavour compounds. The retention of bioactive compounds is also a affected by the chain length of molecules. Long chain length molecules have high retention as compared to short chain molecules. This phenomenon was explained on the basis of 'Thijssen selective diffusion' theory which states that moisture continuous to evaporate (although at slower rate), whereas evaporation of longer molecular size volatiles is diffusion limited (Bhandari et al., 2001).

Chemical groups: Alcohols are best retained compounds by carbohydrates shell (Goubet et al., 1998). In particular, retention rate of benzylic alcohol varied between 45 and 83%, depending on the different carbohydrate used, however this retention rate was much higher as compared to the four esters (ethyl propionate, ethyl butyrate, isoamyl butyrate, and n-butyl pentanoate), for which the retention was between 0.5 and 49.5%, and the retention ability of acids (butyric,

caproic, and lactic acids), was found to be lowest (< 7%) as compared to carbohydrates and esters.

Log P or Hydrophobicity of flavour compounds: Log P or logarithm of octanol water coefficient is a measure of the relative hydrophobicity (positive value)/ hydrophilicity (negative value) of compound. The hydrophobicity of aroma compound is another factor that affecting retention and release of flavour compounds. In practice, the retention of polar (hydrophilic) compounds is very low than non-polar compounds. This is because, the greater solubility of polar compounds in water gives higher diffusivity through the matrix than with nonpolar compounds (Bhandari et al., 2001).

2.3.2 Experimental investigations on controlled release

Two approaches commonly used to observe the release of the encapsulated core are: in-vitro and in-vivo analysis. During in vitro analysis, gastric juice is simulated in the laboratory and controlled release is observed. This approach was adopted in an investigation (Carpenter et al., 2019); curcumin was encapsulated through the double layer emulsion formation using ultrasonication. The emulsion was stable; therefore, release of curcumin was restricted because of the strong impedance of the protein sequence against pepsin (gastric enzyme) through the gastric medium. This observation helped them to confirm the stability of the emulsion system before intestinal digestion. Therefore, the study was extended to observe release under simulated intestinal conditions where lipid and the emulsifying agent got digested, resulting in the release of curcumin.

Boland et al. (2004) demonstrated the release of eleven flavours encapsulated in different shells such as gelatine, pectin gels and starch. They found that the texture of the gels has a significant effect on flavour release. The study concluded that gelatine gel exhibits a considerable increase in flavour release in the saliva medium. In contrast, starch and pectin gels exhibited a decrease in flavour release in a similar environment within the food matrix.

Dong et al. (2011) studied the release rate of peppermint flavour encapsulated in a mixture of gelatine-GA. Complex coacervation method was used for encapsulation with trans-glutaminase as a hardening agent. The release rate of flavour was rapid when exposed to hot water conditions, which further dropped down. Also, the core-shell ratio has a significant influence on the release rate.

Roos et al. (2003) explained that the air phase at equilibrium (thermodynamic parameter) and the resistance to mass transport from product to air (kinetic parameter) controls the rate of flavour release. However, the volatility is mainly affected by the texture of flavour and its contribution in release becomes significant under non-equilibrium conditions. Whereas in a

strong mass transfer governed condition, the diffusion process controls the release rate of encapsulated core compounds. It is well-known that diffusion rates of volatile flavour compounds are invariant for liquid and semi-solid; hence, the release rates are nearly equal under mass transfer-controlled conditions.

2.4 Ultrasonic atomization approach for emulsification

Emulsions are important part of many sectors not limited to paint, cosmetic, home care products, paint and food. Many researchers have attempted to find the best approach to obtain stable emulsion (Mcclements 2007). High energy emulsification approaches like ultrasound, high-pressure homogenization is always preferred over low energy approaches like phase inversion for emulsification. Many scientific reports support the fact that with high energy approach it is possible to obtain an emulsion that is stable for a long duration. However, the energy consumption in such techniques is high and this issue needs to be addressed. In this regard ultrasound atomization is emerging as an energy-efficient ultrasonic atomization approach for emulsification.

Avvaru et al. (2006) carried out ultrasonic atomization of different Newtonian and non-Newtonian liquids with different viscosities. And explained the atomization phenomenon based on cavitation hypothesis. Their study reported that, the average droplet size for the pseudo-plastic liquid was smaller in comparison with viscous Newtonian liquid. The study also observed that droplet size distribution is wide for highly viscous fluids. A correlation was proposed based on the experimental data to predict the droplet size. Rajan and Pandit 2001 also developed a correlation to predict droplet size for the liquid processed with ultrasonic atomisation. Their investigation demonstrated that with ultrasonic atomization spherical droplets with small size are produced in comparison with conventional atomization techniques and developed a method to measure the droplet size by capturing the ejected droplets on a filter paper and carrying out its image analysis which is further adopted by several researchers. The correlation proposed by them is robust considering all the process parameters and overcoming the previous correlations. Ramisetty et al. (2013) performed the ultrasonic atomization of ten different liquids such as water glycerol, methanol, CMC and their various concentrations. The effect of various parameters like viscosity of liquid, surfactant concentration, power dissipation during atomization, frequency of atomization, flowrate to atomizer on droplet size and its distribution were evaluated. The velocity of the droplets disintegrated from the liquid sheet was measured using the motion of droplets as streaks (figure 2.3). The varying pattern of flow of atomization for different power of atomization is shown in figure 2.4.

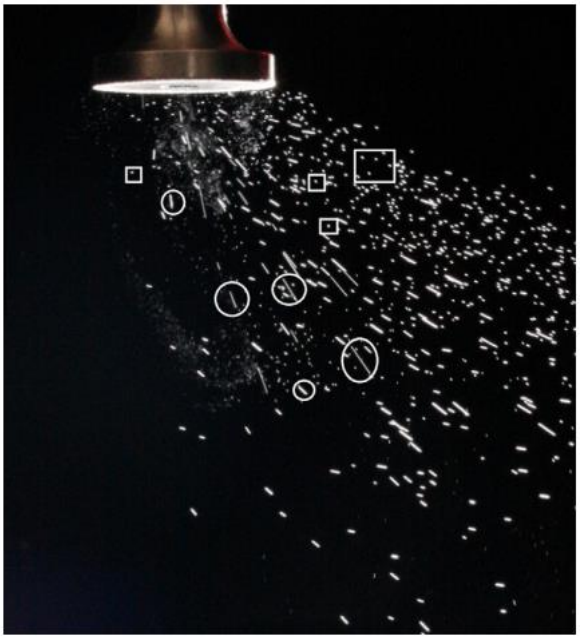

Figure 2.3: Droplets ejection at high velocity streaks caused due to the cavitation effect. Streaks are depicted in circles and droplets are depicted in squares Ramisetty et al. (2013).

Figure 2.4: Variation in the flow pattern of atomization with change in power of atomization (a) 3 W and (b) 9 W Ramisetty et al. (2013).

The study performed to observe the effect of atomizer frequency on particle size illustrated that increase in atomizer frequency is beneficial for reducing the droplet size and making the narrow distribution. As the viscosity of fluid being atomized increases, droplet size decreases due to obstacle in atomization and forming the thin liquid sheet on atomizing surface.

The first equation to predict the mean diameter of the droplets emerged from atomizer tip due to the formation of unsteady capillary waves was proposed by Lang in the year 1962. Various fluids and their properties considered in proposing equation are water, oil, and molten waxes at forced vibration frequencies of 10–800 kHz.

$$d_{p=}0.34\left(\frac{8\pi\sigma}{\rho f^{2}}\right) \qquad (2.4)$$

Where, d_p is the mean diameter of particles which is correlated with the liquid properties of density (ρ) and surface tension (σ).

Following the investigation by Lang, Peskin and Raco (1963) explored a new correlation considering other addition factors such as amplitude of forced vibration and liquid layer thickness present on the vibrating surface. The equation proposed by them is as follow:

$$\frac{d_p}{\pi A_m} = \left[\left(\frac{2\sigma}{\rho\omega_0^2 A_m^3}\right) 2\tan h\left(\frac{\pi A_m}{d_p}\right)\left(\frac{t}{A_m}\right)\right]^{\frac{1}{3}} \quad (2.5)$$

Further research using ultrasonic atomization for two fluid atomization by two groups namely Sindayihebura et al. (1997) and Dobre and Bolle (1999) led to development of new correlations. The factors considered for two fluid atomization are buoyancy force, drag force, gravity force, internal and external viscous forces and interfacial force. Considering all the parameters reported earlier, Rajan and Pandit developed a robust correlation using dimensionless numbers.

$$dp = \left(\frac{\pi\sigma}{\rho f^2}\right)^{\frac{1}{3}} [1 + (N_{we})^{0.22} (N_{0h})^{0.166} (N_{In})^{-0.0277}] \quad (2.6)$$

Where,

N_{we} = Weber number = $\frac{\text{Aerodynamic forces}}{\text{Surface tension forces}} = \frac{fQA}{\sigma}$ (2.7)

N_{oh} = Ohnesorge number = $\frac{\text{Internal liquid viscosity}}{\text{Surface force} * \text{vibrational amplitude}} = \frac{\eta}{f\rho\ A_m^2}$ (2.8)

N_{In} = Intensity number = f (ultrasonic intensity) = $\frac{f^2 A_m^4}{cQ}$ (2.9)

Similar study was performed by Barreras et al. (2002) conducted experiments with transient high-frequency ultrasonic atomizer for water. They investigated the characteristics of ultrasonic water atomization when stimulated with waves in the MHz ranges. They observed that the remaining liquid mass accumulated over the ultrasonic transducer curtailed, the atomization properties changed, and a second peak of larger droplets arose in the size distribution curve. Thus, from literature it is clear that there is high thrust for research to exploit ultrasonic atomization as energy efficient approach for emulsification. Burton et al. (2014), for the first time considered ultrasonic atomization approach for the preparation of vegetable oil in water emulsion instead of sonication. They proposed two possible mechanisms for emulsification through ultrasonic atomization as depicted in figure 2.5 below.

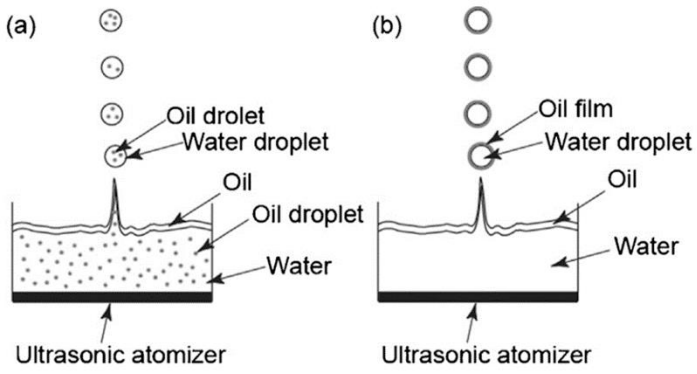

Figure 2.5: Possible mechanisms for emulsification through ultrasonic atomization.

In the first mechanism it is proposed that emulsified oil-in-water solution is atomized into droplet, whereas second proposal describes that very thin oil film coats each atomized droplet. The prepared emulsion at different oil concentrations were used as cutting fluids in micro-milling operations.

From the comprehensive literature review following observations and gaps in the literature are outlined as below.

2.5 Observations and Gaps identified from the literature

- Very few studies reported ultrasound assisted extraction and encapsulation of ginger oil with detail study of effect of process parameters.
- The potential of ANN and Taguchi approaches for food processes is not well understood for process optimization.
- Very few investigations have reported the release study of encapsulated bioactive compound.
- Application of ultrasound atomization for emulsification in food sector is not explored.

2.6 Objectives of present research work

In view of the aforementioned aspects, the objectives of present research work are outlined as follows:

- Ultrasound assisted intensified extraction of ginger oil from *Zingiber Officinale Roscoe* herb.
- An integrated Taguchi and ANN approach for the ultrasonic encapsulation of ginger oil in gum Arabic: Process optimization.
- ANN based modelling of peppermint flavour encapsulation process with ultrasound approach.
- Encapsulation and release studies of encapsulated ginger oil.
- Novel ultrasonic atomization approach for the synthesis of sunflower oil in water emulsion.

2.7 Summary

The exhaustive literature review on different extraction and encapsulation process parameters considered in food processing is presented. The review has reported the core and shell materials used in encapsulation. The properties of core and shell material, the effect of different combination of shell material in different ratios on encapsulation efficiency was observed from the literature. The health beneficial properties of extracted and encapsulated bioactive compounds are reported. The objectives of the present research work based on the research gaps identified are outlined.

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Chapter 3

Ultrasound-assisted intensified extraction of ginger oil from *Zingiber Officinale Roscoe* herb

3.1. Introduction

From the decades bioactive compounds are being used to cure and get relief from chronic diseases using several plant-derived bioactive compounds. Studies have highlighted the importance of minor bioactive compounds in plant-origin foodstuffs that exert antioxidant and/or PAF inhibitory activities in the prevention of cardiovascular diseases. Out of several bioactive compounds of health benefit, ginger is being used since dawn of medicine and food processing. The food preservative action of ginger is due to its antioxidant property which inhibits the oxidation of fats, oils and fat containing food products giving longer life to the food. Ginger also has distinctive characteristic flavour and can also be used as flavour enhancer in the various food matrices. Ginger has food preservative properties and it has also several beneficial effects on human health. For instance, it can be used as a natural drug molecule to prevent various diseases such as cancer, fevers, colds, upset stomachs, nausea, menstrual complaints, and rheumatism respiratory discomforts and so on. Ginger is also used as an anti-microbial in food processing to prevent the growth of harmful bacteria. Hence the use of ginger is favoured in all the ages of time.

This chapter addresses the ultrasound-assisted extraction of ginger oil from *Zingiber Officinale Roscoe herb*. The effect of various extraction parameters such as solvents, sonication time, feed to solvent ratio, applied sonication voltage on extraction of ginger oil was investigated. The quality of the extract was assessed based on extraction yield and UV spectroscopy analysis.

3.2. Materials and methods

Ginger root available in the local area of Warangal, India was used for the study. Ultrasound probe reactor procured from Dakshin Ultrasound Mumbai (20 kHz frequency)was used for the extraction of ginger essential oil from *Zingiber Officinale Roscoe herb*. The diameter of selected probe of ultrasound reactor chosen for the present study is 19 mm. Different polar and non-polar solvents in particular methanol, n-hexane, water and toluene were purchased from Merck.

3.3. Extraction of ginger oil with ultrasound approach

Process of extraction

Figure 3.1 depicts the process and mechanism of extraction. For an extraction of ginger oil, ginger pieces were cleaned and washed to remove the soil attached to them. The dried ginger pieces were crushed without peel off because peeled ginger has significant loss of active ingredients and essential oil content (He et al. 1998), followed by drying in the sunlight. The dried ginger powder was again crushed to make a powder, convenient for an extraction. The extraction of *Zingiber Officinale* was performed with various extracting solvents namely, methanol, n-hexane, water and toluene. To begin the extraction process, dry ginger powder was mixed with different solvents separately and solutions were sonicated in the ultrasound probe sonicator (Dakshin Ultrasound Mumbai operated at 20 KHz frequency, and probe tip diameter =19 mm). The ultrasound was used in pulse mode i.e. the sonication waves were passed through solution for two secs and will remain off for consequent one sec. The different extracts were obtained with the variation of sonication time, sonication power and feed to solvent ratio. Following this the samples were centrifuged at speed of 8000 rpm, for 10 minutes. The supernatant and bio-mass were separated. After extraction the supernant was concentrated in a rotary evaporator at 50 º C.

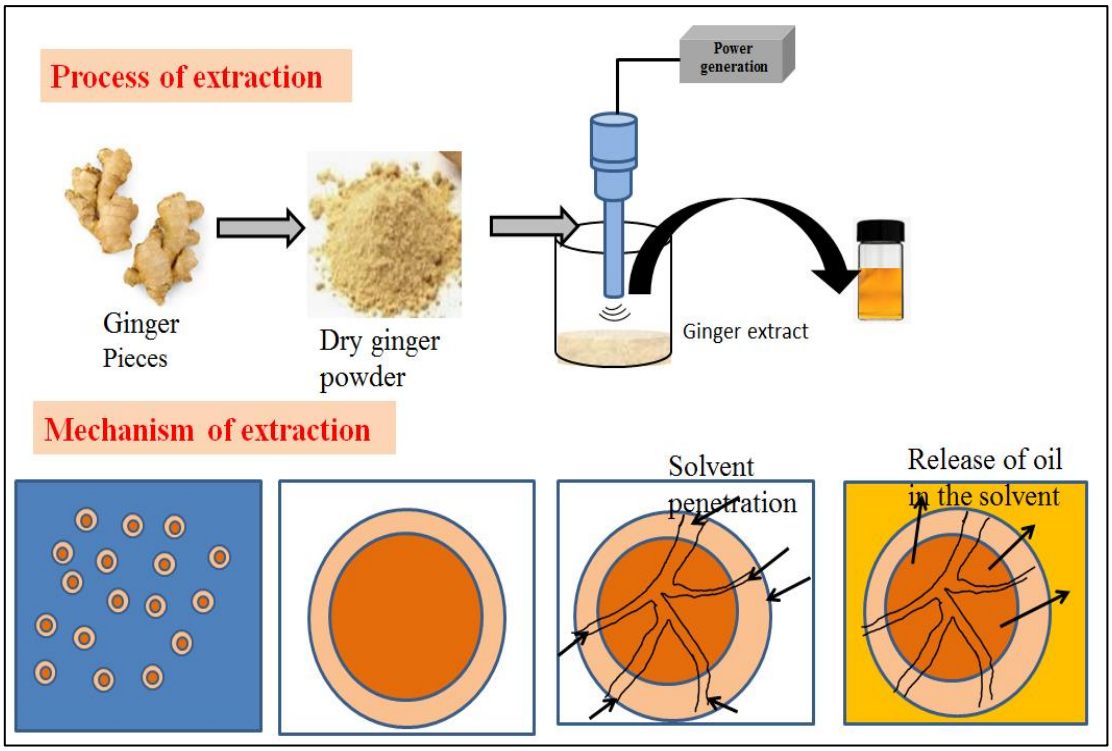


Figure 3.1: Flowchart for process and mechanism for extraction of ginger oil from *Zingiber Officinale* root with ultrasound approach.

Mechanism of extraction

Ultrasonic waves passing through the medium leaves a number of physical and chemical effects. Microstreaming, turbulence, biological cell rupture are the physical effects and free radical formation is a chemical effect (Tiwari 2015). These physical and chemical effects are attributed to cavitation phenomenon (Kentish and Ashokkumar, 2011). During the extraction with acoustic cavitation, cavities attacks on the walls of bioactive compound causing the reduction in particle size (Vilkhu et al. 2008). Also rupture of bioactive compound' s cell wall take place due to mechanical shear. The reduced particle size and ruptured cells enhances the mass transfer (contact between extracting solvent and ginger powder). This brings the solubilisation of oil from ginger powder to solvent. Extraction with sonochemical approach occurs at low temperature so the active ingredients are preserved against deterioration (Wen et al. 2018).

3.4. Characterization

3.4.1. Estimation of extraction yield

To estimate the extraction yield, different extracts obtained were centrifuged to separate the solvent and biomass residue. Further, these supernants were concentrated using rotary evaporator and weight of the oil was measured. The extraction yield was estimated by the formula given below.

$$Extraction\ yield = \frac{g\ of\ ginger\ oil\ obtained}{100\ g\ of\ dry\ ginger\ powder\ used\ in\ extraction} \quad (1)$$

3.4.2. Fourier transform infrared analysis

FTIR spectra was obtained on equipment (Shimazu 8400S, 177 Tokyo, Japan). The spectrometer was equipped with a germanium attenuated total reflection (ATP) accessory. The samples were prepared by mixing 10 µL of each sample with 100 mg of KBr powder. These mixtures were converted to tablets using the KBr compressor. The spectra of the prepared tablets were recorded at 500-4000 cm^{-1} in 64 scans at a resolution of 4 cm^{-1}.

3.4.3. UV-visible spectroscopy analysis

UV-visible spectra of the extracts were obtained by "UV-Vis Carry 7000" spectrophotometer within a wavelength range of 250 nm – 350 nm. To capture the UV-spectra, 2.5 mL of respective solvent was added in a UV quartz cuvette followed by the addition of 200 µL of the obtained extract and UV absorbance spectra were recorded.

3.5. Results and Discussion

3.5.1. Effect of different solvents on the extraction yield

The extracting solvents plays important role in an extraction; hence, it is necessary to choose the solvent carefully. The goal in the selection of solvent is to extract maximum active ingredients with the usage of minimum quantity of solvent (Kamarudin et al. 2016). The solvent removal process after extraction should be easy (Azwanida 2015). With this aim extraction of *Zingiber Officinale* was performed with various extracting solvents namely methanol, water, hexane, and toluene. Initially extraction was carried out with dry and wet ginger to check their efficacy in extraction. Figure 3.2 shows the dry and wet ginger before and after extraction respectively.

Figure 3.2: Dry and wet ginger before and after extraction

From the quality of extract obtained with wet and dry ginger, it was confirmed that dry ginger powder when used in extraction yields better as compared to wet ginger. Hence, it was decided to use dry ginger for further studies. Further to determine the best solvent, extraction was performed using four solvents namely methanol, hexane, toluene and water. Physicochemical properties of different solvents such as polarity index, vapour pressure, viscosity of solvent, and surface tension of the chosen solvents are recorded in table 3.1 below.

Table 3.1: Physicochemical properties of different solvents used in extraction.

Solvents	Polarity index	Vapour pressure (hPa)	Viscosity of solvent (cP)	Surface tension (nM/m)	Boiling Point (ºC)
Methanol	5.1	128	0.543	22.7	64.7
Water	10.2	17.535	0.890	72	100
Toluene	2.4	29	0.55	27.9	110.6
Hexane	0.1	160	0.297	18.43	69

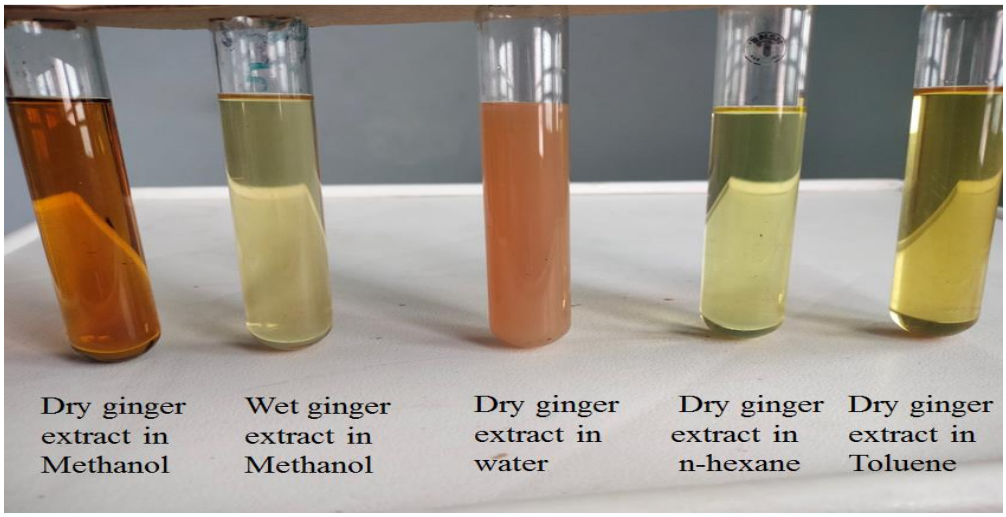
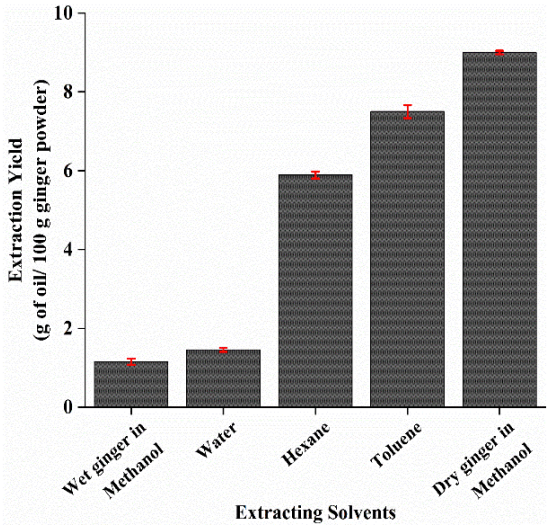


Figure 3.3: Pictorial representation of quality of ginger extract obtained with different solvents

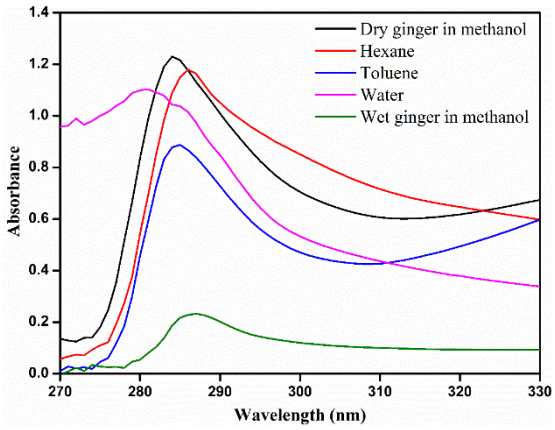
Figure 3.3. shows the pictorial representation of extracts obtained with different solvent. It is clear from the images that methanol is the best extracting solvent for extraction. This was further confirmed quantitatively by estimating an extraction yield. Highest extraction yield of 9 g of oil/ 100 g ginger powder was obtained with methanol solvent followed by toluene and hexane of 7.5 and 5.89 g of oil/ 100 g ginger powder respectively (table 3.2). Extraction of lower molecular weight compounds (polyphenols) is usually performed by employing methanol (figure 3.4 (a)). The extraction yield of water and wet ginger in methanol found to be very less. Different extraction yield with different solvent is due to the polarity and viscosity difference of different solvent. Higher the polarity, higher the extraction yield of antioxidant compounds (Fernández-Ponce et al. 2012; Wijngaard et al. 2012). Thus, with methanol highest extraction yield is obtained. Beside highly polar nature, the lower extraction yield with water as a solvent, might be due to higher viscosity of water in comparison with methanol which causes the difficulty in penetration of solvent in the ginger powder as compared to solvents with less viscosity. The maximum UV-absorbance for all the extracts was obtained shown between 280-292 nm for all solvents except water (figure 3.4 (b)). The absorbance with water solvent was obtained at 280 nm. It is clear from the literature that UV absorbance in the range of 282 – 287 nm wavelength corresponds to extraction of 6-gingerol, 6-shogaol, 8-gingerol, 10-gingerol (figure 3.4 (c)) (Liu et al. 2014).

