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Advances in Pharmacology and Pharmacy

Advances in Pharmacology and Pharmacy is an international peer-reviewed journal that publishes original and high-quality research papers in all areas of pharmacology and pharmacy. As an important academic exchange platform, scientists and researchers can know the most up-to-date academic trends and seek valuable primary sources for reference.

Aims & Scope

The subject areas include, but are not limited to the following fields:

- Behavioral Pharmacology
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Poly(CnO) and Poly(CcO) Organo-Particles Produced from Coconut Oil (CnO) and Cocoa Oil (CcO): Synthesis, Characterization, Bio and Anticancer Activity

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ABSTRACT

With the increasing number of cancer cases in recent years, the solution methods suggested for these cases are also of great importance. Within the scope of the presented study, we aimed to develop an alternative material in cancer immunotherapy by synthesizing poly(coconut oil) (p(CnO)) and poly(cacao oil) (p(CcO)) organo-particles from coconut and cocoa oils. The structural features of these particles synthesized using the redox polymerization technique were elucidated by various characterization methods. The chemical structure and functional groups of p(CnO) and p(CcO) organo-particles were determined by Fourier Transform Infrared Spectroscopy (FT-IR). Organo particle size and zeta potential values were determined by the dynamic light scattering (DLS) method using Zetasizer device. The morphological features of the particles were determined by Scanning Electron Microscopy (SEM). Bioactivity properties were determined by antioxidant, antimicrobial and biocompatibility analyses. In this study, the effects of p(CnO) and p(CcO) organo-particles on cytotoxicity and apoptotic processes against L-929 fibroblast and Capan-1 pancreatic cancer cell lines were also investigated. p(CnO) and p(CcO) organo-particles Capan-1 cell lines were determined to have significant cytotoxic activity at all doses studied. An increase in cell number was observed in L-929 fibroblast cells treated with p(CnO) and p(CcO) organo-particles. As a result, findings have been obtained that the p(CcO) organo particle triggers the apoptotic mechanism. On Capan-1 pancreatic cancer cells, p(CcO) was found to have a fatal impact akin to that of doxorubicin. It is anticipated that combining p(CcO) with doxorubicin could potentially lead to better outcomes than using doxorubicin alone.

Keywords Capan-1, Cocco Oil, Coconut Oil, L-929, Organo-Particle

1. Introduction

Herbal essential oils consist of a mixture of many compounds. Therefore, their chemical composition is quite complex. Essential oils are generally composed of hydrocarbons and their oxygenated derivatives. These derivatives include alcohols, acids, esters, aldehydes, ketones, phenol and phenol ethers, quinones, lactones, furan derivatives, oxides, amines, and sulfur compounds. Since essential oils have a

wide range of uses, they have been the subject of many studies and research recently [1, 2]. Obtaining and assessing the pure, mainly the primary active ingredients of medicinal plants and their essential oils, are crucial today. According to research findings, these plants' essential oils offer antibacterial, antioxidant, and anticarcinogenic properties. It is stated that there may be benefits to employing essential oils in industrial, medicinal, and cosmetic settings by looking at their pharmacological characteristics and constituent parts [1, 2]. Essential oils are among the drugs used in treatment for a long time. Several herbal oils used in traditional medicine were determined by pharmacological research that has an explanation of their bioactive properties, including antibacterial, antioxidant, and anti-carcinogenic properties. These naturally occurring antioxidant components can scavenge free radicals and offer protection against degenerative diseases, cancer, and other illnesses [1, 3].

Cancer, which has many different types, is an important disease that threatens human life. Healthy body cells can divide. They use these divisibility properties to repair injured tissues and regenerate dead cells. However, their divisibility is limited. Each cell has a certain number of divisions throughout its life. On the other hand, cancer cells multiply by dividing uncontrollably. Cancer cells can spread to other parts of the body through the blood or lymph circulation and continue to grow by forming colonies in the areas they go to. The spread of cancer cells to other parts of the body is called metastasis. In the therapy of cancer, controlling the malignant cell's ability to proliferate and die is crucial. The majority of current research on bioactive plants is focused on developing novel medications that are anticipated to be efficacious against various forms of cancer [4, 5].

This has had a significant impact on the development of drug systems, particularly the use of herbal and herbal oils in the treatment of cancer, in the search for new drugs. Herbal and herbal oils have many physical, psychological, and spiritual effects on individuals. These oils are concentrated droplets derived from plants that are hundreds of times more potent than the plants themselves. They are used to cure injured tissue, control growth, and prevent infection [6].

Herbal Oils constitute 30% of the factors affected by diet in cancer development. A relationship has been observed between high fat intake and increased cancer rates. Experimental studies conducted in later years have shown that the amount and type of fat intake are important in terms of cancer development. When the composition of cocoa beans is considered, the majority of the beans consist of fat. In general, cocoa beans contain 54% fat, 12% protein, 5% moisture, 1.46% ash, 1.09% theobromine, and 0.44% caffeine. In cocoa butter, more than 95% of the fatty acids are made up of three fatty acids, palmitic acid (hexadecanoic, 16:0), stearic acid (octadecanoic, 18:0) and oleic (octadec-cis-9-enoic, 18:1) acid (18:1), and contain polar lipids, sterols, and 150-200 ppm tocopherol. The fact that approximately 90% of the triacylglycerols in cocoa butter are symmetrical triacylglycerols is an important factor in the functionality of this oil. There is no other natural oil that has the properties of cocoa butter [7]. Coconut oil is rich in medium-chain fatty acids, and these acids are in the form of Medium Chain Triglycerides, which are well digestible. The oil obtained from dried coconut fruit consists of 90% saturated triglycerides. Fatty acid groups include caproic acid, myristic acid, palmitic acid, caprylic acid, capric acid, and lauric acid. It is reported that coconut oil has the potential to protect/treat various chronic diseases and infectious diseases, including cardiovascular diseases, diabetes, and cancer [8-11].

Although cancer and cancer treatments are among the important problems of human life, increasing and developing technological systems play an important role in the solution of these diseases. Especially the fact that plant-derived materials show positive results in these treatments and are promising both economically and in terms of treatment causes the increase of these studies. In this study, p(CnO) and p(CcO) based organo-particles were synthesized using coconut and cocoa butter, which have important bioactive properties, and the bioactive properties and cytotoxic effects of these particles on Capan-1 and L-929 cancer cells were focused on.

2. Material and Method

Purchased Cocoa Oil (CcO) and Coconut Oil (CnO) from regional vendors. Throughout the whole experiment, distilled water (Human II-UV) was utilized. N,N,N',N'-tetra methyl ethylene diamine,

ammonium persulfate(APS), ethanol, acetone, Tween 80, and ethylene glycol dimethacrylate (EGDMA) were also acquired from SigmaAldrich. AP Institute and ATCC bought L-929 fibroblast cells and Capan-1 pancreatic cancer cells. The culture dishes were cleaned and washed using phosphate buffer, and the growth medium is DulbeccoModifiedMedium, FetalBovineSerum, Penicillin/Streptomycin, and TrypsinEDTA. Trypan blue was utilized to count the cells. Using the tetrazolium salt MTT (Serva, Israel), cell viability was assessed in the cytotoxicity assay. 2-propanol(Sigma Aldrich) and MTT(Life Science). Serva(Israel) provided the PI, Hoechst 33342, and RNaseA needed for the double staining test.

2.1. The Preparation of Organo-Particles

As described by Alpaslan et al. in the literature, organo particles were synthesized [12-15]. Poly(Coconut Oil) (p(CnO)) and poly(Cocoa Butter) (p(CcO)) based organoparticles were synthesized using the redox polymerization technique in an emulsion environment. In summary, 8 milliliters of water, 2 milliliters of ethanol, and 1 milliliter of tween 80 were added to the reaction vessel and stirred continuously for 10 minutes at room temperature with a magnetical stirrer. Next, 0.5 milliliters of either cacao or coconut oil (CcO) were added. As a crosslinker, accelerator, and initiator, respectively, EGDMA (10% mol), TEMED (10 μ L), and APS (3% mol) were employed. The entire reaction mixture was stirred thoroughly for three hours at a stirring speed of 1000 rpm at a temperature of 50°C. After the polymerization reaction was completed, the synthesized organo-particles were centrifuged with acetone at 9000 rpm for 30 minutes, and this process was repeated three times using acetone for complete cleaning. The particles obtained after centrifugation were dried in an oven at 40°C to a constant weight and stored in closed containers at 4°C for further research. SEM images of the synthesized organo-particles are shown in Figure 1. Particles synthesized from coconut and cocoa oil were called p(CnO) and p(CcO) organo-particles.

2.2. Characterization of Organo-Particles

Using dynamic light scattering, the zeta potential and particle size of the organo-particles were determined. Using a thermo-gravimetric analyzer, the thermal behaviors of organo-particles were investigated. FTIR examination was performed using an Attenuated Total Reflection (ATR) integrated Fourier Transform Infrared Spectroscopy. X-ray SEM Ultra Field Emission-Dependent Spectroscopy was employed to track the morphological characteristics of the organo-particles [13]. Each oil sample was analyzed using a Shimadzu GCMS-branded gas chromatography device with an FID detector. In DB-23, a silica capillary column (30 m x 0.25 mm) was used as the column. Chromatographic data were analyzed on the computer. After waiting at 60°C for 5 minutes, the column temperature was adjusted to reach the final temperature of 230°C with an increase of 4°C/min and remained at this temperature for 10 minutes. The injection mode was selected as split. The split ratio is 1:20. Injector and detector temperatures are 80 and 250°C, respectively. It was used as a carrier gas, and its flow rate was 1 mL/min. The injection volume was set at 1 μ L. The oil sample was diluted 1/10 with methanol. Each essential oil component was identified by comparing the GCMS retention indices (RI) by determining the retention times of the homologous series of n-Alkanes (C8–C18) in the apolar column. These components were compared in the NIST and WILEY spectrum libraries.

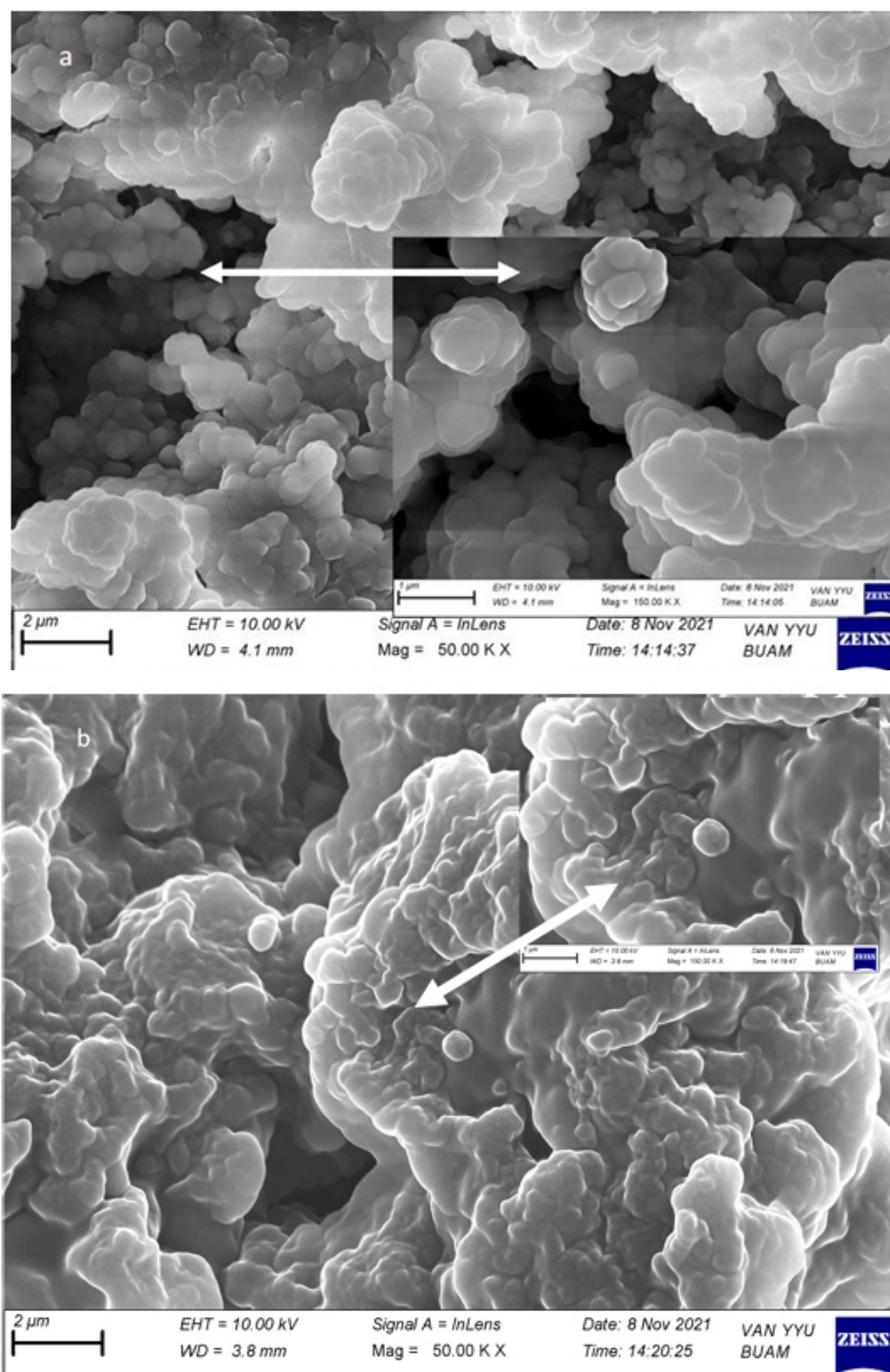


Figure 1. Image of SEM (a) p(CnO) and (b) p(CcO) particles

2.3. Bioactive Analysis

The Folin technique described in the literature was used to study the antioxidant capabilities of the p(CnO) and p(CcO) organo-particles. The data were analyzed using the antimicrobial, blood clotting, and hemolysis analyses documented in the literature. The antibacterial properties of p(CnO) and p(CcO) organo-particles were evaluated against Gram-positive (*Escherichia coli*) and Gram-negative (*Bacillus cereus*, *Staphylococcus aureus*) bacteria [13, 14]. The data were analyzed using cell culture, cytotoxicity

assays, apoptosis, and necrosis by double staining analysis documented in the literature [13, 14].

2.4. Cell Culture

For anticancer activities, mouse normal fibroblast cell lines (L929) and human pancreatic adenocarcinoma cell lines (Capan-1) were employed. L929 fibroblast cells can be used for various purposes in cancer testing. Although these cells are of mouse origin, they can be used as model systems in studies with human cancer cells or human tissue samples. The use of this cell line in biocompatibility tests for humans has been accepted in EN ISO 10993-5 due to its high similarity rate. This is why the L929 cell line was employed. The cells were incubated in flasks in a 37°C carbon dioxide oven with DMEM-F12 or RPMI culture media supplemented with 10% FCS and 1% antibiotic.

Until the cells achieved a sufficient number, the media was changed every two days. Trypsin was used to extract the cultures once they attained a specific number (5.000 cells/well), and well plates were then injected with them. Media containing serum and antibiotics were switched out for new media containing serum and antibiotics once the cell count reached a predetermined level. Then, it was applied to ascertain the cytotoxic, apoptotic, and necrotic actions.

3. Results and Discussion

Determining the qualities of essential oils by the GC-MS technique is of great importance in terms of their saturated, unsaturated, or essential fatty acid contents. The composition of fatty acids shows characteristic differences specific to plant species. The specific fatty acid composition of each oil plant is not constant and varies depending on many factors. The qualities and quantities of fatty acids determine the way the oils are used, thus leading to the production of oils according to their intended use. GC-MS data for cocoa oil and coconut oil are given Table 1. The synthesis mechanism of organo-particles can be explained by the redox polymerization technique between fatty acids, flavonoids, and the EGDMA crosslinker. The polymerization reaction occurs with the formation of hydrogen or covalent bonds between many functional groups, such as OH, NH₂, and COOH

Table 1. Cocoa oil and Coconut oil GC-MS oil composition data

Number	Cacao Oil	Retention Time
1	Spiro[4.4]Nonane, 1-Methylene- (CAS)1-Methylenespiro(4,4)Nonane	5.30
2	10-Dimethylaminomethyl-2,8,12,18-Tetramethyl-3,7,13,17-Tetraethyl-21H,23H Nickel Complex	7.09
3	L-Menthone	16.17
4	Cyclohexanone, 5-Methyl-2-(1-Methylethyl)-, (2S-Cis)- (CAS)D-Isomenthone	16.30
5	Cyclohexane, 1,1'-(1-Methylpropylidene)Bis- (CAS)2,2-DICYCLOHEXYLBUTANE	16.65
6	Neo-Menthol	17.86
7	Neo-Menthol	18.00
8	Citronellyl Valerate	18.13
9	Citronellyl Acetate	18.19
10	(+)-Caran-Cis-4-Ol	19.49
11	2-Methylamino-N-Phenyl-Acetamide	22.85
12	Allo-2-Carbomethoxy-Tropanol	23.57
13	Alpha-D-Glucopuranosid, Methyl-4-O-Heptyl-	23.85
14	Tricyclo[4.2.2.0 1,5]Decan-7-One	25.75
15	(Tetrahydroxycyclopentadienone)Tricarboyliron(0)	25.90
16	2h-1,2-Oxaborin, 3,6-Dihydro-2,3,3-Tripropyl- (CAS)1,6,6-Tri-N-Propyl-1,2-Boroxacyclohex-4-Ene	26.44
17	1-(2'-Ethenyl-1'-Cyclohexenyl)-2,2-Dimethyl-1-Propanol	26.67
18	Phenol, 2-Methoxy-3-(2-Propenyl)- (CAS)Phenol, 3-Allyl-2-Methoxy- (CAS)3-Allylguaiacol	29.17
19	(3as,9as,9br)-6,6,9aa-Trimethyl-Trans-Perhydronaphtho[2,1-B]Furan	31.94
20	Carbamic Acid, (1-Phenylethyl)-, 2-Methyl-5-(1-Methylethyl)Cyclohexyl Ester (CAS)Menthyl N-(1-Phenylethyl)-Carbamate	32.10
21	Hexadecanoic Acid, Methyl Ester (CAS)Methyl Palmitate	32.37
22	9-Hexadecenoic Acid, Methyl Ester, (Z)- (CAS)Methyl Palmitoleate	32.95
23	Trans-8-Endo-Methoxybicyclo[4.3.0]-3-Nonene-7-Exo-Carboxaldehyde	33.08
24	Hexadecanoic Acid, Ethyl Ester (CAS)Ethyl Palmitate	33.29

Table 1 continued

25	Phenol, 2-Methoxy-4-(2-Propenyl)-, Acetate (CAS)Aceteugenol	33.53
26	Ethyl 9-Hexadecanoate	33.85
27	1a,3,6,9-Tetrahydro-4h-Quinolizine-1,7,9(And 1,1,7)-D3	33.93
28	Octanedioic Acid, 3-Methyl-, Dimethyl Ester (CAS)3-Methyloctanedioic Acid-Dimethyl Ester	34.10
29	Propyl Hexadecanoate	35.13
30	Tetradecanoic Acid, Ethyl Ester (CAS)Ethyl Myristate	35.25
31	1-(2'-Formylethyl)-2-Oxocyclooctan-1-Carbonsaure-Ethylester	35.71
32	Thiourea, N-Phenyl-N'-(1-Phenylethyl)- (CAS)N-Phenyl-N'-(.Alpha.-Methylbenzyl)Thiourea	35.81
33	Octadecanoic Acid, Methyl Ester (CAS)Methyl Stearate	36.30
34	9-Octadecenoic Acid, Methyl Ester (CAS)Methyl Octadec-9-Enoate	36.75
35	Octadecanoic Acid, Ethyl Ester (CAS)Ethyl Stearate	37.10
36	9-Octadecenoic Acid (Z)-, Ethyl Ester (CAS)Ethyl Oleate	37.52
37	8,11-Octadecadienoic Acid, Methyl Ester (CAS)Methyl 8,11-Octadecadienoate	37.68
38	Ethyl Linoleate	38.42
39	9,12,15-Octadecatrienoic Acid, Methyl Ester (CAS)Methyl 9,12,15-Octadecatrienoate	38.81
40	9-Octadecenoic Acid, Methyl Ester (CAS)METHYL OCTADEC-9-ENOATE	39.07
41	Elaidmic Acid, Isopropylester	39.18
42	Ethyl Linoleate	39.54
43	Eicosanoic Acid, Methyl Ester (CAS)Arachidic Acid Methyl Ester	39.89
44	9,12-Octadecadienoic Acid (Z,Z)-, 2,3-Dihydroxypropyl Ester (CAS)1-Monolinolein	40.09
45	11-Eicosenoic Acid, Methyl Ester (CAS)METHYL 11-EICOSENOATE	40.32
46	Butanoic Acid, Hexyl Ester (CAS)Hexyl Butanoate	40.59
47	8-Octadecenoic Acid, Methyl Ester, (E)- (CAS)Trans-8-Octadecenoic Methyl Ester	40.81
48	15-Octadecenoic Acid, Methyl Ester (CAS)Methyl Octadec-15-Enoate	41.55
49	Docosanoic Acid, Methyl Ester (CAS)Methyl Behenate	43.17
50	2-Furanoctanoic Acid, 5-Hexyltetrahydro-, Methyl Ester (CAS)Methyl 9,12-Epoxystearate	44.90
51	Oxiraneoctanoic Acid, 3-Octyl-, Methyl Ester, Cis- (CAS)Methyl Cis-9,10-Epoxystearate	45.35

Table 1 continued

52	6,9-Octadecadienoic Acid, Methyl Ester (CAS)Methyl 6,9-Octadecadienoate	45.71
53	3-Oxabicyclo[3.3.0]Octan-2-On, 7-Methylen-4-Menthoxo-	46.03
54	4,5-Diethyl-2,2-Dimethyl-3-Isopropenyl-Delta.-3-1,2,5-Oxasilaboroln	46.63
55	10-Undecenoic Acid, Methyl Ester (CAS)Methyl 10-Undecenoate	47.02
56	(3ars,4RS,6asr,2'Z)-Hexahydro-4-(Pent-2'-Enyl)-2H-Cyclopenta[B]Furan-2-One	47.69
57	2(3h)-Benzothiazolone (CAS)2-Hydroxybenzothiazole	48.02
58	Chlorodecaborane	49.37
59	1h-Purin-2-Amine, 6-Methoxy- (CAS)2-Amino-6-Methoxypurine	49.68
60	Bicyclo[2.2.1]Heptan-2-Ol, 4-Chloro-1,7,7-Trimethyl-, Exo- (CAS)4-Chloroisoborneol	49.79
61	Pyrrolidine, 1-(1-Cyclohepten-1-Yl)- (CAS)1-(1-Pyrrolidinyl)-1-Cycloheptene	49.83
62	2-[2-(5,6-Dimethyl-1h-Benzimidazol-2-Ylthio)Acetamido]Benzoic Acid	50.38
63	2,8-Decadien-1,10-Dioic Acid, Diethylester	50.66
64	Trans-2,5-Bis(Chloromethyl)-P-Dioxane	51.33
65	.Alpha.-(4-T-Butylphenyl)Propanoic Acid	51.93
66	Acetic Acid 7-Hydroxy-1,3,4,5,6,7-Hexahydro-2h-Naphthalen-4a-Ylmethyl Ester	52.11
67	Benzaldehyde, 2,3-Dimethoxy- (CAS)2,3-Dimethoxybenzaldehyde	52.30
68	Ergosta-7,22-Dien-3-Ol, Acetate, (3.Beta.,5.Alpha.)- (CAS)3.Beta.-Acetoxy-5.Alpha.-Ergost-7,8-22,23-Diene	52.58
69	Cholest-5-Ene-3,20-Diol, 3-Acetate, (3.Beta.,20r)- (CAS)	52.74
70	2-(4-Chlorophenylthio)-N'-(2,3-Dimethoxybenzylidene)Acetylhydrazide	52.93
71	Manool	53.06
72	Hexadecanoic Acid, Trimethylsilyl Ester (CAS)Palmitic Acid-Monotms	53.49
73	Bufo-20,22-Dienolide, 14,15-Epoxy-3-[(8-Methoxy-1,8-Dioxooctyl)Oxyl]-, (3.Beta.,5.Beta.,15.Beta.)- (CAS)3.Beta.-Suberyloxyresni	53.70
74	2,2'-Sulfinylbis(4-Methylphenol)	53.85
Number	Cocconut Oil	Retention Time
1	Isopulegol 1	17.03
2	Cyclohexanol, 5-Methyl-2-(1-Methylethyl)- (CAS) 3-P-Menthanol	17.83

Table 1 continued

3	Cyclohexanol, 5-Methyl-2-(1-Methylethyl)- (CAS) 3-P-Menthanol	17.93
4	Phenol, 2-Methoxy-4-(2-Propenyl)- (CAS) Eugenol	29.07
5	Phenol, 2-Methoxy-3-(2-Propenyl)- (CAS) Phenol, 3-Allyl-2-Methoxy- (CAS) 3-Allylguaiacol	29.17
6	Exo-8-(2-Propenyl)-Endo-8-Methyl-3-Oxa-Bicyclo[4.2.0]Oct-5-Ene	29.46
7	1,3-Benzodioxole, 2-Propyl- (CAS) 2-N-Propyl-1,3-Benzodioxole	29.56
8	Phthalazine, 1,2,3,4,5,6,7,8-Octahydro-1-[(4-Methoxyphenyl)methyl]-2,3-Dimethyl- (CAS) 1,2,3,4,5,6,7,8-Octahydro-1-(4-Methoxy-	29.59
9	3,3'-(3,7-Diphenylperhydro-1,5-Diaza-3,7-Diphosphocine-1,7-	29.62
10	Phenol, 2-Methoxy-4-(2-Propenyl)- (CAS) Eugenol	29.65
11	Phenol, 2-Methoxy-4-(1-Propenyl)-, (E)- (CAS) (E)-Isoeugenol	29.72
12	Phenol, 2-Methoxy-4-(2-Propenyl)- (CAS) Eugenol	30.79
13	2h-Pyran-2-One, Tetrahydro-6-Propyl- (CAS) .Delta.-Octalactone	31.09
14	2,4-Dimethoxy-Adamantane	32.13
15	Hexadecanoic Acid, Methyl Ester (CAS) Methyl Palmitate	32.37
16	4-Isobenzofuranol, Octahydro-3a,7a-Dimethyl-, 4-Nitrobenzoate, (3a.Alpha.,4.Beta.,7a.Alpha.)-(+,-)- (CAS) 2.Alpha.,3.Alpha.-D	33.02
17	P-Phenylenediamine, N'-Ethyl-N,N-Dimethyl- (CAS) N,N-Dimethyl-N'-Ethyl-P-Phenylenediamine	33.15
18	Hexadecanoic Acid, Ethyl Ester (CAS) Ethyl Palmitate	33.29
19	Phenol, 2-Methoxy-4-(2-Propenyl)-, Acetate (CAS) Aceteugenol	33.53
20	9-Hexadecenoic Acid, Methyl Ester, (Z)- (CAS) Methyl Palmitoleate	33.65
21	Ethyl 9-Hexadecenoate	33.85
22	11-Dodecenoic Acid, 10-Hydroxy-, Methyl Ester	33.97
23	11-Dodecenoic Acid, Methyl Ester (CAS) Methyl Dodec-11-Ynoate	34.12
24	Tetradecanoic Acid, 5,9,13-Trimethyl-, Methyl Ester (CAS) Methyl 5,9,13-Trimethyltetradecanoate	34.39
25	Propyl Hexadecanoate	35.12
26	Ethyl 9-Decenoate	35.25
27	Isoxazole, 5-Amino-3-Butyl-4-Propyl- (CAS) 3-N-Butyl-4-N-Propyl-5-Aminoisoxazole	35.68
28	9-Octadecenoic Acid (Z)-, Ethyl Ester (CAS) Ethyl Oleate	35.72

Table 1 continued

29	.Delta. Decalactone	36.09
30	Octadecanoic Acid, Methyl Ester (CAS) Methyl Stearate	36.29
31	Cis-Jasmone	36.52
32	Zectane	36.55
33	9-Octadecenoic Acid, Methyl Ester (CAS) Methyl Octadec-9-Enoate	36.75
34	11-Octadecenoic Acid, Methyl Ester (CAS) Methyl 11-Octadecenoate	36.89
35	Octadecanoic Acid, Ethyl Ester (CAS) Ethyl Stearate	37.09
36	3-Octadecenoic Acid, Methyl Ester (CAS) Methyl Octadec-3-Enoate	37.16
37	13,16-Octadecadienoic Acid, Methyl Ester (CAS) Methyl-13,16-Octadecadienoate	37.24
38	9-Octadecenoic Acid (Z)-, Methyl Ester (CAS) Methyl Oleate	37.29
39	9-Octadecenoic Acid (Z)-, Ethyl Ester (CAS) Ethyl Oleate	37.51
40	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester (CAS) Methyl Linoleate	37.67
41	N-Cyclooct-4-Enyl-Acetamide	38.29
42	Ethyl Linoleate	38.41
43	Naphth[1,2-B]Oxirene, Decahydro-1a,7-Dimethyl- (CAS) 1,7-Dimethyl-7,8-Epoxybicyclo(4.4.0)Decane	38.56
44	Tridecanedial	38.63
45	9,12,15-Octadecatrienoic Acid, Methyl Ester (CAS) Methyl 9,12,15-Octadecatrienoate	38.81
46	2(Z)-4-Methyl-3-Pentenyl-Butenedial	38.98
47	(2Z-Z)-Dehydrocholesterol-1-Ether	39.07
48	9-Octadecenoic Acid (Z)-, Ethyl Ester (CAS) Ethyl Oleate	39.17
49	Eicosanoic Acid, Methyl Ester (CAS) Arachidic Acid Methyl Ester	39.88
50	Ethyl Linoleate	40.07
51	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester (CAS) Methyl Linoleate	40.13
52	11-Eicosenoic Acid, Methyl Ester (CAS) Methyl 11-Eicosenoate	40.31
53	9-Octadecenoic Acid (Z)-, 9-Octadecenyl Ester, (Z)- (CAS) 9-Cis-Octadecenyl 9-Cis-Octadecenoate	40.81
54	2h-Benzocyclohepten-2-One, Decahydro-9a-Methyl-, Trans- (CAS)	41.22

Table 1 continued

55	2,6,10,15,19,23-Hexamethyl-Tetracos-2,10,14,18,22-Pentaene-6,7-Diol	46.93
56	Bicyclo[2.2.1]Heptan-2-One, 1,7,7-Trimethyl-, Oxime (CAS) 2-Hydroxymunobornane	46.95
57	2(3h)-Benzofuranone, Hexahydro-3a,7a-Dimethyl-3-Methylene-, Cis-	475.07
58	Valerylaldehyde-2,4-Dnp-D1	48.39
59	Isopropyl Myristate	49.29
60	Phosphonic Acid, [1-(Acetylmino)Ethyl]-, Bis(Trimethylsilyl) Ester (CAS) 1-Aminoethylphosphonic Acid-N-Acetyl-Ditms Ester	49.38
61	Benzenecarbothioic Acid, 2-(1-Methylethyl)Hydrazide (CAS) Benzoic Acid, Thio-, 2-Isopropylhydrazide (CAS)	49.45
62	Phosphonic Acid, [[2-(1-Methyl-1-Propenyl)-1,3-Dioxolan-2-Yl]Methyl]-, Diethyl Ester, (E)- (CAS) (3-Methylethylen-2-Cetal-3-Pe	49.49
63	D(17a)-Homo-C,18-Dinorcard-20(22)-Enolide, 14-Hydroxy-17a-Methylene-3-Oxo-, (5.Beta.)- (CAS) C-Nor-D-Homocardenolide Derivativ	49.53
64	1-(3,4-Methylenedioxybenzylidene)Semicarbazide	49.80
65	4-Pyridinecarboxylic Acid, 3-Hydroxy-5-(Hydroxymethyl)-2-Methyl- (CAS) 4-Pyridoxic Acid	50.09
66	.Alpha.-D-Glucopyranose, 2-Amino-3,6-Anhydro-2-Deoxy-1,4-Bis-O-(Trimethylsilyl)- (CAS) 2-Amino-3,6-Anhydro-A-Glucopyranose-1,4	51.79
67	Cholest-5-En-3-Ol (3.Beta.)-, Nonanoate	52.60
68	Succinic Acid, (5-Hexenyl)- (CAS)	53.18
69	Tramat	53.27
70	N,N'-Bis(Piperonylidene)Ethylenediamine	54.00
71	3.Beta.-Fluoro-5.Xi.-Hydroxy-6-Keto-17.Beta.-Androstane	54.05
72	Dodecane, 1-Bromo- (CAS) 1-Bromododecane	54.17
73	Azafrin Methyl Ester	54.37
74	Methyl 2-[(2-Oxocyclohexyl)Methyl]Propenoate	54.59
75	Trans-7.Beta.-Carbomethoxy-Decal-1-One	54.87

3.1. Characterization of P(CnO) and P(CcO) Organo Particles

It can be possible to efficiently control the reaction rate, particle size, and morphology using the emulsion polymerization process. The one-step emulsion polymerization method was used to create the organoparticles p(CnO) and p(CcO), whose SEM images are shown in Figure 1.

The Zeta potential occurs between the particle and the liquid in which the particle is located. It generates both attracting and repulsive forces among the particles. In a liquid, particles with opposite charges attract one another, while those with the same charge repel one another. The particle's zeta potential value determines the strength of this attractive and repulsive force. Conversely, low zeta potential particles lack the force necessary to resist one another, which causes them to group to form agglomerates [16, 17]. The zeta potentials of p(CnO) and p(CcO) were 169.77 mV and -120.02 mV, respectively. The hydrodynamic diameters of synthesized p(CnO), and p(CcO) organo-particles were between 20–520 nm and 300–506 nm, respectively. The interactions of functional groups of organo-particles were examined by FTIR analysis, and related spectra are shown in Figure 2 and Table 2. The most fundamental bands in the FTIR spectra of coconut oil, cocoa oil, p(CnO), and p(CcO) organo-particles are summarized in Table 2. It was observed that some peaks seen in the FTIR of coconut oil and cocoa oil and p(CnO) and p(CcO) organo-particles decreased in intensity, and some peaks shifted to lowfrequency regions, and these peaks deepened. These changes indicate that the particles were successfully synthesized. In addition, these findings were found to be compatible with the findings of previous studies.

A thermogravimetric analyzer was used to examine the changes in the masses of p(CnO) and p(CcO) organoparticles with increasing temperature. Thermogravimetric analysis is an instrumental analytical technique that enables us to investigate and comprehend how sample structure varies with temperature. As a result, it describes the gradual separation of free and bound water, somewhat volatile chemicals, and other compounds from the sample's structure. The decomposition temperatures of the p(CnO) and p(CcO) organo-particles were thermograms of the analysis made to determine them and are given in Figure 3. As seen in Figure 3, it was observed that p(CnO) and p(CcO) organo-particles reached constant weight after approximately 470°C, and there was a total mass loss of 85% and 91%, respectively.

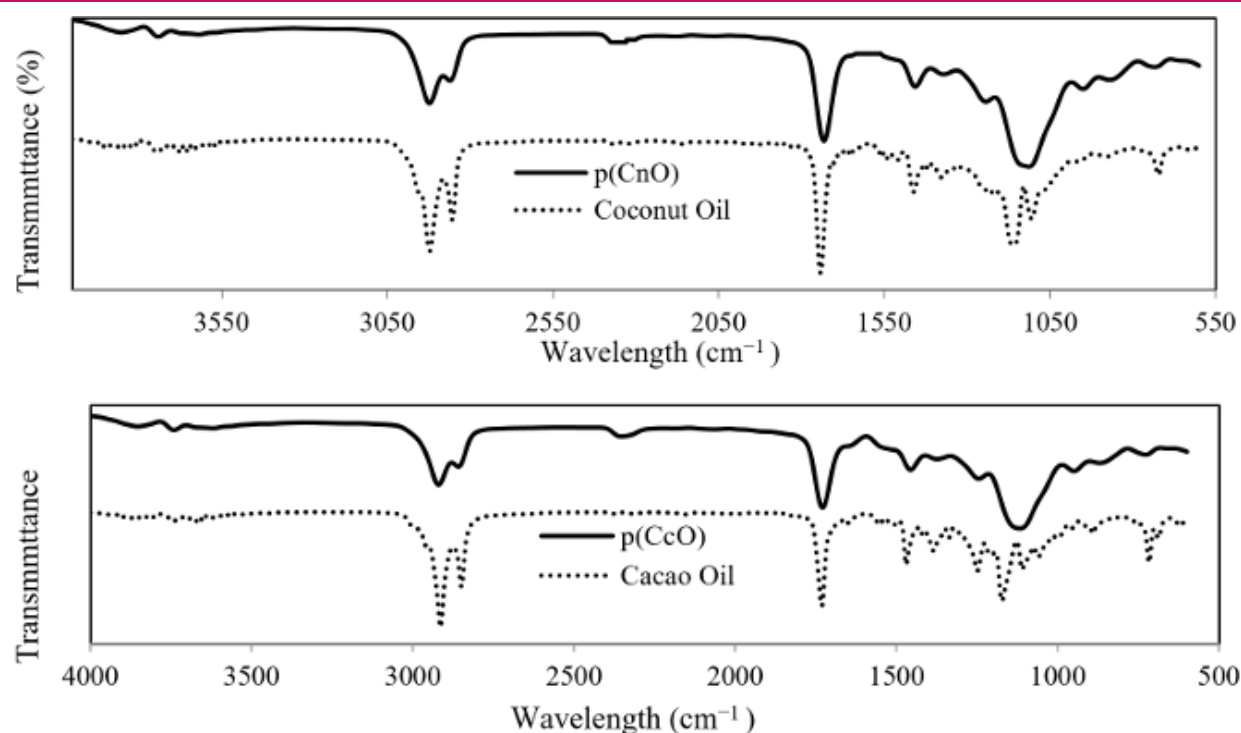


Figure 2. FT-IR spectra of Coconut oil, Cacao oil and, p(CnO) and p(CcO) organo-particles

Table 2. FTIR peak table of coconut oil, cacao oil, p(CnO), and p(CcO) organo-particles

Cacao Oil		Coconut Oil	
Wavelength (cm ⁻¹)	Vibration type	Wavelength (cm ⁻¹)	Vibration type
2913	C-H stretch	2920	CH ₂ stretch
2851	C-H stretch	2853	CH ₂ stretch
1733	C=O stretch	1741	C=O stretch
1471	C-H stretch	1460	CH ₂ and CH ₃ stretch
1385	C-H ₂ stretch	1378	CH ₃ stretch
1172	O=C-O stretch	1158	C-O stretch
1111	C-O strong	1104	CH stretch
		722	CH ₂ stretch
p(CcO)		p(CnO)	
Wavelength (cm ⁻¹)	Vibration type	Wavelength (cm ⁻¹)	Vibration type
2920	C-H stretch	2920	CH ₂ stretch
2861	C-H stretch	2856	CH ₂ stretch
1725	C=O stretch	1734	C=O stretch
1460	C-H stretch	1458	CH ₂ and CH ₃ stretch
1114	C-O strong	1111	CH stretch

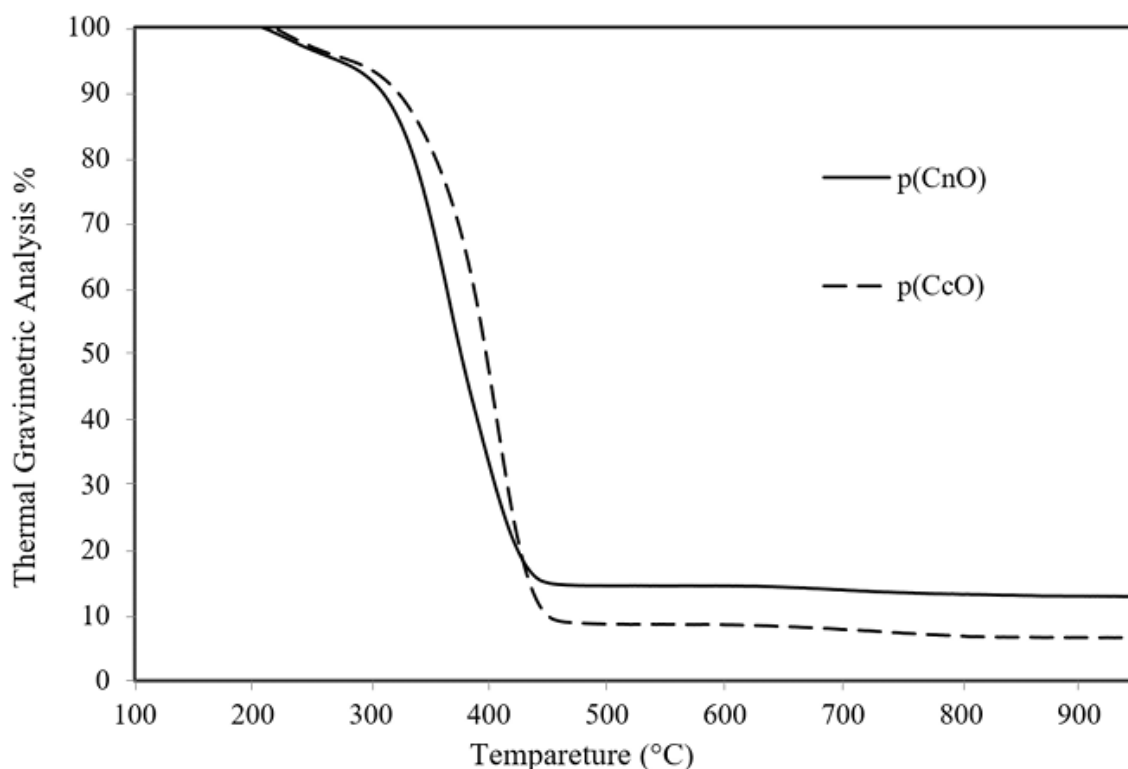


Figure 3. TGA graphic of p(CnO) and p(CcO) organo-particles

Herbal oils, flavonoids, alkaloids, saponins, tannins, and resins produced in medicinal and aromatic plants are secondary metabolites. Research has demonstrated the effectiveness of flavonoids, phenolic compounds, essential oils, and their derivatives, as well as herbal remedies, in averting autoxidation. Its modes of action include scavenging free radicals, combining with metal ions to create compounds, and limiting or blocking the production of oxygen.

As results in Table 3, the total-phenol values of coconut oils, cocoa oil, p(CnO) and p(CcO) organo-particles were measured to be 929.6 mg g⁻¹, 2014.0 mg g⁻¹, 665.9 mg g⁻¹, and 858.8 mg g⁻¹, respectively. It has been determined that coconut oil and cocoa butter have antioxidant properties, and the organo-particles obtained from these oils exhibit similar antioxidant properties. Additionally, when the antioxidant properties of other materials in the literature are examined, it was found that as p(AG-m-CnO)1 980 mg L⁻¹, p(AG-m-CnO)2 is 997 mg L⁻¹, p(AG-m-CnO)3 1032 mg L⁻¹, p(AG-g -CnO)1 1202 mg L⁻¹, p(AG-g-CnO)2 1003 mg L⁻¹, p(AG-g-CnO)3 1399 mg L⁻¹, coconut oil 5818 mg L⁻¹ (gallic acid) [15]. It was determined that the organo-particles we synthesized showed weaker antioxidant activity than the particles examined in the literature. This is because the antioxidant value of commercially using vegetable oils varies in each production batch. Therefore, it is envisaged that a comparison should be made by taking. This is due to the fact that every production batch's antioxidant value for vegetable oils utilized in commerce differs.

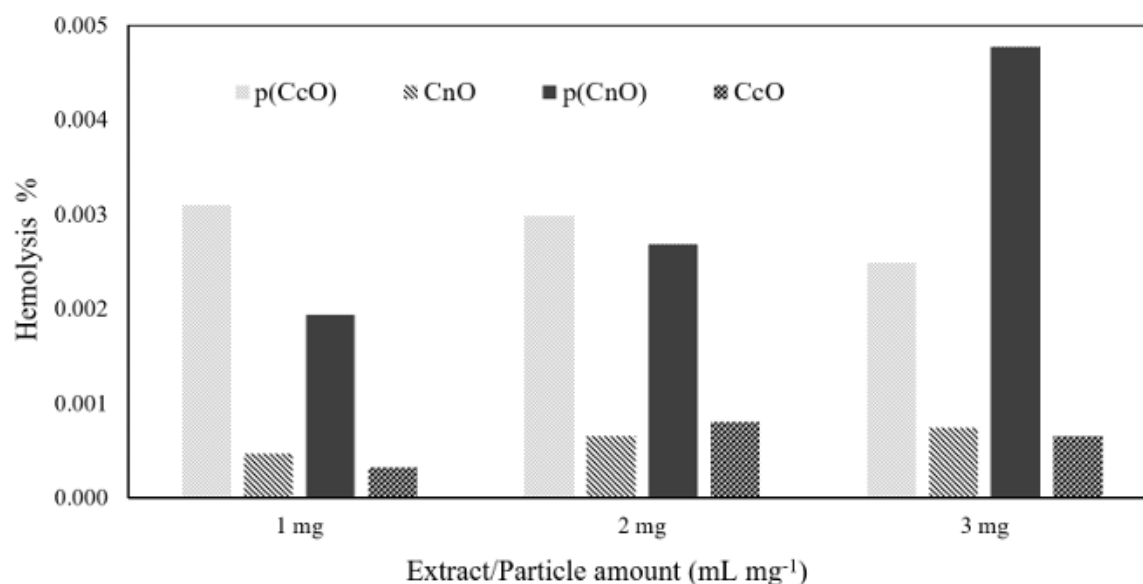
Table 3. Total phenol content values of coconut oil, cacao oil, p(CnO), and p(CcO) organo-particles

Substance	Antioxidant
	Total phenol (mg g ⁻¹)
CnO	929.6
p(CnO)	665.9
CcO	2014.0
p(CcO)	858.8

After being implanted into the body, organo-particles come into direct touch with tissue and blood, which is why it's critical to assess their hemocompatibility. If the hemolysis rate was less than 5%, it was classified as very compliant, up to 10% compliant, and more than 20% discordant. As seen in Figure 4, upon examining the hemolysis and BCI, it was observed that the p(CnO) and p(CcO) organo-particles were hemocompatible. In this case, the synthesized p(CnO) and p(CcO) organo-particles can be used easily in the body. Hemolysis values of various quantities (1,2,3 mL) of coconut oil were 0.0005 % CnO, 0.0007 % CnO, 0.0007 % CnO, and various quantities of p(CnO) organo-particles were found as 0.0017 % p(CnO), 0.0029 % p(CnO), 0.0048 % p(CnO). The hemolysis of organo-particles and cacao oils was measured as follows: 0.0031 % p(CcO), 0.0030 % p(CcO), 0.0025 % p(CcO), 0.0003 % CcO, 0.0007 % CcO, 0.0007 % CcO, respectively. The BCI of particles, coconut oil, and cacao oils was measured as follows and can be used easily in the body.

BCI values of various quantities (1,2,3 mL) of coconut oil were 6.1 % CnO, 7.7 % CnO, 3.3 % CnO; various quantities of p(CnO) organo-particles were found as 7.8 % p(CnO), 7.6 % p(CnO), 8.1 % p(CnO). BCI values of various quantities of cacao oil were 4.2 % CcO, 2.5 % CcO, and 3.5 % CcO; various quantities of p(CcO) organoparticles were found as 6.7 % p(CcO), 7.5 % p(CcO), 6.9 % p(CcO). The very low percent hemolysis readings in this study's evaluation of the particle blood compatibility findings show blood compatibility. As the particle concentration rose, it was noted that blood coagulation levels (BCI) did not significantly alter. The hemolysis results indicate that the structural modifications that the coconut and cacao oils experienced to become organoparticles did not affect the biocompatibility qualities.

The kind, concentration, and nature of herbal oils, as well as the microorganism they target, can all alter their antimicrobial efficacy. Figure 5 shows that, out of the test microorganisms, *S. aureus* most usually impacted the p(CnO) organo-particles, followed by *B. cereus* and *E. coli*. It was shown that the growth of *B. cereus*, *S. aureus*, and *E. coli* was less affected by different concentrations of p(CcO) organo-particles. The antifungal, antibacterial, and antioxidant properties of herbal oils were attentiongrabbing [18, 19]. The phenolic and terpenoid components in them are what give them their antibacterial properties (lauric acid, myristic, caprylic, capric acid oleic and palmitic acid, c-nonolactone,) coconut oil and cocoa oil structure [20, 21]. The phospholipid layer in the cell membrane becomes more sensitive due to the phenolic chemicals included in essential oils, which increases the permeability of the membrane. As a result, they inhibit microorganisms by either disrupting the enzyme systems or allowing internal components to seep out of the cell [22].



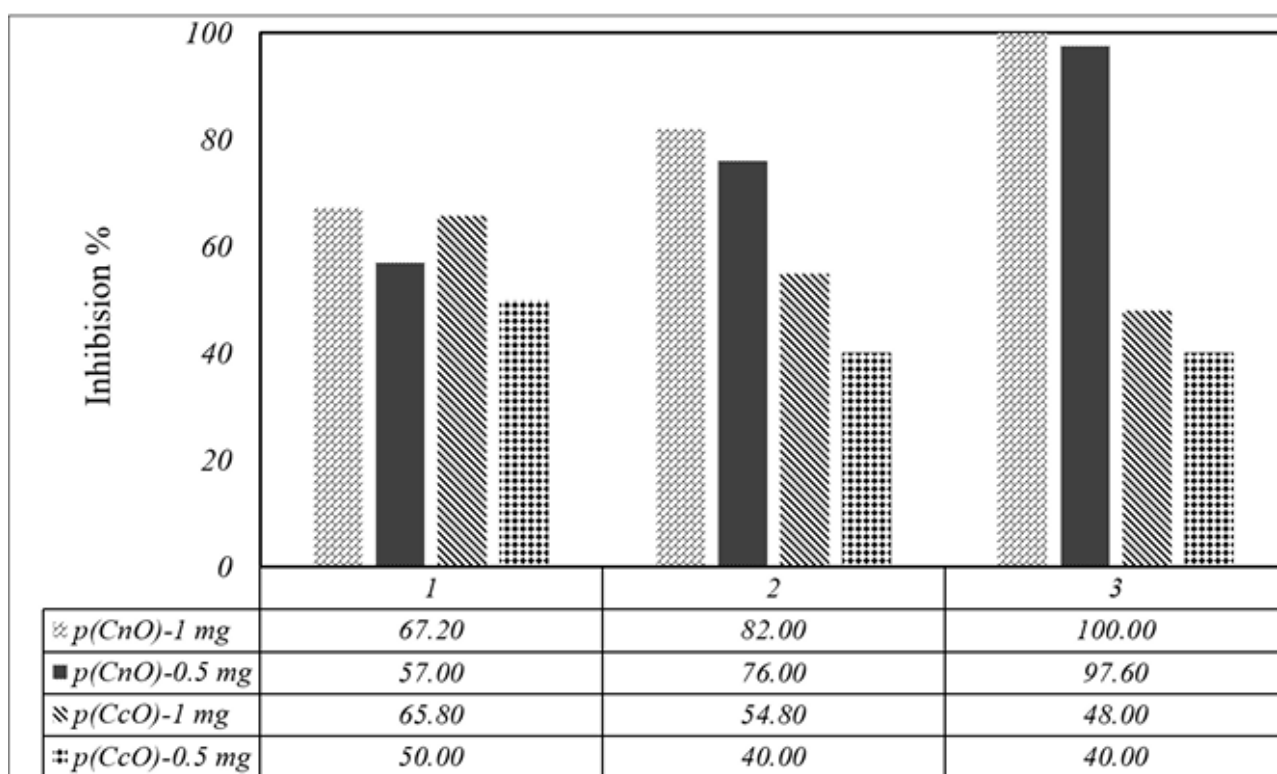
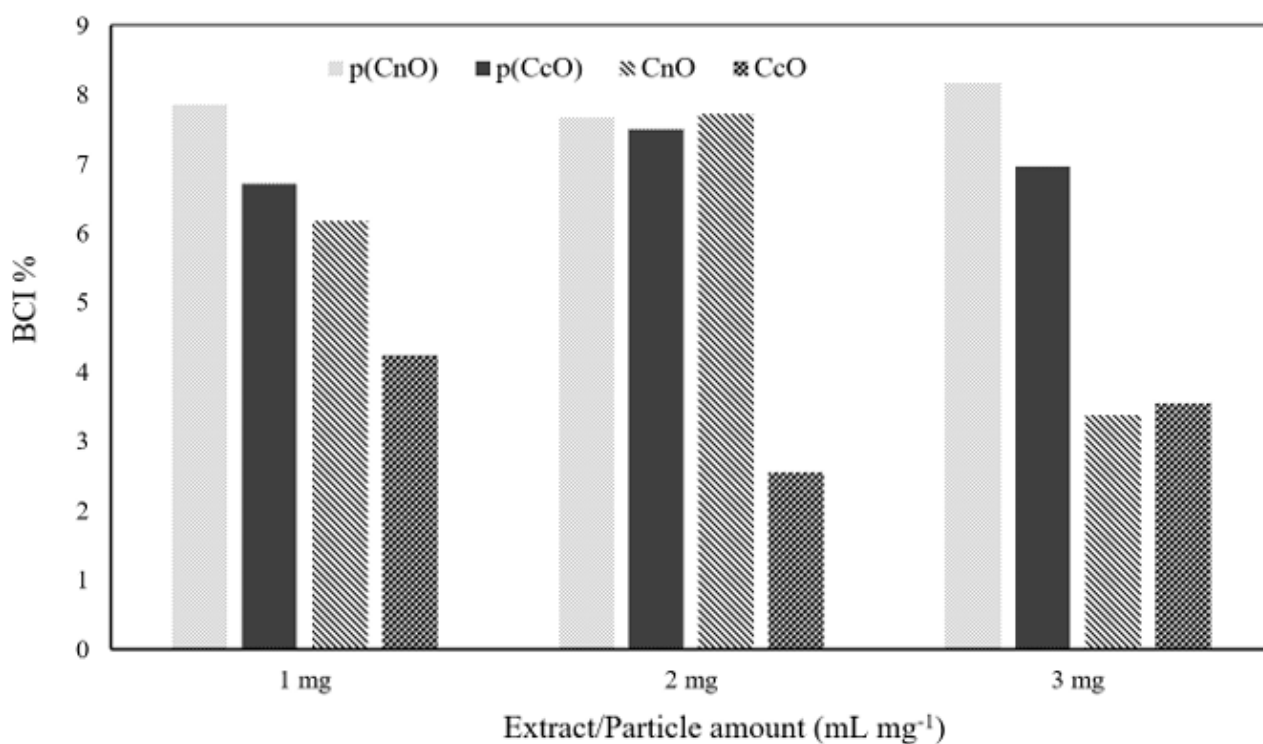


Figure 5. Antimicrobial inhibition % of the p(CnO) and p(CcO) organo-particles

3.2. Cell Culture

The % viability values for the L-929 and Capan-1 cell lines were calculated based on the absorbance values that were obtained following the cessation of the MTT treatment. During the computations, it was

determined that p(CnO) and p(CcO) organo-particles did not have a toxic effect on L-929 cells ($158.9 \pm 1.24\%$ and $113.45 \pm 4.33\%$). While $50.25 \pm 3.22\%$ viability was measured in doxorubicin application, this rate increased to $114.15 \pm 1.95\%$ and $100.05 \pm 2.96\%$ with p(CnO) and p(CcO) organo-particles as well as doxorubicin application (Figure 6). In Capan-1 cells, the viability was $114.12 \pm 6.43\%$ and $69.99 \pm 3.62\%$ after p(CnO) and p(CcO) organo-particles treatments, while it was calculated as $103.16 \pm 8.24\%$ and $32.19 \pm 0.29\%$ when they were administered together with doxorubicin. While the viability was $51.23 \pm 3.98\%$ with the application of doxorubicin alone, it was found to be $32.19 \pm 0.29\%$ when it was applied together with p(CcO) organo-particle (Table 4 and Figure 7).

3.3. Double Staining Results

Different characteristics of necrotic and apoptotic cells can be seen using dyes like Hoechst and propidium iodide (PI). Furthermore, ribonuclease A (RNase A) aids in emphasizing the traits of apoptotic cells. The fluorescent dye Hoechst illuminates the nucleus of cells because it binds to DNA. Chromatin condenses as the cell's nucleus shrinks during apoptosis. With Hoechst staining, this is seen as smaller, brighter nuclei. Apoptosis is a type of controlled cell death in which the nucleus is extracted from the surrounding medium by forming a 180 kB pocket at the time of cell death. Blue blips of the same size are seen in the DAPI filter during imaging. By gaining more access to DNA, ribonuclease A (RNase A) degrades ribonucleic acids in apoptotic cells and aids in their identification. Living cells' outer membranes prevent propidium iodide (PI) from passing through, but when the membrane is broken, particularly during necrosis, PI can pass through and enter the cell. PI staining is therefore used to detect damaged or dead cells. The integrity of the cell membrane is maintained during apoptosis, preventing PI from entering the cell. PI-stained cell nuclei produce erratic stains and show up more clearly on the FITC filter. Following the application, the cells were analyzed using the double staining procedure to calculate the apoptotic and necrotic indices of p(CnO) and p(CcO) combined with doxorubicin. There was no apoptotic or necrotic death in the L-929 cell line for all materials. For p(CnO), there were no deaths in the Capan-1 cell line. However, for p(CnO) and p(CnO)+dox, the apoptotic index was $14.3 \pm 2.1\%$ and $42.6 \pm 2.3\%$, respectively, at a concentration of 1 mg mL⁻¹. Apoptotic resuscitation was performed in p(CcO) +dox application. Observed necrotic values are deaths not higher than 5% (Figure 8).

When the obtained MTT and double staining results were compared, it was seen that the results were consistent within themselves. It showed no toxic effect at any concentration in L-929 fibroblast cells, which were p(CcO) healthy. When co-administered with doxorubicin p(CcO), it also inhibits the toxic effect of doxorubicin on L-929 cells. It has been determined that p(CcO) causes death in Capan-1 cells, which, at a concentration of 1 mg mL⁻¹, were pancreatic cancer cells. The death rate increases from approximately 30 % to approximately 70 % when applied together with doxorubicin, which was used as a cancer drug. It is a common situation that drugs used in cancer cause many complications due to side effects, damage to healthy cells, and weakening of the immune system. For this reason, it was thought that the application of p(CcO) together with cancer drugs would eliminate or minimize these effects.

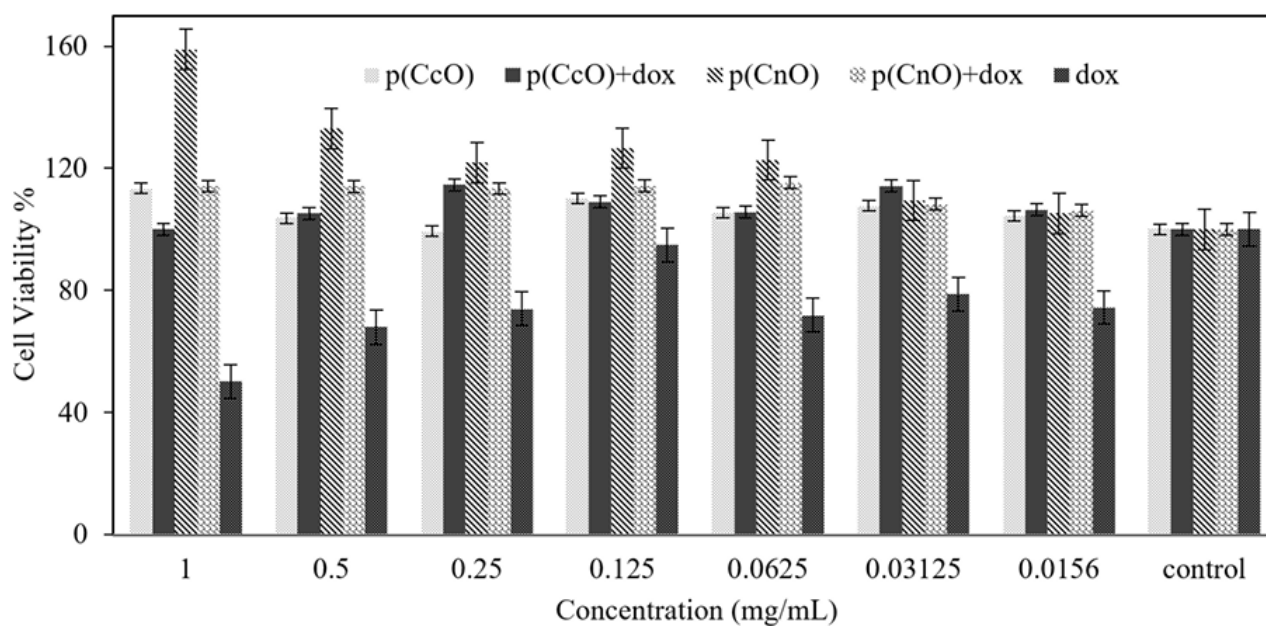


Figure 6. Viability% data of L-929 cells after application

Table 4. Apoptotic and necrotic index results (%)

	Concentration (mg/mL)	Concentration (1 mg/mL)	Capan-1	L929
p(CnO)	1	%Apoptosis	0	0
		%Necrosis	0	0
	0.25	%Apoptosis	0	0
		%Necrosis	0	0
p(CnO)+ dox	1	%Apoptosis	0	0
		%Necrosis	0	0
	0.25	%Apoptosis	0	0
		%Necrosis	0	0
p(CcO)	1	%Apoptosis	14.3±2.1	0
		%Necrosis	1.2±0.2	0
	0.25	%Apoptosis	0	0
		%Necrosis	0	0
p(CcO)+ dox	1	%Apoptosis	42.6±2.3	0
		%Necrosis	2.6±0.9	0
	0.25	%Apoptosis	5.6±0.1	0
		%Necrosis	0	0

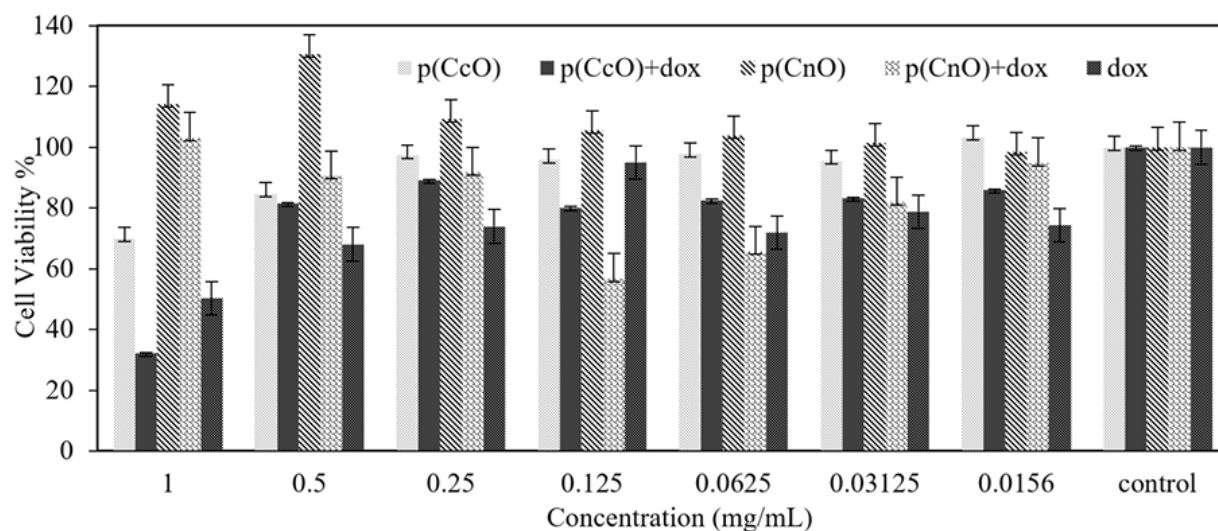


Figure 7. Viability% data of Capan-1 cells after application

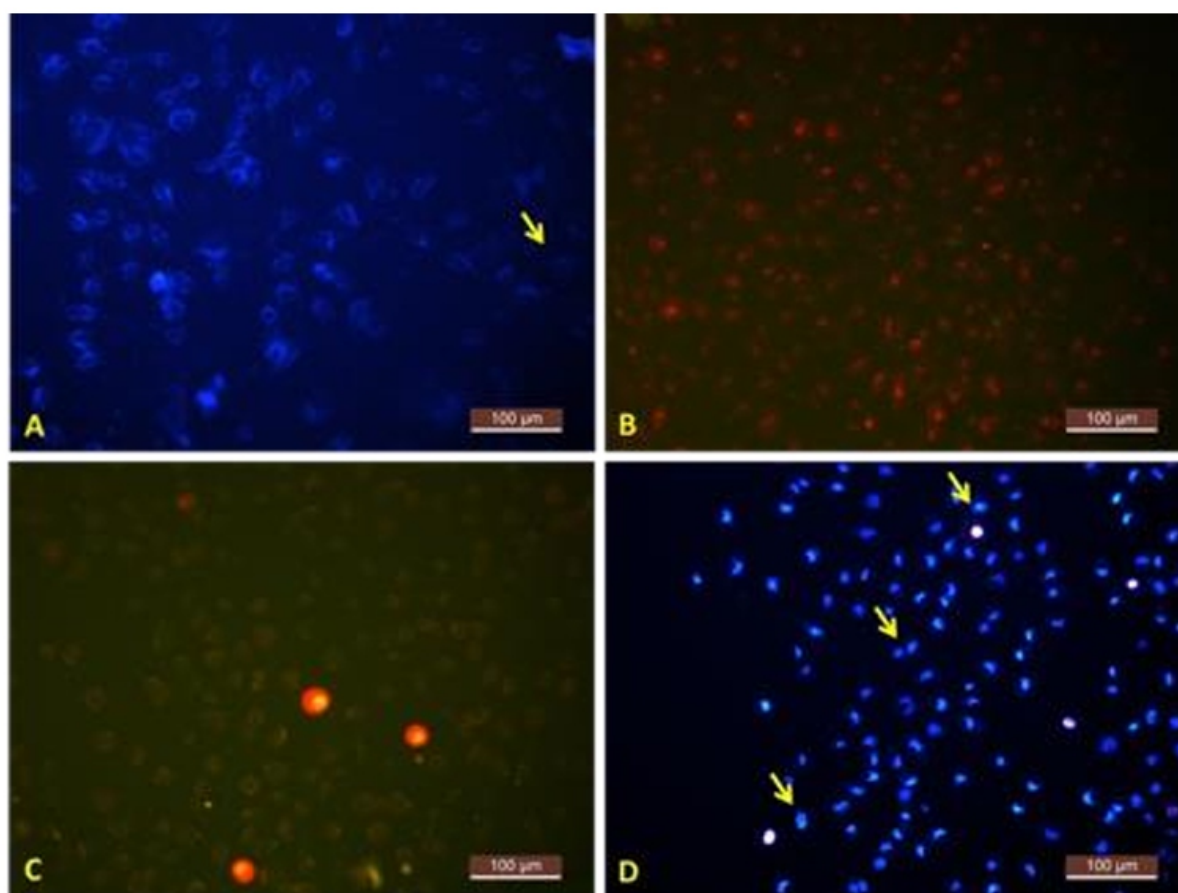


Figure 8. Apoptotic and necrotic cells in L-929 and Capan-1 cell lines (A: L-929 cells after administration of p(CcO)+dox at a concentration of 1.00 mg mL⁻¹, appearance in the DAPI filter, arrows indicate live cells. B: Images of p(CnO) applied to L-929 cells at a concentration of 1.00 mg mL⁻¹, appearance in the FITC filter. C: Capan-1 cells after administration of p(CnO)+dox at a concentration of 1 mg mL⁻¹, appearance in the FITC filter. D: Capan-1 cells after administration of p(CcO) at a concentration of 1.00 mg mL⁻¹, appearance in the DAPI filter) Photographs were obtained by FITC and DAPI filters using a Leica inverted fluorescent microscope (200× magnification)

aromatherapy: A systemic review," *Asian Pacific Journal of Tropical Biomedicine*, vol. 5, no. 8, pp. 601-611, 2015, doi: 10.1016/j.apjtb.2015.05.007.

N. Y. Saad, C. D. Muller, and A. Lobstein, "Major bioactivities and mechanism of action of essential oils and their components," *Flavour and Fragrance Journal*, vol. 28, no. 5, pp. 269-279, 2013, doi: 10.1002/fff.3165.

L. R. Lizarraga-Valderrama, "Effects of essential oils on central nervous system: Focus on mental health," *Phytother Res*, vol. 35, no. 2, pp. 657-679, Feb 2021, doi: 10.1002/ptr.6854.

L. Wayteck, K. Breckpot, J. Demeester, S. C. D. Smedt, and K. Raemdonck, "A personalized view on cancer immunotherapy," *Cancer Letters*, vol. 352, no. 1, p. 12, 2014.

J. Zhou, "Advances and Prospects in Cancer Immunotherapy," *New Journal of Science*, vol. 1, pp. 1-13, 2014, doi: 10.1155/2014/745808. P. F. d. Oliveira et al., "Cytotoxicity screening of essential oils in cancer cell lines," *Revista Brasileira de Farmacognosia*, vol. 25, no. 2, pp. 183-188, 2015, doi: 10.1016/j.bjp.2015.02.009.

R. D. Abigor, W. N. Marmer, T. A. Foglia, P. Uadia, and P. Uadia, "Production of Cocoa Butter-like Fats by the Lipase-Catalyzed Interesterification of Palm Oil and Hydrogenated Soybean Oil," *Journal of the American Oil Chemists' Society*, vol. 80, no. 12, p. 3, 2003.

L. Boateng, R. Ansong, W. B. Owusu, and M. Steiner-Asiedu, "Coconut oil and palm oil's role in nutrition, health and national development: A review," *Ghana medical journal*, vol. 50, no. 3, p. 7, 2016.

K. Blowman, M. Magalhães, M. F. L. Lemos, C. Cabral, and I. M. Pire, "Anticancer Properties of Essential Oils and Other Natural Products," *Evid Based Complement Alternat Med.*, vol. 2018, p. 12, 2018.

M. Sharma, K. Grewal, R. Jandrotia, D. R. Batish, H. P. Singh, and R. K. Kohli, "Essential oils as anticancer agents: Potential role in malignancies, drug delivery mechanisms, and immune system enhancement," *Biomedicine & Pharmacotherapy*, vol. 146, p. 10, 2022.

B. Bayala et al., "Anticancer activity of essential oils and their chemical components - a review," *Am J Cancer Res.*, vol. 4, no. 6, p. 16, 2014.

D. Alpaslan, T. Ersen Dudu, and N. Aktas, "Synthesis of Poly(ginger oil) Organo Particles as a Metal Free Catalysis and Their Use in Hydrogen Production from Sodium Borohydride Methanolysis," *Journal of Polymers and the Environment*, vol. 1, p. 12, 2022, doi: 10.1007/s10924022-02636-6.

D. Alpaslan, A. Turan, T. E. Dudu, B. M. Bozer, N. Aktas, and M. Turk, "Characterization of p(PmO), p(LO) and p(RO) organoparticles, their bioactivity properties and their effect on pancreatic cancer Capan-1 cell," *Materials Chemistry and Physics*, vol. 1, p. 127871, 2023, doi: 10.1016/j.matchemphys.2023.127871.

D. Alpaslan, T. E. Dudu, B. M. Bozer, N. Aktas, and M. Turk, "p(thyme oil) and p(clove oil) organoparticles with biocompatible, anticancer, antioxidant, and antibacterial properties against Capan-1 and L-929 cells," *The Canadian Journal of Chemical Engineering* vol. 1, p. 12, 2023, doi: 10.1002/cjce.25061.

D. Alpaslan, T. Olak, A. Turan, T. Ersen Dudu, and N. Aktas, "Use of Coconut Oil-Based Organo-Hydrogels in Pharmaceutical Applications," *J. Polymers and the Environment*, vol. 30, pp. 666-680, 2022, doi: 10.1007/s10924-021-02219-x.

K. E. Sapsford, K. M. Tyner, B. J. Dair, J. R. Deschamps, and I. L. Medintz, "Analyzing nanomaterial bioconjugates: a review of current and emerging purification and characterization techniques," *Anal Chem*, vol. 83, no. 12, pp. 4453-88, Jun 15 2011, doi: 10.1021/ac200853a.

A. Degen and M. Kosec, "Effect of pH and impurities on the surface charge of zinc oxide in aqueous solution," *Journal of the European Ceramic Society*, vol. 20, p. 6, 2000.

I. M. Helander et al., "Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria," *J. Agric. Food Chem.*, vol. 46, p. 5, 1998.

4. Conclusions

Advances in nanotechnology have brought many vital innovations in targeted drug delivery in cancer. This approach has the potential to reduce the harmful effects of medications on healthy cells while simultaneously increasing the intracellular concentrations of these chemicals in cancer cells. For the first time in the literature, p(CnO) and p(CcO) organo-particles from coconut oil and cocoa oil were synthesized in one step by free radical polymerization. p(CnO) and p(CcO) organo particle characterization and antioxidant, antimicrobial, and biocompatibility analyses were performed. In our study, the effect of p(CnO) and p(CcO) organo-particles on cancerous cells was examined. Nanotechnology, which is an interdisciplinary science, offers significant advantages in cancer.

The study investigated whether newly synthesized p(CnO) and p(CcO) organo-particles have cytotoxic and anticancer effects on L-929 cells lines and Capan-1 pancreatic cancer cells. In our study to evaluate the effectiveness of p(CnO) and p(CcO) organo-particles in normal cells, L-929 fibroblast cells were treated with different concentrations of p(CnO) and p(CcO) organoparticles ranging from 1.56 mM to 100 mM. It was determined that p(CnO) and p(CcO) organo-particles increased cell viability in fibroblast cells. The lethal effect of p(CcO) on Capan-1 pancreatic cancer cells was found to be similar to doxorubicin. In light of the findings obtained, it is predicted that the use of p(CnO) and p(CcO) organoparticles, which we have successfully synthesized, together with cancer drugs in cancer treatment will have positive effects. It is also thought that it will minimize the side effects of drugs used in cancer treatment.

Ethical Approval

No human or animal application was made in the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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REFERENCES

B. Ali, N. A. Al-Wabel, S. Shams, A. Ahamad, S. A. Khan, and F. Anwar, "Essential oils used in

-
-
- M. Szczerbanik, J. Jobling, S. Morris, and S. Holford, "Essential oil vapours control some common postharvest fungal pathogens," Australian Journal of Experimental Agriculture, vol. 47, p. 6, 2007.*
- M. Božík, P. Hovorková, and P. Klouček, "Antibacterial Effect of Carvacrol and Coconut Oil on Selected Pathogenic Bacteria," Scientia Agriculturae Bohemica, vol. 49, no. 1, pp. 46-52, 2018, doi: 10.2478/sab-2018-0008.*
- M. Zuzarte and L. Salgueiro, "Essential Oils Chemistry," in Bioactive Essential Oils and Cancer, 2015, ch. Chapter 2, pp. 19-61.*
- M. R. Moreira, A. G. Ponce, C. E. d. Valle, and S. I. Rour, "Inhibitory parameters of essential oils to reduce a foodborne pathogen," LWT - Food Science and Technology, vol. 38, p. 5, 2005.*

Lipidic Nanotechnology Carriers: A Secure and Excellent Fungicide and Hepatoprotective Transporting Devices

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ABSTRACT

Throughout the past three decades, there has been a notable rise in the incidence and variety of invasive fungal infections due to modifications in medical and surgical care, especially in intensive care units that employ invasive catheters for observing, as well as the use of more potent immune suppression and antibiotic agents. The types, benefits, and limitations of various nanoformulations for the topical and intravenous delivery of antifungal medications are investigated in the current investigation. A couple of instances of NPs used in the delivery of medicines include solid lipid nanoparticles, nanostructured lipid carriers, polymeric NPs, polymeric micelles, phospholipid vesicles (liposomes, deformable liposomes, ethosomes, transfersomes, transethosomes, etc.), nonphospholipid vesicles (noisome and plastics) and dendrimers. Collectively with these features hepatic diseases persist to be a major global health concern and one of the greatest risks to the general population. Even with all of the excellent advances in modern medicine, there are currently no fully effective drugs that improve liver function, provide total organ protection, or promote liver cell regeneration. Therefore, pharmaceutical substitutes for the therapy of liver conditions need to be explored to make them more secure and successful. Focusing liver illnesses may benefit from the use of liposomes, metallic nanoparticles, ceramic nanomaterials, polysaccharides, Carbon-nanotubes, multifunctional NPs, and dendrimers can be made much more effective when these are paired with particular targeted agents.

Keywords NPs-nanoparticles, Solid Lipid Nanoparticles, Hepatic Diseases, Antifungal Medications, Metallic Nanoparticles

1. Introduction

Throughout the past three decades, there has been a notable rise in the chronicity and variety of invasive fungus-related illnesses due to modifications in laparoscopic and healthcare settings, particularly in emergency rooms that employ devices that are invading to observing, in conjunction with using more potent immune suppression added to antimicrobial agent [1]. Around 2025 percent of the human population is thought to be affected by superficial mycoses. Extensive contagions involving fungi also become a notable participant in fatalities and death in individuals receiving organ transplants, immune-compromised those diagnosed with acquired immunodeficiency syndrome, myeloid & lymphatic tumors, severe bone marrow failure, acute myeloid leukemia, preterm neonates and the elderly [2]. Candidemia-related mortality has reduced annually since its peak, thanks to recent breakthroughs when managing invasive candidiasis. Despite this, systemic candidiasis continues to rank as the 4th most

common nosocomial bloodstream infection in hospitals [3]. Major advancements in the identification of novel antifungal medications have led to the release of improved generations that provide superior therapeutic results for those individuals who are at threat [4]. An azole of the original class medicines, among these are itraconazole and fluconazole launched around the nineties after a more than 20-year hiatus. Azole of the following class medications, comprising voriconazole, posaconazole, and isavuconazole, as well as echinocandins (anidulafungin, caspofungin, and micafungin) became available during the decade of 2000 [5].

Particles with a diameter of 1–1000 nm are referred to as nanoparticles (Nps). However, items with a size between one and one hundred nanometres are referred to as "nanomaterials" or "nanoscale". For this review, particles utilized in drug delivery applications ranging in size from 1 to 1000 nm shall be referred to as NPs. When compared to their larger-scale counterparts, NPs have unique chemical, physical, and biological features, making them promising drug-delivery vehicles [6]. Keeping a closer eye on how nanotechnologies are used in biomedical research, those substances can be used for several applications including dressings for injuries, laser ablation treatment, drug delivery, therapy, modification of genes, immunizations fabricated devices heat exhaustion and medicinal automation. These include their large surface-to-volume ratio, their simplicity of functionalization through the incorporation of biologically compatible substances as a hat, and their distinct visual magnetic features [7].

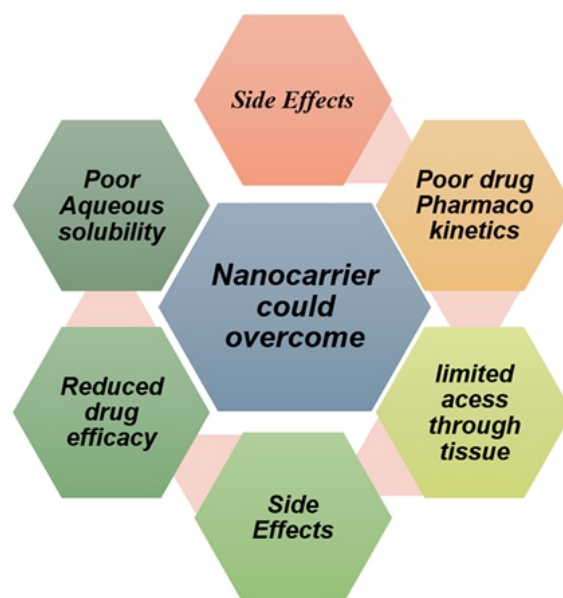


Figure 1. Unfavorable drug properties that can be improved through amalgamation into various nano-formulations

Figure 1 illustrates how nanocarriers lessen adverse effects and increase tissue penetration while improving medication solubility, effectiveness, and pharmacokinetics. The current study examines the kinds, advantages and restrictions of several nanoformulations for the cutaneous and central administration of antifungal drugs. Additionally, it highlights how these NPs may be used to combat aggressive as well as peripheral fungal illnesses. The difficulties that this research faces, as well as the roadblocks to the clinical application of a few potential nanoformulations, were explored.

2. Nanoparticles (NPs) as a Means of Dispensing Antifungal Agents

Solid lipid nanoparticles, nanostructured lipid carriers, polymeric NPs, polymeric micelles,

phosphoglyceride vesicles (liposomes, deformable liposomes, ethosomes, transferosomes, transethosomes, etc.), nonphosphoglyceride vesicles (niosomes and plastics) and dendrimers are a few examples of NPs used in drug delivery [8]. In accordance with Figure 2, dendrimers, polymeric nanoparticles, solid lipid nanoparticles, phospholipid vesicles, and non-phospholipid vesicles are the components of antifungal nanoformulations.

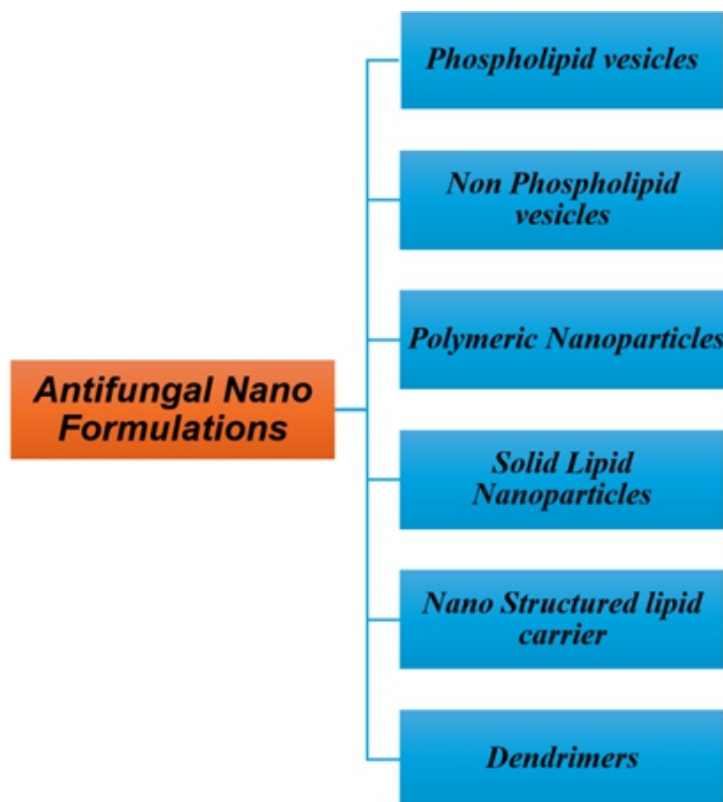


Figure 2. Various antifungal Nano formulation

3. Phospholipid-based Vesicles

3.1. Liposomes

Liposomes are a set of concentric phospholipid membranes encased in an aqueous shell operating as reliable drug-transporting vehicles for the pair of water loving and repelling medications due to their unique structure. Biocompatibility, minimum noxious effects, extended release of drugs, low pharmacological adverse effects, significant loading ability, enhanced bioavailability of drugs, and stability are some of the other appealing characteristics of liposomes as drug delivery vehicles. Cholesterol is generally integrated into the lipid bilayers of liposomes to increase membrane stiffness, stabilize vesicles and regulate the pace of drug release [9].

3.1.1. Amphotericin B

The initial and most effective commercially available antifungal nanoformulation is liposomal AmB (AmBisome®) [10]. To address the nephrotoxicity and infusion-related adverse effects of AmB deoxycholate injection (Fungizone®) was created. Treatment for patients with endophthalmitis,

Candida meningitis, or disseminated histoplasmosis with AIDS now involves liposomal AmB [11].

3.1.2. Nystatin

Nystatin is a polyene antifungal medication with a broad spectrum of activity that has been effectively introduced into liposomal medications to treat systemic fungal infections. It comes from *Streptomyces noursei* and has a wider range of effects than AmB (Amphotericin B). Because of its limited oral absorption, nystatin is solely utilized topically. Furthermore, Parenteral dosing cannot occur in the presence of thrombophlebitis, high temperature, shivers or nausea. According to in vitro experiments liposomal nystatin exhibited an equivalent level of activity compared with that of the unbound drug [12]. The liposomal formulation allowed for intravenous nystatin administration. In Hale-Stoner rodents, the highest tolerated dosage was raised concerning 4-16 mg/kg of body mass per day. Mice diagnosed with *Candida albicans* displayed a substantial rise in their survival rates when liposomal nystatin was administered [13]. Figure 3 depicts the structural elements and drug encapsulation techniques of four different types of nanocarriers: liposomes, ethosomes, transferosomes, and transethosomes.

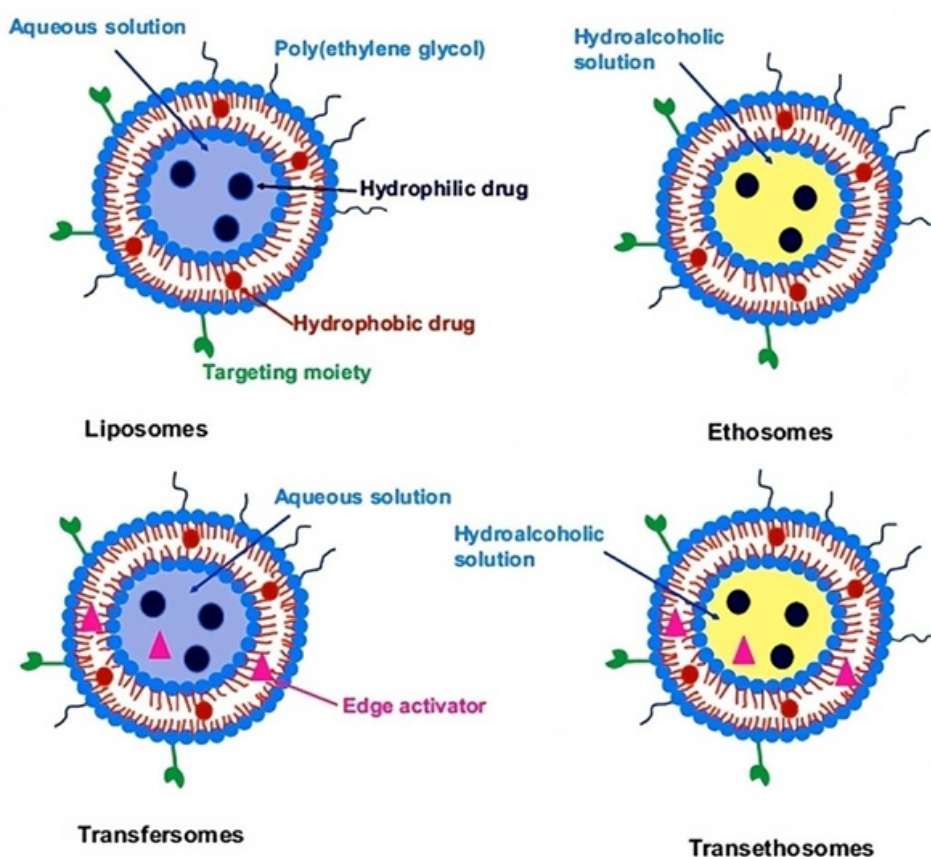


Figure 3. Phospholipid based drug delivery systems

3.2. Transferosomes

Transferosomes are ultra-deformable, self-optimized containing lipids and biocompatible softeners for transdermal administration. Liposomes' low penetration into cornified layers inspired the change of the makeup of their twin layer to get around this constraint, notwithstanding their advantages and achievements. Wetting agents with a great degree of transportation like edge-activating agents include dipotassium glycyrrhizinate, sodium cholate, polysorbate 20, polysorbate 60, polysorbate 80, and Sorbitan Tri stearate 65, Sorbitan monooleate 80, etc. The edge activator is in charge of deteriorating and increasing the deformability of vesicle lipid bilayers. In terms of medication penetration and skin contact, it was discovered that these pliable vesicles outperformed conventional liposomes [14].

3.3. Ethosomes

Ethosomes are soft vesicles that are made up of phospholipids, ethanol, and water. Typically, these consist of 100 percent water, 20–45 percent ethanol, and 2-3% phospholipids Touitou et al., first proposed them in 2002 as a way to overcome the poor epidermal invasion of traditional liposomes [15]. Ethosomes had a higher penetration impact than ethanol by itself, grade alcohol phosphoglyceride solution, give direction to drinking alcohol, the vesicular configuration and moisture barrier work in concert. Ethanol's propensity to lubricate ethanolic triglycerides and interpersonal fats of stratum granulosum allows ethosomes to penetrate the skin more effectively than liposomes [16].

3.4. Transethosomes

A novel class of vesicular carriers known as transethosomes merges the perks of ethosomes and collapsible liposomes. It accommodates an edge activator or permeability accelerator in addition to the typical ethosome makeup. Because transethosomes are a relatively novel drug carrier there is little research on their use for antifungal drug delivery, contrasted with the ethosomes, medicine flexible liposomes and typical liposomes, polyethylene glycol solution, voriconazole transethosomes exhibited considerably higher skin permeability. Furthermore, as compared to other vesicular carriers, the transethosomes improved voriconazole skin deposition in the dermis/epidermis layers both in vitro and in vivo [17].

3.5. Niosomes

Niosomes and liposomes share characteristics in that they both have double layers formed up of non-ionized single-alkyl chain detergents rather than amphiphilic lipids. The water-resistant tail of the surfactant is submerged within the bilayer and the hydrophilic head confronts both architecture of vesicles. Although water-repellent pharmaceuticals are implanted in the hydrophobic bilayers, hydrophilic drugs can be integrated into the watery core. To increase the rigidity of the bilayer and stop drugs from releasing too soon, cholesterol is introduced. Similar to liposomes, niosomes have several advantages over liposomes, including higher chemical stability, lower cost and the capacity to keep them in a controlled environment for storage [18].

3.6. Polymeric Nanoparticles

The subject of drug delivery, notably cancer chemotherapy, has been transformed by degradable and/or

bio-adaptable polymer-assembled nanoparticles (Nps). NPs have demonstrated a strong capacity to improve medication therapeutic qualities while reducing side effects and toxicity. Many cytotoxic medication formulations based on polymeric NPs have already entered clinical trials and many more are in the works. Based on their molecular makeup and production method polymeric, NPs are categorized into distinct categories i.e., three categories: polymeric micelles, nanospheres, and nanocapsules [19]. Figure 4 demonstrates a few polymeric nanocarrier structural compositions, namely nanospheres, and polymeric micelles. nanocapsules,

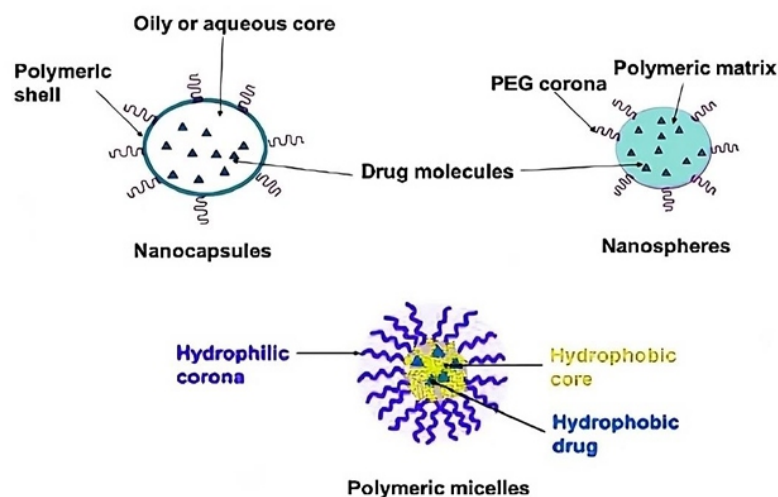


Figure 4. Polymeric nanoparticles

3.7. Lipid Nanoparticles in Solid State (SLNs) and Fat soluble Transporters with Nanostructure (NLCs)

SLNs (Solid lipid nanoparticles) stand for therapeutically appropriate lipids suspended in an aqueous surfactant solution, forming dispersion nanostructured carriers for drugs. They were first made available as an alternative to polymeric NPs at the beginning of the nineties. Since the lipids in SLNs remain solid at human body temperature & ambient temperature release of drugs is facilitated with a prolonged process of medication dispersion via rigid fat. SLNs have some benefits to providing medication such as improved biological barrier permeability the Chemical endurance of lipids, the capacity to alter their outer layer, the space available for delivering several medications, and protection from integrated molecule breakdown. SLNs on the other hand have some drawbacks such as inadequate ability to load drugs and drug ejection upon preservation. Whenever the molecules of lipids rearrange to form an ideal crystal lattice through storage, drug evacuation happens because less space exists for therapeutic incorporation. Two-generation carrier lipids with nanostructured structures of SLNs were introduced to overcome these constraints. Better medication loading, controlled release of drugs, and stability over time are achieved by NLC's (Nanostructured lipid carriers) solid lipid matrix, which is made up of solid lipid and oil in specific ratios at ambient temperature and the temperature of the body [20]. The creation of crystallized phospholipid structures that are extremely organized is inhibited by the fluid lipid inside NLCs, which are the source of SLN's flaws. ITZ (Itraconazole) put into NLCs, for example, exhibited a 99.98 percent encapsulation efficiency and stable circumstances for storage for more than six months. Additionally, it was discovered that SLNs comprised entirely of solid or fluid lipids achieved encapsulation efficiencies of 59 and 42 percent respectively for AmB (Amphotericin B). These formulations on the other hand had an AmB ejection rate of 80%. AmB encapsulation efficiency was

dropped to 12% as long as a combination of liquid and solid forms of fats was utilized suggesting improved formulation stability [21].

3.8. Dendrimers

Dendrimers are three-dimensional hyperbranching and spherical macromolecules with multiple arms extending from a distinct focal core, originating from the ancient Greek word dendron, meaning "tree." Dendrimers have a lot of possibility of delivering antifungal medication because of their distinctive properties such as structural plasticity, multivalency, and low polydispersity. Hydrophobic fissures formed by dendrimer branches are conceivably utilized to remediate the hydro-based solubility of water-repellent medications by encapsulating them. At pH 7.4 poly (propylene imine) dendrimers increased AmB aqueous solubility by a factor of 25. Furthermore, one can use ionized surfaces made up of dendrimers as focal areas for attaching targeting or imaging moieties or integrating ionized medicines via ionic interactions. Dendrimers' potential as antifungal medication delivery strategies is still lacking as indicated by the small number of studies in this field and AmBisome, to name a few. Peptide-conjugated poly amidoamine dendrimers (PADRE-PAMAM) seemed additionally employed to improve AmB localization to epitope presentation cells (APC (Antigen presenting cells), macrophages, and dendritic cells) [22]. AmB effectiveness was increased by 83% with PADRE-PAMAM and its targeting to APC by tenfold in vivo experiments resulting in lower AmB dose and toxicity [23].

4. Difficulties in Clinically Translating Nps

The results of the aforementioned study unequivocally show that different NPs compositions can function as efficient antifungal drug delivery vehicles. Flavonoids and phenols were found in the water-based extract of *Stephania japonica* leaflets by botanical compounds examination. The medicinal qualities that this breed exhibits are mostly due to these types of chemicals. The antimicrobial, fungicide, and antioxidant properties of "SJ-Aq" (Aqueous extract of *S. japonica*) are probably attributed to its chemical components particularly to its flavonoids some of which are phenolic chemicals with notably significant amounts [24]. Despite decades of research and hundreds of articles published on this subject, AmB is the sole antifungal medication accessible economically in compositions that employ NPs. This wide disparity could be attributable to a variety of factors, the most significant of which include industry-related issues, NPs limits, and issues with clinical investigations & preclinical research. The majority of NPs research takes place in academia, with little backing from the pharmaceutical sector. In the pharmaceutical sector, two significant obstacles to Nps manufacturing are control of quality and elevated nanostructures having coverings on their surfaces or many elements necessitating complicated manufacturing and assessment stages, which might drastically elevate the production costs and render the scale-up process impossible [25].

5. Hepatoprotection

As an integral component of the body, the liver is vital for several processes, such as the metabolic processes, secretion, detoxification, and storage of both internal and external chemicals. Hepatic illnesses remain one of the most serious hazards to public health as a result of these functions, and they continue to be a problem throughout the world. Nevertheless, despite tremendous breakthroughs within contemporary medicine, there exist zero effective medications that enhance the performance of the liver, offer complete organ defense, or assist in the regenerative process of liver cells. Therefore,

pharmaceutical substitutes for the therapy of liver conditions need to be explored to make them more secure and successful. It is critical to shield the liver's tissues from the harmful consequences of ingested hepatotoxins or to reverse changes in antiradical defense mechanisms, medications capable of doing so are known as hepatoprotectives [26]. Hepatoprotective activity may be easily evaluated/screened in experimental animals using a variety of model systems of liver damage. Conditions for liver damage are implemented in all test model systems and using the material or preparation being tested, an effort is made to combat this poisoning [27].

5.1. Hepatoprotective Medicaments

Hepatoprotective properties are found in a significant range of plant-based medications, either directly or indirectly. Herbal medications for the treatment of liver problems have become increasingly popular in recent years all over the world [28]. The herb's hepatoprotective qualities could be linked to the phenolic compounds, flavonoids, and saponins [29]. Flavonoids' ability to scavenge free radicals is responsible for their hepatoprotective properties [30]. Many different plants and combinations have been shown to have liver-protective properties. In contemporary therapy, no meaningful reliable hepatoprotective medication is currently available, notwithstanding major advancements. This has led to a great deal of global emphasis being paid to the invention of plant-derived hepatoprotective drugs that are effective towards a range of liver-related conditions [31].

5.2. Hepatoprotective Molecules Used in Emergency Medicine

When treating an acetaminophen/paracetamol overdose, N-acetylcysteine is the hepatoprotective medication of choice [32]. It is currently shown that a key factor in the liverprotective effects of plant-based extracts and polyherbal compositions (PHF) is their capacity as antioxidants. When treating illnesses involving oxidative damage, NMAE (Natural Methylated Alkaloid Extract) could be beneficial [33]. Silymarin is used intravenously to treat Amanita mushroom toxicity. In Polycystic rodents, excessive amounts of allicin are found to provide significant liverprotective and antioxidant functions [34]. Rats with a combination of quercetin and curcumin showed no liver dysfunction and its cytoarchitecture did not alter, indicating preserving the liver [35].

5.3. Plants that May Have Liver-protective Constituents [36]

Mongolian milkvetch
Curcuma domestica
Cruciferous vegetables, cabbages, or mustard plants.
Marian thistle
Andrographis paniculata

6. Conclusions

Infections caused by fungi are becoming a global problem, causing major morbidity and death rates. Ostensibly, there is an assortment of efficient antifungal medicines on the market, but their therapeutic benefits are restricted due to excessive toxicity or unfavorable physicochemical features. Because their advantageous qualities, including the potential to surpass numerous limitations, stem from their compactness, numerous functions, and biological compatibility. Liposomal, solid lipid nanoparticle, and Phospholipid-based tiny carriers known as lipid carriers with nanostructured structures were studied

the most when it comes to the distribution of antifungal medication correlated with various nanocarrier types. Numerous such nanoparticles (such as liposomes) were been tested in clinical studies to treat invasive mycoses. Indeed, a major advancement in the medicinal application of this powerful antifungal medication with little or no side effects was made possible by the availability of liposomal amphotericin B. Nanocarriers have a lot of promise for delivering drugs to specific cells. To present, a vast number of formulations for liver targeting using polymeric nanocarriers as targeting ligands have been developed. Only a few delivery systems for liver-targeted drugs are currently on the market. The presence of flavonoids, alkaloids, terpenoids, glycosides, and steroids in plants is thought to be responsible for their hepatoprotective properties. Plant active extracts, fractions, or a combination of fractions/extracts could be very effective medications.

REFERENCES

- Correa-Moreira D., Baptista B. O., Giosa D., Oliveira M. M. E., "Emerging fungal pathogens: perspectives," *Front Fungal Biology*, vol. 5, pp. 1369062, 2024. DOI: 10.3389/ffunb.2024.1369062.
- Bouz G., Doležal M., "Advances in Antifungal Drug Development: An Up-To-Date Mini Review," *Pharmaceuticals (Basel)*, vol. 14, no. 12, pp. 1312, 2021. DOI: 10.3390/ph14121312.
- Al-Ahmadey Z. Z., Al-Ahmadi S. S., Aljohani E. D., AlRashidi N. H., "Candida Bloodstream Infection and Antifungal Susceptibility Over Three Years in a Single Center from Medinah, Saudi Arabia," *Microbiology Research Journal International*, vol. 33, no. 2, pp. 1-7, 2023. DOI: 10.9734/mrji/2023/v33i21363.
- Garg A., Sharma G. S., Goyal A. K., Ghosh G., Si S. C., Rath G., "Recent advances in topical carriers of anti-fungal agents," *Heliyon*, vol. 6, no. 8, pp. e04663, 2020. DOI: 10.1016/j.heliyon.2020.e04663.
- Lombardo D., Kiselev M. A., "Methods of Liposomes Preparation: Formation and Control Factors of Versatile Nanocarriers for Biomedical and Nanomedicine Application," *Pharmaceutics*, vol. 14, no. 3, pp. 543, 2022. DOI: 10.3390/pharmaceutics14030543.
- Yusuf A., Almotairy A. R. Z., Henidi H., Alshehri O. Y., Aldughaim M. S., "Nanoparticles as Drug Delivery Systems: A Review of the Implication of Nanoparticles' Physicochemical Properties on Responses in Biological Systems," *Polymers*, vol. 15, no. 7, pp. 1596, 2023. DOI: 10.3390/polym15071596.
- Naik A. Y., Sundarrajan P., "Applications of nanomaterials in biomedical science," *Annals of Phytomedicine*, vol. 12, no. 1, pp. 171-179, 2023. DOI: 10.54085/ap.2023.12.1.54.
- Sivadasan D., Ramakrishnan K., Mahendran J., Ranganathan H., Karuppaiah A., Rahman H., "Solid Lipid Nanoparticles: Applications and Prospects in Cancer Treatment," *International Journal of Molecular Sciences*, vol. 24, no. 7, pp. 6199, 2023. DOI: 10.3390/ijms24076199.
- Matos C. M., "Liposomes: The Brave Old World," *International Journal of Molecular Sciences*, vol. 24, no. 5, pp. 4343, 2023. DOI: 10.3390/ijms24054343.
- Faustino C., Pinheiro L., "Lipid Systems for the Delivery of Amphotericin B in Antifungal Therapy," *Pharmaceutics*, vol. 12, no. 1, pp. 29, 2020. DOI: 10.3390/pharmaceutics12010029.
- Noor A., Preuss C. V., "Amphotericin B," *StatPearls [Internet]*, Treasure Island (FL): Stat Pearls Publishing, 2024. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK482327/>. DOI: 10.9734/mrji/2023/v33i21363.
- Panda P., Mohapatra S., Sahoo R., "Formulation And In Vitro Evaluation of Nystatin's In Liposomal Drug Delivery System," *International Journal of Pharmaceutical Sciences*, vol. 1, no. 9, pp. 31-40, 2023. DOI: 10.5281/zenodo.8312639
- Sousa F., Nascimento C., Ferreira D., Reis S., Costa P., "Reviving the interest in the versatile drug

nystatin: A multitude of strategies to increase its potential as an effective and safe antifungal agent," *Advanced Drug Delivery Reviews*, vol. 199, pp. 11496, 2023. DOI: 10.1016/j.addr.2023.114969.

Simrah, Hafeez A., Usmani S. A., Izhar M. P., "Transfersome, an ultra-deformable lipid-based drug nanocarrier: an updated review with therapeutic applications," *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 397, no. 2, pp. 639-673, 2024. DOI: 10.1007/s00210-023-02670-8.

Emanet M., Ciofani G., "Ethosomes as Promising Transdermal Delivery Systems of Natural-Derived Active Compounds," *Advanced Nano Biomed Research*, vol. 3, no. 2300020, 2023. DOI: 10.1002/anbr.202300020.

Chauhan N., Vasava P., Khan S. L., Siddiqui F. A., Islam F., Chopra H., Emran T. B., "Ethosomes: A novel drug carrier," *Annals of Medicine and Surgery (Lond)*, vol. 82, pp. 104595, 2022. DOI: 10.1016/j.amsu.2022.104595.

Nayak B. S., Mohanty B., Mishra B., Roy H., Nandi S., "Transethosomes: Cutting edge approach for drug permeation enhancement in transdermal drug delivery system," *Chemical Biology & Drug Design*, vol. 102, no. 3, pp. 653-667, 2023. DOI: 10.1111/cbdd.14254.

Ghafahehbashi R., Memarzadeh F., Mansouri A., Akbarzadeh I., Abtahi M. S., Hejabi F., Ren Q., "Current advances in niosomes applications for drug delivery and cancer treatment," *Materials Today Bio*, vol. 23, pp. 100837, 2023. DOI: 10.1016/j.mtbio.2023.100837.

Elmowafy M., Shalaby K., Elkomy M. H., Alsaidan O. A., Gomaa H. A. M., Abdelgawad M. A., Mostafa E. M., "Polymeric Nanoparticles for Delivery of Natural Bioactive Agents: Recent Advances and Challenges," *Polymers*, vol. 15, no. 5, pp. 1123, 2023. DOI: 10.3390/polym15051123.

Lopez K. L., Ravasio A., González-Aramundiz J. V., Zacconi F. C., "Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) Prepared by Microwave and Ultrasound-Assisted Synthesis: Promising Green Strategies for the Nanoworld," *Pharmaceutics*, vol. 15, no. 5, pp. 1333, 2023. DOI: 10.3390/pharmaceutics15051333.

Khan S., Sharma A., Jain V., "An Overview of Nanostructured Lipid Carriers and its Application in Drug Delivery through Different Routes," *Advanced Pharmaceutical Bulletin*, vol. 13, no. 3, pp. 446-460, 2023. DOI: 10.34172/apb.2023.056.

Mhlwatika Z., Aderibigbe B. A., "Application of Dendrimers for the Treatment of Infectious Diseases," *Molecules*, vol. 23, no. 9, pp. 2205, 2023. DOI: 10.3390/molecules23092205.

Dannert C., Mardal I., Lale R., Stokke B. T., Dias R. S., "DNA Condensation by Peptide-Conjugated PAMAM Dendrimers, Influence of Peptide Charge," *ACS Omega*, vol. 8, no. 47, pp. 44624-44636, 2023. DOI: 10.1021/acsomega.3c05140.

Krishnarao K. L., Rajeswari T. R., "Phytochemical investigation and evaluation of the antioxidant, antibacterial and antifungal activities of *Stephania japonica* L. leaves extract," *Annals of Phytomedicine: An International Journal*, vol. 12, no. 1, pp. 477-485, 2023. DOI: 10.54085/ap.2023.12.1.59

Nami S., Aghebati-Maleki A., Aghebati-Maleki L., "Current applications and prospects of nanoparticles for antifungal drug delivery," *Excli Journal*, vol. 20, pp. 562584, 2021. DOI: 10.17179/excli2020-3068.

Saikat Mitra, Mashia Subha Lami, Tanvir Mahtab Uddin, Rajib Das, Fahadul Islam, Juhaer Anjum, Md. Jamal Hossain, Talha Bin Emran, "Prospective multifunctional roles and pharmacological potential of dietary flavonoid narirutin," *Biomedicine & Pharmacotherapy*, vol. 150, pp. 112932, 2022. DOI: 10.1016/j.biopha.2022.112932.

Martinez-Lopez S., Angel-Gomis E., Sanchez-Ardid E., Pastor-Campos A., Picó J., Gomez-Hurtado I., "The 3Rs in Experimental Liver Disease," *Animals*, vol. 13, no. 14, pp. 2357, 2023. DOI: 10.3390/ani13142357.

Arman M., Chowdhury K.A.A., Bari M.S., "Hepatoprotective potential of selected medicinally important herbs: evidence from ethnomedicinal, toxicological and evaluations," *Phytochemistry Reviews*, vol. 21, pp. 1863–1886, 2022. DOI: 10.1007/s11101-022-09812-5.

Ramasamy Thilagavathi, S. Sameema Begum, Sowfika Dharshini Varatharaj, Arun Kumar Balasubramaniam, Joselin Susan George, "Recent insights into the hepatoprotective potential of medicinal plants and plantderived compounds," *Phytotherapy Research*, vol. 27, no. 5, pp. 2102–2118, 2023. DOI: 10.1002/ptr.7821.

Kim M., Jee SC., Sung JS., "Hepatoprotective Effects of Flavonoids against Benzo[a]Pyrene-Induced Oxidative Liver Damage along Its Metabolic Pathways," *Antioxidants (Basel)*, vol. 13, no. 2, p. 180, 2024. DOI: 10.3390/antiox13020180.

Pandey B., Baral R., Kaundinnyayana A., "Promising hepatoprotective agents from the natural sources: a study of scientific evidence," *Egyptian Liver Journal*, vol. 13, p. 14, 2023. DOI: 10.1186/s43066-023-00248-w.

Licata A., Minissale MG., Stankevičiūtė S., SanabriaCabrera J., Lucena MI., Andrade RJ., Almasio PL., "N-Acetylcysteine for Preventing AcetaminophenInduced Liver Injury: A Comprehensive Review," *Frontiers in Pharmacology*, vol. 13, p. 828565, 2022. DOI: 10.3389/fphar.2022.828565.

Jyothilekshmi S., Valsa A.K., Ramadasan, Kuttan, "Protective effect of the polyherbal formulation Nalpamaram on the oxidative stress induced by ethanol," *Annals of Phytomedicine*, vol. 11, no. 2, pp. 296–301, 2022. DOI: 10.54085/ap.2022.11.2.34.

Nazia Begum, Rahathunnisa Begum, Kandavalli Manipriya, B. Veeresh, "Hepatoprotective and antioxidant activity of allicin on the polycystic ovarian syndrome in rats," *Annals of Phytomedicine*, vol. 11, no. 2, pp. 400–404, 2022. DOI: 10.54085/ap.2022.11.2.48.

Rao SS., Patel UD., Makwana CN., Ladumor VC., Patel HB., Modi CM., "Effect of quercetin and curcumin in rats sub-acutely exposed to cadmium chloride: haematobiochemical changes, oxidative stress parameters and histopathological changes in intestine, liver and kidney of rats," *The Journal of Phytopharmacology (Pharmacognosy*

and Phytomedicine Research), vol. 10, no. 5, pp. 399–408, 2021. DOI: 10.31254/phyto.2021.10520

Shahparan Islam Shawon, Rashmia Nargis Reyda, Nazmul Qais, "Medicinal herbs and their metabolites with biological potential to protect and combat liver toxicity and its disorders: A review," *Heliyon*, vol. 10, no. 3, 2024. Doi:<https://doi.org/10.1016/j.heliyon.2024.e25340>.

Advancements in Optimizing Microsphere Preparation: A Comprehensive Review of Past Research Utilizing Factorial Design Methodology

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ABSTRACT

Microspheres, as versatile carriers for controlled drug delivery, have garnered significant attention in pharmaceutical research because they facilitate drug release kinetics and increase therapeutic efficacy. This abstract presents a comprehensive overview of the preparation of microspheres utilizing factorial design as an optimization strategy. Factorial design, a powerful statistical tool, offers a systematic approach to investigate multiple variables simultaneously, thereby efficiently identifying critical factors influencing the characteristics of microspheres. By systematically varying factors such as polymer type, solvent composition, stirring rate, and drug-to-polymer ratio, factorial design enables the exploration of their individual and interactive effects on microsphere properties. This abstract highlights the key components of factorial design, including the selection of factors and levels, experimental design, data analysis using statistical techniques like analysis of variance (ANOVA), and interpretation of results. Moreover, it elucidates the advantages of factorial design over traditional onevariable-at-a-time methods, such as reduced experimentation time, improved understanding of interactions, and enhanced robustness of the optimization process. Furthermore, this abstract discusses the application of factorial design in optimizing various characteristics of microspheres, containing particle size, morphology, drug entrapment efficiency, and drug discharge profile. By systematically optimizing these parameters, factorial design facilitates the development of microspheres tailored to specific therapeutic requirements, thereby enhancing drug delivery efficacy while minimizing side effects. This abstract underscores the significance of factorial design as a methodical and efficient method for optimizing microspheres in pharmaceutical research.

Keywords Controlled Drug Delivery, Factorial Design, Microspheres, Optimization, Pharmaceutical Research

1. Introduction

The idea behind microencapsulation technology originated as a different approach to medicine delivery in the 1940s and 1960s. The 1980s saw the breakthrough development of polymer/membrane technology as part of the ongoing search for a more sophisticated system. Additionally, bioactive molecules can be attached to liposomes, bioerodable polymers, implants, and a variety of particulate carriers (such as nanoparticles and microspheres) to provide precise targeting and site-specific delivery [1]. A microsphere is a spherical particle with a core material that varies in size and has a diameter within the micrometer range, usually between 1 μm and 1000 μm (1 mm). They are characterized by their free-

flowing powder. The potential of microspheres as drug delivery systems has been thoroughly investigated. It has been that these systems may shield delicate macromolecules from acidic and enzymatic destruction, while also enabling the formulated medication to be delivered to specific tissues and with regulated release. Drug carriers include soluble polymers, cells, cell ghosts, lipoproteins, liposomes, microcapsules, and microparticles composed of unsolvable or decomposable natural and synthetic polymers. The carriers can be conjugated with certain antibodies that specifically target certain distinctive components of the region of interest, making them pH or temperature-sensitive, slowly degradable, and uniformly targeted. Microspheres are rough sphere-shaped, solid particles with 1 to 1000 μm diameters. They can also take the form of microcrystalline particles or pharmaceuticals disseminated in a particular solution. Microspheres and microcapsules are frequently used interchangeably. Medications that go into the bloodstream from side to side of the gastrointestinal tract (GIT) and have a brief half-life are promptly eliminated by blood circulation. So that controlled or sustained-release drug delivery systems are discovered and formulated. These will steadily discharge the drug into the gastrointestinal tract and uphold a constant level of medication strength in the plasma for a prolonged amount of time.

1.1. Advantages

The merits of microspheres are as follows:

- They offer protection for unstable medicine both before and after delivery.

They decrease the drug's concentration at a location other than the target organ or tissue.

- Lower toxicity and dosage.

- Reduce the size of particles to increase poorly soluble medication solubility.

- Offer a prolonged and consanguineous therapeutic impact.

- A decrease in stomach discomfort.

- Boost bioavailability.

- Extended half-life in biology.

1.2. Disadvantages

The demerits of microspheres are as follows [2]:

- These dosage forms shouldn't be eaten or crushed.

- Low drug loading (up to 50%) for parents with controlled release.

- If the carrier has a harmful impact, it might be challenging to fully eliminate it from the body after injection.

- Microspheres delivered by parents may interact or combine to create complexes with the blood component.

- Variations in the frequency of release between doses.

- Because controlled-release preparations often have greater drug loads, any compromise in the dosage form's discharge properties might result in dosage dumping, treatment failure, and even toxicity.

2. Types of Microspheres

2.1. Bio Adhesive Microsphere

Bio adhesion is the adherence of medication to mucosal membrane, for example, the nasal, rectal,

ophthalmic, or buccal. This type of microsphere that is bioadhesive in nature has the ability to keep the medicament for a long duration of time and have superior therapeutic effects.

2.2. Magnetic Microsphere

This particular delivery method, which localizes the medication to the illness location, is crucial. In this case, a lesser quantity of a drug that is magnetically targeted or directed can substitute the greater quantity of a drug that is freely flowing in this situation. Therapeutic magnetic microspheres and diagnostic magnetic microspheres are the types of magnetic microspheres.

- a) Therapeutic magnetic microspheres are used commonly to deliver chemotherapeutic agents to treat liver tumours and it is also used to deliver proteins and peptides.
- b) Diagnostic microspheres are the nanoparticles of supra-magnetic iron oxides that can be used to identify intestinal loops from other abdominal structures and to image liver metastases

2.3. Floating Microsphere

Floating microspheres are mainly used in gastroretentive drug delivery systems. Floating microspheres are also called hollow microspheres, microballoons, or floating microparticles. Floating microspheres are essentially tiny, hollow particles without a core. These cells flow freely and have a size ranging from 1 to 1000 μm .

2.4. Radioactive Microsphere

The subset of microspheres that interact radioactively is usually handled similarly to non-radioactive microspheres. Nonetheless, the radioactive microsphere in question always contains one or more radionuclides in addition to the matrix material that characterizes the microsphere and confers upon its targeting characteristics within a certain tissue or organ. Radioactive microspheres can provide high radiation doses to a particular area in small quantities without harming the surrounding natural tissue.

2.5. Polymeric Microspheres

Polymeric microspheres are mainly two types:

- a) Biodegradable polymeric microspheres.
- b) Synthetic polymeric microspheres.

The residence duration of biodegradable polymers when in contact with mucosal membranes is extended. The degree and extent of drug discharge are regulated by the polymer concentration and the sustained discharge pattern. The main drawback is the complexity and difficulty in controlling drug release associated with the drugtrapping efficiency of biodegradable microspheres in clinical settings. They do, however, provide a broad variety applications in microsphere-based therapy. The main benefit of utilizing biodegradable polymers is that, once they've completed their duties, they break down in a way that is conducive to biology. Synthetic polymeric microspheres are shown to be harmless, non-toxic, and biocompatible when utilized as bulking agents, fillers, embolic particles, drug transport vehicles, and other therapeutic applications.

3. Drug Delivery Mechanism of Microspheres

3.1. Diffusion Controlled Release

Under this process, drug molecules move from higher concentration areas within the microsphere to lower concentration areas outside the microsphere via diffusing through the polymer matrix. The size of the medication and the permeability of the polymer are two examples of parameters that affect the rate of diffusion.

3.2. Erosion-controlled Release

Certain microspheres are engineered to deteriorate or dissolve over time as a result of physiological fluids or enzymes. The medicine that is enclosed is released when the microspheres disintegrate. The composition of the polymer may be changed to regulate the rate of erosion. 3.3. Swelling Controlled Release When the microspheres absorb body fluids or water, they enlarge and release the medicine under the control of swelling. Drug release may result from the swelling process' disruption of the microsphere's structure.

3.4. Mechanical Disruption

It is possible to design microspheres so that they will release medication in response to mechanical deformation, such as shearing or compression pressures. Drug delivery systems that are transversal frequently use this method. The particular medication, the preferred release profile, and the microspheres' characteristics all influence the mechanism selection.

A major problem in the creation of pharmaceutical formulations is creating microspheres with precise control over release kinetics, because a complex interaction of various processes results from the release of drug from microspheres (Figure 1).

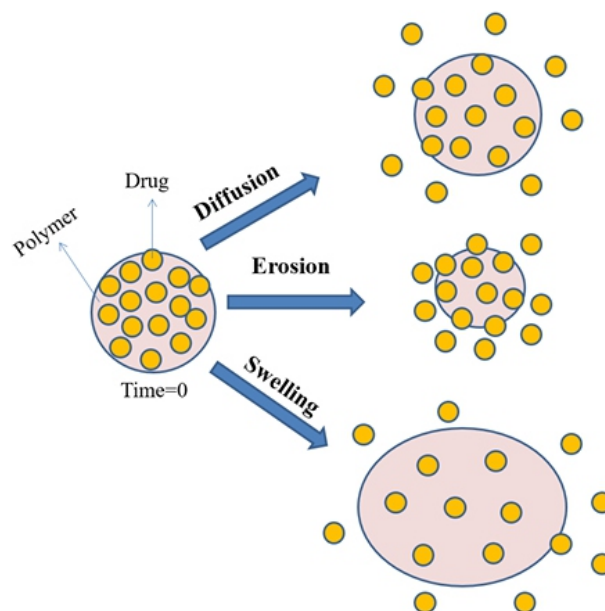


Figure 1. Different types of drug delivery mechanisms of microsphere

4. Methods of Preparation

4.1. Single Emulsion Technique

The natural polymers are first dispersed or dissolved in an aqueous medium. Subsequently, the mixture is put into a non-aqueous, oil-based medium. The next step of preparation involves cross-linking the broken globule. Materials can be crossed over using two different techniques: heat or chemicals (Figure 2), linking chemicals like formaldehyde, chloride, glutaraldehyde acid, etc. [3].

4.2. Double Emulsion Technique

This technique of microsphere synthesis is a great fit for water-soluble medications. It entails producing a double w/o/w emulsion or several emulsions. Both synthetic and natural polymers can be employed with this technique. Initially, the aqueous protein solution is dissolved within the lipophilic organic continuous phase. The active ingredients may be present in this protein solution [4]. Protein present in the continuous phase is eventually wrapped by the solution of the polymer that is composed of the interspersed aqueous phase. The aqueous polyvinyl alcohol solution (PVA) is then mixed with the primary emulsion and homogenized, or sonicated. This will give rise to double emulsion. By solvent evaporation or solvent extraction, the remaining solvent evaporated (Figure 3).

4.3. Solvent Evaporation Technique

The process of making microparticles with this technique involves extracting the organic phase with an organic solvent. Water is a miscible organic solvent used in the process, and water extraction gets rid of the organic phase. This will decrease the expansion time of the microspheres. Other methods include directly adding the appropriate medicament. Elimination affects several factors which include the volume of the emulsion, the solubility profile of a polymer in water, the amount of solvent, the temperature of the water, and others [5].

4.4. Method of Phase Separation and Coacervation

The purpose of this process is to reduce the solubility of the polymer in the early phases of the natural phase to influence the creation of a phase known as the coacervates, which is rich in polymers. Using this technique, an incompatible polymer is mixed with the drug-containing polymer solution. The first polymer absorbs the drug particles, which causes phase separation. A polymer solidifies as a result of non-solvent addition. The technique is used to produce the polylactic acid (PLA) microspheres. It's a polymer that doesn't work with butadiene. The dispersion of the particle size, polymer coating, and cluster of the generated particles are all influenced by the rate of coacervate synthesis. The process variables are crucial as a result (Figure 4). Agglomeration must be avoided by vigorously churning suspended material with a stirrer running at the right speed because agglomerates of polymerized globules are formed as a result of the formation of microspheres [6].

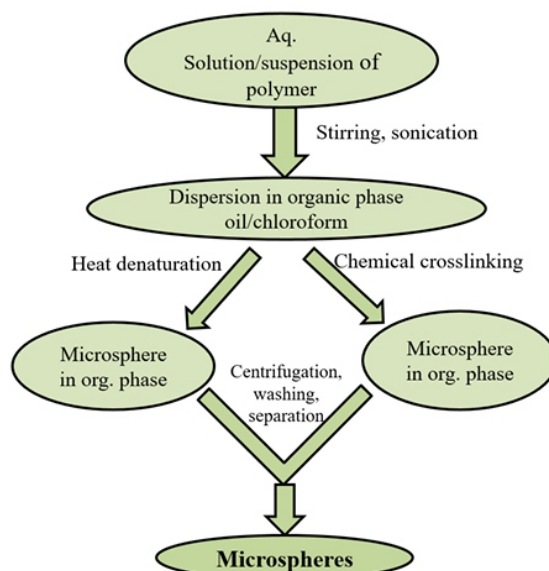


Figure 2. Preparation of microsphere by single emulsion method

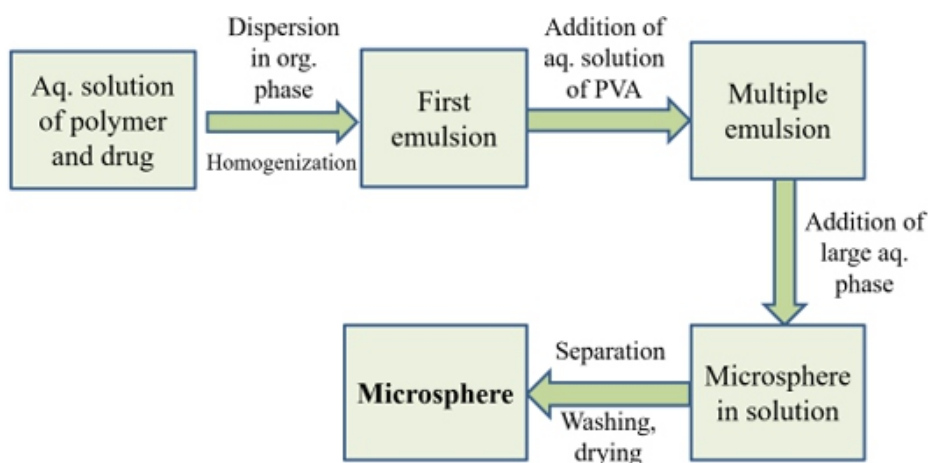


Figure 3. Double emulsion method for the preparation of microsphere

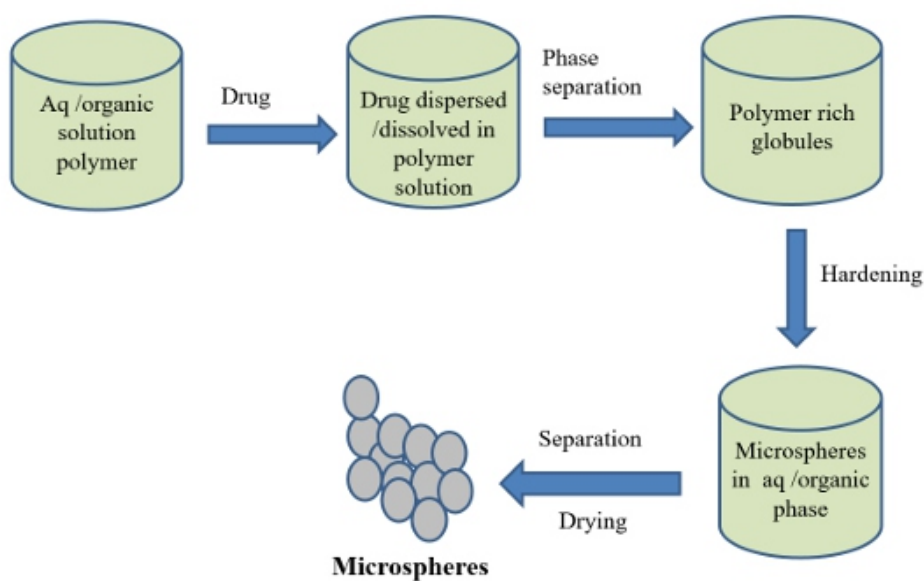


Figure 4. Microsphere preparation by phase separation and coacervation

4.5. Spray Drying

It is a closed, single-step system approach that works well with a range of materials, containing heat-sensitive materials. Components of the polymer coating and medication are either suspended. It can also dissolve or be suspended within a coacervate or emulsion system. The drug and polymer are dissolved using methylene chloride. For example, polylactide microspheres can be made in a polymer solution or dispersed in an appropriate solvent. In this method of preparation, the size of the microspheres is influenced by several factors, including the nozzle's size, the temperature in the drying and gathering chambers, the polymer supply rate, and dimensions within two chambers.

4.6. Hot Melt and Wax Coating Method

Melted wax is used to dissolve or disperse the product, encasing the main ingredients. By blending the waxy paste or mixture like frozen liquid paraffin intensely with cold water, the mixture is released. It takes at least an hour to heat the water. The material is agitated for a minimum of sixty minutes. Subsequently, the external layer is decanted, and the microspheres are submerged in an insoluble solvent being allowed to air dry. Commonly used surface ingredients are Beeswax and carnauba wax and it is best to combine the two to achieve the desired effects (Figure 5).

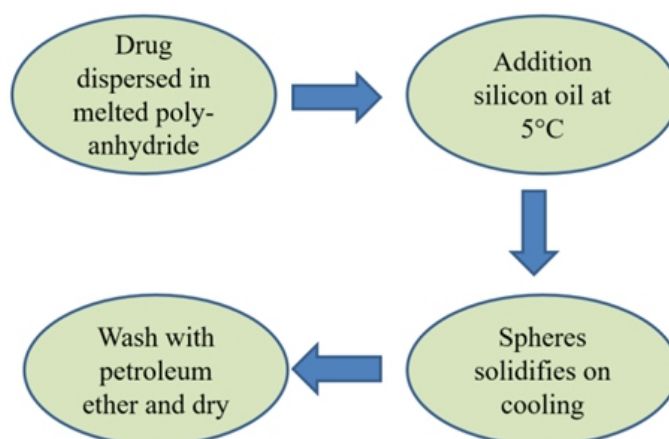


Figure 5. Hot melt and wax coating method for the preparation of microsphere

4.7. Ionic Gelation Technique

Ionic gelation is a technique that relies on the susceptibility of polyelectrolytes to form hydrogel beads, also known as gel spheres, through cross-linking when counter ions are present. Hydrophilic circular cross-linked polymeric agents that are known as gel spheres exhibit significant gelation and thickening in simulated biological fluids, as well as drug release controlled by polymer relaxation. In the first step, the drug-filled polymeric solution is transferred into an aqueous solution containing polyvalent cations to form hydrogel beads. Ionically crosslinking the moiety, then cations move through drugloaded hydrophilic compounds, which will form a 3D structure. Under mild circumstances, biomolecules can also be added to these gel spheres to preserve their three dimensional shapes (Figure 6).

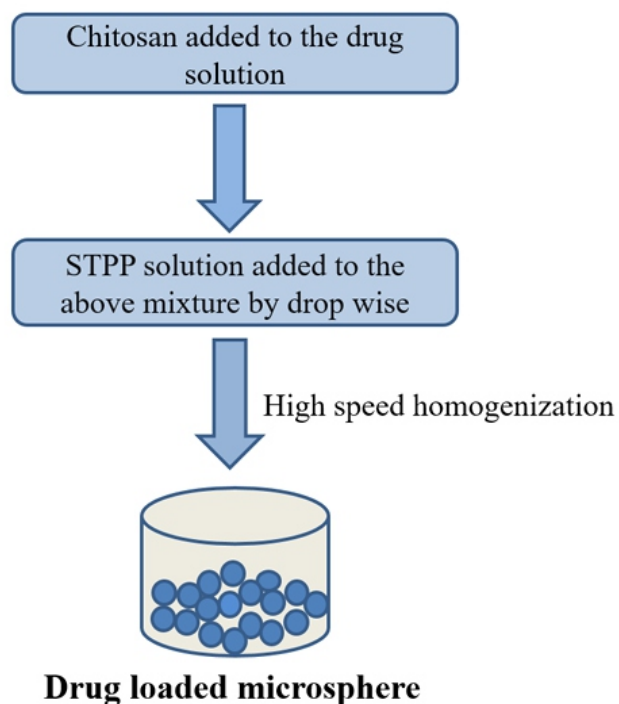


Figure 6. Ionic gelation method for preparation of microsphere

5. Evaluation

5.1. Percentage Yield

Microspheres were dried completely and weighed accurately. % yield of the microsphere was performed by using the provided formula eq. 1.

$$\%Yield = \frac{\text{weight of microsphere}}{\text{weight of polymer} + \text{weight of drug}} \times 100 \quad (1)$$

5.2. Optical Microscopy

The particle size was ascertained using this technique in conjunction with an optical microscope. Measurement is to be done under 450x (10x eyepiece and 45x objective), for which 100 particles were computed.

5.3. Scanning Electron Microscopy (SEM)

SEM analysis was done to examine the particles. X-ray diffraction analysis (EDXA) is used for the examination of elemental structure of samples, for identifying specific elements. Using a focused electron beam, the sample was scanned in parallel lines in this method. After that, microspheres were put on a sample holder for SEM characterization. Before that, coat the microspheres with a conductive metal, such as Pt or Zr. A fine-tuned electron beam was then directed toward the sample for scanning. The surface properties of the material were determined by using the secondary electrons that escaped from the surface of the sample.

5.4. Flow Characteristics

To analyze the flow properties of particulate materials such as microspheres, three key metrics are commonly used: Carr's compressibility index, Hausner's ratio, and the resting angle of repose. Each of these measures provides insight into how well the particles can move and settle under different conditions, which is crucial for applications like drug delivery [7].

Hausner's ratio is a critical metric for evaluating the flow characteristics of microspheres. Tapped density divided by bulk density is called Hausner's ratio. The density of the particles is compacted by tapping or vibrating the container until no further volume change occurs. It reflects how the particles pack together under external forces. The density of the particles when loosely packed in a container without any compaction is called bulk density. It represents the initial packing state of the material [8].

Hausner's ratio provides valuable information about the compressibility and cohesiveness of particles. The lowest Hausner's ratio implies better flow properties. A value around 1 suggests that the particles have minimal friction and resistance to movement, implying good flowability. Conversely, a higher Hausner's ratio indicates poorer flow properties, with values significantly above 1 suggesting higher friction and interparticle forces, leading to poor flow characteristics [9].

The flow qualities of the particles can be interpreted based on the value of Hausner's ratio. If the ratio is less than 1.11, the flow qualities are excellent, meaning the particles are likely to move freely and easily, making them ideal for processes requiring good flow. A ratio between 1.12 and 1.18 indicates favorable flow qualities, suitable for most practical applications. A ratio greater than 1.18 suggests poor flow qualities, with particles likely to be cohesive and resist movement, which can pose challenges in handling and processing [10].

For microspheres, those in mucoadhesive drug delivery systems, flowability is a crucial factor. Good flow properties ensure uniform filling and dosing, which is essential for consistent medication administration. Hausner's ratio is thus a vital parameter to determine if the microspheres have the necessary flow characteristics for effective drug delivery [11].

To measure Hausner's ratio, the following steps are typically followed. First, the bulk density is measured by filling a volumetric cylinder with the sample without compacting and measuring the volume and weight to calculate the bulk density. Tapped density is measured by tapping the cylinder with the sample until the volume stabilizes (no further volume reduction), then measuring the final volume and weight. By using these values, Hausner's ratio is calculated [12].

Hausner's ratio serves as a reliable indicator of microsphere flow properties. A lower ratio signifies better flowability, which is essential for applications like drug delivery, where consistent and predictable behavior of the particles is crucial. Ensuring that microspheres exhibit good flow characteristics can significantly enhance the efficiency and effectiveness of drug administration (eq.2).

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad (2)$$

5.5. Thermodynamic Evaluation

Thermal analysis techniques regularly apply scheduled temperature variations for heating and cooling, along with predetermined specimen atmospheres and pressures, to analyze these changes. Small variations in gas evolution, thermal increase or shrinkage, weight gain or loss, Young's modulus, heat, and enthalpy are a few of the most commonly observed characteristics.

5.6. Drug Content

The mixture was filtrated. Take it in a volumetric flask, and the volume is then adjusted. The medication was assessed employing spectrophotometry following the appropriate dilution.

5.7. Entrapment Efficiency

The drug-loaded microspheres were mixed with distilled water using a stirrer, filtered, and examined spectroscopically. The ratio of actual drug content to theoretical drug content establishes the entrapment effectiveness.

6. Quality-by-Design (QbD)

Understanding the link between product performances, attributes, and processes is essential to QbD. Formulations are often produced by-product specifications' quality control testing requirements. The product must pass quality control testing if it is approved for use in commerce. The batch that fails these tests, creates regulatory issues and a clear financial impact so that it is either reprocessed or rejected.

QbD starts through predetermined goals and necessitates knowledge of by what means process and formulation factors affect the final product's quality. The quality of the product can be verified by final product testing, but it cannot be a component of process control or consistency [13].

Product design and development, manufacturing process development, CQA identification, risk assessment and management, design space establishment, and control strategy definition are crucial elements of QbD-based manufacturing process factors are managed to produce consistently high-quality products known as the control strategies. For continuous product monitoring and improvement, information about the lifecycle management of products is used [14].

The target product profile provides an overview of a dosage form's therapeutic goals. Key elements of innovator product labeling establish TPP for a generic product. A product must have TPQP, or performance-based quality characteristics, to fulfill TPP. TPP is translated into quantitative testing by TPQP, including assay, impurities, content homogeneity, dissolution, stability, and bioequivalence. This section contains tests that are essential to the dosage form's success in terms of achieving therapeutic objectives [15].

6.1. Product Design and Development

For pharmaceutical development, physicochemical and pharmacological characteristics of the active pharmaceutical ingredient (API) are the essential characteristics. Achieving desirable patient needs and identifying characteristics that a pharmacological product should have to employ the anticipated therapeutic reaction are the goals of the QbD-based product development program. To achieve these predetermined goals, product development must always be methodical, scientific, and risk-based. The experience component adds value to this comprehensive approach at different phases. It is easier to identify CQA, which has to be managed to consistently deliver the intended output when one has a solid grasp of the product and its production process [16]. Knowledgebased product development may be achieved with the effective use of statistical techniques like experiment design, appropriate RA, and management tools. Additionally, creating flexible and relevant regulatory product standards is made easier with an understanding of CQA. QbD is facilitated and enhanced manufacturing capabilities may be achieved using development expertise.

6.2. Design of Experiments

While experiment design is a useful implement for selecting experiments effectively and methodically to provide accurate and clear information, it is not a replacement for experience, competence, or intellect. A structured method that determines the relationship between factors affecting a process and the output of that process is called experimental design [17]. Regression modelling, response surface modelling, screening experiments, comparing experiments may all be done with experiment design.

6.3. Common Experimental Designs

A certain amount of reasoning or experience-based knowledge should be used to determine which aspects are dependent and independent. Incorporating all pertinent variables is crucial. Instead of choosing reasonable high and low values for selected factors, unworkable and unattainable levels should not be incorporated into the design [18].

Completely randomized designs: In this case, the response variable derived only from the various values of a primary component, without accounting for any additional factors.

Randomized block design: The design in which there is only one main component, and blocking is utilized which reduces the effect of uncontrollable but unimportant elements.

Full factorial designs: All levels of each element appear alongside every level of every other factor. The variables are set at their higher or lower levels. For “n” number of independent factors containing two levels, then the number of experimental runs needed is equal to 2^n .

Fractional factorial designs: Rather than doing every run as in full factorial design, a carefully selected subset of the runs is chosen. As the number of components rises, full factorial design can easily grow to a very larger design.

Plackett–Burman designs: These designs are considered highly efficient when the variable's primary impacts are the only ones that matter. Their number for the trial run is a multiple of four. A minimum number of runs is sufficient to examine a very large number of variables.

Response surface designs: Process behavior can be fully described by a quadratic or cubic model. The curvature in the response surface is caused due to the existence of quadratic and perhaps cubic components in the response. The pharmaceutical sector always finds that quadratic models are adequate. When more than four components are present, sometimes threelevel factorial designs fit a quadratic equation but need a significant number of runs. In contrast, twolevel factorial designs are not suitable for a quadratic equation. A second-order model is typically utilized to calculate the response when a first-order model is insufficient and the experiment is near the optimal response zone. Some special designs fit the second

order model to the response with a minimum number of runs to analyze response surfaces. Box-Wilson central composite designs (CCD) and Box-Behenken designs (BBD) are the two types of classical quadratic designs. CCD is used to create a second-order

(quadratic) model for the response variable. Linear regression of the design is used to obtain the findings. Aimed at the design, the factor levels are often coded. These are fractional factorial or two-level complete factorial designs. Several center points and other selected runs enhance the designs. They make it possible to find every regression parameter required to match a response to a second-order model. The axial points, also known as star points, are separated from the center point by a symbol, “ α .” In a CCD, there will be twice as many axial points as independent variables. A CCD can be face-centered (requiring three levels of each factor), circumscribed (requiring five levels of each factor and having circular, spherical, or hyperspherical symmetry), or inscribed (a scaled-down circumscribed CCD with

each factor level of the design divided by α to generate this design). Any design should aim for rotatability, which provides equivalent estimating precision in all directions. If a CCD provides consistent variance of the calculated factors corresponding to every new observation point at the same distance from the center point, then the CCD is said to be rotatable. Extensively BBD is used to fit second-order models to the response. These designs are for three-level variables. Also, two-level factorial designs and unfinished block designs are used to create the design. These patterns are almost rotatable. One benefit of BBD is that they only need three tiers. In this design, there are no runs at the corner points and no runs when all factors are at the +1 or -1 levels. Corner point runs can occasionally be costly or difficult. However, this design's capacity to block orthogonally is restricted when compared to CCD. A graphical method for displaying a 3D surface in a two-dimensional format is called a contour plot. Contours are the plotted constant z-slices, represented in a two-dimensional format. A contour plot is a substitute or alternative for the 3D surface plot. The lines show the iso-response values, while the vertical and horizontal axes show the independent components. In the part that describes design space, figures are used to demonstrate the response surface and contour plots.

Three-level full factorial designs: For every aspect, three tiers are taken into account here. Three times the number of independent elements under consideration, n , equals the number of experimental runs. Level codes in this case are -1, 0, and +1. In contrast to the two-level designs, a third level facilitates the investigation of the quadratic connection between each element and the answer [19].

6.4. Multivariate Tools for Design, Data Acquisition, and Analysis

A scientific knowledge of the relevant multifactorial interactions is obtained via the application of information management systems and multivariate mathematical approaches. Response surface methods, process modeling, pattern recognition tools, and experiment design are examples of multivariate mathematical approaches. Statistical analysis may be used to assess the reliability and applicability of model predictions. When these strategies are applied properly, variables about the process and product are recognized and evaluated [20].

7. Application of Microspheres

7.1. Microspheres in Vaccine Delivery

Using microspheres to administer vaccines offers a promising approach to enhancing immunity against microbes and their hazardous components. An ideal vaccine must meet stringent criteria for protection, efficacy, and cost-effectiveness. Traditional vaccine delivery methods often face challenges in maintaining these standards, particularly regarding the application mode and the level of antibody response generated. One innovative solution is to employ biodegradable microspheres for intravenous vaccine delivery. This approach addresses many shortcomings of traditional vaccines, such as stability and targeted delivery, by ensuring controlled release and enhanced immune response. Microspheres are particularly advantageous in chemotherapy, especially for delivering anti-tumor drugs. Their potential as drug delivery systems stems from their ability to target tumor cells more effectively. Injecting microspheres into leaky vasculature associated with tumors can enhance endocytic activity, allowing for more efficient drug uptake by cancer cells. This targeted approach minimizes the adverse effects typically associated with chemotherapy, as the drug is concentrated at the tumor site rather than affecting the entire body. To further improve the efficacy of microsphere-based chemotherapy, "stealth"

microspheres are developed by coating them with soluble polyoxyethylene. This coating helps evade the immune system, allowing the microspheres to circulate longer in the bloodstream and increase their chances of reaching the tumor cells. Stealth microspheres offer a significant advantage over non-stealth microspheres, as they reduce the likelihood of being recognized and cleared by the immune system. However, non-stealth microspheres can still be beneficial in certain chemotherapy contexts, depending on the specific requirements of the treatment regimen [21].

7.2. Microsphere in Chemotherapy

Microspheres have significant potential as drug delivery systems for anti-tumor drugs. Their ability to target and deliver drugs efficiently makes them a valuable tool in cancer therapy. One of the key advantages of using microspheres in this context is their ability to exploit the leaky vasculature commonly found in tumors. When microspheres are injected into this leaky vasculature, they can enhance endocytic activity, leading to increased uptake of the drug by tumor cells. To further improve their efficacy, microspheres can be modified to become "stealth" microspheres by coating them with soluble polyoxyethylene. This coating helps the microspheres evade immune system, specifically the reticuloendothelial system (RES), allowing them to remain in circulation longer and increasing the likelihood of reaching and penetrating the tumor cells. Stealth microspheres are designed to avoid detection and clearance by the body's immune defenses, thereby enhancing the delivery and effectiveness of the encapsulated drug. The non-stealth microspheres can accumulate within the RES and can also be advantageous for cancer chemotherapy. While stealth microspheres are designed to evade the RES, non-stealth microspheres are more readily taken up by the RES, which includes organs such as the liver, spleen, and lymph nodes. This characteristic can be exploited to target certain types of cancers that metastasize to these organs or to modulate the immune response in a way that supports anti-tumor activity [22]

7.3. Ophthalmic Drug Delivery

Microspheres composed of polymers exhibit a range of favorable biological features, making them excellent candidates for various drug delivery applications. These features include bio-adhesion, permeation-enhancing qualities, and interesting physicochemical properties, all of which contribute to their effectiveness as drug carriers. One of the key advantages of polymer-based microspheres is their bio-adhesive properties, which ensure that the drug remains in close contact with the target tissue, enhancing the duration of drug action and improving therapeutic outcomes. Additionally, these polymers possess permeation-enhancing qualities that facilitate the movement of drugs across biological barriers, such as the corneal epithelium in the eye, which is crucial for effective ophthalmic drug delivery. The physicochemical properties of polymer-based microspheres, such as particle size, surface charge, and hydrophilicity, can be tailored to optimize drug delivery, influencing the stability, release profile, and bioavailability of the encapsulated drug [23].

7.4. Gene Delivery

Due to their GI tract adhesion and transport properties, microspheres may function as an efficient oral gene carrier. It is also useful in the administration of vaccines meanwhile resistance to the bacterium or virus is a prerequisite for getting one. The product poses a risk. The shortcomings of traditional vaccinations might be made up for by biodegradable intravenous vaccine delivery systems.

Biodegradable polymer microspheres have been utilized to encapsulate various parenteral vaccines, such as tetanus and diphtheria vaccinations [24].

7.5. Oral Delivery

Polymer-containing microspheres can form films, making them a viable alternative to traditional drug tablets in the form of film shapes. Due to sensitivity to pH and the reactivity of primary amines, these microspheres are well suited for oral delivery which allow for targeted and controlled release of the drug. Polymers such as chitosan and gelatin are commonly used in these microspheres, enhancing their effectiveness in the gastrointestinal tract. Chitosan, for instance, has excellent mucoadhesive properties and can react with the acidic environment of the stomach to provide a controlled release, while gelatin offers biocompatibility and the ability to form gels that protect the drug and improve its stability. Bioavailability and patient compliance of a drug can be improved by developing polymer-containing microspheres as oral drug delivery systems [25].

7.6. Nasal Drug Delivery

Bioadhesive capabilities and the ability to spread quickly upon contact with the nasal mucosa microspheres have applications in nasal drug delivery. Microspheres can enhance the residence time of the drug's remains in the nose and improve its bioavailability. When microspheres, liposomes, and gels connect with the nasal mucosa, their bioadhesive properties enable them to adhere to the mucosal surface effectively. This adherence ensures that the drug remains in close contact with the absorption site for an extended period, facilitating sustained release and absorption. The polymers used in these delivery systems, such as starch, dextran, albumin, gelatin, and chitosan, contribute to their bioadhesive properties and overall effectiveness. Starch, dextran, and albumin are commonly used polymers in these drug delivery systems. They provide structural integrity and enhance the bioadhesive properties of the microspheres, liposomes, and gels. Gelatin and chitosan are particularly effective due to their excellent biocompatibility and biodegradability. Gelatin, derived from collagen, offers a versatile platform for drug delivery, while chitosan, derived from chitin, is wellknown for its mucoadhesive properties, which are particularly beneficial for nasal drug delivery [26].

7.7. Buccal Drug Delivery

Chitosan and sodium alginate are the commonly used polymers for buccal drug delivery. These polymers possess mucosal or bioadhesive properties that can enhance drug absorption through the buccal mucosa. Chitosan is known for its excellent mucoadhesive properties. Mucoadhesive ability prolongs the residence time between the drug and the mucosa, facilitating enhanced drug absorption. Chitosan's positive charge can also interact with negatively charged mucosal surfaces, further enhancing its bioadhesive properties. Sodium alginates can form viscous gels in the presence of divalent cations, such as calcium ions, which are abundant in saliva. These gels can adhere to the buccal mucosa, providing sustained drug release and enhancing drug absorption. Additionally, sodium alginate's mucoadhesive properties contribute to its ability to prolong residence time in the buccal cavity, allowing for improved drug delivery [27]. The earlier attempts made on microspheres using the QbD approach are listed in Table 1.

Table 1. Past work done on microspheres using factorial design

Drug	Design	Independent variables	Dependent variables	References
Acetoclofenac	Plackett Burman design	Ethyl cellulose (EC) (X_1), stirring speed (SS) (X_2), and continuous phase volume (X_3)	Entrapment efficiency (% EE) (Y_1) and Particle size (PS) (Y_2)	[28]
Acetazolamide	Box Behnken design (BBD)	Drug: polymer ratio (D:P) (X_1), surfactant level (X_2), and SS (X_3)	% EE (Y_1), Q_{6h} (Y_2), and PS (Y_3)	[29]
Acyclovir	3^2 full factorial design (FFD)	EC (X_1), and carbopol 940 (X_2)	% EE (Y_1), Q_{1h} (Y_2), $t_{90\%}$ (Y_3), and mucoadhesive strength (MS) (Y_4)	[30]
Acyclovir	3^2 FFD	D:P (X_1), and feed rate (X_2)	% Yield (Y_1), and EE (Y_2)	[31]
Amoxicillin	3^2 FFD	Polymer (X_1), Emulsifying agent (X_2), and rpm (X_3)	% EE (Y_1), and PS (Y_2)	[32]
Carvedilol	3^2 FFD	Drug-to-polymer ratio (X_1), and SS (X_2)	PS (Y_1), EE (Y_2), t_{50} (Y_3), % Drug release (DR) after 5h(Q_{5h}) (Y_4), and Q_{12h} (Y_5)	[33]
Cefpodoxime Proxetil	3^2 FFD	SS (X_1) and ES100 (X_2)	% DR (Y_1), EE (Y_2), and PS (Y_3)	[34]
Clotrimazole	3^2 BBD	D:P (X_1), surfactant amount (X_2), and SS (X_3)	PS (Y_1), and % EE (Y_2)	[35]
Diclofenac Sodium	3^2 BBD	Chitosan (X_1), tripolyphosphate (X_2), and cross-linking time (X_3)	PS (Y_1) and EE (Y_2)	[36]
Diltiazem hydrochloride	2^3 FFD	Polycarbonate (X_1) and SS (X_2)	% DR (Y_1), and % EE (Y_2)	[37]
Esomeprazole	3^2 FFD	Eudragit L (X_1) and SS (X_2)	% DR (Y_1), and EE (Y_2)	[38]
Ibuprofen	2^3 FFD	Sodium alginate (X_1), magnesium stearate (X_2), and calcium chloride (X_3)	% DR (Y_1)	[39]
Ivabradine HCl	3^2 FFD	Polymer (X_1) and SS (X_2)	% drug loading (Y_1), PS (Y_2), and % CDR (Y_3)	[40]
Metoprolol tartrate	2^3 FFD	EC (X_1), SS (X_2), and different volumes of continuous phase (X_3)	% EE (Y_1), and PS (Y_2)	[41]
Nicorandil	3^2 FFD	D:P (X_1), and volume of Gallic acid (X_2)	% EE (Y_1), PS (Y_2), and Q_{8h} (Y_3)	[42]
Nifedipine	3^2 FFD	EC (X_1), and carbopol 934P (X_2)	% EE (Y_1), % CDR at 1h (Y_2), $t_{90\%}$ (Y_3), and MS (Y_4)	[43]
Nifedipine	3^2 BBD	Polymer (X_1), SS (X_2), and glutaraldehyde (X_3)	PS (Y_1), EE (Y_2), and flow assets (Y_3)	[44]
Quetiapine fumarate	3^2 BBD	EC (X_1), type of HPMC (X_2), concentration of HPMC (X_3), chitosan (X_4), and rpm (X_5)	PS (Y_1), EE (Y_2), Q_{24h} (Y_3), and MS (Y_4)	[45]
Quetiapine fumarate	3^2 FFD	Sodium lauryl sulfate (X_1) and D:P (X_2)	% yield (Y_1), % EE (Y_2), PS (Y_3), and % <i>in-vitro</i> DR at 10h (Y_4)	[46]
Rasagiline mesylate	Central composite design (CCD)	Polymer (X_1) and SS (X_2)	% EE (Y_1), PS (Y_2), and % DR (Y_3)	[47]
Selegiline hydrochloride	CCD	Karaya gum level (X_1), and SS (X_2)	% DR (Y_1), % EE (Y_2), and PS (Y_3)	[48]
Sitagliptin Phosphate Monohydrate	3^2 FFD	Polylactic co-glycolic acid (X_1), and feed speed (X_2)	PS (Y_1), and % DR (Y_2)	[49]
Stavudine	3^2 FFD	D:P (X_1) and SS (X_2)	EE (Y_1), PS (Y_2), and time to 80% DR (Y_3)	[50]
Terbinafine	3^2 BBD	Polyvinyl alcohol (X_1), Dichloromethane (X_2), and SS (X_3)	EE (Y_1) and PS (Y_2)	[51]

8. Conclusions

In conclusion, the application of factorial design in the preparation of microspheres for controlled drug delivery represents a significant advancement in pharmaceutical research. By systematically exploring the effects of multiple formulation variables on microsphere properties, the factorial design enables the optimization of key parameters such as the size of the particle, morphology, drug entrapment efficiency, and drug delivery profile. Through its systematic and efficient approach, factorial design not only

reduces experimentation time but also enhances the understanding of interactions between factors, leading to the formation of extra robust and efficient drug release systems. Overall, the incorporation of factorial design methodology offers a promising avenue for the rational design and optimization of microsphere-based drug release systems, which can improve therapeutic outcomes and patient care.

REFERENCES

- [1] Li Q, Chang B, Dong H, Liu X. "Functional microspheres for tissue regeneration". *Bioactive Materials*. Vol. 25, no. 7, pp. 485-499, 2023.
- [2] Pawar A, Lohakane P, Pandhare R, Mohite P, Munde S, Singh S, Chidrawar V. "Chitosan fortified repaglinide gastro-retentive mucoadhesive microsphere with improved anti-diabetic attribute". *Intelligent Pharmacy*. Vol. 12, no. 3, pp. 441-449, 2014.
- [3] Pawar KS, Sonawane MP, Nikam V, Rathod A, Tamboli A. "Floating Microsphere as Gastro-Retentive Drug Delivery System". *Research Journal of Pharmaceutical Dosage Forms and Technology*. Vol. 16, no. 2, pp. 173-177, 2024.
- [4] Chavan PB, Malpure PS, Talele GS, Kadam AR. "The Latest Advancements: A Comprehensive Review of microballoons for Enhanced Gastroretention". *Asian Journal of Pharmaceutical Research and Development*. Vol. 11, no. 3, pp. 222-229, 2024.
- [5] Cheng Q, Xie M, Li G, Xue W, Zeng L, Ma D. "BacteriaLoaded Gastro-Retention Oral Delivery System for Alcohol Abuse". *ACS Biomaterials Science & Engineering*. Vol. 9, no. 3, pp. 1460-1471, 2024.
- [6] Mundarinti S, Ahad HA. "Past decade attempts on gastro retentive microspheres using factorial design: A comprehensive literature". *Int J Pharm Phytopharmacol Res*. Vol. 11, no. 2, pp. 24-30, 2021.
- [7] Babu GN, Muthukarupam M, Ahad HA. "Neem fruit mucilage impact on acyclovir release at different intervals: A central composite design screening". *Int J Pharm Res Allied Sci*. Vol. 10, no. 4, pp. 31-41, 2021.
- [8] Bumbak F, Cook S, Zachleder V, Hauser S, Kovar K. "Best practices in heterotrophic high-cell-density microalgal processes: achievements, potential and possible limitations". *Applied microbiology and biotechnology*. Vol. 1, no. 1, 2011, pp. 31-46.
- [9] Aboshyan-Sorgho L, Besnard C, Pattison P, Kittilstved KR, Aebischer A, Bünzli JC, Hauser A, Piguet C. "Nearinfrared→ visible light upconversion in a molecular trinuclear d_{fd} complex". *Angewandte Chemie-International Edition*. Vol. 50, no. 18, pp. 4108-4112, 2011.
- [10] Hauser AS, Chavali S, Masuho I, Jahn LJ, Martemyanov KA, Gloriam DE, Babu MM. "Pharmacogenomics of GPCR drug targets". *Cell*. Vol. 172, no. 1, pp. 41-54, 2018.
- [11] Mowry EM, Waubant E, McCulloch CE, Okuda DT, Evangelista AA, Lincoln RR, Gourraud PA, Brenneman D, Owen MC, Qualley P, Bucci M. "Vitamin D status predicts new brain magnetic resonance imaging activity in multiple sclerosis". *Annals of neurology*. Vol. 72, no. 2, pp. 234-240, 2012.
- [12] Townsend G, LaPallo BK, Boulay CB, Krusienski DJ, Frye GE, Hauser C, Schwartz NE, Vaughan TM, Wolpaw JR, Sellers EW. "A novel P300-based brain-computer interface stimulus presentation paradigm: moving beyond rows and columns". *Clinical neurophysiology*. Vol. 121, no. 7, pp. 1109-1120, 2010.
- [13] Kumar LS, Ahad HA. "Quality by Design based Quercetin Hydrate Nanoemulsions for Enhanced Solubility by Reducing Particle Size". *Ind J Pharm Edu Res*. Vol. 57, no. 3, pp. 965-970, 2023.
- [14] Abdul AH, Bala AG, Chintaginjala H, Manchikanti SP, Kamsali AK, Dasari RRD. "Equator assessment of nanoparticles using the design-expert software". *International Journal of Pharmaceutical Sciences and Nanotechnology (IJPSN)*. Vol. 13, no. 1, pp. 4766-4772, 2020.

-
-
- [15] Hales D, Vlase L, Porav SA, Bodoki A, Barbu-Tudoran L, Achim M, et al. "A quality by design (QbD) study on enoxaparin sodium loaded polymeric microspheres for colon-specific delivery". *European Journal of Pharmaceutical Sciences*. Vol. 100, no. 1, pp. 249-261, 2017.
- [16] Hossain KM, Patel U, Ahmed I. "Development of microspheres for biomedical applications: a review". *Progress in biomaterials*. Vol. 4, no. 1, pp. 1-9, 2015.
- [17] Benoit JP, Faisant N, Venier-Julienne MC, Menei P. "Development of microspheres for neurological disorders: from basics to clinical applications". *Journal of controlled release*. Vol. 65, no. 1-2, pp. 285-296, 2000.
- [18] Dastidar DG, Saha S, Chowdhury M. "Porous microspheres: Synthesis, characterisation and applications in pharmaceutical & medical fields". *International journal of pharmaceutics*. Vol. 548, no. 1, pp. 34-48, 2018.
- [19] Mateus AY, Barrias CC, Ribeiro C, Ferraz MP, Monteiro FJ. "Comparative study of nanohydroxyapatite microspheres for medical applications". *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*. Vol. 86, no. 2, pp. 483-493, 2008.
- [20] Saralidze K, Koole LH, Knetsch ML. "Polymeric microspheres for medical applications". *Materials*. Vol. 3, no. 6, pp. 3537-3564, 2010.
- [21] Wei W, Wang LY, Yuan L, Wei Q, Yang XD, Su ZG, Ma GH. "Preparation and application of novel microspheres possessing autofluorescent properties". *Advanced Functional Materials*. Vol. 17, no. 16, pp. 3153-3158, 2007.
- [22] Shagymgereyeva S, Sarsenbekuly B, Kang W, Yang H, Turtabayev S. "Advances of polymer microspheres and its applications for enhanced oil recovery". *Colloids and Surfaces B: Biointerfaces*. Vol. 233, no. 1, pp. 113622, 2023.
- [23] Yang C, Zhang Z, Gan L, Zhang L, Yang L, Wu P. "Application of biomedical microspheres in wound healing". *International Journal of Molecular Sciences*. Vol. 24, no. 8, pp. 7319, 2023.
- [24] Huang Y, Chen Z, Liu Y, Yuan J, Zheng Y, Nie Y, Zou X, Huang Y, Zeng Z. "Preparation and application of diacetone-based functionalized polymeric microspheres". *European Polymer Journal*. Vol. 196, no. 1, pp. 112297, 2023.
- [25] Huang Y, Chen Z, Liu Y, Yuan J, Zheng Y, Nie Y, Zou X, Huang Y, Zeng Z. "Preparation and application of diacetone-based functionalized polymeric microspheres". *European Polymer Journal*. Vol. 196, no. 1, pp. 112297, 2023.
- [26] Zhang M, Peng L, Dong Z, Yan J, Wang C, Sun Y, Zhao L. "Highly efficient and selective recovery of Au (III) by cellulose microspheres bearing nucleobase and their applications in gold slag treatment". *Separation and Purification Technology*. Vol. 314, no. 1, pp. 123669, 2023.
- [27] Tello RP, Wang S, Fontana F, Correia A, Molinaro G, Cerdà SL, Hietala S, Hirvonen J, Barreto G, Santos HA. "Fabrication of hydrogel microspheres via microfluidics using inverse electron demand Diels–Alder click chemistry based tetrazine-norbornene for drug delivery and cell applications". *Biomaterials Science*. Vol. 11, no. 14, pp. 4972-4984, 2023.
- [28] Choi HS, Kim YH, Kim HK, Kim KB. "Assembly of graphene-wrapped ZIF-8 microspheres and confined carbonization for energy storage applications". *Journal of Power Sources*. Vol. 560, no. 1, pp. 232702, 2023.
- [29] Behera S, Ghatuary CK, Nayak AK, Hasnain MS. "Losartan potassium-loaded linseed polysaccharide alginate-calcium silicate bio mucoadhesive-floating beads for gastro retentive delivery". *Polymer-Plastics Technology and Materials*. Vol. 63, no. 8, pp. 75-89, 2024.
-
-

-
-
- [30] Ahad HA, Chinthaginjala H, Priyanka MS, Raghav DR, Gowthami M, