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FORMULATION AND EVALUATION OF MICROEMULSION BASED ALLICIN FOR THE TREATMENT OF STAPH Infection

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ABSTRACT:

Microemulsions have several advantages over emulsions, such as low manufacturing costs, high solubility, good penetrating power, clarity and transparency, the ability to be sterilised through filtration, and thermodynamic stability over an extended length of time. One of the most important components in the creation of a microemulsion was surfactants. Combining surfactants and co-surfactants can enhance the dispersion of oil in water. The skin acts as an amazing barrier against bacterial infections. Many bacteria touch or reside on the skin, yet most of the time they are unable to create an infection. In the event that bacterial skin infections do develop, they can impact any area of the body, no matter how big or tiny. Depending on how bad they are, they could be harmless or even deadly. Bacterial skin infections can arise when bacteria enter the skin through hair follicles or microscopic skin breaks brought on by burns, sunburns, animal or insect bites, wounds, or pre-existing skin diseases. People are prone to developing bacterial skin illnesses following a variety of activities, such as gardening in contaminated soil or swimming in a contaminated pond, lake, or ocean. Antibiotics used topically are drugs used to treat bacterial infections.

Keywords: Microemulsions, Skin infections, Bacteria, Antibiotics, Co-surfactants

1.Introduction

In comparison to emulsions, microemulsions have a number of advantages, including thermodynamic stability over a long period of time, clarity and transparency, the capacityto be sterilized through filtration, low manufacturing costs, high solubility, and good penetrating power. Surfactants were one of the most crucial elements in the formation of a microemulsion [1]. The dispersion of oil in water can be improved by using co-surfactants and surfactants in combination. The skin serves as an impressive defence against infections caused by bacteria. Although several bacteria come into contact with or inhabit the skin, they often lack the ability to

produce an illness [2]. Bacterial skin infections can occur in any area of the body, ranging from a small area to the entire surface. These conditions can range from harmless to life-threatening, dependent on their intensity. Bacteria infection of the skin can occur when bacteria enter the skin via hair shafts or small skin openings produced by scrapes, punctures, operations, burns, sunburn, animals or insect-borne wounds, or already present skin diseases. Participating in certain operations, such as farming in soil that is polluted or bathing in a pond, reservoir, or ocean that has been polluted increases the likelihood ofindividuals acquiring bacterial skin illnesses [3]. Topical antibiotics are medications used topically to treat bacterial infections. Topical medicines can be administered to the skin with ease and at high concentrations to reach effective levels locally with low systemic harm. High local antibiotic concentrations that can be attained using topical preparations can aid in the eradication of bacteria in bacterial biofilms [4, 5]. Famous antibacterial, antifungal, antioxidant, antigiardial, and antidiabetic agentsinclude herbal extracts and essential oils. According to reports, several herbal ingredients, including essential oils and oil-based formulations, are effective antibacterial agents that can be utilised to stop food rotting.The potential of oil components to harm bacterial membranes and subsequently cause cell lysis may be the cause of the antibacterial activity.[6] Due of the antibacterial qualities of essential oils, aromatherapy has been utilised to treat significant skin conditions. These activities include antibacterial, antibacterial, anticancer, antimutagenic, antidiabetic, antiviral, and anti-inflammatory properties.[7] Therefore, an effort is made in the current work to examine the synergistic antibacterial effects for treating staph infection and research the effect of allicin to be prepared as microemulsion.

1.1 Drug resistance Antibiotics are medications that are utilized to assess and manage bacterial illnesses. Antibiotic-resistant arises whenever bacteria adapt in response to antibiotic therapy.[9].

ESKAPE Pathogens: In the year 2014, the WHO published its initial evaluation on the monitoring of microbes that are resistance to treatment. The report revealed that this issue is increasingly becoming a global problem, putting at risk our ability to effectively treat common diseases acquired in hospitals or communities. The pathogens of ESKAPE are a group of resistance to drugs gram-positive microbes that cause infectious disorders. This group includes six nosocomial pathogens: Enterococcus faecium, Acinetobacterbaumannii, and Enterobacter spp. These pathogens are both resistant to multiple drugsand highly virulent. They are becoming a growing concern for public health policies worldwide[10]. This term describes their capacity to circumvent the impacts of antimicrobial drugs or the absence of more modern and suitable medications [4]. Due to its ability to cause numerous and diverse infectious illnesses, as well as its enormous impact on mortality and morbidity, S. aureus appears to be the most crucial toxigenic bacterium within the Staphylococcus genus. These bacteria belonging to the gram-positive group are capable of flourishing in challenging environments and producing numerous types of antibioticresistance [11].The most notable factors include the production of Amp C-lactamase, the lowering ofporin channels, the development of extended-spectrum lactamase, and the alteration oftopoisomerase II and IV [12-14]. It is crucial to bear in mind that one strain can possessmany mechanisms for resistance, and any of these pathways can effectively counteracta broad spectrum of antimicrobials. The mechanisms of resistance in S. aureus havebeen associated with an elevated mortality rate in patients affected by this illness.Moreover, the growing and unselective demand for antimicrobial agents in medicalfacilities or by individuals who self-administer medication could possibly exposesusceptible individuals to bacteria that are resistant to many drugs. The decrease in theusage of antibiotics due to concerns about

susceptibility and associated toxicity has ledto a renewed focus onr search into phytotherapy [15]. Scientists are actively involvedin the exploration and assessment of antibacterial properties in specific plants as aregional substitute for pharmaceuticals and therapeutic approaches to address this issue.Most of them are indigenous to Peru or thrive in the Amazon region. However,it is probable that just under one percent of the many types have beenexamined for compounds that may have potential medical advantages [16].

Infections through Staphylococcus aureus: The staphylococcus aureus germs are the primary cause of staphylococcal infections in the skin. This may result in erythema, blistering, and formation of boils on your skin. Diseases can occur everywhere on your body, especially the skin of your face, but they are most commonly found around your nostrils and mouth. [17] 1.3.1 Staph infection: Staphylococcus aureus bacteria are responsible for causing infections caused by staph. Many individuals in good health often harbour these types of microorganisms on their bodies or within their nasal passages. These bacteria generally do not cause any problems or only lead to mild infections in the skin. Nevertheless, if the bacteria disseminate extensively within your body and infiltrate the bloodstream, bones, cartilage, hearts, infections caused by staph might result in fatality. An increasing number of people who are generally in good condition are becoming infected with potentially lethal staph infections. Treatment usually involves the use of medicines and the thorough washing of the affected region. Conventional antibiotics are no longer effective against some staph diseases or can contribute to the development of antibiotic resistance in particular illnesses. When attempting to treat antibiotic-resistant staph infections, doctors may need to utilize medications that have supplementary detrimental effects [18].

2. Material and Methodology

2.1Materials

List of Chemicals Used Through Study Along With Their manufacturers

CHEMICAL	**SOURCE**
Allicin	Garlic
Carbopol 71	CDH fine chemicals, India
PEG 400	Qualigensfine chemicals, India
Distilled water	Lab
Capmul MCM	Abitec Corporation, USA
Tween 80	CDH Fine Chemicals India
PEG-400	CDH Fine Chemicals India

List of Equipment Used Through outthe Study Along With Their Manufacturers

INSTRUMENTS	**SOURCE**
Magneticstirrer(5MLHDX)	RemiEquipmentsPvt.Ltd,India
Electronicweighingbalance	Mettler,Japan
Vortex	RemiCM-101cyclomixer,India
UVspectrophotometer(UV-1601)	LabindiaUV3200,India
Centrifuge	RemiR8CLaboratoryCentrifuge,India
Brookfieldviscometer	DVII+Pro,U.S
Abbe'sRefractometer	GuruNanakInstruments,India,SerialNo. 9522
pHmeter	(EutechpHTutor,EffemTechnologies,India)
Oven	WidsonsScientificwork,India
Particlesizeanalyzer	MalvernZetasizer, MalvernInstruments, Worcestershire,UK
TA-XT2TextureAnalyzer	StableMicroSystem,UK
DeepFreezer	Vestfrost, India
FranzDiffusion cell	RamaScientific, India

2.2Methodology:

Samplecollection:

The garlic was collected from the local market of Bareilly, near to Somics Lifesciences Pvt. Ltd. Bareilly**.**

Extraction of Allicin:

By combining 400 mg to10 mL of water and shaking for two minutes, allicin was extracted.

Purified allicin was extracted using the solid phase extraction (SPE) method from the aqueous garlic extract. The C18 SPE cartridge was utilised. Preformulationstudies:

Organoleptic properties: The organoleptic attributes encompass the visual look, colour, and odour of the medicinal element.

Melting point: The standard MP process, known as ClassIa, Apparatus I, involves placingthecapillarycontaining0.1 grammeofthe drugonto theheatingblock, which is settoatemperature5°Clowerthantheanticipatedmeltingpoint.Thetemperatureisthen increased gradually at a rate of 1 +/- 0.5 °C per minute until the drug has completely melted. The range of melt points is determined and recorded once the substance has completely melted.

UV-VisSpectral analysis:

Lamda max determination: The solution of 10 mg/ml of drug was prepared in methanol, and then incubated at room temperature in shaker for 24 hours. The samples were filtered and then absorbance was taken at different range from200 nm – 600 nm.

- **Standard calibration curve preparation:** 100mg/ml stock solution was prepared and thenthe workingsolution ofdifferent concentrations wereprepared and theabsorbance was taken at their Lamda max.

Drug solubilityin differentsolvents: 0.01 gram drugwas dissolved in 10 ml different solvents and then incubated at room temperature for 24 hours. Then the solutionwasfilteredandthenabsorbancewastakenatitsLamdamax..

Drug solubilityindifferentpH: 10 mg/ml stock solution was prepared and then the different pH was maintained by using HCl and NaOH, then incubatedat room temperaturefor24hours.Thenthesolutionwasfilteredandthenabsorbancewastakenat its Lamda max.

FTIR: FT-IR spectra of the samples will be acquired using an FT-IR instrument. The spectrum of the FT-IR will be acquired throughout the range of 400–4000 cm−1 with a magnification of 4.0 cm−1 and a total of 20 scans will be combined. Samples will be manufactured by thoroughly combining themwith KBr in an amount of1:100, and then forming them into KBr discs.

Preparationofmicroemulsion:

Acosurfactantandsurfactantratiowascreated,andacorrespondingmixturewas created. Allicinwasaddedtothemixture.Withtheaidofamagneticstirrer,each liquidwasfully blended to produce a homogeneous dispersion or solution. In this composition, double distilled water was employed to avoid incorporating surface- active contaminants.

Waterandambienttemperaturewereusedtotitratethemixturewhileitwascontinuously stirred. The amount of aqueous phase added when the combination reached the point where it became cloudy. Three different pseudo-phases' integratedpercentages were computed.

AllS/Coratioswerecalculatedusingthesameprocess.Followingthecalculationofthe proportion of each phase needed to createmicroemulsions, phasediagrams were created. The medicated microemulsions were created once the pseudo ternary phase diagram hadbeen prepared.

Table:Formulation of microemulsion

Formula No.	Stirring Time (min)	Percentage (%)				
		Allicin	Tween 80	PEG 400	Carbopol 71	Water (ml)
F1	20	10	10	5	0.3	100
F2	25	10	20	5	0.7	100
F3	30	10	30	5	0.9	100
F4	35	10	20	6	0.3	100
F5	40	10	30	6	0.7	100

Evaluation of microemulsion:

Physical appearance: The prepared microemulsions were examined for colour, uniformity, coherence, texture, as well as pH.

Refractiveindex: Thistriglycerinephysicalpropertyisdeterminedbytheangleatwhich alightbeambendsafterpassingthroughatinylayerofmeltedfat.Afewdropsofoilwere depositedononefaceoftheglassprismofarefractometer,whichwasthengentlyspread, closed, and tightened. The prism and oil are given enough time to reach a constant temperature. Next, a demarcation line was read to determine the refractive index.

Spreadabilty: Utilising two 7.5 cm long glass slides,spreadability was tested. One glass slide was filled with precisely 350 mg of Microemulsion. Another glassslide was positioned on top of it, 5 cm above the ground. After1 minute, the upper slide with the 5 g weight was removed, and the diameter of the circle that had extended in cm was recorded. The type of sample is shown by the detected diameter.

Viscosity: The temperature was measured precisely at 25±1 °C usinga Brookfield the viscometer.

Surface Morphology: The scanning electronmicroscope, also known as the SEM, will be used for assessing the surface shape of the most optimal microemulsion compositions.

Invitro drug release: Utilising a diffusion membrane, a drug release research was conducted in vitro. The phosphate buffer pH 7.4 microemulsion equivalent to0.2gm was put in the dialysis bag. Throughout the experiment, the diffusion was kept at 370.5°Cwith stirring at 100 rpm. Every 1, 2, 3, 4, 5, and 6 hours at predetermined intervals, 5 ml of sample was removed, and the same volume was
refilled with buffer solution to maintain the sinkstate.AUV spectro photo meter wasused to analyse the samples that were collected at maximum.

Stability studies: The microemulsion that was created underwent a stability trial for a duration of three weeks at ambient temperature.

Antimicrobialstudies:

Sub-culturing of bacterial strains: The pathogenic bacterial strains gram positive suchas*S.aureus*and*E.coli*weretaken.Initiallythepre-culturedplatesofpathogenswere revived by spreading and streaking in agar plates, and then the brothofthese pathogenic strains were prepared and used during various analyses.

Preparation of antimicrobial extract by solvent extraction: Firstly, the newly collectedplant material is washed, thenit is dried and cut into littlepieces or crushed. 50 millilitresofvariousorganicsolventshavebeenmeasuredinseparatecontainers,andthen 5 grammes of a fresh plant sample was put to each container. Subsequently, the material andsolventwereintroducedintoaSoxhletapparatus,whereextractingwasconductedfor a duration of 3-4 hours. Subsequently, the extracts were put into bowls that had been weighedbeforehand.Thebowlsweremeticulouslysealedwithaluminiumfoil,withsmall perforationsconstructedtofacilitatethedecompositionofsolvents inahot airovenset at 50℃.

Antibiogramanalysis: Sterilenutritionalagarmediawasmadeusingautoclavingand thenput20mlintoeverysterilepetriplate.Themediawasthenpermittedto gather.After solidifying, 20µl of the pathogen culture was dispersed onto plates labelled as S. aureus and E. coli. Afteradurationof3-4minutes,fourwellswithadiameterof8mmwerecreatedusing a sterile borer. Subsequently, 50µl samples were placed into each well. The bacteria on the plates were placed in an incubator and kept at atemperature of37℃ forthe duration of one night

Resultanddiscusion

Samplecollection: ThegarlicwascollectedfromthelocalmarketofBareilly, near to Somics Life sciences Pvt. Ltd. Bareilly.

Figure 1:garlic.

ExtractionofAllicin:

5gofrawgarlicwastakenandpeeledoff.MThegarlicwaswashedinsterilizedwaterand 2-5 minutes. The 5g garlic was crushed in 20 ml of distilled water and then incubated at 50℃for1hour.Allicinwasextractedusingthesolidphaseextraction(SPE)methodfrom theaqueousgarlicextract.AfterconditioningtheSPEcolumnwiththreecolumnvolumes (18mL)ofmobilephase(methanol:water50:50),thecolumnwaswashedwith6mLof methanol. Using the same mobile phase (methanol: water), allicin was eluted and collected. The eluted sample were allowed to air dried and stored for further use.

Extracted Allicin

Preformulationstudies:

Organolepticproperties ofallicin

Table:Organolepticpropertiesofextractedallicin

S. No.	Test	Outcome
1	Appearance	Powder
2	Color	Yellow
3	Meltingpoint	22

Lamdamaxdetermination:

Thesamples were filtered andthenabsorbance wastakenat different range from200nm – 400 nm. 220 nmwas detected asthe lamda max for allicin.

Table:Lamdamaxdeterminationoftheextractedallicin

S.No.	Wavelength(nm)	Absorbance
1	200	0.551
2	220	0.896
3	240	0.151

4	260	0.069
5	280	0.051
6	300	0.023
7	320	0.059
8	340	0.009
9	360	0.009
10	380	0.009
11	400	0.009

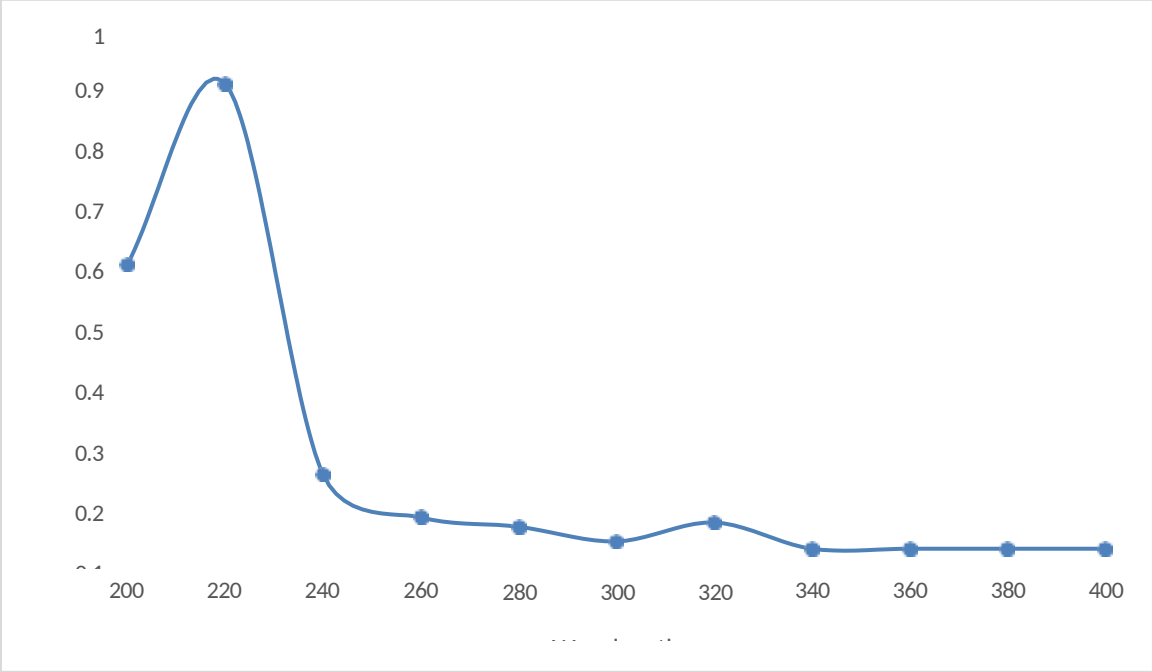


Figure 2:Lamd amaxestimation

Standard calibration curve preparation:

One prepared sample of all the drug was scanned for the absorbance wavelength of UV spectra and authentication. The absorbance wavelengths were found tobe 220 nm respectively. The calibration plots for, ALLICIN, was a straight line with the R^2 value of 0.9756.TheUVwasfoundtobesensitiveenoughtoanalyze1µg/mlofthesamples.Hence it was

employed in the solubility analysis as well asthe determination of drug content.

Table:Calibration curve preparation

S.No.	Concentration(ug)	Absorbance
1	10	0.012
2	20	0.045
3	40	0.069
4	60	0.154
5	80	0.198
6	100	0.252

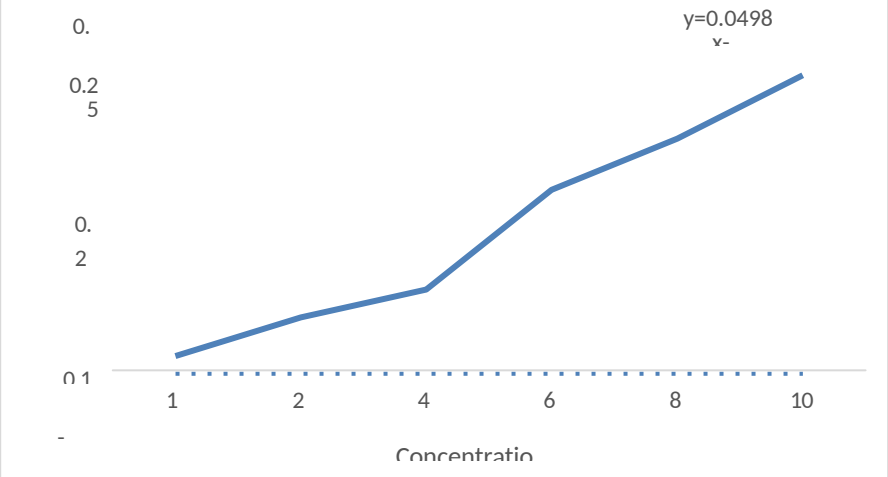


Figure3:Calibration curve

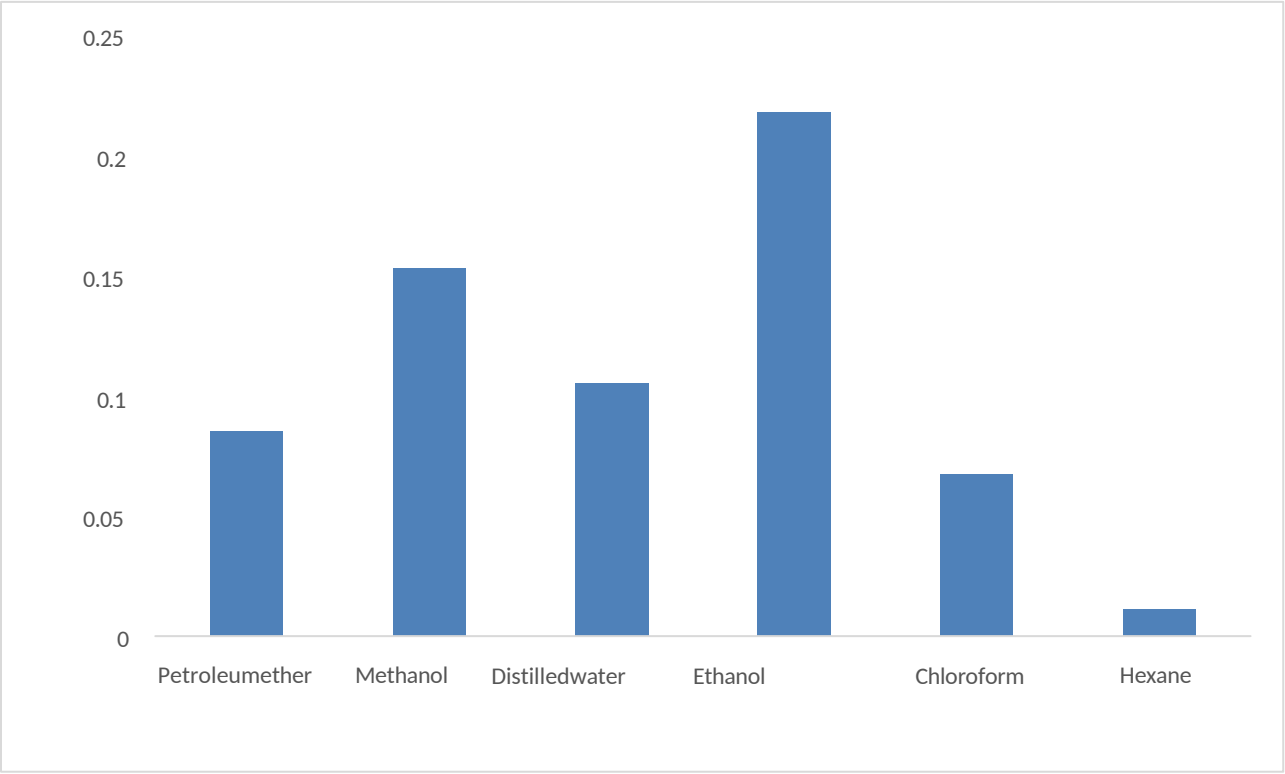
Drugsolubility in different solvents:

0.01 gram drug was dissolved in 10 ml different solvents and then incubated at room temperature for 24 hours. Then thesolution was filtered andthen absorbance was taken at 220 nm.

Table: Solubility in differentsolvents

S.No.	Solvent	Absorbance
1	Petroleumether	0.085

2	Methanol	0.153
3	Distilledwaster	0.105
4	Ethanol	0.218
5	Chloroform	0.067
6	Hexane	0.011



Solubility in differentsolvents

Table Solubility at different pH

S.No.	pH	Absorbance
1	1	0.005
2	3	0.469

3	7	0.355
4	9	0.218
5	11	0.257
6	14	0.288

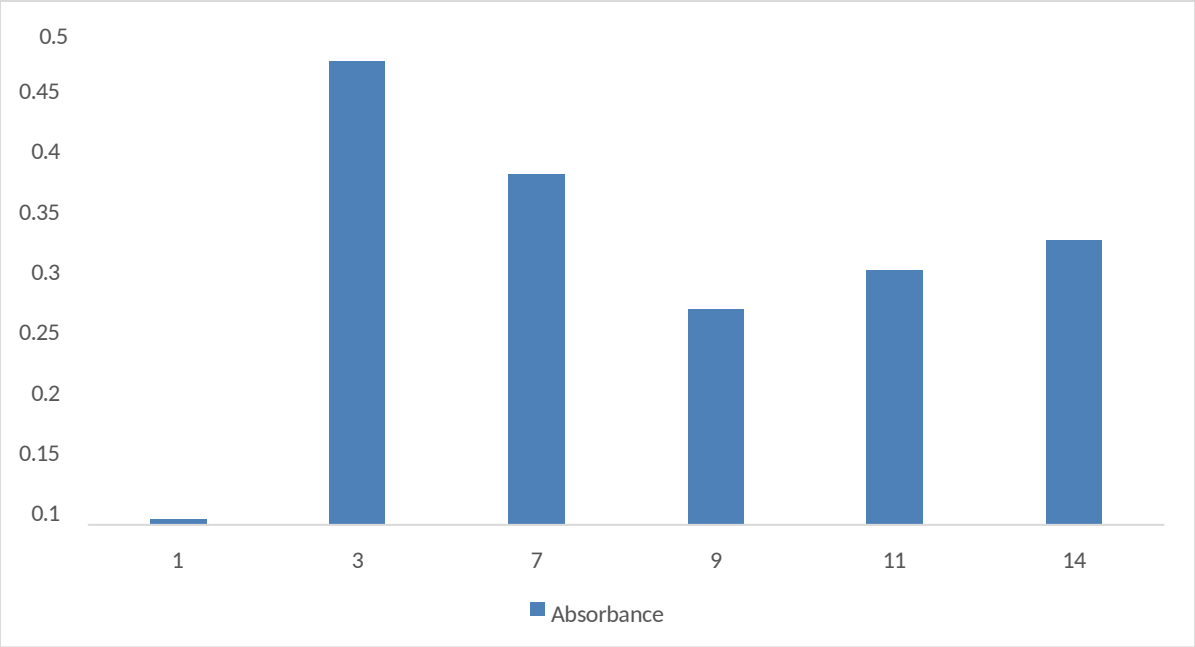


Figure 4 Solubility at different pH

FTIR Analysis

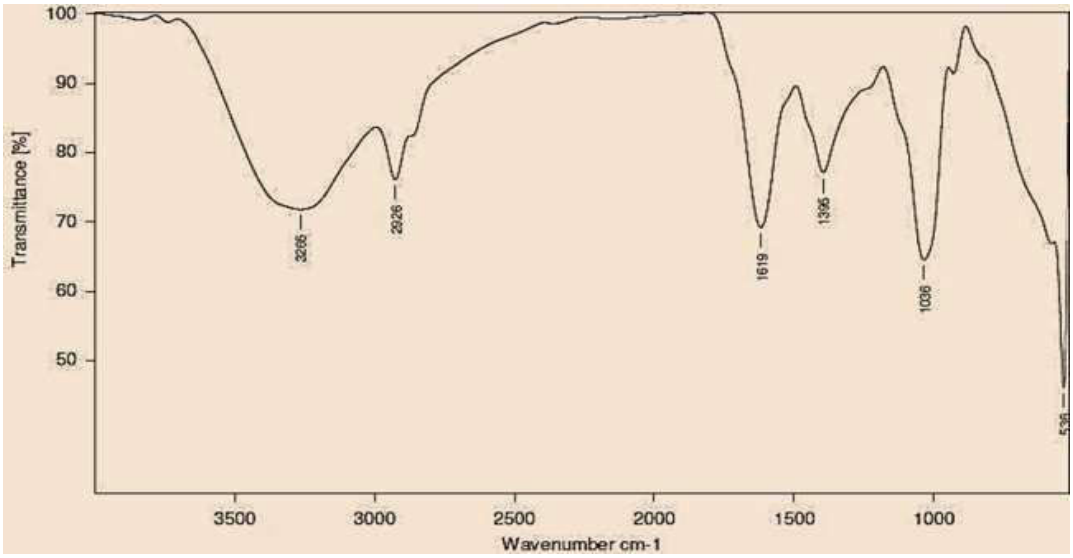


Figure 5:FTIRspectrumofAllicin

Screening of surfactantandco-surfactant

The soluble levels of allicin in the surfactants solutions were determined to be , as shown intheFigure.Tween80aswellasPEG400werechosenasthesurfactantandanadditional surfactant, respectively, based on the findings of this investigation.

TheselectionofTween80asasurfactantwasbasedonoilsolubilizingpotential,as wellas minimum toxicity profile and it's inexpensive. The role of cosurfactant is to reduce the interfacial tension between the water and oil phase, and for this purpose, PEG 400 was selected. The preparation of microemulsion was carried out by microtitration with water without any application of high energy due to its thermodynamic stability.

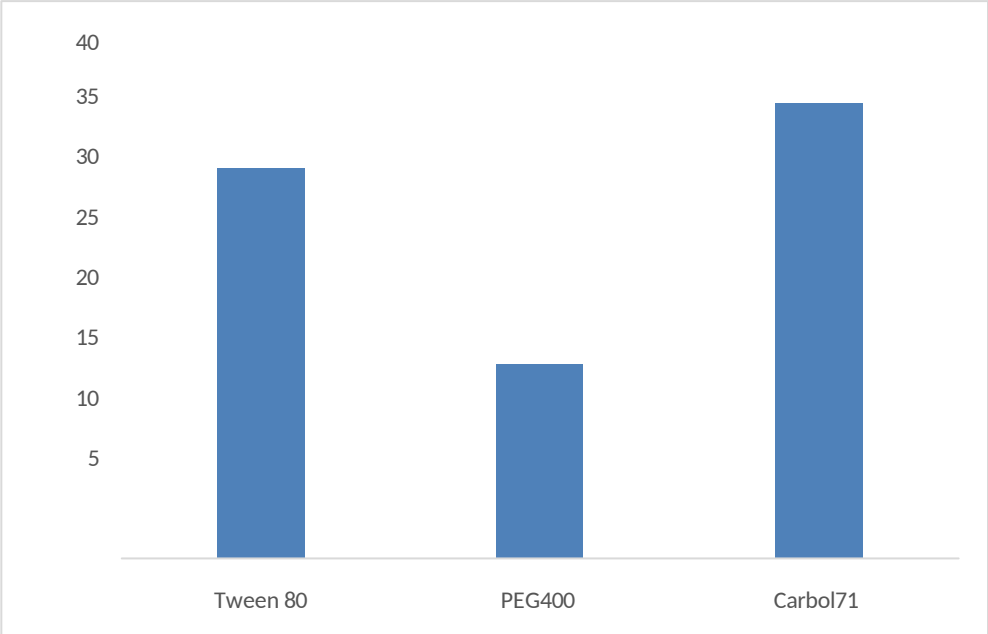


Figure6 :Screening of surfactantandcosurfactanton the basis of oil Solubilizing capacity

Comparative antibacterial efficacy of her bal drugs

Comparativeantibacterialefficacyofindividualdrugs,aswellasacombinationof drugs,wereanalyzedon*S.aureusforsatphinfections* asshowninFigureandbe24.06±0.249mm,21.5±0.294mm,25.96±0.286mm,and26.36±0.309mm respectively.

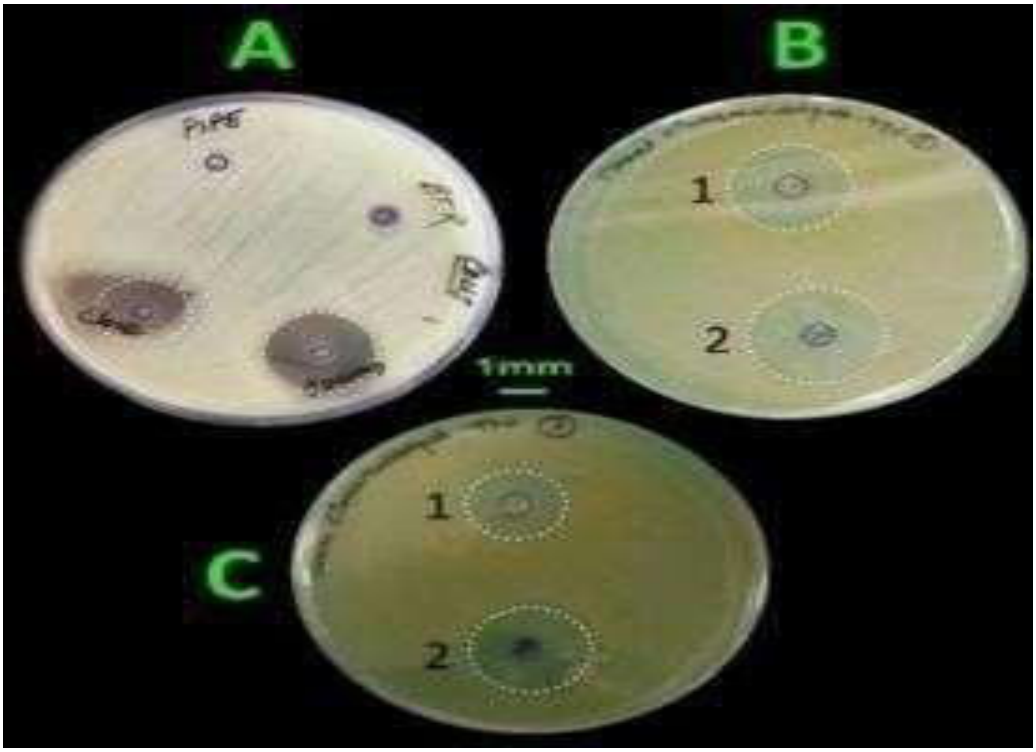


Figure7:Antibacterial efficacy on S.aureus

Evaluation of microemulsion:

Refractiveindex(RI)anddrugcontent.

Therefractiveindexofallicinewasfoundtobe1.482±0.0033.

Thermodynamicstability

The developed formulation i.e. allicin passed the heating-cooling cycletest and freeze thawcycletest.Attheendofallthesetestsnophaseseparation,droplet aggregation,or drug precipitation were observed.

Spreadability

Spreadabilitywasfound3.4±1.3g.cm/secforthebestformulation.

Viscosity

Theviscocity(cps)was observed 17.8 ±4.2; 18±0.8 and 13.4±1.3 for all formulationand found that 13.4±1.3 cps as best formulation.

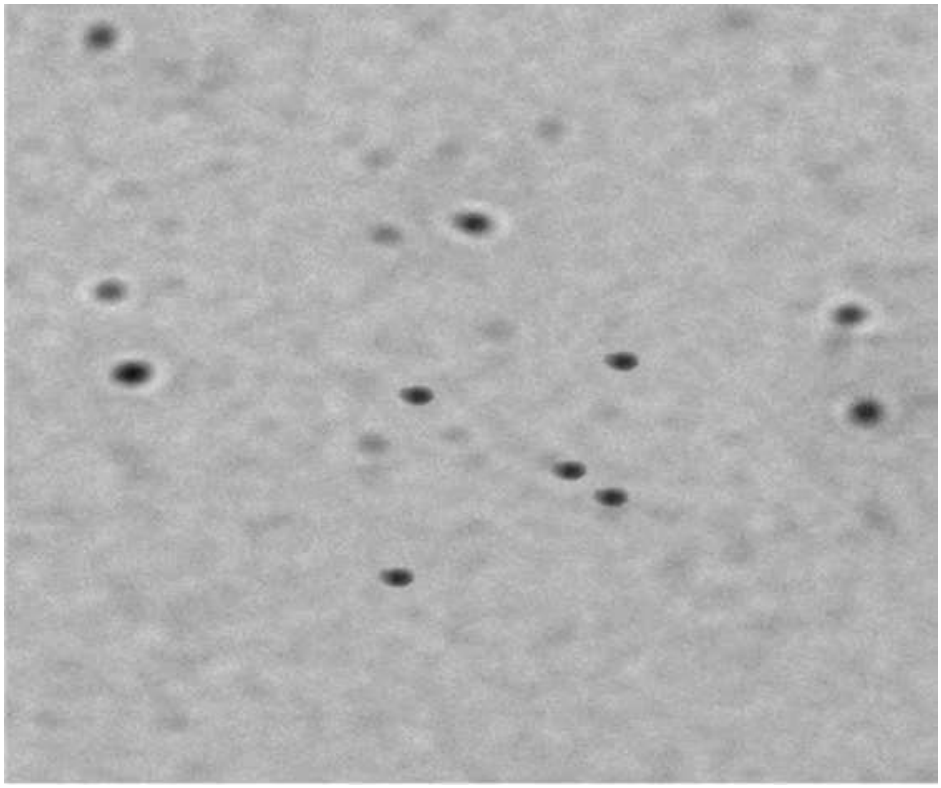
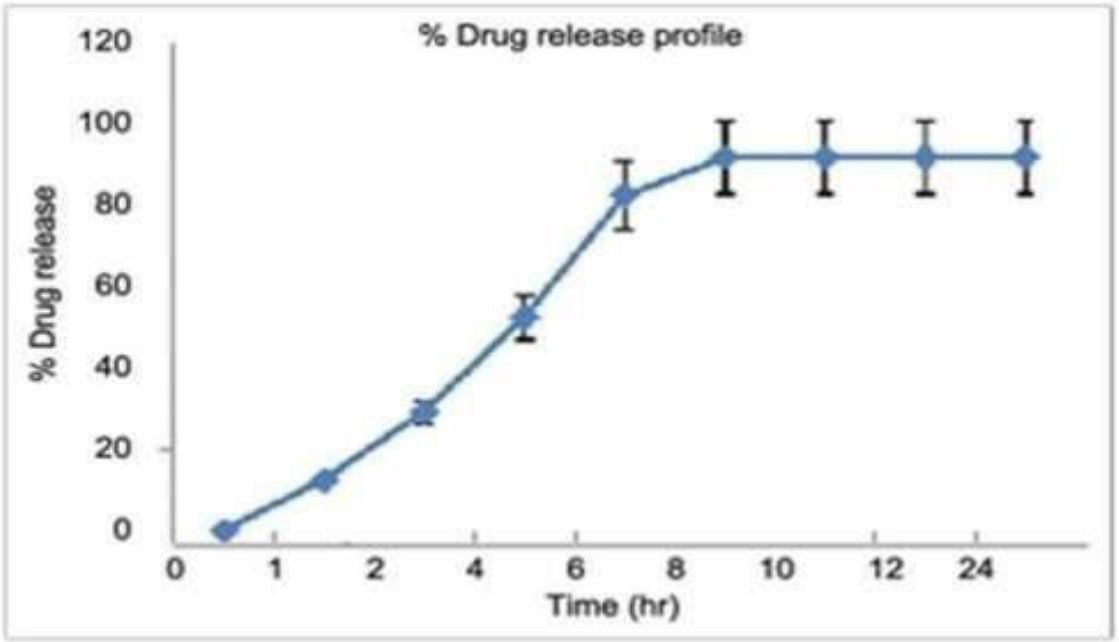


Figure8:SEM formicroemulsion

Invitro drug release

It was observedthatmaximum drug releasefrom microemulsion was achieved within 8hour. The release profile shown in figure.



Antibacterial activity of the developed formulation

The antibacterial activity potential of allicin wasassessedonsatph infections as depicted in Figure. Discretely, Figure A illustrates the untreated planktonic cellsof S.aureus in 100 times dilution, however, Figure B and Figure C indicate the untreated bacterial culture for 24 and 48 h respectively.

Likewise, Figure A, show the structure of untreated satph infections with the proper integrity and shape of the bacterial cells along with the appearance of biofilm among the cells denotes with the help of arrows. In Figure B, the disruptionofthecellwallwas observed upon the treatment of bacterial culture with allicin based microemulsion. Hence, the prepared formulation, allicin based microemulsion, was found to be effective against S. aureus as well as inhibition of their bio film formation capacity.

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