Table 3.2: Effect of different solvents on extraction yield

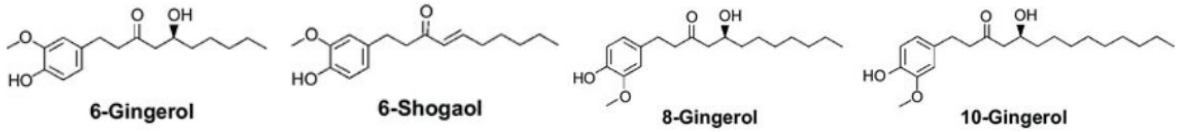
Solvent used	Extraction yield (g of oil/100 g of ginger powder)
Wet ginger in methanol	1.21
Water	1.32
Hexane	5.89
Toluene	7.5
Dry ginger in methanol	9.0



(a)



(b)



(c)

Figure 3.4: Effect of extracting solvent (a) extraction yield (b) UV absorbance (c) Molecular structure of different compounds extracted

3.5.2. Effect of sonication time on extraction yield

To observe the influence of irradiation time towards the extraction yield, initially extraction of ginger powder was carried out for different sonication times such as 10 min, 20 min, 30 min and 40 min. With the increase in sonication time of 10 min to 30 min extraction yield increased from 1.64 g of oil/100 g to 7.5 g of oil/100 g (table 3.3). However, further increase in sonication time up to 40 min has minimal contribution in increasing the extraction yield. This indicated that 30 min of sonication time is optimum to increase the extraction yield. The increase in sonication time increases the number of cavitation cycle consisting of compression and

rarefaction. The increased number of cycles leads to intense collision between the ginger powder and ultrasonic waves that results in the fracture of the cell wall of the ginger powder, and decreases in particle size (Wang and Yuan 2016). The smaller particle size is beneficial to improve the solvent penetration in the compound to be extracted (Özcan et al. 2021). However, after threshold limits, the maximum absorption capacity of ginger powder might have reached, showing minimal increase in extraction yield. The shift in UV absorbance peak towards higher wavelength at 40 min sonication time might be due to liberation and degradation of active compounds ((figure 3.5 (b)).

Table 3.3: Effect of sonication time on extraction yield

Sonication time (min)	Extraction yield (g of oil/100 g of ginger powder)
10	2.64
20	5.45
30	9.0
40	9.15

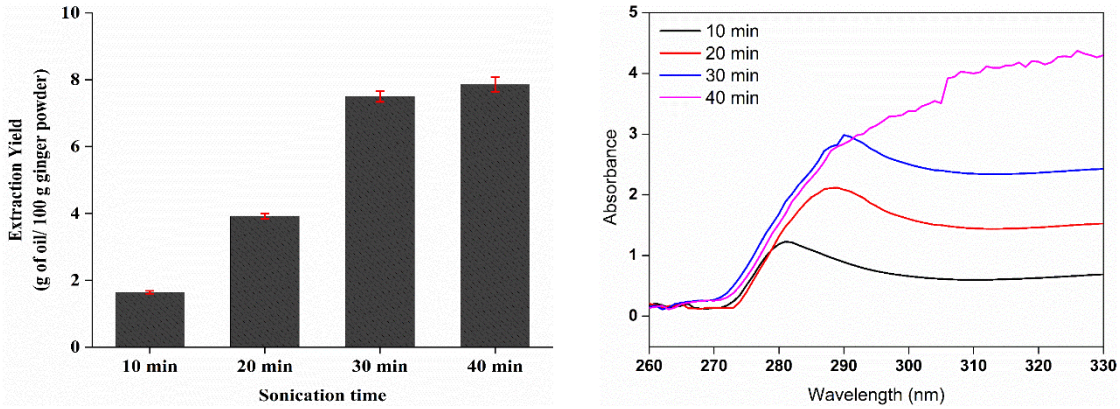


Figure 3.5: Effect of sonication time (a) extraction yield (b) UV absorbance

3.5.3. Effect of ginger powder to solvent ratio on extraction yield

Solvent is major component in extraction. In general, higher the solvent available for extraction higher will be the extraction yield due to increased mass transfer (Jadhav et al. 2022). However, usage of higher solid to extracting solvent ratios typically would induce dilute solutions. Therefore, it is important to optimize the solid (ginger powder)-solvent ratio during extraction of solute from any given source. Optimization of ginger powder to solvent ratio will make sure the efficient utilisation of solvent extraction process. The advantage of ultrasound in extraction is due to green approach and less requirement of solvent. To observe the effect of ratio of solid

to solvent, it was varied as 1:2, 1:3, 1:5, 1:10 and 1:20. It was observed that the release of the oil from the ginger powder increases with an increase in the seed to solvent ratio. The extraction yield increases from 2.33 to 9.67 g of oil/ 100 g ginger powder with increase in ginger powder to solvent ratio from 1:2 to 1:10 ((figure 3.6 (a) (table 3.4). The solid-solvent ratio of 1:20 (w/v) has marginal contribution in increasing extraction yield as compared to 1:10 ratio. Hence, the solid-solvent ratio of 1:10 (w/v) was used for further experiments. Figure 3.6 below shows the extraction yield obtained at different ginger powder to solvent ratio and corresponding UV-absorbance spectra ((figure 3.6 (b). The increase in absorbance with increasing solvent can be attributed to increase in concentration.

Table 3.4: Effect of ginger powder to solvent ratio on extraction yield

Sonication time (min)	Extraction yield (g of oil/100 g of ginger powder)
10	2.64
20	5.45
30	9.0
40	9.15

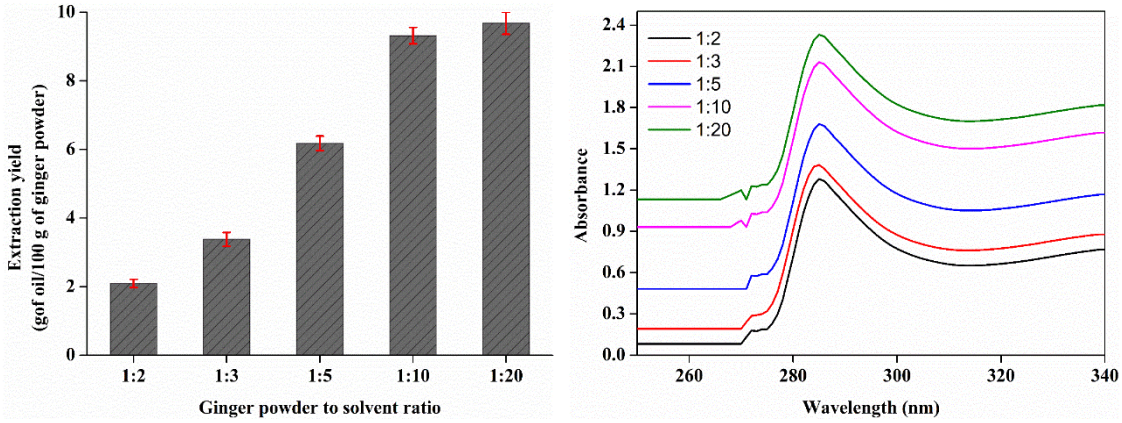


Figure 3.6: Effect of ginger powder to solvent ratio (a) extraction yield (b) UV absorbance

3.5.4. Effect of applied sonication power on extraction yield

Table 3.5: Energy consumption of the ultrasonic horn with variation of applied voltage.

Voltage (v)	Current (A)	Power (w)	Energy (J/mL)
100	0.268	26.8	47.96
130	0.412	53.56	96.13
160	0.556	88.96	159.85
200	0.748	149.6	269.00

The energy supplied and consumed during sonication was estimated as per the method reported by Jadhav et al. 2018. The power consumption of the ultrasonic horn was variable. Current cosnumed for corresponding variation of voltage applied was displayed on the control panel screen and thus power delivered to the horn was estimated by equation 3.1 below,

$$P = V \times I, \quad (3.1)$$

Where, V- Voltage supplied, I- current dissipiated by the horn

The conversion efficiency of energy from electrical to mechanical was calorimetrically estimated to be 15%. Energy per unit reactant volume (E) provided to the horn was estimated as by equation 3.2,

$$E = P \times 0.2 \times \frac{t}{v} \quad (3.2)$$

To investigate the efficacy of applied sonication power on the extraction yield, experiments were performed at four different applied voltages as 100 mV, 130 mV, 160 mV and 200 mV. The corresponding power consumption is shown in table above. The extraction yield corresponding to these applied voltages are 1.23, 2.48, 5.21 and 9.65 g of oil/ 100 g ginger powder. It was observed that at lower applied voltage rate of extraction is very low and the extraction yield increases with increase in sonication power. The intensity of compression and rarefaction cycles depends on the applied voltage. The bubbles formed during each cavitation cycle intensely collapse in short period of time. These shockwaves increase the local temperature and pressure. Thus, the created environment is highly suitable for breaking of the cell wall and making solvent penetration easy (Sivakumar et al. 2007). Another reason for higher extraction yield can be attributed to increased rate of cavity formation, growth and collapse at higher power supplied. Therefore, particle gets ruptured within a short period of time at higher applied voltage as compared to lower applied voltage (Zhang et al. 2008). The

UV absorbance increases significantly beyond 160 mv and 200 mV showing higher concentration of extracted oil in the solvent.

Table 3.6: Effect of applied sonication power on extraction yield

Applied sonication power (J/mL)	Extraction yield (g of oil/100 g of ginger powder)
47.96	1.23
96.13	2.48
159.85	5.21
269.00	9.65

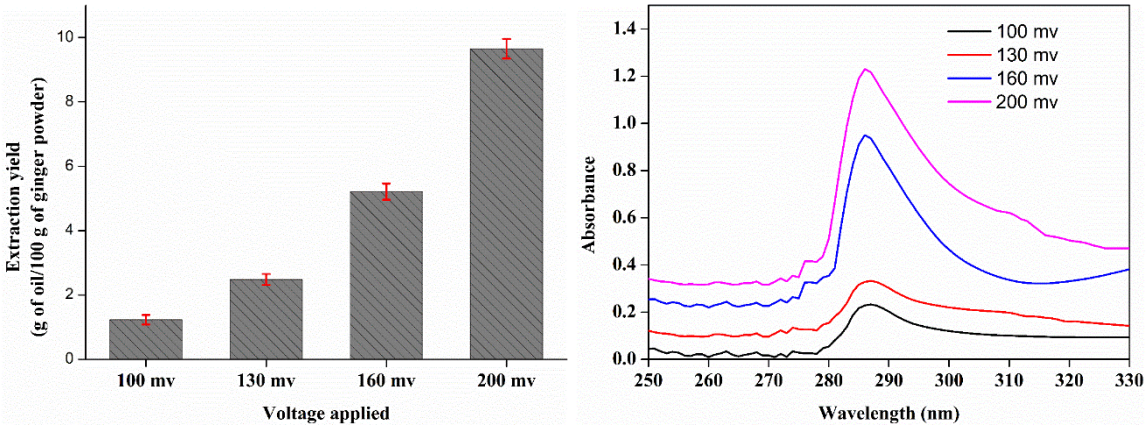


Figure 3.7: Effect of sonication voltage on (a) extraction yield (b) UV absorbance

3.5.5. Fourier transforms infrared spectroscopy (FTIR) analysis

The FTIR analysis was done out to observe weather ultrasonic waves passing through the solution leaves any adverse effect on the active inpatients of extracted ginger oil. For the FTIR analysis the samples (tablets) were prepared as per the procedure detailed in section 3.3. The spectra of the prepared tablets were recorded at 500-4000 cm^{-1} in 64 scans at a resolution of 4 cm^{-1}. The FTIR spectra of ginger extract obtained at different sonication, with 1:10 ginger powder to solvent ratio and methanol solvent are shown in figure 3.8 below. The trend of spectrums for 10 min, 20 min, 30 min and 40 min is almost similar. The transmittance peak obtained in the range of 3000 to 3600 cm^{-1} indicates the presence of aliphatic O-H groups. However, at 40 min of extraction time, the aliphatic O-H absorption was missing. This might be because of the dehydration of gingerol to shogaol as there is absence of aliphatic O-H group in shogaol structure. The peaks of CH_2 and CH_3 stretching were well distinct at 2940 cm^{-1} and 2832 cm^{-1}. The absence of peak in the 2000 to 2500 cm-1 indicated no triple bond region informing no triple bond in the extracted compounds. This observation is in agreement with

the molecular structures of gingerol and shogaol as shown in figure 3.2.(c). The peak in the rage of 1350-1269 cm^{-1} is attributed to primary OH in plane bond. The peak at 1030 cm^{-1} corresponds to C-O bond. These observations are in lined with the previously reported observation (Aris and Morad 2014; Karthickeyan 2018)

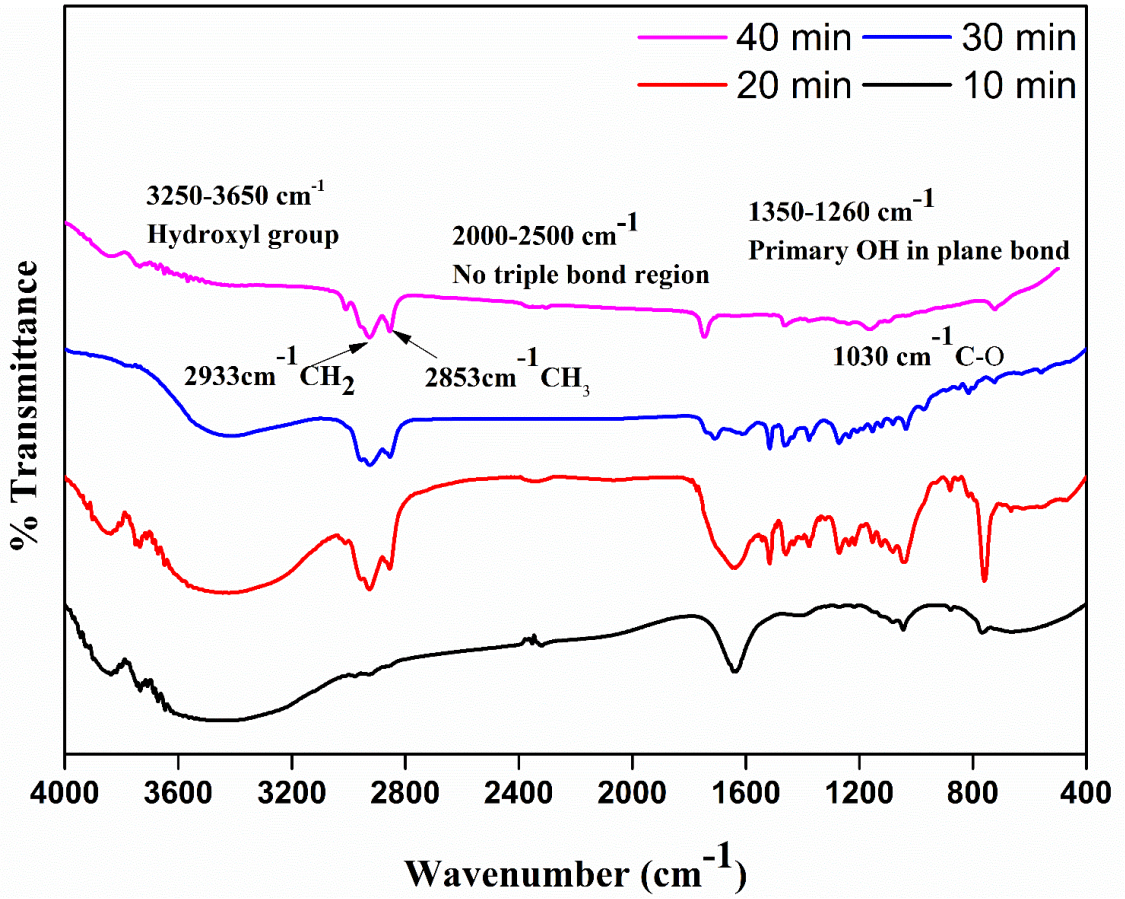


Figure 3.8: FTIR spectra of ginger extract at different sonication time

3.6. Summary

This study suggests ultrasound approach as a green and intensified approach for the extraction of ginger oil from the *Zingiber Officinale root.* The effect of various parameters on extraction yield was evaluated. Four process parameters namely extracting solvent, sonication time, applied voltage and ginger powder to solvent ratio are studied in the present study. The methanol was found to be the best extracting solvent due to high polarity and low viscosity, complete extraction occurred in 30 min, beyond which the increase in sonication time has very negligible effect on extraction yield. The increases in solvent helped to increase extraction yield, 1:10 was found to be the optimum feed to solvent ratio. As the applied sonication power increases, the rate of compression and rarefaction cycles increases which causes the rapid

formation growth and intense collapse of cavities in the solution. Thus, breakage of cell wall of ginger powder is facilitated making more solvent accessible for extraction. The changes in stretching and vibration with all parameters were observed with FTIR analysis. Foe all the extractions carried out, extraction of active ingredients namely 6-gingerol, 6-shogaol, 8-gingerol, 10-gingerol was confirmed with UV-spectroscopy.

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Chapter 4

An integrated Taguchi and ANN approach for the ultrasonic encapsulation of ginger oil in gum arabic: Process optimization

4.1. Introduction

The aim of encapsulation is to stabilize the active compound by forming a protective layer on the active compound. The encapsulation helps to preserve the physical, chemical and biological properties and gives a controlled release at desired site and time. Various parameters influence the encapsulation process. To evaluate the effect of all process parameters is a complex, time-consuming and costly process. ANN and Taguchi are increasingly being used analytical techniques to understand and predict the complex process of concern. The design of experiments (DOE) with Taguchi orthogonal array is a best choice to observe the effect of all parameters with a minimum number of experiments. Whereas, ANN is being popularly used technique to discover and find multivariate dependencies amongst the process parameters. Selection of the data for training is a crucial step in the ANN network development; moreover, a wide range of data needs to be trained. Hence in the present study, the data required for training the ANN is selected from the designed data set used in the Taguchi approach. This chapter explains the preservation method for extracted ginger oil with encapsulation approach. An integrated Taguchi and ANN approach to learn the effect of various process parameters on the process of an encapsulation and prediction of encapsulation process are investigated.

4.2. Experimental Section

4.2.1. Materials

The ginger oil and GA were purchased from Fisher Scientific Mumbai, India. Chemicals namely polysorbate 80 (mw = 6418) purchased from Oxford laboratory, Thane India. 99 % ethanol was procured from Merck Mumbai, India. All the emulsions were prepared using distilled water (DIW).

4.2.2. Encapsulation of ginger oil in GA

To preserve the bioactivity of ginger oil from the deterioration by surrounding, it was encapsulated using GA shell material with ultrasound approach. Ultrasound probe reactor supplied by Dakshin Ultrasound Mumbai with 20 kHz irradiation frequency (Maximum Power

250W) and probe with tip diameter of 19 mm was used throughout the experiments to prepare all the emulsions.

Initially, for the preparation of emulsions, GA was mixed with the DIW with mechanical agitation at 40 ºC. This GA solution was then subjected to sonication, to which ginger oil was added dropwise to form an emulsion. Different emulsions were prepared with the variation of ginger oil concentration (wt. %), GA concentration (wt. %) and sonication time (min). A jacketed reactor was used in all the emulsion preparations to prevent the deterioration of the components due to the thermal effects of power ultrasound. The prepared emulsions were dried using laboratory atomizer at different temperature and flowrate to atomizer. The atomized samples were collected on hot petri dish maintained at different temperature (130 ºC, 150 ºC and 170 ºC). The temperature of petri dish was maintained to desired temperature using hot plate. The atomized product was exposed to temperature conditions for less than 20 sec in order to avoid the degradation of active compound. Figure 4.1 illustrates the mechanism of ultrasonic encapsulation of ginger oil in the GA shell material. The details of process parameters used in encapsulation process are designed with Taguchi standard L^{27} orthogonal array are given in table 4.2.

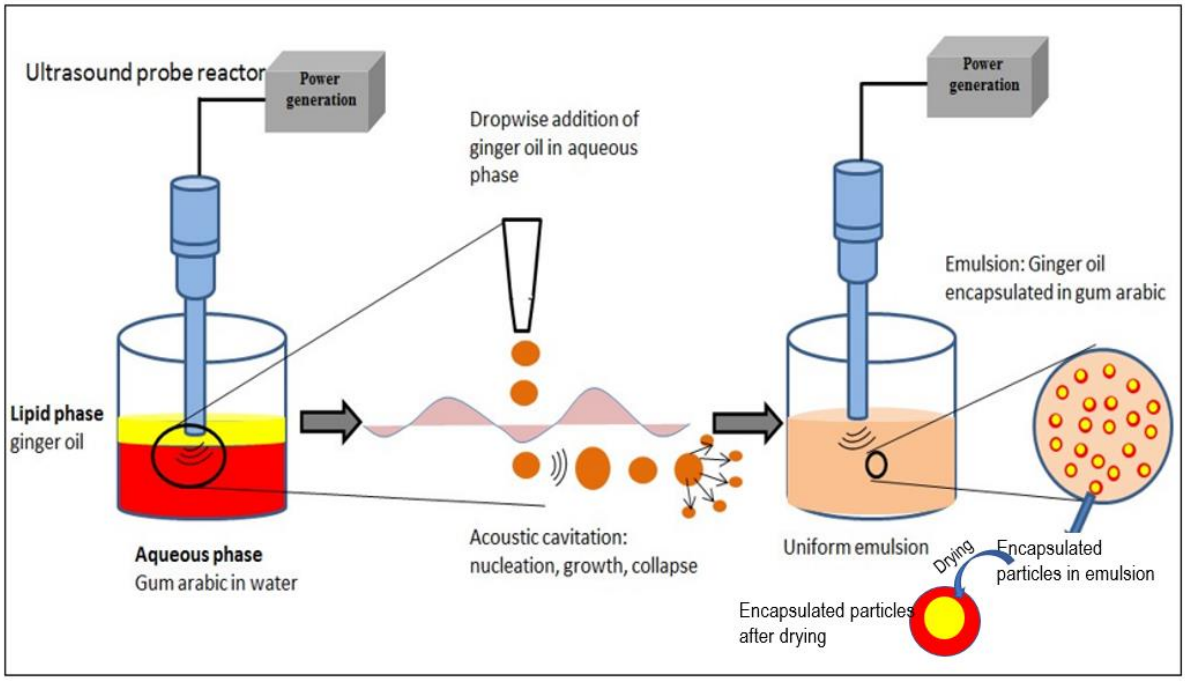


Figure 4.1: Mechanism of ultrasonic encapsulation of ginger oil in GA shell material.

Mechanism of encapsulation with ultrasound approach:

When ultrasonic waves pass through the medium gas nuclei in the solution grow in size this is termed as expansion cycle **or rarefaction cycle (low pressure)**. In expansion cycle vacuum is created and thus giving rise to the dissolved gases. Another half cycle known as compression **(high-pressure) cycle**, the bubbles shrink and their contents expelled into the host fluid. At any complete cycle (i.e. Expansion and compression) the amount of gas absorbed inside is always greater than the amount expelled. With this net amount of gas taken in the bubble bubbles grow to a critical size. The situation reaches when critical size frequency of bubble vibration becomes equals to the ultrasonic wave frequency at that instant the bubble collapse occurs. Thus, the phenomenon of formation growth and intense collapse gives rise to extreme environment (10,000 K temperature and 1,000 atm pressure) are formed (Bhanvase, et al. (2011); Bhanvase et al. (2012); Bhanvase and Sonawane (2014)).

Thus, the energy is supplied by ultrasound causes the dispersion of peppermint flavour (oil phase) in the form of smaller droplets in continuous phase that is, GA solution. The formation of droplets of dispersed phase in continuous phase is can also be attributed to shearing action due to intense environment created by ultrasonic irradiation, responsible to the disruption of the oil droplets to nanometer size in the liquid (Behrend and Schubert, 2000).

Many physical changes occur during acoustic cavitation that include micro streaming, turbulence, and so on are responsible for the generation of the emulsion. However, no chemical changes were observed with the direct exposure of peppermint oil to acoustic cavitation (confirmed from FTIR patterns) which shows the properties were found to be unaffected with acoustic cavitation.

The range of process parameters used in encapsulation process and used for design of experiments using Taguchi L^{27} orthogonal array are given in table 4.1 below.

Table 4.1: Taguchi approach for design of experiments: 5 factors 3 levels

Level for various control factor				
Control factor	Unit	Level 1	Level 2	Level 3
A: Ginger oil	Wt. %	1	4	7
B: GA	Wt. %	10	20	30
C: Sonication time	(min)	10	20	30
D: Drying temperature	(ºC)	130	150	170
E: Flowrate	(mL/min)	1	5	10

The details of experiments designed from above range of parameters are given in table 4.2.

Table 4.2: Experimental parameters in encapsulation process of ginger oil in GA: Taguchi L^{27} orthogonal array

L^{27} (2^{13})	Ginger Oil (wt. %)	GA wt. %	Sonication time (min)	Drying temp. (°C)	Flowrate to atomizer (mL/min)	Encapsulation Efficiency (%)	Product yield (%)	Particle size (nm)
1	1	10	10	130	1	48	50.7	220
2	1	10	10	130	5	43	39.9	227
3	1	10	10	130	10	38	32	239
4	1	20	20	150	1	65	53	185
5	1	20	20	150	5	63	48	192
6	1	20	20	150	10	60	42	203
7	1	30	30	170	1	70	56	186
8	1	30	30	170	5	65	53	194
9	1	30	30	170	10	63	50	200
10	4	10	20	170	1	76	62	196
11	4	10	20	170	5	73	55	205
12	4	10	20	170	10	69	49	213
13	4	20	30	130	1	61	60	201
14	4	20	30	130	5	57	57	205
15	4	20	30	130	10	54	50	210
16	4	30	10	150	1	58	60	245
17	4	30	10	150	5	54	54	237
18	4	30	10	150	10	51	50	218
19	7	10	30	150	1	80	59	213
20	7	10	30	150	5	75	55	205
21	7	10	30	150	10	70	46	198
22	7	20	10	170	1	82	81	235
23	7	20	10	170	5	77	72	242
24	7	20	10	170	10	66	65	252
25	7	20	20	130	1	84	67	240
26	7	30	20	130	5	80	65	253
27	7	30	20	130	10	75	61	258

4.3. Characterization

4.3.1. Estimation of encapsulation efficiency of ginger oil in GA shell

Encapsulation efficiency was assessed according to the procedure explained by Sangolkar et al. 2019 with a slight modification. Briefly, to determine the amount of oil loaded in the core shell structure, 0.5 g of dry emulsion powder was dissolved in 50 mL of 60:40, ethanol: water mixture. This mixture was stirred at 400 rpm for 1 min to remove the oil on the surface of powder which will disintegrate within a short time. To find out the amount of oil that has encapsulated, the core-shell structure must be broken. This was carried out by stirring the powder solution for 3 h at 45 ºC and subsequently cooled to room temperature. The samples stirred for 1 min and the samples stirred for 3 h, were centrifuged at 8000 rpm for 12 min separately. The UV absorbance of supernant of centrifuged samples was recorded at a wavelength of 282 nm. Encapsulation efficiency was evaluated using the formula 4.1 given below.

$$\%\ \text{Encapsulation efficiency} = \frac{\text{oil in the core} - \text{oil at surface}}{\text{Total oil added}} \times 100 \quad (4.1)$$

4.3.2. Estimation of product yield

The product yield was estimated using the weight of powder obtained and the total weight of GA and ginger oil used for emulsion preparation using formula 4.2 given below.

$$\%\ \text{Product yield} = \frac{\text{Dry powder obatined (g)}}{\text{Ginger oil added initially (g)} + \text{GA added initially (g)}} \times 100 \quad (4.2)$$

4.3.3. Particle size analysis

The particle size analysis (PSA) of encapsulated powder was carried out using Malvern Zetasizer (Nano S90 version 7.02). The laser diffraction technique is applied to evaluate the particle size distribution. It is based on the principle of angular variation in the intensity of light scattered when a beam of a laser passes across a dispersed solution of particles. Clearly, large particles have small angle scattering and small particles have wide-angle scattering. The scattering data is used to measure the particle size.

4.3.4. Fourier transform infrared radiation (FTIR) analysis

The components of emulsion are food-grade; hence it is very much essential to know the effect of exposure of sonication waves to emulsion components. Therefore, FTIR analysis was carried out to observe the stretching and vibrations in the ginger oil, GA and ginger oil- GA

encapsulation system. For the analysis, the spectrometer (Shimazu 8400S, 177 Tokyo, Japan) was equipped with a germanium attenuated total reflection (ATP) accessory. The samples were prepared by blending 10 mg of each sample with 100 mg of KBr powder. These mixtures were converted to tablets with the KBr compressor. The spectra were obtained at 500 cm^{-1} to 4000 cm^{-1} in 64 scans at a resolution of 4 cm^{-1}.

4.3.5. Transmission electron microscopy analysis (TEM) of core-shell system

The freshly prepared emulsion was used for transmission electron microscopy (TEM) analysis. The encapsulation of ginger oil in GA was confirmed by TEM analysis. FEI-TechnaiTE-20 and JEOL JEM-2100F field emission transmission electron microscope was used for the analysis. The sample for TEM was precisely prepared by putting a 5-7 µL prepared emulsion drops onto the copper grid and stained to observe the contrast in magnified images. The sample grid was dried in the air and images were captured at 50,000× magnifications. The instrument was operated at 200 kV.

4.4. Design of experiment (DOE): Taguchi approach

The conventional experimental plan to observe the effect of all parameters is a complex process and practically not feasible because it requires many experiments. Taguchi's approach reduces the number of experiments and hence the time and cost of experiments decrease significantly (Davis and John 2018; Antony 2006). During Taguchi analysis, the experimental outcomes are converted into a signal-to-noise (S/N) ratio using the equation 4.3 and 4.4. given below. Out of numerous types of S/N ratios, three types of S/N ratios commonly employed are smaller-the-better, higher-the-better and nominal-the-best (Moral et al. 2018; Sharma and Balan 2013). In the present work, the S/N ratio of higher-the-better is employed for evaluating encapsulation efficiency and product yield and the criteria of smaller-the-better is employed for particle size. S/N ratio and ANOVA signify the effect of process parameters on the encapsulation process.

$$S/N = -10 * \log(\Sigma(1/Y2)/n) \quad \text{——} \quad \text{Higher-the-better} \qquad (4.3)$$

$$S/N = -10 * \log(\Sigma(Y^2)/n)) \quad \text{——} \quad \text{Smaller-the-better} \qquad (4.4)$$

Where, y is experimental output and n is number of observations. All sets of experiments were performed as per the process conditions mentioned in table 4.2. In addition, the plots of mean response characteristic obtained from the Minitab-17 software and the % contribution of the

inputs was examined using ANOVA analysis. Following assumptions are made in Taguchi analysis.

Assumptions made in Taguchi analysis (Surendran et al. 2015)

- The additive assumption implies that the individual or main effects of the independent variables on performance parameter are separable.
- The results obtained are only relative and do not exactly indicate what parameter has the highest effect on the performance characteristic value.

4.5. Artificial neural network (ANN) development

ANN is the most popular modelling techniques, able to solve the complex processes that are difficult for human brain (Panerati et al. 2019). ANN is a technique that trains the existing experimental data most accurately and it seizes non-existing correlations (Ranade et al. 2021). The idea of data processing in ANN is originated with the intention to mimic the biological nervous systems. The procedure of ANN development training is shown in figure 4.2 below. In the network development, Levenberg-Marquardt ((LM) algorithm was used to develop and train the feed forward network because it has fast learning ability (Tarafdar et al. 2019, Socha and Blum 2007). All the networks were developed using the 'nntool' vizard in MATLAB. Each network was trained for three different combinations of transfer functions viz. Purelin-Purelin, Logsig-Purelin and Tansig-Purelin. The model of ANN has three subsets namely input, hidden and an output layer. During the operation, all the nodes of consequent layers are interconnected. To observe the effect of number of neurons on model performance, it was varied from 4 to 10. The experimental parameters (Ginger oil (wt. %), GA (wt. %), drying temperature (ºC) and flowrate (mL/min)) were provided to an input layer. The weights are applied on input data which are connected to adjacent hidden layer. The multiplication between input parameter and assigned weights is done. In the next step, the addition of the individual product data is performed which gives the value of neuron in the hidden layer. Similar operation is performed to obtain the value of output layer. The value of each output neuron is calculated based on the formula 4.5 given below.

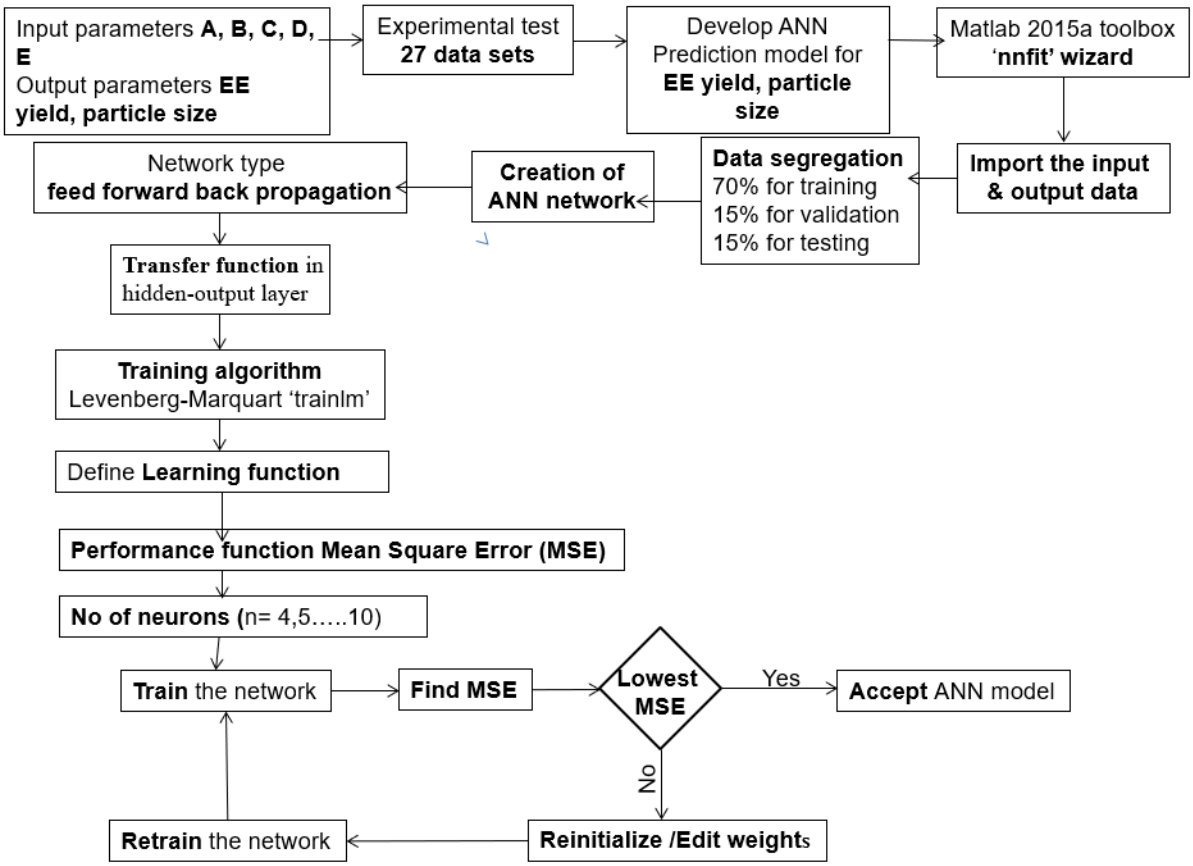


Figure 4.2: ANN network development and training procedure

In above figure A, B, C, D and E refers to five input parameters namely ginger oil concentration (wt. %), GA concentration (wt. %) sonication time (min), flowrate to atomizer (mL/min) and drying temperature (°C).

$$\text{Output} = (W_{01} * X_0) + (W_{11} * X_1) + (W_{21} * X_2) + (W_{31} * X_3) + (W_{41} * X_4) + \beta \quad (4.5)$$

The weights are reinitialized every time till the desired response is obtained from the output layer. The performance of the model is validated through regression coefficient (R^2) and mean square error (MSE). R^2 and MSE are estimated using equations 4.6 and 4.7 respectively.

$$R^2 = 1 - \frac{\sum_{I=1}^{N_{train}} (X_{Pred} - X_{Exp})^2}{\sum_{i=1}^{N_{train}} (X_m - X_{Exp})^2} \quad (4.6)$$

$$MSE = \frac{\sum_{i=1}^{N_{Test}} (X_{Pred} - X_{Exp})^2}{N_{Test}} \quad (4.7)$$

Assumptions in ANN

- Artificial neurons are arranged in layers, which are sequentially arranged. (Comparing with animal brain, this assumption might not hold true as in animal brain the neurons are not arranged sequentially. They are connected randomly in nature whereas in ANN these are arranged sequentially).
- Neurons within the same layer do not interact or communicate to each other.
- All inputs enter into the network through the input layer and passes through the output layer.
- All hidden layers at same level should have same activation function.
- Artificial neurons at consecutive layers are densely connected.
- Every inter-connected neural network has its own weight and bias associated with it.

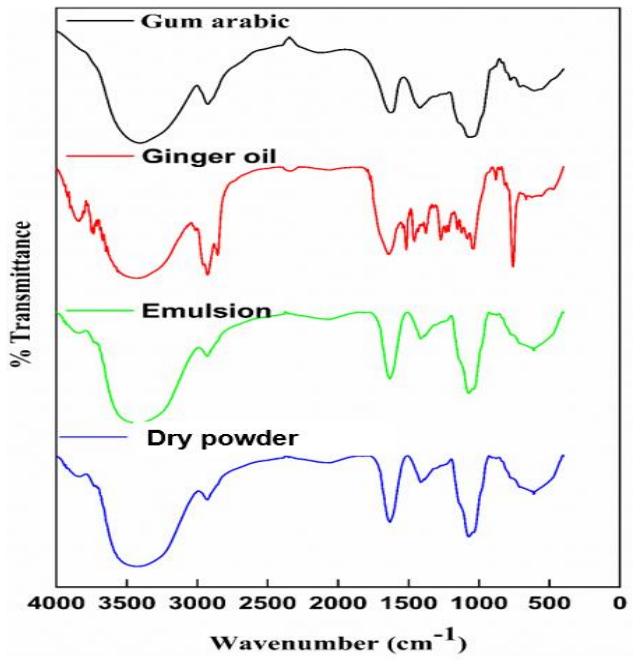
4.6. Results and discussion

For the encapsulation of ginger oil in GA, ultrasonic irradiations were employed. An intense environment is created due to compression and rarefaction cycles. In these cycles, cavity grows over a number of cycles and collapses at resonating frequency with ultrasonic wave frequency (Krasulya et al. 2016; Wu et al. 2013). This phenomenon causes uniform dispersion of lipid phase in an aqueous phase giving stable emulsion. FTIR analysis and TEM analysis of encapsulation system are explained in this section. Also, the effect of input parameters on encapsulation process is explained using Taguchi and ANN approaches.

4.6.1. FTIR analysis of emulsion and its constituent components

FTIR is a promising tool to characterize and identify the effect of direct exposure of sonication waves in emulsion solutions. It was observed that all the stretchings remained unaffected due to sonication treatments. FTIR spectra obtained for ginger oil, GA, ginger oil in GA emulsion and dry powder are shown in figure 4.3. The absorptions at 3433 cm^{-1} and 2935 cm^{-1} found in the ginger oil sample are due to the stretching of a hydroxyl group. The band at 1469 cm^{-1} and 1180 cm^{-1} are owing to aromatic ring stretch and dialkyl ether stretch respectively. The symmetric stretching at 1657 cm^{-1} and 1452 cm^{-1} is due to CO_2 bond in GA. The position of peaks in emulsion and encapsulated spray dried powder is at nearly same wavelength. The absorption at 3827 cm^{-1} and 3418 cm^{-1} are due to the stretching of hydroxyl groups. The characteristics bands observed at 2927 cm^{-1} and 1415 cm^{-1} are attributable to C-H stretching and band at 1933 cm^{-1} arises from C-H bending. The weak band at 1633 cm^{-1} is due to C=C

stretching. The bending and stretching characteristic for GA, ginger oil agrees with the earlier studies (Jafari et al. 2019, Mahdavi et al. 2018, Thongson et al. 2004).



Wavenumber (cm^{-1})	Chemical groups
GA	
3423.32	O-H stretching, characteristic of glucoside ring
2925.14	C-H stretching
1621.86	COO- symmetric stretching
1431.54	COO- asymmetric stretching
1064	Finger print of carbohydrates
(Ginger oil)	
3250-3650	aliphatic O-H groups
2933	CH_2
2853	CH_3
1350-1260	Primary OH bond
1030	C-O bond

Figure 4.3: Fourier transform infrared analysis ginger oil, GA, ginger oil-GA emulsion, and ginger oil-GA dry powder.

4.6.2 Transmission electron microscopy analysis for ginger oil-GA emulsion

TEM analysis was carried out to observe the morphological characteristics such as core-shell structure and droplet size of an emulsion. The obtained images from TEM analysis are shown in figure 4.4. TEM analysis shows the encapsulation of ginger oil in GA biopolymer. The core dark black colour refers to ginger oil, whereas outer grey coloured ring structure refers to shell boundary; these observations are similar to previous findings (Sangolkar et a. 2019; Thongson et al. 2004). The affinity of GA with water brings its hydrophilic end to the surface of oil, and thus shell-like structure gets formed surrounding the ginger oil (Su et al. 2008). The particles are spherical to oval in shape and are uniformly distributed. The average particle size observed was below 200 nm, with a shell thickness of about 30 nm. The size of the encapsulation product evaluated from TEM analysis is following the size evaluated from the particle sizes analysis.

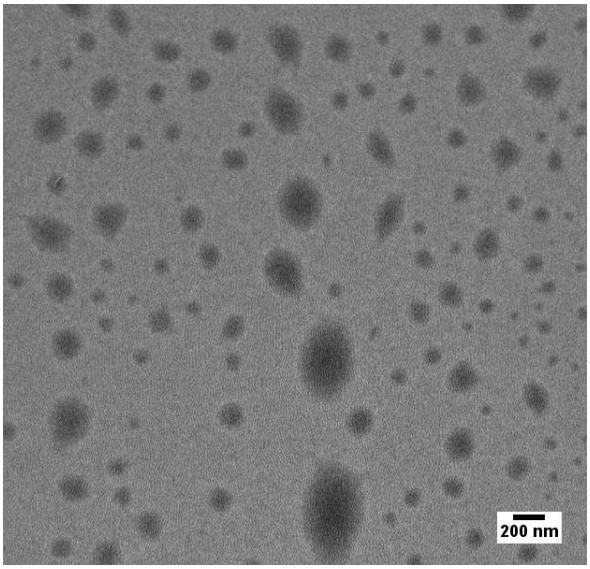


Figure 4.4: Transmission electron microscopy image of emulsion with encapsulated ginger oil in GA shell

4.6.3. Effect of process parameters on encapsulation efficiency

The consequence of different parameters, namely ginger oil and GA concentration, sonication time, also drying conditions like temperature and flowrate on encapsulation efficiency was observed. Figure 4.5 depicts the effect of individual parameter on all the output parameters.

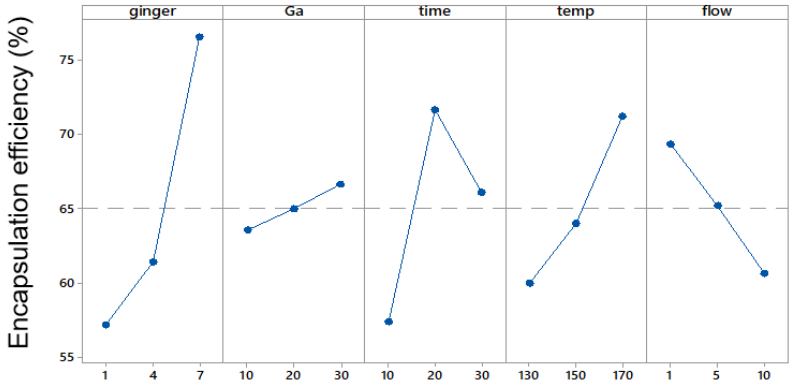


Figure 4.5: Taguchi plot effect of individual process parameter on encapsulation efficiency.

An ANOVA table 4.3 (a, b) was developed to discover the rank of significant factors and their percentage contribution. From the ANOVA analysis, it is observed that in the case of encapsulation efficiency ginger oil concentration is having strongest influence (p = 49.61 %), followed by sonication time (p = 24.66 %), drying temperature (p = 15.53 %) and flowrate to atomizer (p = 9.02 %). GA concentration has a negligible effect on encapsulation efficiency (p = 1.48 %).

Table 4.3. (a): Taguchi Analysis: encapsulation efficiency

Level	Ginger Oil (wt. %)	GA wt. %	Sonication time (min)	Drying temperature (ºC)	Flowrate to atomizer (mL/min)
1	57.22	63.56	57.44	60.00	69.33
2	61.44	65.00	71.67	64.00	65.22
3	76.56	66.67	66.11	71.22	60.67
Delta	19.33	3.11	14.22	11.22	8.67
Rank	1	5	2	3	4

Table 4.3 (b): ANOVA: encapsulation efficiency

Source	Degrees of freedom	Adjusted sum of squares	Adjusted mean of squares	F-value	p-value	Percentage of contribution
Ginger oil	2	1859.85	929.926	303.42	0.000	49.61
GA	2	43.63	21.815	7.12	0.006	1.16
Sonication time	2	924.74	462.370	150.86	0.000	24.66
Drying temp	2	582.30	291.148	95.00	0.000	15.53
Flowrate to atomizer	2	338.30	169.148	55.19	0.000	9.02
Error	16	49.04	3.065			0.163
Total	26		1874.407			

From the individual effect plot (figure 4.5), it can be concluded that the increase in ginger oil concentration from 1 to 4 % increases encapsulation efficiency marginally from 57.22 to 61.44 %. However, when ginger oil concentration increases further up to 7 % concentration, encapsulation efficiency increases rapidly to 76.56 %. This can be attributed to higher ginger oil concentration favourable for encapsulation efficiency. To observe the influence of sonication time on encapsulation efficiency, the sonication time was varied as 10 min, 20 min and 30 min. Taguchi analysis revealed that encapsulation efficiency increases as sonication time increases from 10 min to 20 min, however, further sonication might have caused the agglomeration of particles; thus, 20 min sonication time can be considered as an optimum sonication time (Sivakumar et al. 2014; Jiang et al. 2009). Increase in drying temperature, increases the encapsulation efficiency due to effective drying. As the feed flowrate to the atomizer increases, the encapsulation efficiency decreases from 69.33 % to 60.67 %. This

might have occurred, because the sufficient time required to dry the emulsion is not availed at a higher flow rate hence the product coming out has some moisture left in it, which causes the decrease in the encapsulation efficiency. Another reason for this trend is that, at higher flow rate some of the emulsion comes out from atomizing surface without atomizing and large-sized particles are produced and large-sized particles have less encapsulation efficiency than small-sized particles (Reineccius 2004; Zhang et al. 2021). The regression equation to predict the encapsulation efficiency for unknown inputs is given below in equation 4.8 below.

Regression Equation: Encapsulation efficiency (%) = 3.4 + 3.222 ginger oil (wt. %) + 0.156 GA (wt. %) + 0.433 sonication time (min) + 0.2806 drying temp (ºC) - 0.961 flowrate to atomizer (mL/min) (4.8)

4.6.4. Effect of process parameters on product yield

Product yield for encapsulated ginger oil in GA shell was estimated with formula 4.2 mentioned in section 4.2.2.

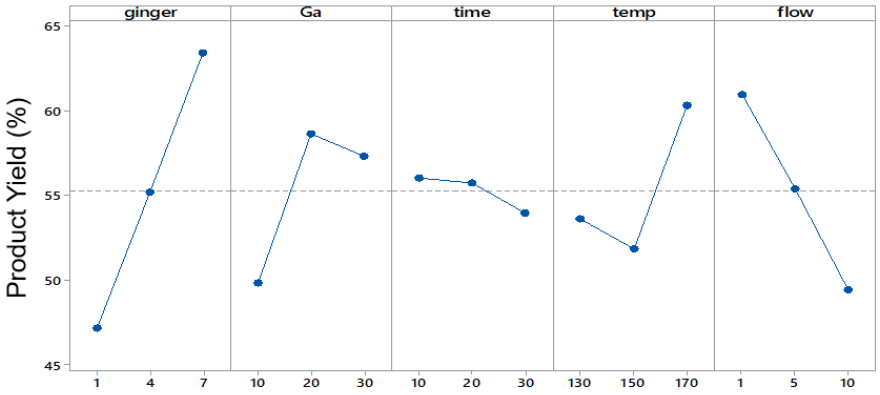


Figure 4.6: Taguchi plot effect of individual process parameter on product yield

Table 4.4 (a): Taguchi Analysis: product yield

Level	Ginger Oil (wt. %)	GA (wt. %)	Sonication time (min)	Drying temperature (ºC)	Flowrate to atomizer (mL/min)
1	47.18	49.94	56.07	53.69	61.02
2	55.29	58.73	55.78	51.89	55.44
3	63.44	57.33	54.07	60.33	49.44
Delta	16.27	8.89	2.00	8.44	11.58
Rank	1	3	5	4	2

Table 4.4 (b): ANOVA: product yield

Source	Degrees of freedom	Adjusted sumof squares	Adjusted mean of squares	F-value	p-value	Percentage of contribution
Ginger oil	2	1190.77	595.384	117.35	0.000	46.04
GA	2	407.08	203.539	40.12	0.000	15.74
Sonication time	2	22.55	11.273	2.22	0.141	0.87
Drying temp.	2	358.06	179.028	35.29	0.000	13.84
Flowrate to atomizer	2	597.74	298.869	58.91	0.000	23.11
Error	16	81.17	5.073			
Total	26		1293.166			

The regression equation to predict the product yield for unknown input parameters is shown below in equation 4.9.

Regression equation: Product yield (%) = 20.66 + 2.711 ginger (wt. %) + 0.374 GA (wt. %)- 0.103 time (min) + 0.1678 drying temp (ºC) - 1.277 flowrateto atomizer (mL/min) (4.9)

The effect of each parameter on product yield estimated using Taguchi analysis is depicted in figure 4.6. The rank and percentage contribution for product yield developed from ANOVA analysis are shown in table 4.5 (a, b). Ginger oil concentration have highest influence (p = 46.04 %) on product yield followed by feed flowrate to atomizer (p = 23.11) GA concentration (p = 15.74 %) and drying temperature (p = 13.84 %). Sonication time displayed negligible effect (p = 0.87 %) on product yield. It was observed that, increase in GA beyond 20 wt. % decreases the encapsulation efficiency. This might have occurred because viscosity of emulsion increases with increase in GA, causing difficulty in atomization. This was observed by uneven atomized flow, forming chunk of particles. Thus, some of the GA might have stick to the walls of atomizer causing decrease in product yield. As the feed flowrate to the atomizer increases, the product yield decreases from 61 % to 49 %. This occurs because the sufficient time is not availed for atomizing the emulsion in the atomizer (Frascareli et al. 2012; Moreira et al. 2009). The regression equation developed from Taguchi analysis to predict product yield is given below (equation 4.9). The plot of experimental vs. predicted product yield obtained from Taguchi and ANN network is depicted in figure 4.8 (b).

4.6.5. Effect of process parameters on particle size

The particle size evaluation of any food system is a very basic parameter as it determines the solubility of food components in the gastrointestinal tract and thus contributes in the bio-availability of the compound. The effect of individual input parameters on particle size was determined using Taguchi analysis (figure 4.7).

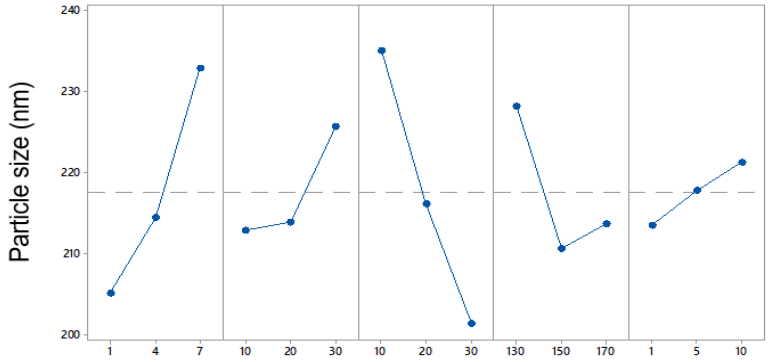


Figure 4.7: Taguchi plot effect of individual process parameter on particle size

Table 4.5 (a): Taguchi Analysis: particle size

Level	Ginger Oil (wt. %)	GA (wt. %)	Sonication time (min)	Drying temperature (ºC)	Flowrate to atomizer (mL/min)
1	205.1	212.9	235.0	228	213.4
2	214.4	213.9	216.1	210.7	217.8
3	232.9	225.7	201,3	213.7	221.2
Delta	27.8	12.8	33.7	17.4	7.8
Rank	2	4	1	3	5

Table 4.5 (b): ANOVA for: particle size

Source	Degrees of freedom	Adjusted sum of squares	Adjusted mean of squares	F-value	p-value	Percentage of contribution
Ginger oil	2	3596.7	1798.37	24.47	0.000	30.96
GA	2	909.0	454.48	6.18	0.010	7.82
Sonication time	2	5125.9	2562.93	34.87	0.000	44.12
Drying temp	2	1565.9	782.93	10.65	0.001	13.47
Flowrate to atomizer	2	273.4	136.70	1.86	0.188	2.35

Error	16	1175.9	73.50			1.27
Total	26		5808.91			

The plot of experimental vs. predicted particle size obtained from Taguchi and ANN network is depicted in figure 4.8 (c). The regression equation to predict the product yield for unknown input parameters is shown below in equation 4.10.

Regression equation: Particle size = 269.4 + 4.630 ginger oil (wt. %) + 0.639 GA (wt. %) - 1.683 sonication time (min) - 0.361 drying temp (ºC) + 0.857 flowrate to atomizer (mL/min) (4.10)

The ANOVA table 4.6 (a, b) shows that sonication time has the highest influence on particle size with a percentage contribution of 44.12 % followed by ginger oil concentration (p = 30.96 %), drying temperature (p = 13.48 %) and GA concentration (p = 7.82 %). Flowrate to atomizer have negligible effect with (p = 2.35 %). The increase in ginger oil concentration from 1 % to 4 % increases particle size marginally from 205 nm to 214 nm. However, further increase in ginger oil concentration increases particle size to a great extent (232 nm). As the GA concentration increases the total solid content of the solution increases, causing an increase in particle size from 212 to 225 nm. The increase in sonication time has proved beneficial for decreasing particle size significantly, the increase in sonication time increases the compression and rarefaction cycles and, thus over a period of time the cavities grows and burst rapidly giving rise to tiny particles. Thus, the overall size of emulsion droplets decreases. Feed flowrate to atomizer has also significant impact on particle size. At a very low flowrate the film thickness formed on atomizing surface is below that necessary to wet the atomizing surface hence the particle size obtained is higher. When the flowrate is sufficient to occupy the atomizing surface the atomization leads to formation of liquid threads and thus lower size particles are obtained. When a flowrate is increased beyond a certain limit the thickness of the film on the atomizing surface increases, and thick film generates particles of a larger size (Moreira et al. 2009). Therefore, for the selected range of flowrates the increase in particle size was observed with flowrate.

4.6.6. Prediction of encapsulation process with ANN

Taguchi analysis proved beneficial in designing the experiments with minimum number of runs and it also helped to determine the indicative rank and contribution of each parameter. However, the outputs predicted by Taguchi have higher deviation from experimental results. Because Taguchi approach has good reproduction of results concerned only with the main

effects of design parameters, including noise factors. Whereas ANN having ability to learn and find multi-dimensional dependencies between the process parameters by learning. ANN model was developed with variation of three combinations of networks namely Purelin-Purelin, Logsig-Purelin and Tansig-Purelin, with number of neurons varied from 4 to 10 for each transfer function. The performance of the model was evaluated in terms of $R^2 \approx 1$ and minimum MSE. The R^2 and MSE values for all the developed network are given in table 4.6.

Table 4.6: The values of R^2 and MSE showing an effect of number of neurons in the hidden layer: Combined model.

Network structure	Transfer function	Training	Validation	Test	Entire data	MSE (e-4)		
5 4 3	Purelin-Purelin	0.9873	0.9933	0.9770	0.9855	35.168	17.038	29.131
5 5 3		0.9871	0.9900	0.9782	0.9851	35.835	15.553	30.375
5 6 3		0.9887	0.9754	0.9735	0.9835	34.059	21.926	34.451
5 7 3		0.9928	0.9017	0.9524	0.9714	41.380	18.521	33.071
5 8 3		0.9929	0.9773	0.9643	0.9787	69.481	20.902	37.980
5 9 3		0.9875	0.9948	0.9735	0.9837	32.603	19.834	40.745
5 10 3		0.9862	0.9751	0.9941	0.9857	31.944	16.909	29.533
5 4 3	Logsig-Purelin	0.9985	0.9588	0.977	0.9875	5.905	20.835	46.683
5 5 3		0.9914	0.9571	0.9904	0.9882	16.017	25.990	30.343
5 6 3		0.9996	0.9560	0.9436	0.9781	18.580	3.928	8.249
5 7 3		0.9775	0.9805	0.9524	0.9714	64.038	18.164	74.621
5 8 3		0.9996	0.9673	0.9507	0.9815	2.022	5.165	98.266
5 9 3		0.9946	0.9802	0.9851	0.9896	8.090	6.889	42.865
5 10 3		0.9952	0.9849	0.9019	0.9818	22.023	35.168	54.560
5 4 3	**Tansig-Purelin**	**0.9976**	**0.9964**	**0.99556**	**0.9964**	**3.041**	**9.167**	**5.137**
5 5 3		0.9635	0.9919	0.98985	0.96543	9.815	14.418	20.680
5 6 3		0.9941	0.9614	0.94047	0.97291	24.684	15.104	122.135
5 7 3		0.9669	0.9679	0.96511	0.96051	103.914	36.100	104.946
5 8 3		0.9994	0.9707	0.95097	0.98757	10.787	23.597	34.164
5 9 3		0.9999	0.9931	0.9726	0.9756	4.551	10.435	20.489
5 10 3		0.9952	0.9739	0.9813	0.9658	9.611	11.423	30.333

The results of ANN modelling were summarized in terms of R2 and MSE. Table 4.6 helped to compare the valued of R2 and MSE for all combination of network from which the best performing network was chosen. The network 5 4 3 with Tansig-Purelin transfer function have good prediction of outcome. Hence this network was used in comparative prediction with Taguchi.

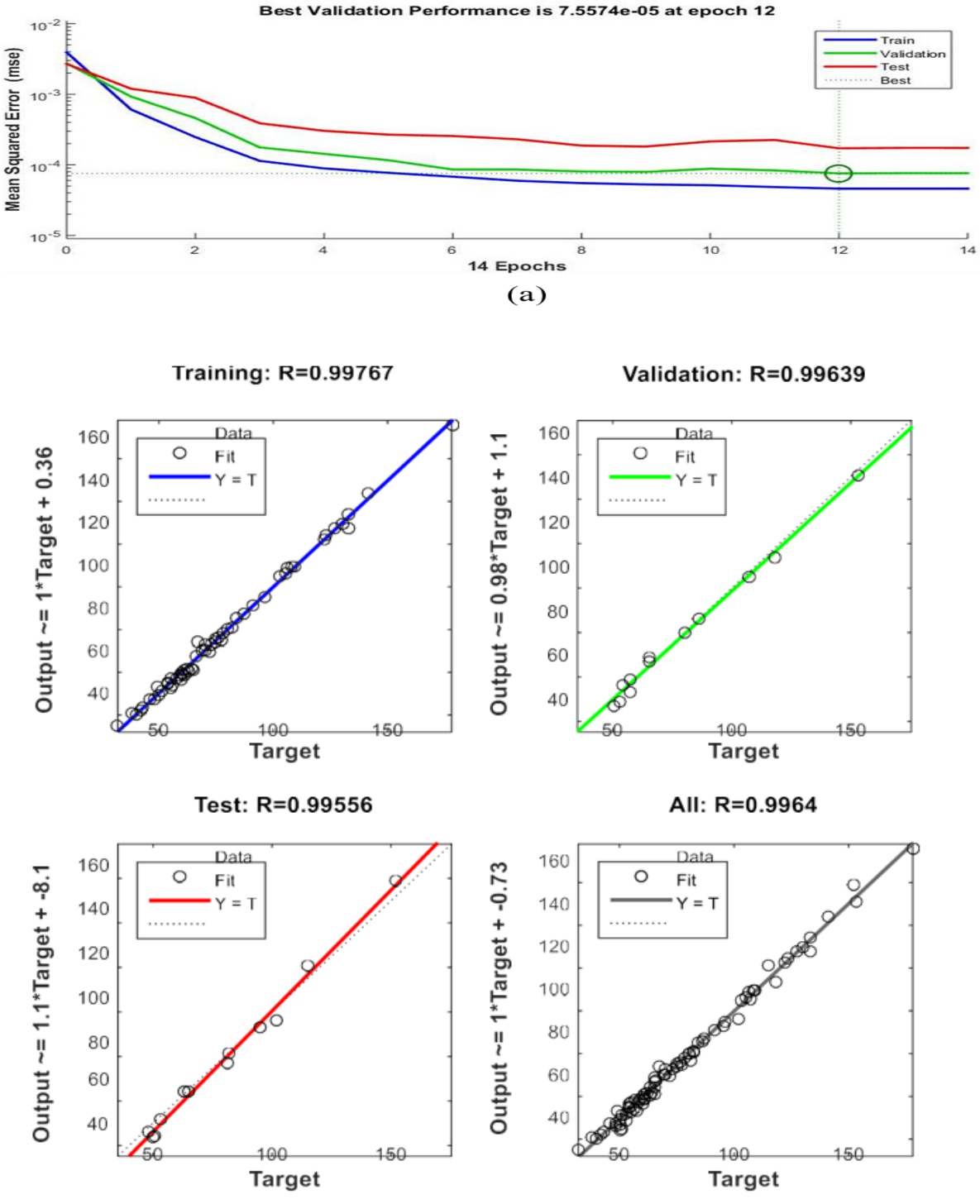
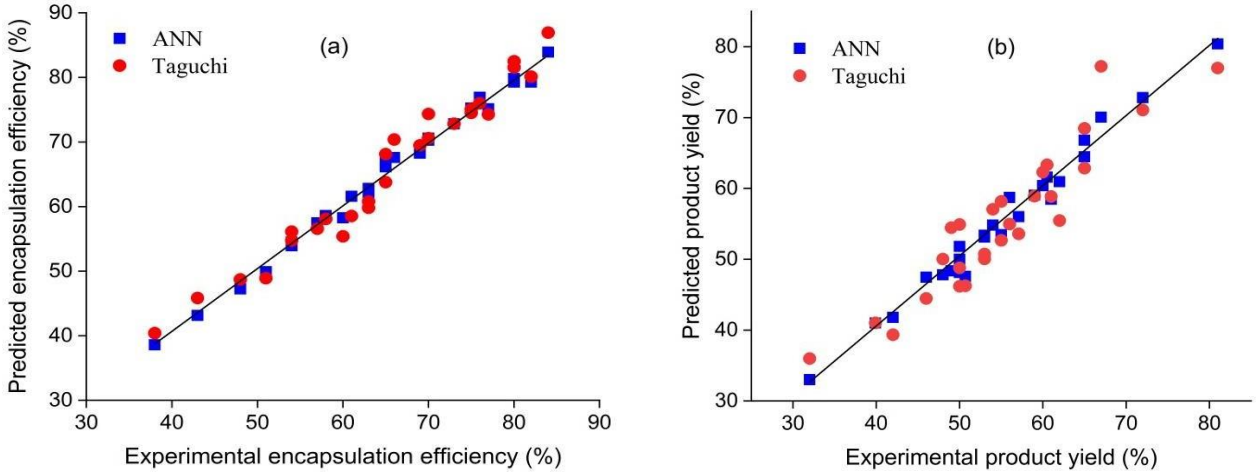


Figure 4.8: (a) Performance plot (b) Regression plots for ANN model

From the table 4.7 it is clear that the Tansig-Purelin function with 4 neurons in the hidden layer has higher R^2 value of 0.9964. The corresponding performance and parity plot obtained from the software are shown in figure 4.8 (a and b) respectively. The corresponding MSE values of 3.041×10^{-4}, 9.167×10^{-4} and 5.137×10^{-4} for encapsulation efficiency, product yield and particle size respectively. These values of error are acceptable and hence the corresponding ANN network can be used in predicting the encapsulation process. From figure 4.9 it is clear that the prediction performance of ANN is much better as compared to Taguchi with a negligible error value.

4.6.7. Comparison of prediction performance of Taguchi and ANN

To assess the performance of Taguchi and ANN, the prediction performance of both approaches was studied. The prediction with ANN was performed using the "simulate" tool in the software. outputs. Also, the outputs were predicted from Taguchi analysis. For this purpose, regression equations, no 4.8, 4.9 and 4.10, developed by software were used. The outputs predicted from ANN and Taguchi were plotted against experimental outputs as shown in figure 4.9. It was confirmed from the comparative plots that the prediction performance of ANN is much better as compared to Taguchi with a negligible error value. Because ANN have the ability to find multivariate dependencies and establish a correlation to predict outputs more accurately. Whereas Taguchi is more widely used to predict the rank and percentage contribution of each input parameter in predicting the output (Olden and Jackson (2002)).



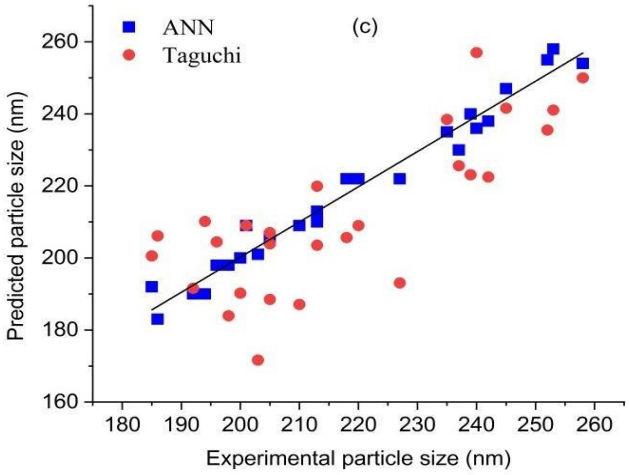


Figure 4.9: Plot of experimental Vs. predicted output from Taguchi and ANN model for (a) encapsulation efficiency (b) product yield (c) particle size.

4.7. Summary

The chapter describes the encapsulation of ginger essential oil in GA carrier. The ultrasound is found to be effective in the encapsulation of lipid compounds like ginger essential oil. The FTIR analysis confirmed that sonication treatment does not produce adverse effect on characteristic bonding of components. Taguchi analysis helped to design the experiments with minimum number of runs simultaneously, it allowed to evaluate the effect of each parameter on encapsulation efficiency, product yield and particle. From Taguchi analysis it can be observed that even though sonication time has not significant effect on encapsulation efficiency and product yield but it is the most important parameter affecting particle size. Hence it is necessary to evaluate the effect of all process parameters. Further, ANN models developed to find multivariate dependencies helped to prove that prediction of ANN is more accurate than Taguchi prediction. Overall, it can be concluded that integrated Taguchi and ANN approach are capable of optimizing the encapsulation process with accuracy and optimum number of experiments.

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Chapter 5

ANN based modelling of peppermint flavour encapsulation process with ultrasound approach

5.1 Introduction

The present chapter gives the investigation on artificial neural network (ANN) based modelling approach for the experimental case study for encapsulation of peppermint flavour in GA previously performed in our research group (Sangolkar et al. 2019). The aim of this chapter is to develop ANN models capable of predicting the existing experimental data with accuracy. Further, the application of the developed and validated ANN model is extended to find the effect of wide range of input parameters on outputs of encapsulation process. With this aim model is extended to carry out interpolation and extrapolation simulations.

5.2. ANN Model development for process prediction and optimization

5.2.1. Peppermint flavour encapsulation process with ultrasound approach

The encapsulation of peppermint flavour in GA shell using ultrasound approach is briefly described in this section. Initially, an aqueous solution of the shell material (GA) was prepared. To this solution, peppermint flavour was added dropwise in the presence of ultrasound. During the experiments, nine different emulsions were prepared by varying flavour concentration, GA concentration temperature of the hot stream entering to the spray dryer and flow rate to spray dryer. Experiments were performed in triplicate and average values were represented. However, in the present investigation, to avail the enough quantity of data for training, validations and testing, the experimental outputs of all three repetitions were taken for ANN network development. Efficacy of the encapsulation process is evaluated with the measure of encapsulation efficiency, product yield and particle size. Therefore, the totals of twenty-seven experimental data sets are available for developing ANN network. The details of parameter conditions for encapsulation process are mentioned in table 5.1 below.

Table 5.1: Details of experimental process parameters used in ANN network development. Data taken from (Sangolkar et al. 2019)

A: Peppermint flavour: (1,1.5 and 2 wt. %)

B: GA: (20,30 and 50 wt. %)

C: Sonication time: 30 min

D: Spray dryer temperature: 150, 160 and 170 ºC

E: Flowrate to spray dryer: 3, 5 and 7 mL/min

	Input parameters					**Process outputs**					
Run	A	B	C	D	E	Encapsulation efficiency (%)		Product yield (%)		Average particle size (nm)	
						Expt.	Prediction	Expt.	Prediction	Expt.	Prediction
1	1	20	30	170	5	43	48	78	75	47	45
2	1	20	30	170	5	46	48	75	75	45	45
3	1	20	30	170	5	49	48	72	75	44	45
4	1	30	30	150	5	79	79	22	20	177	180
5	1	30	30	150	5	81	79	17	20	180	180
6	1	30	30	150	5	85	79	20	20	182	180
7	1	30	30	160	5	79	81	27	25	181	179
8	1	30	30	160	5	80	81	22	25	179	179
9	1	30	30	160	5	84	81	25	25	177	179
10	1	30	30	170	3	89	87	53	56	252	253
11	1	30	30	170	3	90	87	61	56	255	253
12	1	30	30	170	3	84	87	54	56	260	253
13	1	30	30	170	5	81	80	48	52	92	94
14	1	30	30	170	5	78	80	54	52	96	94
15	1	30	30	170	5	83	80	50	52	98	94
16	1.5	30	30	170	5	84	85	52	52	111	109
17	1.5	30	30	170	5	86	85	56	52	109	109
18	1.5	30	30	170	5	85	85	51	52	107	109
19	2	30	30	170	5	84	85	52	56	127	129
20	2	30	30	170	5	86	85	56	56	130	129
21	2	30	30	170	5	86	85	59	56	132	129
22	1	50	30	170	5	87	89	15	17	180	184
23	1	50	30	170	5	90	89	17	17	187	184
24	1	50	30	170	5	91	89	19	17	185	184
25	1	30	30	170	7	64	68	15	14	62	65
26	1	30	30	170	7	69	68	14	14	64	65
27	1	30	30	170	7	66	68	11	14	66	65

5.2.2. ANN model development

An objective of the present work is to develop an ANN model to predict the outputs for wide range of parameters. The mechanisms of emulsification and drying are complex therefore, it is challenging to develop a phenomenological model. In this regard, a validated ANN model will help to predict the process. The steps in the model development are described in detail. Basically, ANN model consists of three layers namely: input layer, hidden layer and output layer as shown in figure 5.1.

All the four networks have five neurons in the input layer that corresponds to five process variables used in encapsulation process viz. peppermint flavour concentration, GA concentration, sonication time, temperature of hot stream entering to spray dryer, and feed rate to spray dryer. Number of neurons in the hidden layer were varied from 4 to 10 for all the networks. However, efficacy of developed model in predicting the process output individually and simultaneously was tested. The initial three networks have only one parameter in the output layer (encapsulation efficiency/ product yield/ particle size), whereas in the fourth network, all the outputs are evaluated together. These four models were referred as encapsulation efficiency model, product yield model, particle size model and combine model. The conceptualization of all developed models is depicted in figure 5.1 (a, b, c and d).

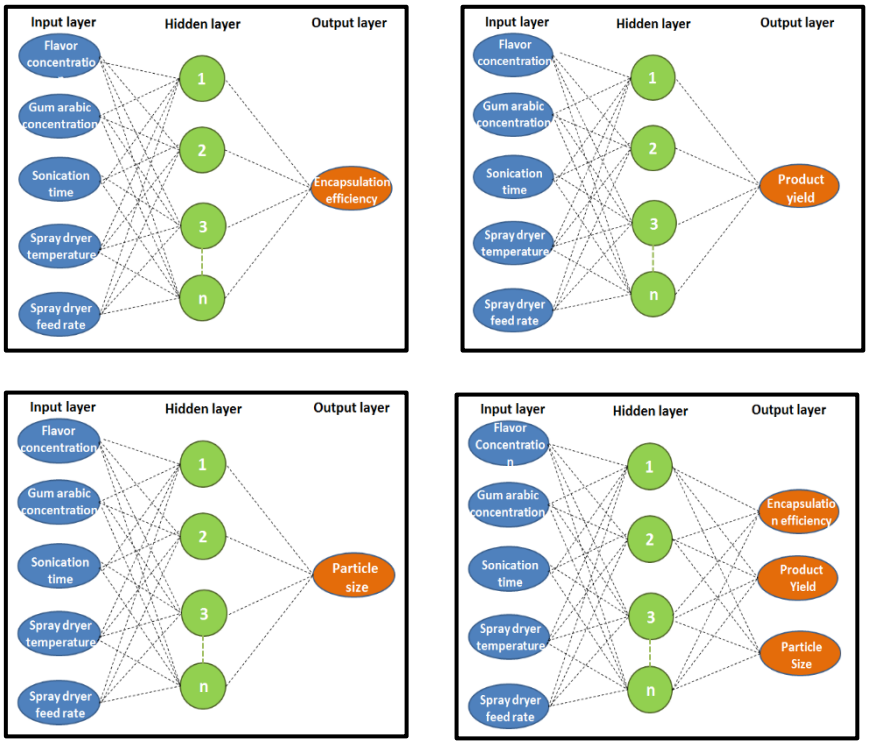

Figure 5.1: Models of the ANN: (a) efficiency model, (b) product yield model, (c) Particle size, (d) model with all output together

5.3. ANN training procedure

The steps in ANN network development and its training are described using flowchart as shown in figure 5.2 below. The input values were given to input layer. Followed by providing the corresponding output values. Based on these input and output values feed-forward ANN network was developed using the 'nntool' vizard in MATLAB (Leppänen et al. 2015). The network was trained using Levenberg-Marquardt ((LM) algorithm. The best performance for all the developed networks is evaluated on the basis of a maximum value of regression coefficient (R^2) and the minimum value of mean square error (MSE). The R^2 and MSE were calculated based on the equation 5.1 and 5.2 below.

$$R^2 = 1 - \frac{\sum_{I=1}^{N_{train}} (X_{Pred} - X_{Exp})^2}{\sum_{i=1}^{N_{train}} (X_m - X_{Exp})^2} \quad (5.1)$$

$$MSE = \frac{\sum_{i=1}^{N_{Test}} (X_{Pred} - X_{Exp})^2}{N_{Test}} \quad (5.2)$$

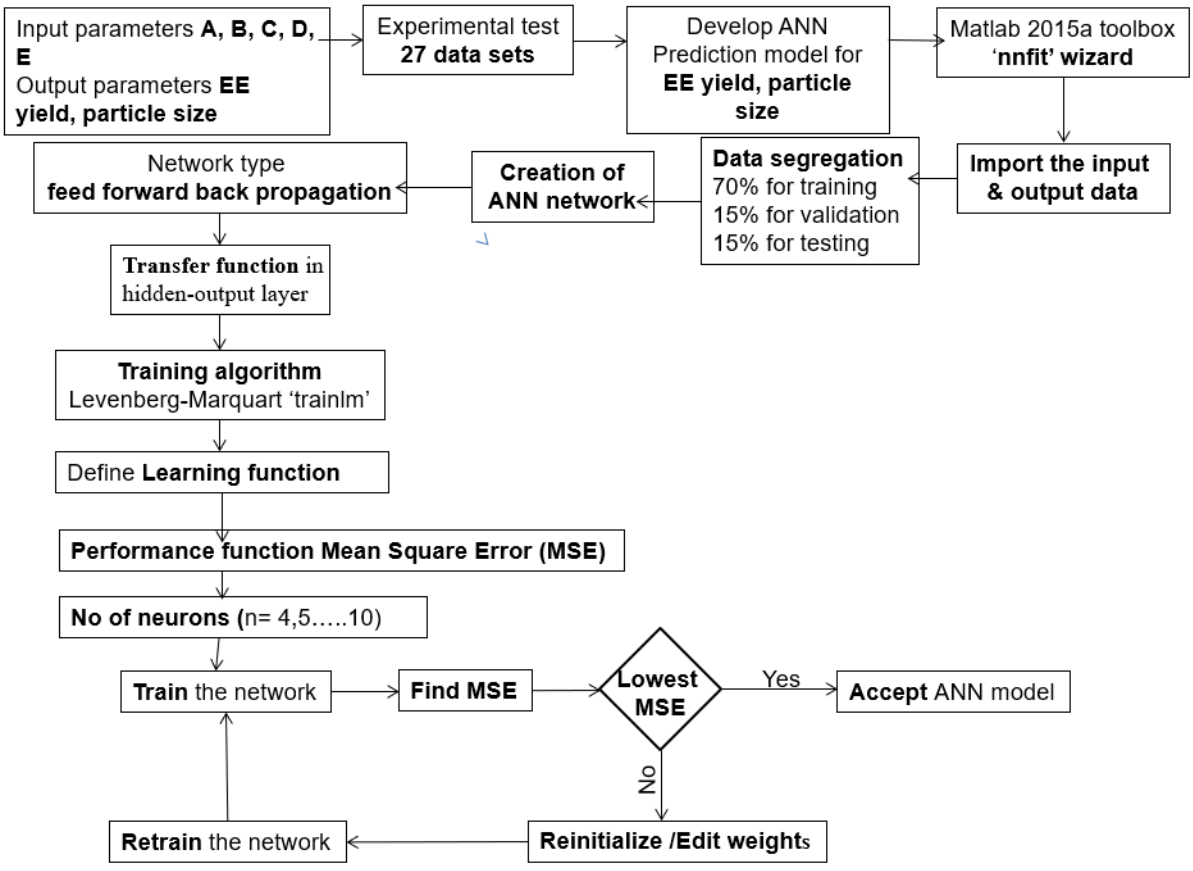

Figure 5.2: ANN network development and training procedure

For the developed network output was predicted and compared with experimental output. Further, to improve the accuracy of prediction (minimum MSE and $R^2 \approx 1$) the models were retrained by varying the combination of transfer functions such as purelin-purelin, logsig-purelin and tansig-purelin. The iteration limit of 5,000 was fixed in order to avoid overtraining and also to reduce the training time for all model's development.

As a thumb rule, neurons in the hidden layer should be greater than or equal to the number of input neurons and it must be less than two-fold the size of the input layer (Lai and Liu 2010). Considering this, number of neurons in the hidden layer were varied from 4 to 10 for each combination of transfer function. For the present study, a single hidden layer is chosen. Since a single hidden layer is capable of approximating a function with continuous mapping from one finite space to another (Guliyev and Ismailov (2016); Heaton (2008); Ranade et al. (2021))

Training an artificial neural network refers to finding the weights and bias in each layer and computing it to compare the predicted output with experimental results. The values of the output were predicted from the developed network by applying weights and bias on each neuron. The weight and bias on each neuron were reassigned in each epoch using, a 'train' function of MATLAB (Frascareli et al. (2012)). During the training, a shallow network model was developed. The training of a network is a batch process, which means that, during the training of epoch, all input vectors are given to the network and then weights are applied (Plumb et al. (2005)). The number of epochs in each set was altered in order to achieve the maximum R^2 and minimum MSE value. During the network development number of failures in each were kept equally to achieve the best fit. The combination of functions for input and hidden layer were chosen in as: purelin – purelin, logsig - purelin and tansig-purelin. The operation was performed for individual output as well as for their combined network model.

Considering the thumb rule, the experimental data available for ANN network development was divided as 70:15:15 for training, testing and validation respectively. The value of each neuron in the hidden layer and output layer was calculated based on equation 5.3 given

$$\text{Output} = (W_{01} * X_0) + (W_{11} * X_1) + (W_{21} * X_2) + (W_{31} * X_3) + (W_{41} * X_4) + \beta \quad (5.3)$$

Where W_{01}, W_{11}, W_{21}, W_{31} and W_{41} are the values of weight applied. The suffix 0, 1, 2, 3 and 4 represents weights from all five inputs and suffix 1 attached to each weight indicates network with first neuron and β is the corresponding value of bias. In the course of the training, the values of weights and bias on each neuron gets revised based on the knowledge of the previous epoch. The network development loop was repeated until maximize R^2 and minimize MSE was obtained.

5.4. Results and discussion

The result and discussion section, elaborates the criteria and guidelines for selecting the parameters in the model development, the optimum number of neurons, and the transfer function in the developed ANN network were investigated. The predicted results were compared with experimental outputs to confirm the model's accuracy and efficacy. The model is further simulated to evaluate its interpolation and extrapolation ability in the prediction of encapsulation process parameters.

5.4.1. Selection and validation of best performing ANN model

As mentioned in the above section, to develop the best performing model, three combinations of function such as purelin-purelin, logsig-purelin and tansig-purelin, were investigated. Each combination is tested with a variation of number of neurons from 4 to 10. For each combination learning rate is also varied as: 0.001, 0.01, 0.09, and 0.9. The R^2 and MSE value for all ANN simulations were noted. The response of ANN networks giving R^2 and MSE for individual input are given below. Table 5.2 illustrates the R^2 values and MSE for different ANN models developed to predict encapsulation efficiency.

Table 5.2: The values of R^2 and MSE, showing an effect of number of neurons in the hidden layer: **Encapsulation efficiency model**

Note: In the network structure column number, the terminology 5 4 1 refers to number of neurons present in the input, hidden, and output layer, respectively.

Network structure	Transfer function	Training	Validation	Test	Entire data	MSE ($\times 10^{-4}$)
5 4 1	Purelin - Purelin	0.8489	0.9424	0.9516	0.841	51.08
5 5 1		0.8108	0.9045	0.9322	0.841 5	50.52
5 6 1		0.8739	0.7662	0.8025	0.842	52.99
5 7 1		0.8509	0.9811	0.9638	0.843	51.07
5 8 1		0.8737	0.9366	0.8653	0.832 6	53.99
5 9 1		0.8463	0.9447	0.703 4	0.839 3	56.34
5 10 1		0.8545	0.8509	0.421 2	0.842 4	51.84
5 4 1	Logsig-Purelin	0.8424	0.9984	0.7929	0.903 6	53.99
5 5 1		0.9918	0.858	0.7921	0.941 2	56.34
5 6 1		0.9938	0.9306	0.922 8	0.985 9	51.84
5 7 1		0.9885	0.992	0.978 6	0.985 9	53.63
5 8 1		0.9843	0.9994	0.968 1	0.986 9	61.30
5 9 1		0.9769	0.9994	0.998	0.987 4	82.15
5 10 1		0.9523	0.9604	0.868 6	0.914 7	56.62
5 4 1	Tansig-	0.9828	0.8412	0.995 3	0.983 8	4.12

5 5 1	purelin	0.9882	0.997 2	0.984 7	0.984 7	5.8
5 6 1		0.9846	0.996	0.962 1	0.980 3	5.20
5 7 1		0.9837	0.9319	0.999 2	0.985 1	5.12
5 8 1		0.9855	0.9944	0.978 6	0.985 6	5.75
5 9 1		0.9863	0.994	0.991 6	0.985 6	5.09
5 10 1		0.9894	0.9939	0.981 3	0.985 8	8.12

The results listed in Table 5.2 show that, the ANN model 5-9-1 i.e. 9 neurons in hidden layer with tansig - purelin transfer functions, exhibits the highest R^2 value of 0.9856 with MSE of 0.000509. This indicated that developed ANN model helps to predict encapsulation efficiency closer to the target value. Therefore, the developed ANN model is precise enough to predict the responses for unknown input process parameters.

In the same fashion Table 5.3 represents the R^2 value and MSE obtained for the prediction of product yield. The ANN model 5-4-1 i.e. 5 neurons in hidden layer with tansig-purelin transfer functions, exhibits the highest R^2 with 0.9822 with MSE about 0.000614. Hence, it can be concluded that the developed ANN model is adequately precise to predict the responses for unknown input process parameters.

Table 5.3: The values of R^2 and MSE showing an effect of number of neurons in the hidden layer: **Product yield model**

Network structure	Transfer function	Training	Validation	Test	Entire data	MSE ($\times 10^{-4}$)
5 4 1	Purelin - Purelin	0.907	0.966 3	0.997 6	0.918 6	71.64
5 5 1		0.936 1	0.996 3	0.956 8	0.913 7	74.30
5 6 1		0.924 1	0.956 5	0.965 7	0.921 6	43.35
5 7 1		0.915 1	0.793 1	0.958 2	0.910 2	53.69
5 8 1		0.927 1	0.977 2	0.722 6	0.921 9	66.92
5 9 1		0.920 9	0.921 8	0.998	0.926 5	62.30
5 10 1		0.927 6	0.915 3	0.886 6	0.913 6	69.77
5 4 1	Logsig-Purelin	0.992 4	0.998 1	0.978 9	0.993 3	38.06
5 5 1		0.973 1	0.995 8	0.999	0.978	15.86
5 6 1		0.994 7	0.974 1	0.992 9	0.991 8	18.41
5 7 1		0.991 3	0.995 3	0.999 7	0.991 8	17.24
5 8 1		0.995 2	0.99	0.989 9	0.992 5	26.32
5 9 1		0.994 4	0.988 8	0.994 9	0.992 5	26.80
5 10 1		0.989 5	0.998 9	0.987 2	0.99	19.61
5 4 1	Tansig-purelin	0.992 1	0.997 3	0.998 4	0.993	6.11
5 5 1		0.995 3	0.963 9	0.999 9	0.992 2	7.40
5 6 1		0.991 6	0.989 1	0.995 5	0.988 2	8.71

5 7 1		0.994	0.998 2	0.991 6	0.990 2	6.72
5 8 1		0.995 1	0.981 1	0.982	0.990 7	8.24
5 9 1		0.992 8	0.997 6	0.996 2	0.992 7	6.74
5 10 1		0.993	0.9958	0.9981	0.9917	7.46

Table 5.4 represents the R^2 value and MSE obtained for the prediction of particle size. The ANN model 5-5-1 i.e. 5 neurons in hidden layer with tansig-purelin transfer functions, exhibits the highest R^2 with 0.9822 with MSE about 0.0072. Hence, the developed ANN model is adequately precise to predict the responses for unknown input process parameters.

Table 5.4: The values of R^2 and MSE showing an effect of number of neurons in the hidden layer: **Particle size model**

Network structure	Transfer function	Training	Validation	Test	Entire data	MSE ($\times 10^{-4}$)
5 4 1	Purelin - Purelin	0.908 5	0.849	0.976 2	0.908 5	66.50
5 5 1		0.916 1	0.952 4	0.992 5	0.908 6	72.78
5 6 1		0.915 9	0.956 1	0.999 4	0.910 2	64.88
5 7 1		0.918 9	0.958 4	0.900 5	0.908 7	78.01
5 8 1		0.904 1	0.997 5	0.999 1	0.909 2	84.20
5 9 1		0.917 3	0.936 7	0.964 5	0.909 3	77.28
5 10 1		0.908 4	0.994 3	0.968 8	0.901 7	68.33
5 4 1	Logsig-Purelin	0.994 9	0.999	0.998 8	0.995 7	11.23
5 5 1		0.999 5	0.997 9	0.998 3	0.999 1	13.11
5 6 1		0.999 4	0.997	0.999	0.999 1	12.56
5 7 1		0.998 9	0.999 9	0.999 5	0.999 2	14.15
5 8 1		0.999 5	0.999 1	0.999 4	0.999 3	10.04
5 9 1		0.999 5	0.999 8	0.997 3	0.999 3	14.98
5 10 1		0.999 3	0.999 8	0.999	0.999 3	14.76
5 4 1	Tansig-purelin	0.908 5	0.849	0.976 2	0.908 5	66.50
5 5 1		0.916 1	0.952 4	0.992 5	0.908 6	72.78
5 6 1		0.915 9	0.956 1	0.999 4	0.910 2	64.88
5 7 1		0.918 9	0.958 4	0.900 5	0.908 7	78.01
5 8 1		0.904 1	0.997 5	0.999 1	0.909 2	84.20
5 9 1		0.917 3	0.936 7	0.964 5	0.909 3	77.28
5 10 1		0.908 4	0.994 3	0.968 8	0.901 7	68.33

5.4.2. ANN model for predicting combine responses in the output layer (encapsulation efficiency, product yield and particle size)

Further, to check the capability of the 5-n-3 ANN model for predicting simultaneous response for encapsulation efficiency, product yield and particle size, ANN models developed with

combinations of different transfer functions. The obtained results in terms of R^2 and MSE are listed in table 5.5. The result shows that the network 5-4-3 i.e. 5 neurons in the input layer, 4 neurons in the hidden layer when simulated to predict all the three outputs together with tansig-purelin transfer functions exhibits the highest R^2 value of 0.999 with MSE value of 0.000604. Thus, it is confirmed that the developed ANN model is precise enough equally to individual models to predict the encapsulation process.

Table 5.5: The values of R^2 and MSE showing an effect of number of neurons in the hidden layer: **Combine model**

Network structure	Transfer function	Training	Validation	Test	All	MSE ($\times 10^{-4}$)		
5 4 1	Purelin - Purelin	0.963 1	0.962 3	0.919 8	0.951 7	52.11	68.22	78.76
5 5 1		0.814 2	0.962 5	0.901 4	0.804 3	45.87	60.62	58.27
5 6 1		0.953 9	0.928 8	0.972 4	0.954	51.02	70.10	74.81
5 7 1		0.944 1	0.917 6	0.981 5	0.940 4	52.25	69.59	98.70
5 8 1		0.948	0.965 3	0.956 8	0.947 1	52.97	98.24	85.17
5 9 1		0.960 5	0.919 7	0.978 1	0.954 8	50.10	64.61	75.28
5 10 1		0.961 2	0.952 7	0.9561	0.956 5	52.41	48.87	62.21
5 4 1	Logsig-Purelin	0.999	0.998 7	0.998 4	0.998 8	17.10	28.83	17.49
5 5 1		0.998 6	0.999 4	0.999 3	0.998 8	16.12	27.60	26.17
5 6 1		0.999 1	0.998 7	0.998 4	0.998 9	24.88	26.48	26.64
5 7 1		0.999	0.998 8	0.999 6	0.999	15.78	17.38	12.07
5 8 1		0.999 4	0.998 5	0.998 5	0.999 1	25.15	16.82	25.79
5 9 1		0.997 3	0.998 7	0.998 6	0.997 7	10.51	38.34	23.26
5 10 1		0.999 5	0.996 7	0.998 1	0.998 9	24.78	17.63	8.34
5 4 1	**Tansig-Purelin**	**0.999 1**	**0.999 1**	**0.999 1**	**0.999 1**	**5.36**	**6.35**	**6.04**
5 5 1		0.999 2	0.996 5	0.998 9	0.898 9	7.48	6.95	7.02
5 6 1		0.998 9	0.998 4	0.997 6	0.998 3	11.09	8.46	10.59
5 7 1		0.999 4	0.993 2	0.986 9	0.995 6	13.34	22.89	10.28
5 8 1		0.999 4	0.997 8	0.997 4	0.997 6	6.19	6.56	6.94
5 9 1		0.999 3	0.971 8	0.940 9	0.997 5	20.29	26.12	15.52
5 10 1		0.999 2	0.998 1	0.999	0.998 9	5.69	7.59	7.38

From the values of R^2 and MSE, it was observed that the combination of tansig-purelin function performs superior over the other two combinations for all the models. The comparison between predicted results for individual models with the combine model revealed that combine model has similar accuracy as that of an individual model together with that it helps to save the processing time; therefore, combine model was adopted for further study. The regression plot obtained from the software for the optimized conditions of combined model showing the good

agreement between experimental and predicted results for training validation, test data set and entire data set are shown in figure 5.3 below.

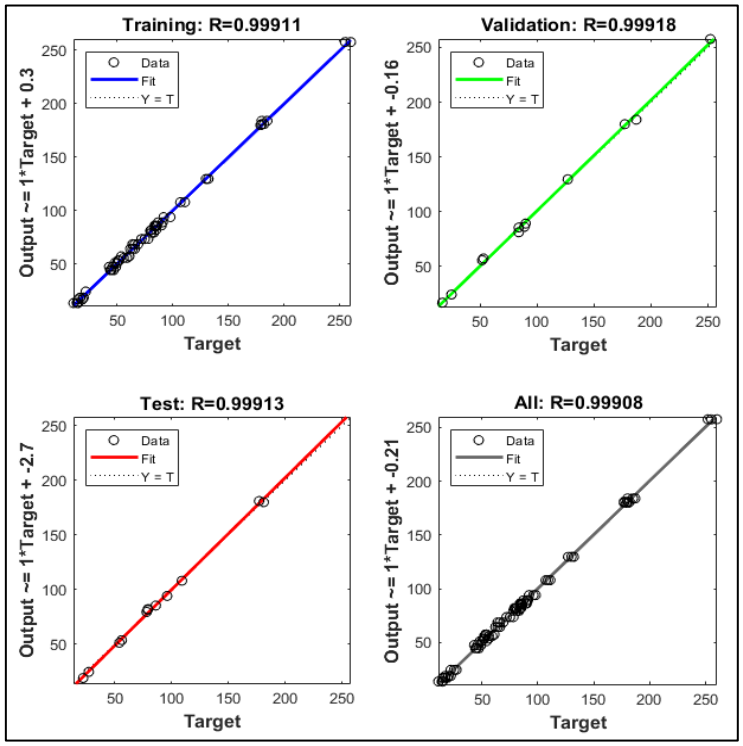

Figure 5.3: (Combine model) Regression plots of training, validation, testing and all data set

The effect of the number of neurons on MSE is shown in figure 5.4. The performance of each model is evaluated in terms of R^2 and MSE for the complete data set and data used for testing. It is clear that the number of neurons does not have a significant effect on the accuracy of the model. This observation is in agreement with the observation reported by Bahmani et al. (2018) data on encapsulation. The negligible effect of number of neurons might be due to careful selection of range of number of neurons in the hidden layer considering thumb rule in the selection of number of neurons as mentioned in section 5.2. The plots of experimental Vs. ANN predicted results for all output parameters are displayed in figure 5.5 below.

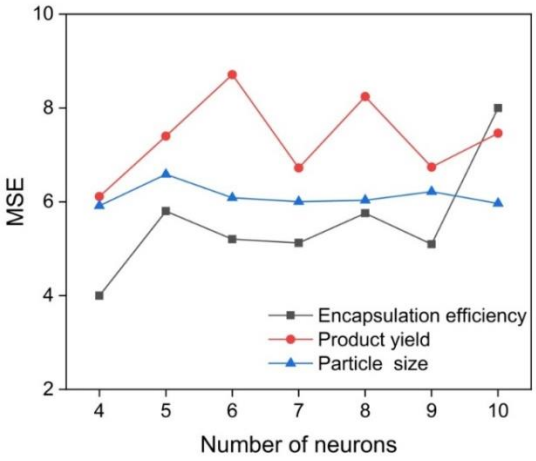

Figure 5.4: Effect of number of neurons on MSE in predicting outputs

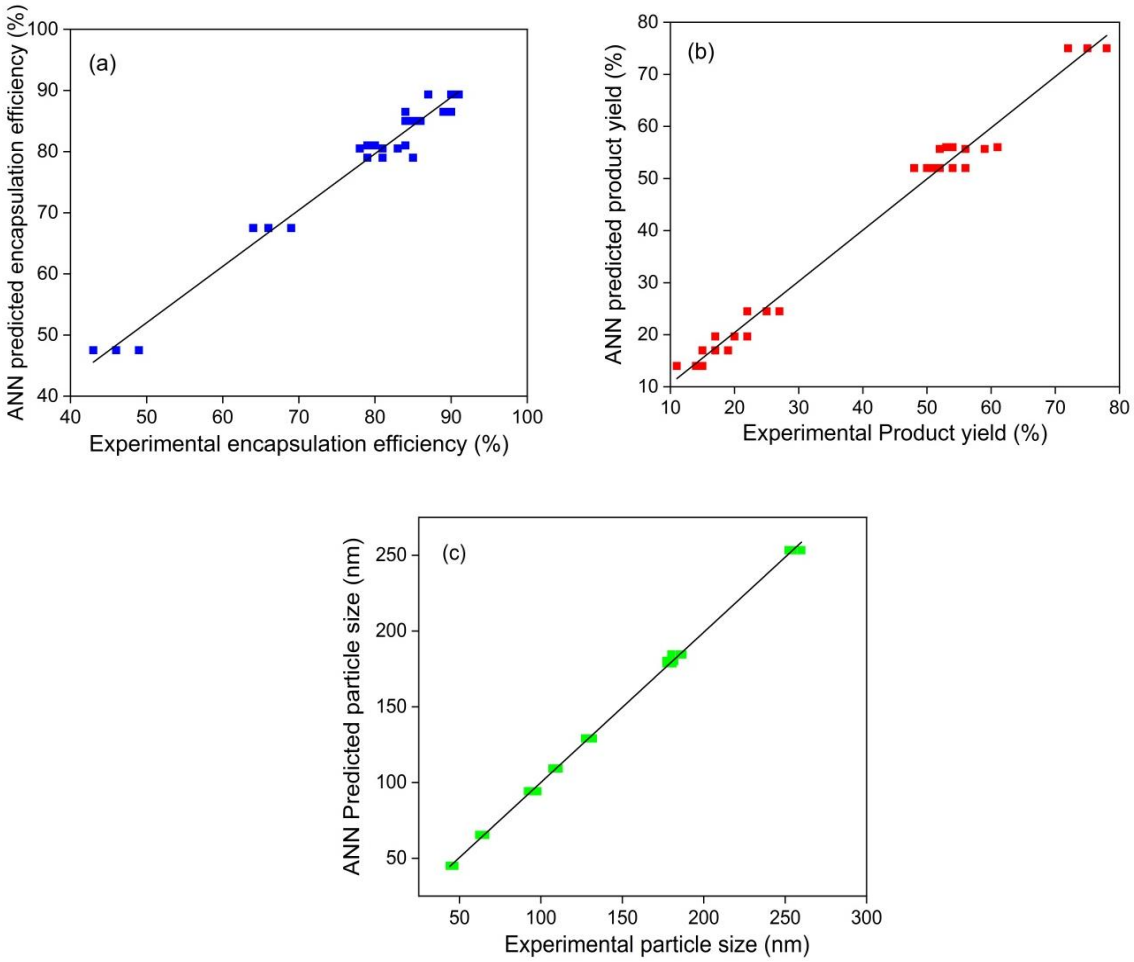

Figure 5.5: Plot of experimental vs. predicted outputs from combine ANN model (a) Encapsulation efficiency (b) Product yield (c) particle size.

5.5. Interpolation and extrapolation

The study was extended to evaluate the interpolation and extrapolation ability of developed models. In the interpolation and extrapolation study, to observe the effect of any individual

process parameter, all others parameters were held constant at an optimized condition. Observations for optimized conditions were taken from table 5.1. The predicted results obtained from interpolation are in agreement with thermotical understanding. However, much of the limitations were observed for extrapolation.

The parameters of the encapsulation process studied in experimental work (Sangolkar et al. 2019) are considered with wide range to evaluate the interpolation and extrapolation ability of the developed ANN network. The outputs are evaluated using 'simulate' wizard in 'nntool' in terms of encapsulation efficiency, product yield and particle size.

5.5.1 Prediction of effect of peppermint flavour and GA concentration

The flavour is an important parameter in food processing. However, it is observed that flavour of the food gets reduces in many food processing steps. Hence, flavour compounds are encapsulated to make-up the lost flavour. The range of core and shell concentration was chosen taking into account the thumb rule, that shell to core ratio should not go beyond 60:40.

The range of flavour concentrations considered in the experimental investigation was 1, 1.5 and 2 %. To observe the effect of low and high flavour concentrations, ANN simulations were performed for different flavour concentration as 0.5, 1.25, 1.75, 2.5, 3 and 5 wt. %. The increase in flavour concentration from 0.5 to 5 % resulted in an increase in encapsulation efficiency from 74 to 81.11 % as depicted in figure 5.6 (a). The increase in encapsulation efficiency can be attributed to an increase in flavour concentration. The marginal increase in encapsulation efficiency might be due to marginal increase in flavour concentration. The similar trend was observed for product yield. With increase in flavour concentration from 0.5 to 5 % there was an appreciable increase in product yield from 44.59 to 60.12 % (Sangolkar et al. 2019).

In an encapsulation system, the type of shell material used and its concentration are the major controlling factor for encapsulation efficiency. Furthermore, the shell materials have significant effect on rheological properties of the emulsion. Therefore, it is very much necessary to evaluate the effect of shell concentration on encapsulation efficiency and product yield. Effect of GA concentration for experimental study was observed at 20, 30 and 50 % wt. %. Therefore, it was decided to predict the output for GA concentration at 15, 25, 35, 45 and 55 %, as depicted in figure 5.6 (b). With an increase in GA concentration from 25 to 35 %, encapsulation efficiency increased from 49.34 to 81 %. This might have happened because of the higher concentration of GA available for encapsulation process. Due to which the shell gets formed easily around the core and thus, it causes an increase in encapsulation efficiency. These

observations are in agreement with the observations reported by Satpathy and Rosenberg (2003). However, further added GA remains unused in encapsulation process

For the considered range of shell material concentration decrease in product yield from 57.33 to 15.64 % was obtained. This trend might have occurred because viscosity of solution increases with shell concentration and higher concentration increases viscosity and stickiness, giving rise to clogging and wall deposition (Chong and Wong (2015)).

5.5.2. Effect of temperature and feed flow rate to spray dryer

Drying is an important step in an encapsulation process to obtain the product in the form of free-flowing powder (Fernandes et al. (2016)). The experimental data was available only for 150 -170 ºC. Hence the ANN predictions were carried out for large range of temperature conditions (130 - 185 ºC). The temperature range was chosen considering the phase change parameters of the emulsion mixture. It was observed from predicted results that encapsulation efficiency and product yield increase with an increase in temperature for considered temperature range. The increase in encapsulation efficiency and product yield occurs with temperature because of facilitated water evaporation from droplets and therefore helps to decrease the water content (Gharsallaoui et al. (2007); Costa et al. (2015)). However, from experimental observation and theoretical understanding, the decrease in encapsulation efficiency and product yield was expected for temperature above 160 ºC because at very high temperatures, drying occurs rapidly on the outer surface of the particle than inner surface. This phenomenon drives the release of flavour. Hence, encapsulation efficiency and product yield are expected to decrease. Thus, an observation from predicted results is inconsistent with intuitive understanding when correlated with experimental results. This outlines the constraint of developed ANN networks in extrapolation study.

The feed flowrate to the spray dryer is also a crucial parameter in the drying process. It will control the residence time available for drying and in turn will have impact on the drying characteristic. Considering this theoretical understanding the range of flowrate to atomizer was selected. Further, to investigate the effect of feed flowrate, it was varied from 1- 10 mL/min. This range was selected considering the time required for drying. Observations revealed that increase in flow rate to spray dryer from 1 to 10 mL/min encapsulation efficiency decreases from 89.25 to 51.22 % and product yield decreases from 75 to 9.1% respectively. As the flowrate increases, the residence time of emulsion in the spray dryer decrease and short residence time at a higher flow rate leads to inefficient drying.

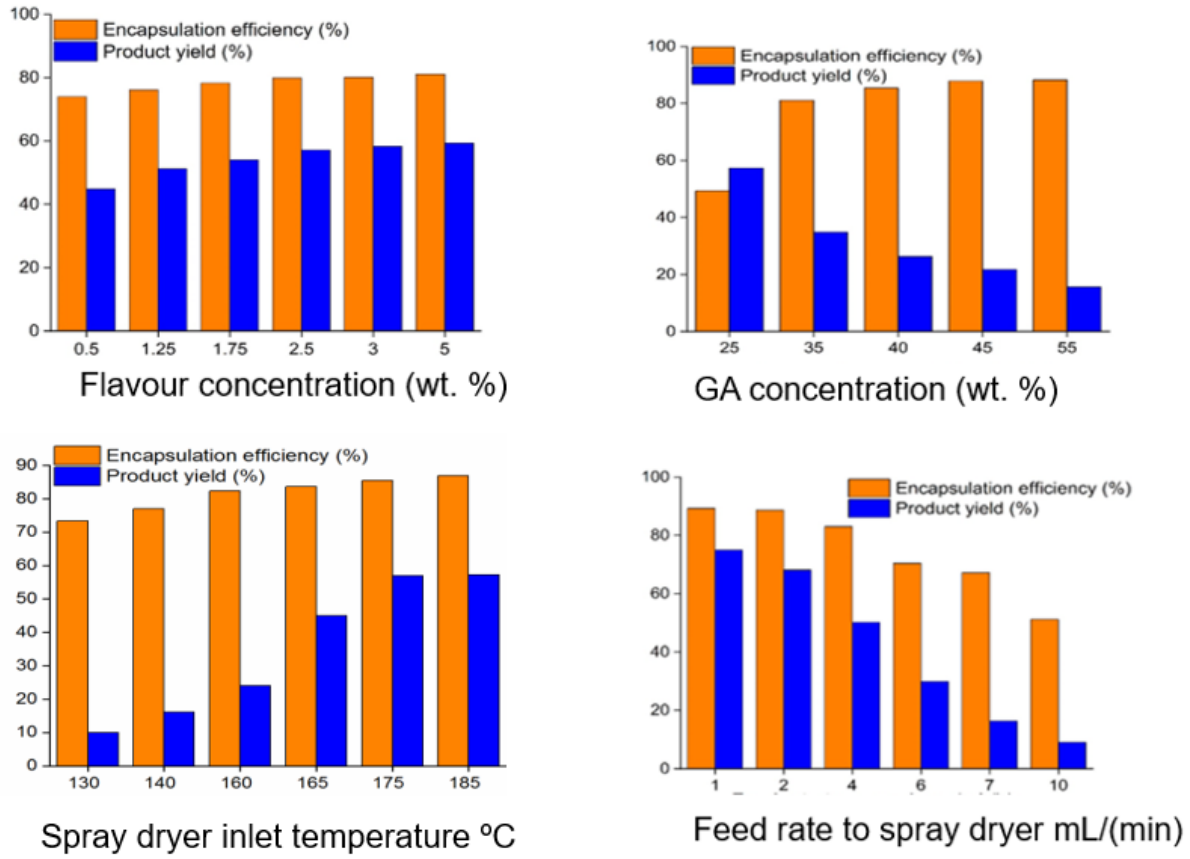

Figure 5.6: Prediction of effect of various input parameters on encapsulation efficiency and product yield on (a) Peppermint flavour (wt. %), (b) GA (wt. %), (c) Inlet temperature to spray dryer ºC (d) Feed rate to spray dryer (mL/min)

5.5.3. Effect of encapsulation process parameters on particle size

Particle size is a key feature in the food the sector. The small size particle results in easy availability of bioactive compounds in opposition to their counterpart (Tang et al. (2012)). This section explains the effect of all the encapsulation process parameters on the particle size of the product. The simulated results are plotted in figure 5.7 (a and b). Increase in peppermint flavour concentration from 0.5 to 5 % causes the particle size increment from 77 to 230 nm. These observations are in inlined with experimental observation and can be attributed to the effect of sonication (Sangolkar et al. (2019)).

With increase in GA concentration, the viscosity of emulsion increases, and viscous solutions form an obstacle in the drying process (Yanjun et al. (2004)). Hence it can be observed from figure 5.7 (a) that particle size got increased linearly from 69 to 207 nm for the corresponding increase in GA from 25 to 55%. This theoretical knowledge helps to correlate between ANN simulated results with experimental results of particle size.

A spray dryer is commonly used to dry semi-solid solutions to achieve particles in nano-micro size. Therefore, it is crucial to understand the effect of spray dryer temperature on particle size. The decrease in particle size is observed from 280 to 21 nm for the increase in temperature from 130 to 185 °C. The decrease in particle size with an increase in spray dryer temperature can be attributed to retarded agglomeration of particles at a higher temperature. Also, at higher temperature, particles are dried away at a higher rate as compared to a lower temperature and hence particle size decreases (Chegini and Ghobadian (2007)). Further, increase in feed flow rate from 1 to 10 mL/min causes exponential decrease in particle size from 465 to 19 nm. At lower feed rate the aggregation of the emulsion on the walls of drying chamber occurs because of slow evaporation. Hence, particle size is more.

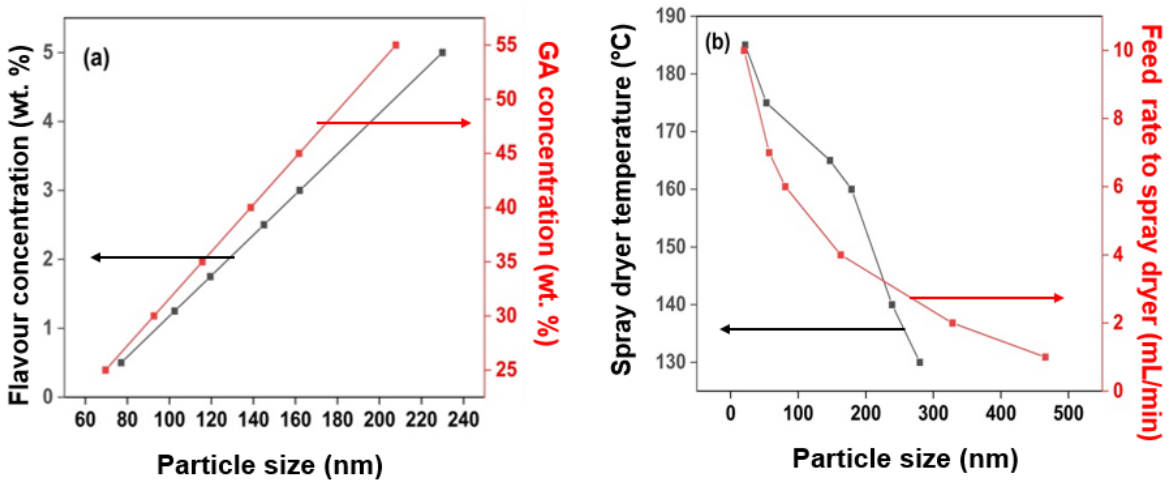

Figure 5.7: Effect of process parameters on particle size (a) Flavour concentration and Gum arabic concentration (b) Spray dryer temperature and feed rate to spray dryer

5.6 Summary

In this work, ANN models were developed to understand its ability to predict the encapsulation process. To develop ANN network, ANN tool in MATLAB was employed. Out of the tested combinations of transfer function in two consequent layers, the prediction performance of tansig-purelin transfer function was more accurate as compared to other two combinations. The same observation was drawn for individual model as well as combine model.

The developed ANN models are capable of predicting the existing experimental data. Further the study was extended to observe the prediction performance of developed networks for the case of interpolation. The obtained results illustrates that the models are suitable for interpolating data on encapsulation efficiency, product yield, and particle size in a consistent

manner with experimental results. Developed ANN model is suitable for extrapolation of flavour concentration, GA concentration and feed flow rate for all the input parameters. Nevertheless, extrapolation results obtained for unknown inputs of spray dryer temperature are inconsistent with theoretical understanding. Conclusively, the encapsulation process of bioactive compounds in food processing can be optimized using ANN approach without massive experiments.

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Chapter 6

Encapsulation and release studies of encapsulated ginger oil

6.1. Introduction

The most fascinating fact about encapsulation lies in its ability of controlled release. Controlled release is the process in which active ingredient breaks the core-shell structure and moves from one environment to other molecular environment in a food and into the surrounding saliva (Silva et al. 2012). In this chapter the controlled release profile of ginger oil encapsulated in GA with ultrasound approach were studied.

The controlled release studies were carried out in phosphate buffer solution (PBS) in order to mimic the gastrointestinal tract conditions. The study was carried out to observe the effect of different emulsion formation conditions and PBS on release profile of ginger oil.

6.2. Materials and methods

6.2.1. Materials

Buffer tablets of pH 3.0, 4.0, 7.0 and 9.2 were procured from S D fine chemicals limited (SDFCL) Mumbai India. All the buffer solutions were prepared using distilled water. Freshly prepared encapsulation solutions were used to evaluate controlled release profile of ginger oil from GA shell.

6.2.2. Experimental set-up for controlled release study

Encapsulation of ginger oil in GA: To study the controlled release of encapsulated ginger oil different emulsions were prepared with the ultrasound assisted encapsulation approach as described in chapter 4. In brief, aqueous solution of GA was prepared in distilled water with mechanical agitation at 40 ºC. This GA solution was then subjected to sonication, to which ginger oil was added dropwise to form an emulsion. To study the release profile, different emulsions were prepared with the variation of GA concentration as 10, 20 and 30 wt. % and sonication time of 10, 20 and 30 min. The range of shell material concentration in the complete solution was chosen considering the thumb rule that core to shell ratio should not exceed 1:4. The details of parameters of emulsion used in the present study are described in the next section of this chapter.

Controlled release study in phosphate buffer solutions

The pH of food products can be as low as 2.0 (for lemon juice) and as high as 7.96 (for egg white). Dissolution media (phosphate buffer solutions) were chosen to represent the pH values of fluids found in the gastrointestinal tract. Therefore, characterization of release kinetics of encapsulated ginger oil was conducted using phosphate buffers of pH 3.0, 4.0, 7.0 and 9.2. To prepare the PBS solution, phosphate buffer tablets of respective pH were used. 1 tablet in 100 mL will form the buffer solution of respective pH. Accordingly, the 1000 mL solution of each pH mentioned above was prepared.

The typical dissolution apparatus used in pharmaceutical drug release studies was used to study the release profile of encapsulated ginger oil from core-shell structure. The experimental set up used in the present study is shown in figure 6.1 below.

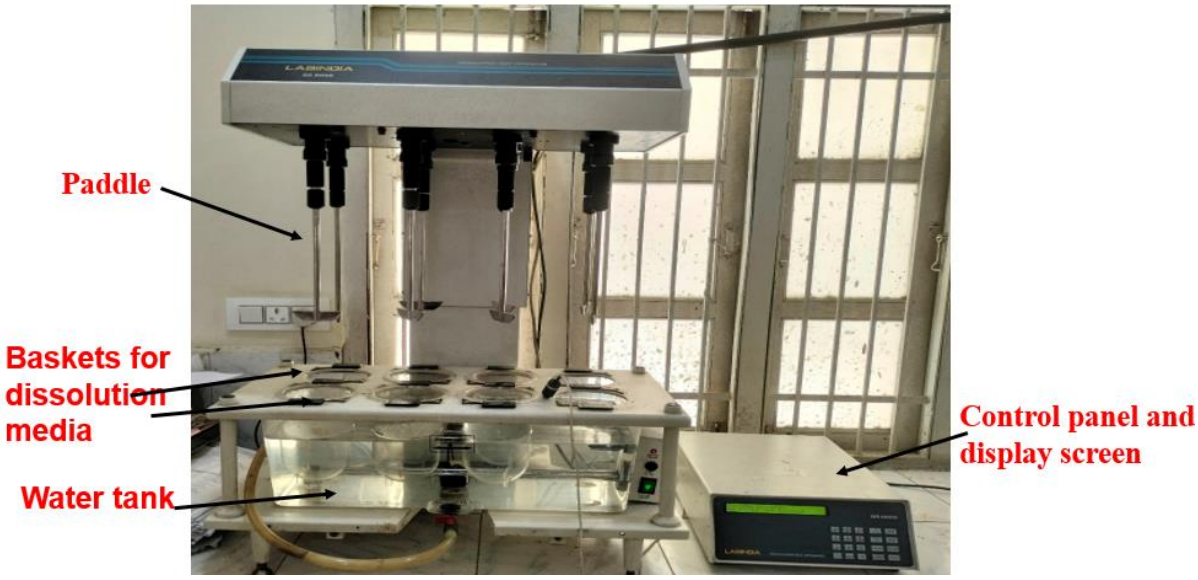

Figure 6.1: Dissolution apparatus: Experimental set-up for release study

Experimental set-up consists of six baskets to hold the dissolution media surrounded by water tank to maintain the temperature of dissolution media. There is an arrangement for paddle to enter in each basket and to mix the dissolution content uniformly. The volume of each basket is 1000 mL. Initially, all the baskets were washed with water and then rinsed with respective buffer solution. Further, 900 mL of prepared PBS solution of different pH was added to each basket. The baskets of the dissolution method were immersed in a water bath. The dissolution medium within the vessels were heated to 37 °C with an acceptable difference of ± 0.5 °C. The paddles in the basket were set for rotating. The care was taken that paddle will rotate smoothly with no wobbling and no vortex will form when the paddle is turning.

6.3. Estimation of cumulative release of ginger oil

To establish release profiles of encapsulated ginger oil 10 mL of emulsion (ginger oil in aqueous GA) was added to each basket. The estimation of release was done according to the method described by Xiao et al. (2011), with slight modifications. At a desired time, 100 mL of sample was withdrawn from each basket followed by supplementing the corresponding fresh PBS to the remaining suspension. 10 mL hexane was mixed with the 7 mL sample obtained to extract ginger oil by vertexing. Upper hexane phase after clear separation into two phases was measured for absorbance at 282 nm. The flowchart of this process is depicted in figure 6.2

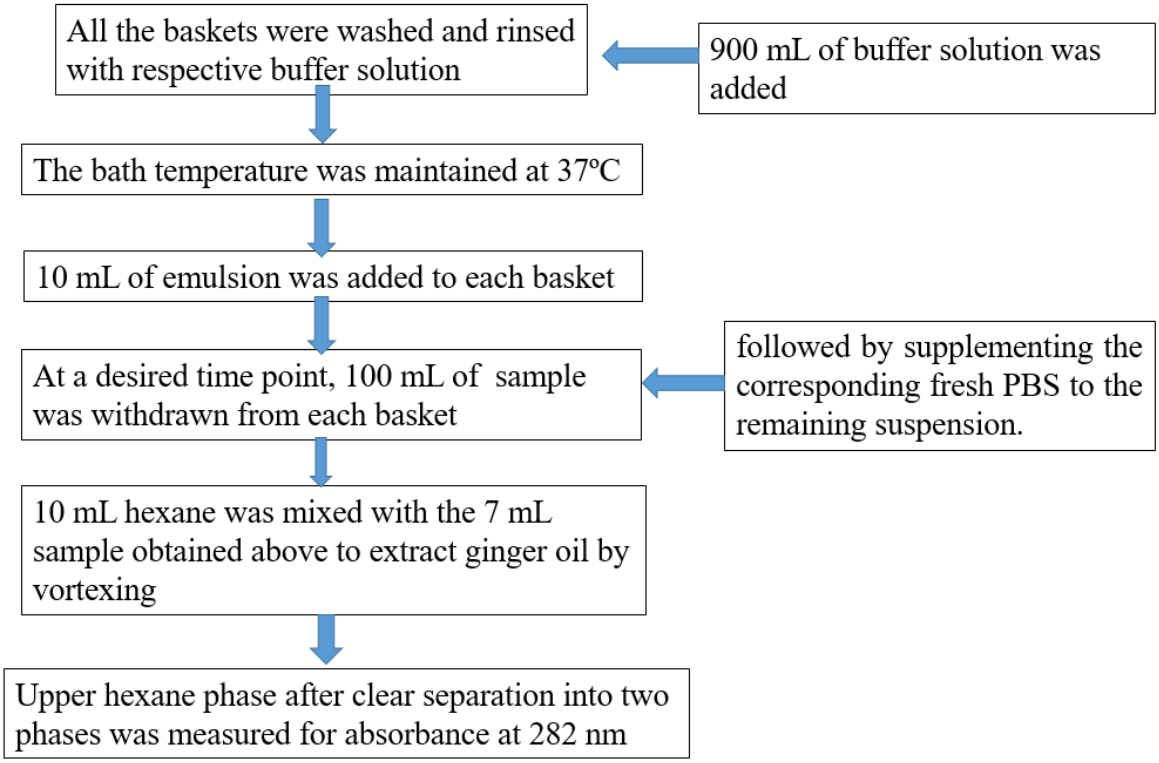

Figure 6.2: Flowchart of the process for release study with dissolution apparatus.

The cumulative release of ginger oil was calculated by the following equation.

$$R_{ti}(\%) = \frac{\sum_{n=1}^{i-1} a_n + 9a_i}{U_o} \times 100 \qquad (6.1)$$

Where,

Rti (%) = Cumulative release of ginger oil at time ti

i = Time of sampling

ai = Concentration of ginger oil measured with UV absorbance at the sampling time t

Uo = Theoretical 100 % release from 10 mL of emulsion

Determination of 100% theoretical release of ginger oil from encapsulation

Uo was determined separately by dissolving 10 mL of emulsion in 900 mL of 70% v/v aqueous ethanol solution. The prefactor 9 results from derivations based on mass balance and reflects the sampling practice when 100 mL of the supernatant was taken from a total volume of 900 mL.

6.4. Results and Discussion

6.4.1. Effect of pH on release of encapsulated ginger oil

The pH of the solution in gastrointestinal tract varies from highly acidic to neutral (Fallingborg 1999). Hence, PBS solutions of pH 3.0, 4.0, 7.2 and 9.2 were prepared. The release of freshly prepared emulsion of ginger oil with 4 wt. %, GA of 20 wt. % concentration sonicated for 20 min was used. In each basket, containing PBS solution of different pH, 10 mL of the emulsion was added and the % cumulative release of ginger oil PBS was measured using the procedure explained in section 6.2. The parameters of emulsification and different pH condition maintained are mentioned in table 6.1 and corresponding cumulative release (%) is give in table 6.2.

Table 6.1: Parameters of emulsion preparation used to observe the effect of pH on release profile.

pH of dissolution media	Ginger oil wt. (%)	GA wt. (%)	Sonication time (min)
3.0	4	20	20
4.0	4	20	20
7	4	20	20
9.2	4	20	20

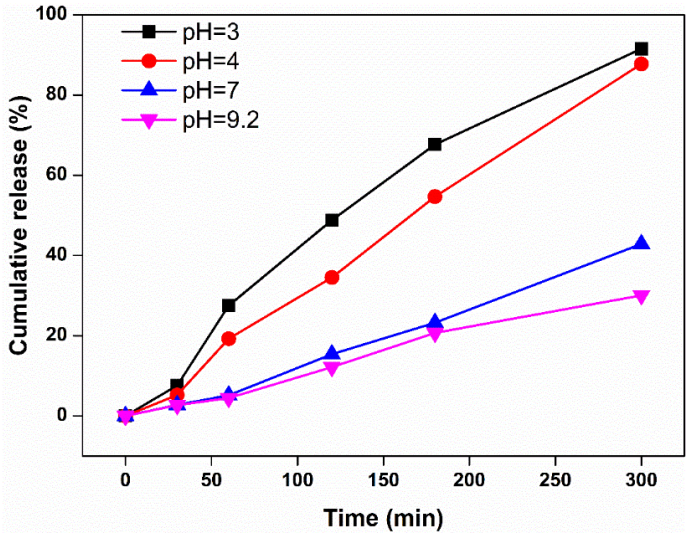

Figure 6.3: Effect of pH on cumulative release of ginger oil

Table 6.2: Cumulative release (%) of ginger oil from encapsulated in GA shell at different pH

pH	Cumulative release (%)					
	0 min	**30 min**	**60 min**	**120 min**	**180 min**	**300 min**
3	0.00	30.00	60.00	120.00	180.00	300.00
4	0.00	7.62	27.50	48.81	67.65	91.50
7	0.00	5.26	19.28	34.51	54.66	87.74
9.2	0.00	2.77	5.15	15.38	23.23	42.85

The cumulative release profile of encapsulated ginger oil performed over a period of time to observe the effect of pH on controlled release is depicted in figure 6.3. The gradual release of ginger oil was observed at all pH. However, faster release of ginger oil was observed at a lower pH (pH 3.0 and 4.0). With pH 3.0, 50 % of the release occurred in less than 125 min. The time required for 50 % release with pH 4.0 increased to 180 min. However, for both the pH of acidic conditions (pH= 3.0 and 4.0) more than 90 % of the release occurred in 300 min. The faster release in acidic pH can be attributed to higher solubility of ginger oil in more acidic solution This observation is in agreement with the observation reported by Chen & Zhong (2015) for release of peppermint oil from zein/GA complex. The similar observation was also reported by Hwang et al. (2013). Whereas the cumulative release of ginger oil for neutral and basic PBS solution was 42 % and 30 % respectively in 300 min of observation period. This accounts less than 50 % oil release at higher pH of PBS solution. Thus, it is clear that ginger oil will have controlled release in acidic environment as compared to basic and neutral environment.

6.4.2. **Effect of GA concentration on release of encapsulated ginger oil**

It is well understood from the previous experiments carried out to encapsulate ginger oil that GA forms a shell-like structure surrounding the ginger oil in the core (Sangolkar et al. 2019). It is also observed from Taguchi analysis and explained in chapter 4 that concentration of GA has impact on the encapsulation efficiency. This clearly indicates that GA concentration in encapsulation will also drive the controlled release profile. Therefore, it is essential to observe the effect of GA concentration on release profile. To observe the effect of different GA concentration on release, three different encapsulations were prepared with 4 wt. % of ginger oil and 30 min sonication time. The GA concentrations in emulsion preparation were varied as 10 wt. %, 20 wt. % and 30 wt. % of the total solution. The release profile of these prepared emulsion with variation GA concentration was investigated in PBS solution of pH 3.0. The

parameters of emulsification and pH condition maintained are mentioned in table 6.3 and corresponding cumulative release (%) is given in table 6.4.

Table 6.3: Parameters considered in the study to observe the effect of GA concentration on release profile

pH of dissolution media	**Ginger oil wt. (%)**	**GA wt. (%)**	**Sonication time (min)**
3.0	4	10	20
3.0	4	20	20
3.0	4	30	20

The GA concentration will have effect in forming the shell around the ginger oil droplets. In general, higher the GA concentration, shell will form around the easily (Yeo and Park (2004)). It is also interesting to note that thickness of shell is also largely affected by the shell concentration. The thickness of the shell surrounding the core is less at 10 wt. % of GA, which increases with increase in GA wt. %. Lower the thickness of the shell, it is easy to break the shell wall due to rotating motion of paddles causing faster release. Another reason for this trend can be due to faster dissolution of thin core-shell morphology in acidic medium. Therefore, it was observed that more than 90 % of cumulative release occurred in less than 200 min and above which gradual release occurred reaching 100 %. At higher GA concentration (30 wt. %) initial slope of release curve is very less which increased significantly above 120 min. This occurs because initially the thick shell was has not broken completely and release occurs from the ruptured section of shell. This thick shell might have broken completely at higher time and release rate increased significantly after 200 min.

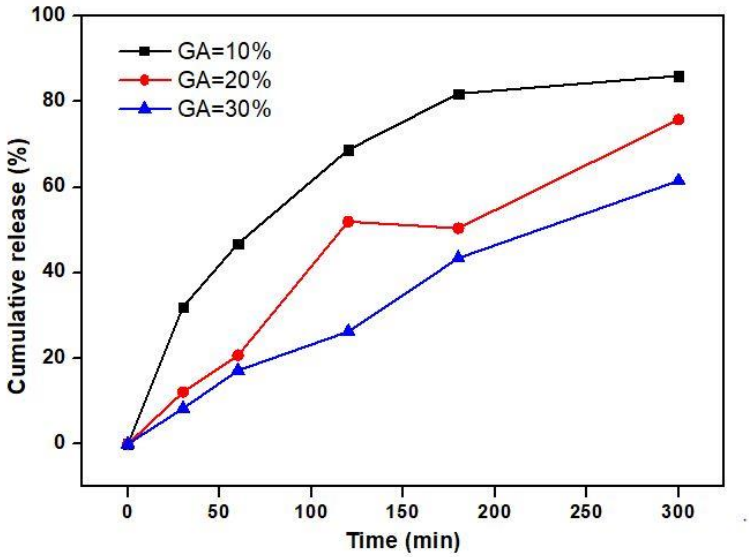

Figure 6.4: Effect of GA concentration on cumulative release of ginger oil at pH 3.0

Table 6.4: Cumulative release (%) of ginger oil from encapsulated in GA shell at different GA concentration (wt. %)

GA (wt. %)	Cumulative release (%)					
	0 min	**30 min**	**60 min**	**120 min**	**180 min**	**300 min**
10	0.00	32.04	46.81	68.73	81.85	86.00
20	0.00	12.12	20.73	51.96	50.42	75.81
30	0.00	8.31	17.19	26.27	43.42	61.50

6.4.3. Effect of emulsification time on release of encapsulated ginger oil

Sonication time is major factor in emulsion preparation (Sivakumar et al. 2014; Jiang et al. 2009). As the sonication waves passes through the medium it breaks down the droplets of ginger oil (dispersed phase). Smaller the size of oil droplet, smaller will be the size of core-shell structure in encapsulation. Therefore, it was observed that with increase in sonication time the size of core-shell structure decreases. Thus, to observe the effect of sonication time on release profile, three different emulsions were prepared with 4 wt. % ginger oil concentration, 20 wt. % GA concentration and sonication time was varied as 10 min, 20 min and 30 min. The release profile of these prepared encapsulation was observed at pH 3. The parameters of emulsification and different pH condition maintained are mentioned in table 6.5 and corresponding cumulative release (%) is give in table 6.6.

Table 6.5: Parameters considered in the study to observe the effect of GA concentration on release profile.

pH of dissolution media	Ginger oil wt. (%)	GA wt. (%)	Sonication time (min)
3.0	4	20	10
3.0	4	20	20
3.0	4	20	30

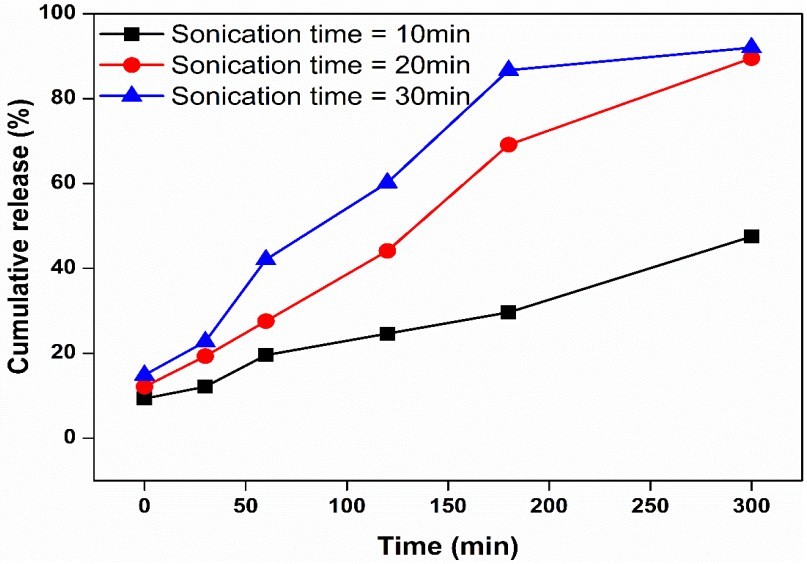

Figure 6.5: Effect of emulsification time on cumulative release of ginger oil at pH 3.0

Table 6.6: Cumulative release (%) of ginger oil from encapsulated in GA shell at different emulsification time (min).

Emulsification time (min)	Cumulative release (%)					
	0 min	30 min	60 min	120 min	180 min	300 min
10	9.35	12.12	19.58	24.62	29.65	47.50
20	12.12	19.35	27.58	44.12	69.12	89.50
30	14.88	22.77	42.08	60.15	86.69	92.00

This phenomenon of size correlation with sonication time is responsible in determining the release profile of encapsulated ginger oil from core shell structure. At lower sonication time the release rate of ginger oil was slower and reached upto 47 % cumulative release in in 300 min. The slower release for the emulsion samples sonicated for 10 min is because of higher particle size. Because at lower sonication time, sufficient energy is not supplied to break the

oil droplets. Therefore, higher size oil droplets give rise to large size of encapsulated particles in the emulsion. Hence, it was observed that for the encapsulation prepared with 10 min sonication time, the slope of release profile is very less and less than 50 % of cumulative release occurred in 300 min of release study. Whereas at higher sonication time 20 min and 30 min slope increased rate 69 % release occurred in 120 min which was 87 % release in 120 min for 30 min sonicated sample. And in 300 min of observation period, 90 % of oil got released for 20 min and 30 min sonicated sample. The observation on controlled release to study the effect of sonication time in emulsion preparation indicates that with smaller particles it is easy to make bioavailability of encapsulated active ingredient (Fontana et al. 2001; Yamauchi et al. 2007).

6.5. Summary

Controlled release is an important and attractive phenomenon considering encapsulation. Therefore, the controlled release study is necessary to perform to understand the release behavior of encapsulated active ingredient. Out of different mechanisms proposed in the literature to observe the controlled release of encapsulated active ingredient, in the present study we have successfully demonstrated the controlled release of ginger oil in phosphate buffer solutions of different pH. The study conducted to observe the effect of pH of PBS indicated that encapsulated ginger oil has faster release in acidic environment as compared to neutral and basic conditions. Further, the study to observe the effect of GA concentration revealed that at lower GA concentration shell breaks easily and controlled release is fascinated. However, when this observation correlated with the observation to see the effect of GA concentration on encapsulation efficiency which indicates that encapsulation efficiency increases with GA concentration. Hence, it is necessary to carefully select the GA / shell concentration during encapsulation process. Sonication time above 20 min is beneficial both to increase encapsulation efficiency and also to provide controlled release.

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Chapter 7

Novel Ultrasonic Atomization Approach for the Synthesis of Sunflower Oil in Water Emulsion.

7.1 Introduction

The alternative approach for incorporating bioactive compounds in food matrix is through emulsification. Basically, emulsions are thermally unstable systems, where droplets of one phase are uniformly dispersed in another continuous phase. In particular, emulsions used in food industries, not only contributes in imparting a mouthfeel and taste to food products but also helps to improve the physical form (appearance, texture, colour) of food and thus takes the attention of consumers (Torrico 2015). The application products of emulsion in the food sector are not limited to mayonnaise, milk, beverages, sauces salad dressings, gravies, peanut butter, ice cream and other whipped dessert toppings.

In the present work we have attempted to synthesize sunflower oil (Helianthus annuus) in water emulsion. It is non-volatile oil, extracted from seeds of sunflower triglyceride. The nutritional components of sunflower oils are linoleic acid. palmitic acid, oleic acid stearic acid. Sunflower oil has several health benefits such as; It is good for heart functioning, oleic acid present in it boosts the blood lipid and coafulant activity. Linoleic acid takes part in building new cells and tissues of the body which is helpful for boosting and maintaining the immune system. Vitamin E contributes in inhibiting the free radical oxidization of body cholesterol (Anushree et al. 2017).

The synthesis of oil-in-water emulsion with ultrasound atomization is discussed in this chapter with effect of different emulsion parameters on the droplet size distribution. The droplet size analysis is also supported with optical microscope image analysis to support the observations.

7.2 Experimental section

Emulsions have significant contributions in many industries like pharmaceutical, cosmetic, home care products, paint and food. Among such a wide application sector, an emulsion in food industries is fascinating and necessarily used to deliver bioactive compounds. Many researchers have attempted to find the best approach to obtain stable emulsion (Tadros 2013; Mcclements 2007). High energy emulsification approaches such as ultrasound, high-pressure homogenization are always preferred over low energy approaches like phase inversion for emulsification. Many scientific reports support the fact that with high energy approach it is

possible to obtain an emulsion that is stable for a long duration (Jasmina et al. 2017). However, the energy consumption in such techniques is high and this issue needs to be addressed (Schultz et al. 2004; Taha et al. 2020). Therefore, in the present work, we present novel, energy-efficient ultrasonic atomization approach for emulsification.

The literature present to date has focused on the use of ultrasonic atomization of different liquids and the parameters associated with these liquids. However, there are very few reports available stating the application of atomization for emulsification in the food sector. Burton et al. 2014 considered an ultrasonic atomization approach for emulsification of vegetable oil and water mixture and obtained stable emulsion but the synthesized emulsions were applied as cutting fluid in micro-milling operation. In the present work, we have reported the use of ultrasonic atomization for emulsification and its application in the food sector.

7.2.1 Materials

Sunflower oil and Tween 20 ($C_{58}H_{114}O_{26}$) surfactant were purchased from Swastik Acids and Chemicals, Nagpur, India. Throughout the experiments distilled water was used as an aqueous phase. For the preparation of emulsions Tween 20 (HLB =16.7) was used as a surfactant. Ultrasound (Dakshin Ultrasound Mumbai operated at 190W power, with 20 KHz frequency was used to prepare coarse emulsion and ultrasonic atomizer (Sonic Vibra cell model: CV25, serial no. 201204081) was used for atomization of coarse emulsion.

7.3 Ultrasonic emulsification

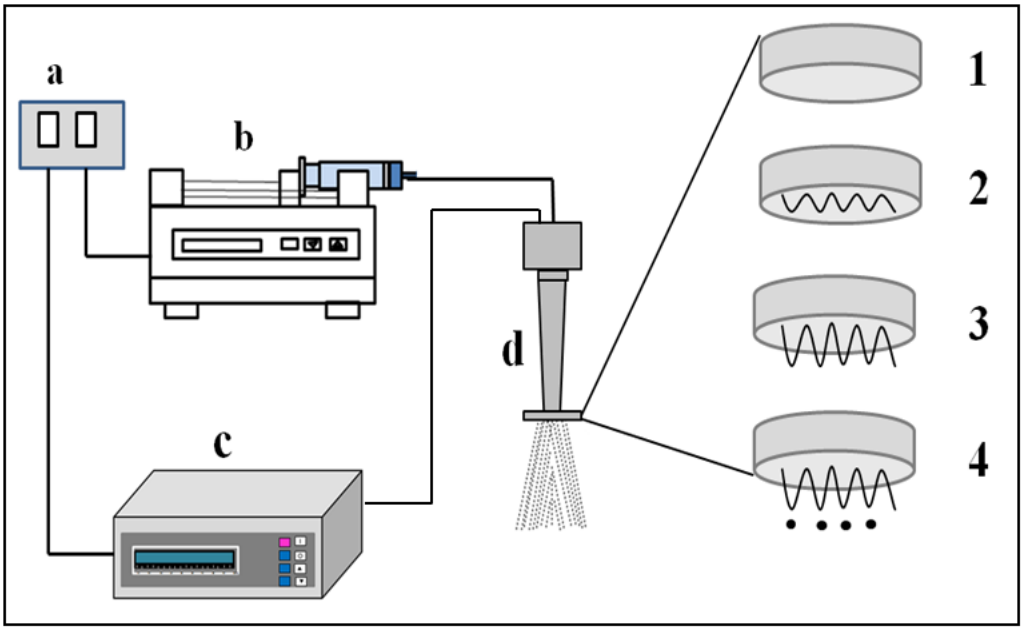
7.3.1 Mechanism of emulsification through ultrasonic atomization

Ultrasonic atomization is preferred in applications where fine control on droplet size is required. There are no moving parts in the atomization system; hence supplied electrical energy is used only to transmit to a piezoelectrically vibrating disk. Hence it is considered as a more energy-efficient approach. The mechanism for tiny particle synthesis through ultrasonic atomization is shown in figure 7.1. Atomization using ultrasound is based on two principal hypotheses, namely Capillary wave hypothesis and the Cavitation hypothesis. The capillary wave hypothesis assumes that capillary waves consist of crests and troughs formed on the vibrating surface. Cavitation hypothesis considers the generation of tiny droplets by virtue of cavitation (cavity formation, growth and intense collapse). Zhang et al. (2021) and Dalmoro et al. 2012 reported that steady capillary waves and low cavitation intensity contribute to narrow droplet size distribution.

In brief, the phenomenon of atomization can be explained as below: When liquid passes through a piezoelectric element, a thin film of liquid is formed. During the vibrating motion

of sonication waves perpendicular to liquid film, liquid film absorbs the vibrational energy. With the absorption of more amount of energy, the crest of these waves becomes taller and trough becomes deeper. Critical amplitude reaches whereat the amplitude of the capillary waves is above that requires sustaining the stability. As a result, the waves collapse, and tiny drops of liquid are ejected from the tops of the degenerating waves normal to the atomizing surface (Deepu et al. 2018 and Tang et al. 2012).

In ultrasonication with probe reactor, the supplied vibration energy is used to break droplets of the dispersed phase and it does not break the droplets of the continuous phase. But during ultrasonic atomization, the mixture is broken into tiny droplets in the air Burton et al. 2014.



(a) Switch Board (b) Syringe Pump (c) Ultrasound Generator (d) Probe containing Piezoelectric transducer

1. Tip of ultrasonic atomizer probe 2. Capillary waves formed on tip surface

3. Waves reached critical amplitude (unstable waves) 4. Mist formation from the surface

Figure 7.1: Schematic of experimental set-up ultrasonic atomization study

Synthesis of sunflower oil in water emulsion

In the present work, attempt was made to synthesize sunflower oil (Helianthus annuus) in water emulsion. Sunflower oil is non-volatile oil, extracted from seeds of sunflower triglyceride. The nutritional components of sunflower oils are linoleic acid, palmitic acid, oleic acid stearic acid. Sunflower oil has several health benefits such as; It is good for heart functioning, oleic acid present in it boosts the blood lipid and coagulant activity. Linoleic acid takes part in building new cells and tissues of the body, which is helpful for

boosting and maintaining the immune system. Vitamin E contributes in inhibiting the free radical oxidization of body cholesterol (Anushree et al. 2017).

The emulsion mixture contains water as an aqueous phase and sunflower oil as a dispersed phase. A water-soluble surfactant Tween 20 (HLB = 16.7) was used to reduce surface tension between two immiscible phases. Initially, a surfactant was added to distilled water and stirred with heating at 40°C to obtain transparent solution. To prepare the emulsion, an aqueous phase was subjected to sonication using ultrasound probe reactor operated at 20 kHz, to which oil phase was added and the mixture was sonicated for 5 min to obtain a coarse emulsion. Different coarse emulsions were obtained by changing the concentration of the oil phase and surfactant concentration.

7.3.2 Ultrasonic atomization of coarse emulsion

The coarse emulsions obtained from the ultrasound probe reactor were subjected to ultrasonic atomization. The coarse emulsions were passed with a syringe pump to the inlet of the atomizer as shown in figure 7.1. Different emulsions were synthesized by varying the ultrasound atomization frequency and flowrate to atomizer. Effect of atomization frequency is evaluated at 20 kHz, and 40 kHz. The flowrate to atomizer are varied as 0.83 mL/min, 1.66 mL/min, 2.50 mL/min and 3.33 mL/min.

7.4 Emulsion characterization

7.4.1. Droplet size characteristic of emulsion

The droplet size of an emulsion is one of the measures for emulsion stability. Hence it was decided to measure the droplet size of all the atomized samples. The droplet size analysis was carried out using a Malvern droplet size analyser (Zetasizer Nano S90 version 7.02). The accuracy to measure the droplet size is a function of the degree of scattering in the emulsions. Therefore, the samples were diluted carefully using distilled water to prevent multiple scattering from emulsion droplets.

7.4.2. Optical microscopy analysis

The distribution of droplets was observed using a light microscope armed with a digital camera. The microscope was operated in transmission mode. Olympus BX53 images were taken using a micro publisher 5.0RTV camera mounted on the microscope attached to system. For the visual observation, nearly 5µL of emulsion sample was taken on the glass slide. To observe the emulsion with high clarity, the sample stage was moved in X and Y direction opening of the light aperture was adjusted. All the samples were analysed at room temperature.

7.5 Results and discussion

Droplet size is the best measure for deciding the quality of an emulsion. The emulsions with small droplets size and narrow droplet size distribution are more stable for a longer period of time. In contrast, droplets with larger size suffer from common problems of agglomeration, coalescence, Ostwald ripening (Urbina-Villalba 2009; Hunter et al. 2008). Because, smaller sized droplets of dispersed phase in continuous phase forms solution of uniform viscosity, whereas the large sized droplets of dispersed phase have tendency of phase separation giving non-uniform viscosity to emulsion. Hence all the prepared emulsions were characterized with droplet size analysis and microscope images. It should be noted that two emulsions with the same average droplet size can have different droplet size distributions. Hence microscopic observation is necessary along with the droplet size analysis to describe emulsion. The effect of process parameters such as oil phase concentration, surfactant concentration, atomization frequency and feed flowrate to atomizer on droplet size is well described in this section.

7.5.1 Effect of oil phase concentration

Four different emulsions were prepared by varying the oil phase concentration as 1 %, 5 %, 10 % and 20 %. For the studied range of oil concentration, viscosity of oil increased as 0.00135, 0.0033, 0.00556 and 0.01047 Pa.S for 1, 5, 10 and 20 % oil concentrations respectively. From the analysis, it was clear that droplet size is a strong function of the viscosity of an emulsion. The average droplet size increases from 16 µm to 107 µm with the rise in oil concentration from 1 % to 20 %, as depicted in figure 7.2. The higher droplet size for highly viscous emulsions is obtained because for the viscous solution, it is difficult to spread emulsion on the entire atomizing surface, therefore, atomization takes place from a smaller area with relatively high operating intensity. These observations are inlined with previously reported literature (Ramisetty et al. 2013). Also, it is observed that the droplet size distribution curve for 20 % oil concentration has two peaks. An appearance of a second peak might be due to the formation and ejection of secondary droplets after detachment of one droplet. The formation of the secondary droplets can be attributed to the effect of cavitation. During cavitation each droplet of liquid absorbs gas in it and grows over a number of cycles, at crtitical atomization frequency, the vibration frequency of bubble is equal to atomization frequency at which bubble collapse intensely forming tiny droplets. This indicates that all the droplets are not same in size. This observation is consistent with the observation from optical microscopy. The particle density per unit surface area also increases with an increase in oil concentration.

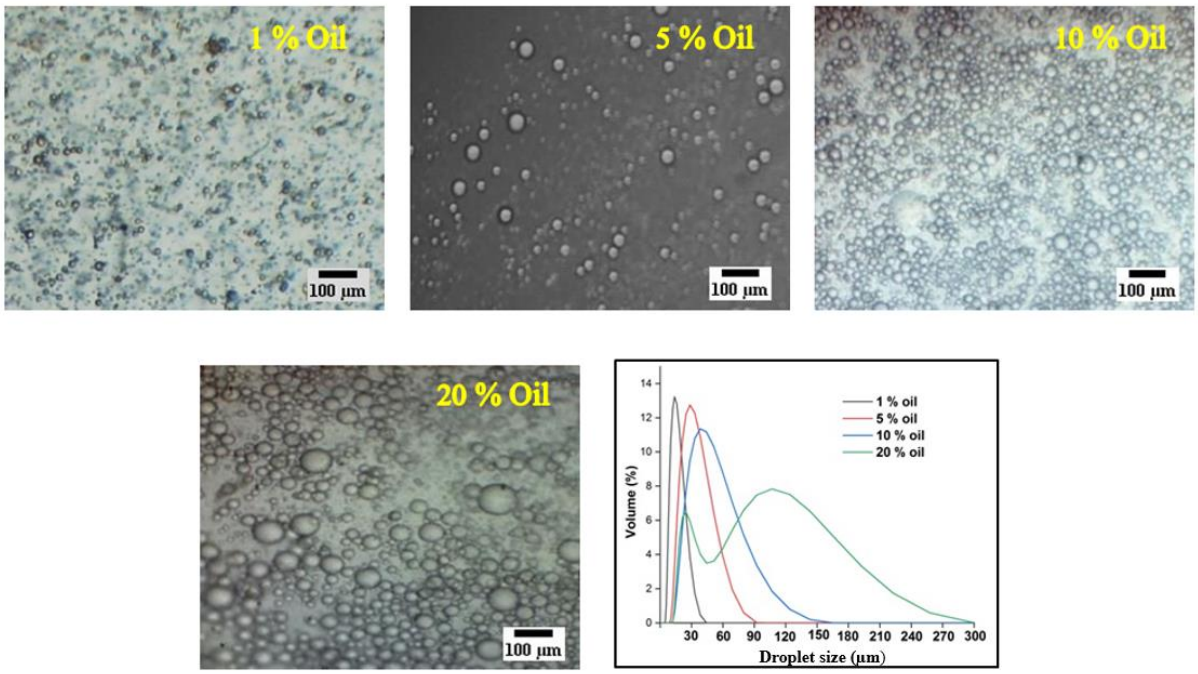


Figure 7.2: Effect of oil concentration on droplet size at 40 kHz frequency, 1 % surfactant concentration and at 1.66 mL/min flowrate.

7.5.2 Effect of surfactant concentration on droplet size and distribution

Surfactant is one of the key parameters in the synthesis of an emulsion. Many investigations demonstrate that surfactant concentration strongly affects the stability of an emulsion. An added surfactant helps to occupy the interface between the two phases and to decrease the surface tension between two phases. It is observed that when surfactant concentration increases from 0.5 % to 1 %, the droplets are more separated, showing less tendency of aggregation. However, after 2 % surfactant concentration, the added surfactant is not used in the micelle formation and shows a negative effect. The particle begins to aggregate at 2 % concentration of surfactant concentration giving instability to emulsions. The observation from microscopy analysis is also in good agreement with droplet size analysis, as shown in figure 7.3. Thus, the increase in droplet size takes place at higher surfactant concentration due to the rapid coalescence of droplets (Rajan and Pandit et al. 2001).

At 0.5 % surfactant concentration phases begin to separate in the first 10 minutes, showing instability of emulsion at low surfactant concentration. Here, the emulsion is unstable due to inadequate surfactant to reduce oil-water interfacial tension. Raising the surfactant concentration to 2 % substantially improved the emulsion stability. Because more surfactant adsorbs at the interface between the oil and water phase. This observation is also supported by microscope images and drop size distribution. However, increasing surfactant concentration to

5 % yielded unstable emulsion. At high surfactant concentration, large number of droplets can be formed. However, destabilization of emulsion takes place because of rapid coalescence between droplets. Furthermore, higher surfactant concentration also increases the viscosity of the emulsion which is not preferable in emulsion, as it obstruct mass transfer rate because of increasing interfacial resistance. These observation of effect of surfactant concentration on emulsion are similar to the observations reported by Jusoh N. and Othman (2016) where they studied effect of surfactant concentration in the range of 1 to 7 % in the preparation of water-in-oil emulsion in liquid membrane prospect.

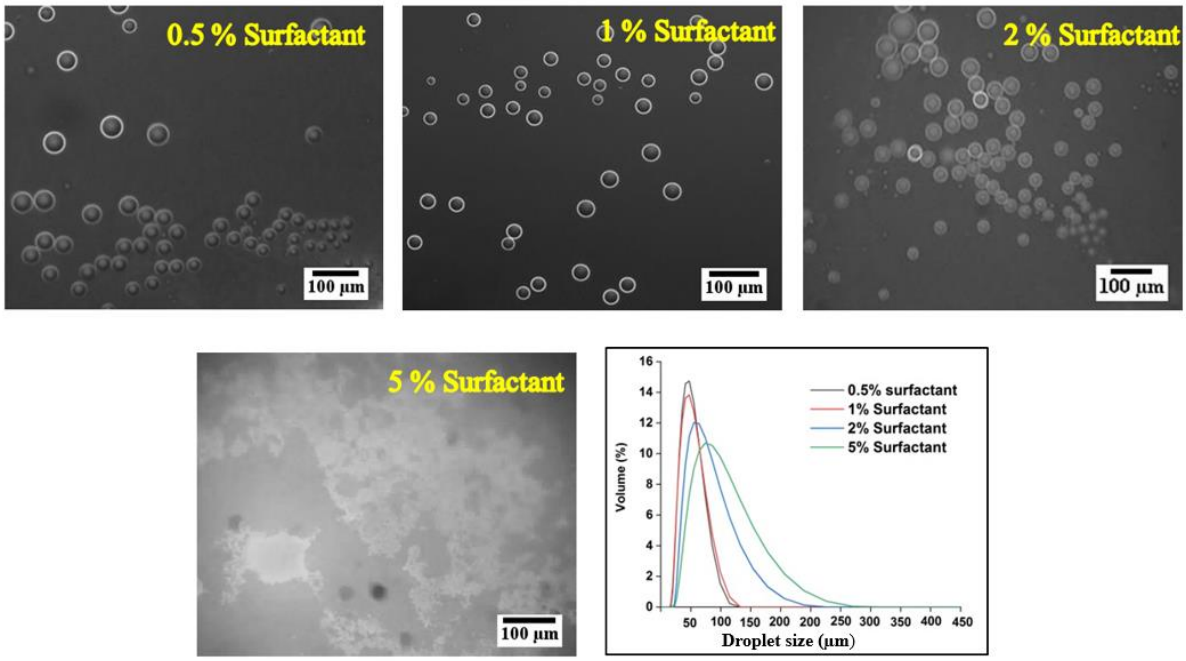


Figure 7.3: Effect of surfactant concentration on droplet size distribution of an emulsion at 5 % oil concentration, 40 kHz frequency of ultrasound atomizer and at 1.66 mL/min flowrate.

7.5.3 Effect of atomizer frequency on droplet size and distribution

To observe the effect of an atomizer frequency, it was varied as 20 kHz, 40 kHz and 130 kHz. It can be seen from figure 7.4 that the corresponding average droplet size for this variation in frequency was found to be 65 µm, 32 µm and 17 µm respectively. The decrease in droplet size with an increase in atomization frequency can be explained on the basis of the capillary wave hypothesis. As the atomization frequency increases, compression in crest and trough points occurs and results in decreased surface area for atomization. The atomizing mixture gets exposed to an increased number of compression phases in the cycle resulting in a reduction in the rate of the crest growth and a corresponding decrease in the droplet size. Another reason for the decrease in size with an increase in frequency can be explained as the

threshold liquid flowrate required to cover the atomizing surface increases with an increase in the frequency (Kudo et al. 2017).

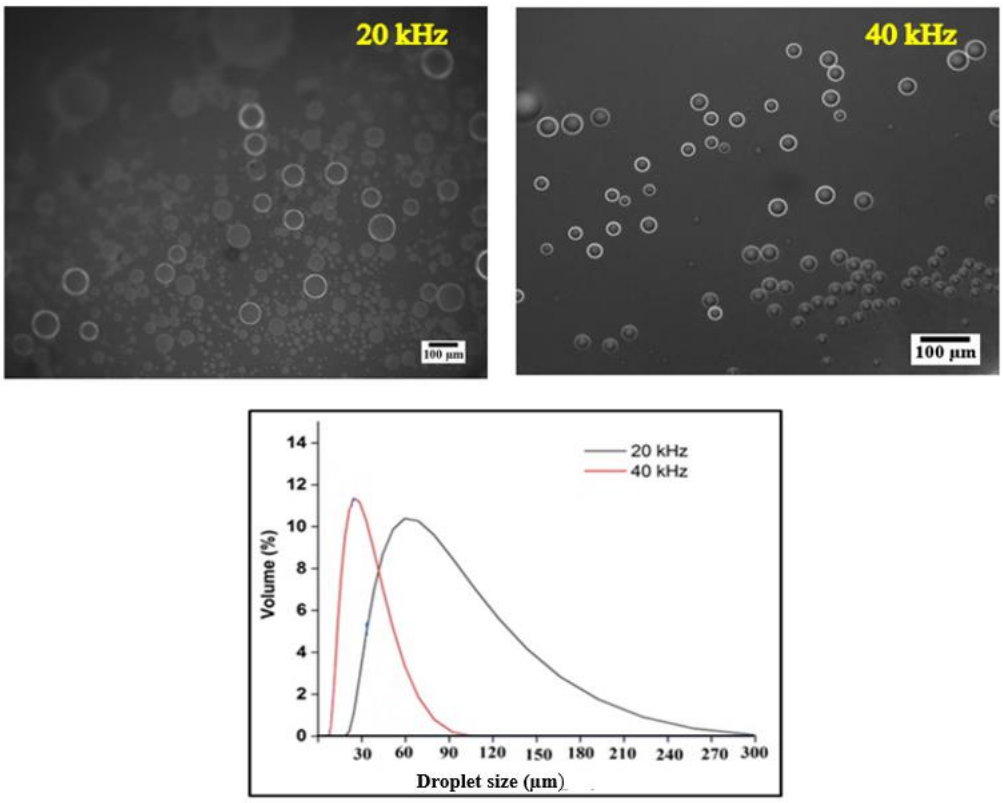


Figure 7.4: Effect of ultrasound atomization frequency on droplet size at 5 % oil concentration, 1 % surfactant concentration and at 1.66 mL/min flowrate.

7.5.4 Effect of flowrate on droplet size and distribution

It can be observed from Fig. 5 that, with the increase in inlet flowrate to atomizer from 0.83 mL/min to 3.33 mL/min the droplet size increased from 17 µm to 45 µm. The reason for this trend can be attributed to an increase in the liquid film thickness on the vibrating surface before actual atomization. The thick film formed causes the deformation of capillary waves making their irregular vibration, when the vibration of these waves is irregular, the droplet size distribution becomes wider. Hence the particle distribution curves for 2.50 mL/min and 3.33 mL/min flowrates are observed to be double-peaked with wide droplet size distribution. Microscopic analysis carried out to observe the droplet size and shape is also agreement with this discussion. For higher flowrates, some of the droplets are very small and some droplets drifted without atomization are found to be of higher size (d'Apuzzo et al. 2013).

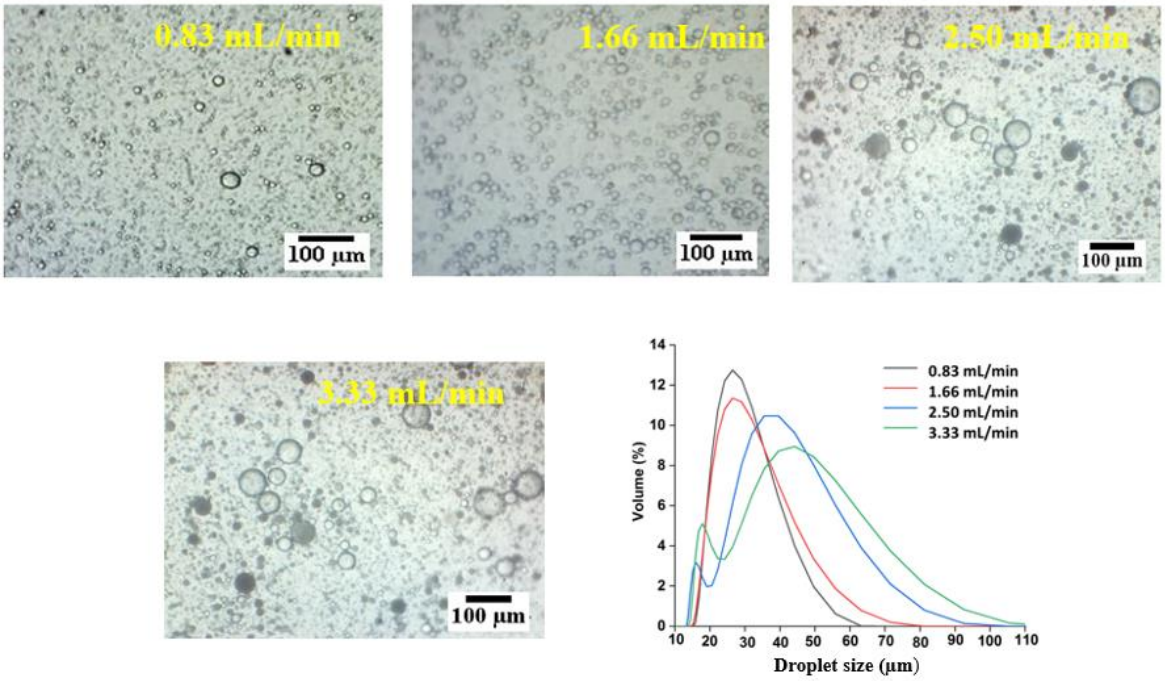


Figure 7.5: Effect of flowrate to atomizer on droplet size at 40 kHz, 5 % oil concentration and 1 % surfactant concentration

7.6 Summary

Ultrasound atomization is an energy-efficient approach for emulsification. Two principal mechanisms for ultrasonic atomization are based on the capillary wave hypothesis and cavitation hypothesis. Droplet size analysis and microscopic observation are the key techniques to assess the quality of the emulsion. The droplets with lower size and narrow distribution are more stable over and preferred in emulsion science against their contrast part of higher droplet size emulsion. The properties of the fluid being atomized have major contribution in determining the droplet size distribution. An increase in oil concentration increases the viscosity of solution hence atomization from the vibrating surface is hindered, causing an increase in droplet size. The increase in surfactant concentration was helpful to stabilize the emulsion however, after 2 % of emulsifier concentration, the rapid coalescence occurs resulting in higher droplet size. The effect of atomization parameters like atomization frequency and flowrate to an atomizer was also evaluated. The increase in atomization frequency was useful to decrease the droplet size. With an increase in flowrate to the atomizer, thickness of film on vibrating surfaces increases. The thin liquid film generates droplets of smaller droplets with narrow distribution and thick film generates wide droplet size distribution. Thus, ultrasound atomization is proved as a novel and efficient approach for the synthesis of sunflower oil in water food emulsion. Overall, it can be concluded that the stability of emulsion composition

such as oil concentration, surfactant concentration and atomization parameters such as atomization frequency and flowrate to atomizer are the major parameters in deciding the quality of emulsion.

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Chapter 8

Conclusion and Future Scope

8.1 Conclusions of the research work

The investigation was carried out to explore ultrasound approach for the extraction of ginger oil from the *Zingiber Officinale root.* Effect of different extraction parameters and sonication parameters was studied in. The methanol was found to be the best extracting solvent due to high polarity and low viscosity, complete extraction occurred in 30 min, beyond which the increase in sonication time has very negligible effect on extraction yield. The increases in solvent helped to increase extraction yield, 1:10 was found to be the optimum feed to solvent ratio. As the applied sonication power increases, the rate of compression and rarefaction cycles increases and hence extraction yield also increased. 9.65 g of oil/100 g of ginger powder was obtained at above optimized conditions.

Further, the encapsulation of ginger essential oil was successfully demonstrated in GA carrier with ultrasound approach. The parametric optimization of encapsulation process was done using integrated Taguchi analysis. Maximum of 82 % encapsulation efficiency, 81 % product yield was obtained at 7 % ginger oil, 20 % GA concentration, 10 min sonicated sample 170 °C 1mL/min flowrate to atomizer. Integrated Taguchi and ANN approach found to be effective in deciding the percentage contribution of individual input parameters on output.

The study was extended to observe the effect of wide range of input parameters for which experimental outcomes are not available. The study of peppermint flavour encapsulation performed in our research group was considered for this purpose. ANN models developed to study interpolation and extrapolation illustrated that a combination of tansig-purelin transfer function has good prediction of output for the known combination of input and output parameters. Also, these models were found to be effective in interpolation and extrapolation of results. The obtained results were in agreement with experimental trend as well as theoretical understanding. However, few limitations were observed for extrapolation when correlated with theoretical understanding for the case of temperature effect on encapsulation efficiency. The next objective of the research was demonstrated the controlled release profile of encapsulated ginger oil. Out of different mechanisms proposed in the literature to observe the controlled release of encapsulated active ingredient, in the present study we have successfully demonstrated the controlled release of ginger oil in phosphate buffer solutions of different pH. The study conducted to observe the effect of pH of PBS indicated that encapsulated ginger oil

has faster release in acidic environment as compared to neutral and basic conditions. More than 90 % cumulative release occurred in 300 min. Further, the study to observe the effect of GA concentration revealed that at lower GA concentration shell breaks easily and controlled release is fascinated. This shows that encapsulation has controlled release property as an asset to use in food processing steps. 20 min sonication time and 20 wt. % GA concentration were found to be effective in giving cumulative release over 80 % in 200 min.

Further, the present work also described ultrasonic atomization as a novel approach for the synthesis of oil-in-water emulsion. The mechanism of atomization with ultrasound is explained in detail. Emulsion quality is largely assessed with particle size. Smaller the particles, higher the stability and vice versa. Hence effect of different parameters on particle size of emulsion was investigated. It was observed that with increase in oil concentration from 1 to 20 %, the thickness of liquid film increases which leads to increase in particle size. The increase in surfactant concentration from 0.5 – 1 % contributed to decrease the particle size, however further increase in surfactant concentration resulted in agglomeration of particles. The increase in atomization frequency from 20 - 130 kHz is beneficial in decreasing particle size from 65 - 17 µm. The increase in flowrate to atomizer increases the particle size. Microscopic observations for all parameters are in agreement with the results obtained from particle size analysis.

8.2 Recommendations and future scope

Based on the conclusion drawn from all the investigation and literature available till dates following recommendations are made for the future work.

- Encapsulation process of combination of different active ingredients with detail process parameter should be studied. In order to meet the requirement of bioactive food preservative and flavouring agent in food processing.
- The comparison of prediction performance of different machine learning approaches for food processing should be studied.
- Controlled release of encapsulated active ingredient with other release mechanisms mentioned theoretically in the literature should be practically exploited.
- The application of ultrasound atomizer for emulsification should be studied in detail with effect of all process parameter, to explore as low energy emulsification approach
- Hydrophobic and hydrophilic interaction of emulsion component should be investigated.

Outcome of the research

Publications in Reputed Journals:

1. **Potdar S. B**., Bhanvase B. A., Saudagar P., Potoroko, I., and Sonawane S. H. (2021). ANN-based modelling of peppermint flavor encapsulation process with ultrasound approach. The Canadian Journal of Chemical Engineering. doi.org/10.1002/cjce.24283.

2. **Potdar S.B.,** Saudagar P, Potoroko I, et al. Recent Advances and Reports on Encapsulation in the Food Matrix: A Review. Current Pharmaceutical Biotechnology. 2022 May. doi: 10.2174/1389201023666220513105654. PMID: 35570555.

3. **Shital Potdar**, Uday Bagale, Irina Potoroko, Vikas S. Hakke, Yadagiri Maralla, Manickam Sivakumar and Shirish Sonawane, "Sonochemical Approach for the Synthesis of Safflower Oil Based Low Fat Emulsion: Effect of Ultrasonic Parameters" Materials Today Proceedings- doi: 10.1016/j.matpr.2021.12.232.

4. Bagale Uday, Tsaturov Aram, Potoroko Irina, **Potdar Shital,** and Sonawane Shirish "In-vitro evaluation of high dosage of curcumin encapsulation in palm-oil-in-water, nanoemulsion stabilized with a sonochemical approach," Karbala International Journal of Modern Science: Vol.8: Iss.-1 , Article 9. doi.org/10.33640/2405-609X.3205.

5. **Shital B. Potdar**, Prakash Saudagar, Irina Potoroko, Shirish H. Sonawane "An integrated Taguchi and ANN approach for the ultrasonic encapsulation of ginger oil in gum arabic: Process optimization" status: Under review- Chemical Papers.

Book chapters:

1. **Potdar S. B.**, Landge V. K., Barkade S. S., Potoroko I., and Sonawane S. H. 2020. Flavour encapsulation and release studies in food In: Shirish Hari Sonawane Bharat A. Bhanvase Manickam Sivakumar (Eds.) Encapsulation of Active Molecules and Their Delivery System (pp.293-321) In print: Elsevier, ISBN: 978-0-12-819363-1.

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Conference Publication:

1. 7th International Scientific Conference **'Food products: chemistry, rheology, technology' CFSCRT 2021**. Organized by Ministry of Science and Higher Education of the Russian Federation; Russian Academy of Sciences; Novel Ultrasonic Atomization Approach for the Synthesis of Oil in Water Emulsion" held on 24-25 June 2021: Oral Presentation.

2. **International Chemical Engineering Conference 2021 (ICheEC 2021)** – 100 Glorious Years of Chemical Engineering & Technology, Department of Chemical Engineering, Dr. B. R. Ambedkar NIT Jalandhar "Sonochemical approach for the synthesis of safflower oil based low fat emulsion: Effect of ultrasonic parameters" September 17 to 19, 2021.

3. **ESS-JSS-AOSS 1st joint Sonochemistry conference** "Intensified ultrasound-assisted extraction of ginger oil from *Zingiber Officinale Roscoe herb"* held on Nov 8-10, 2021.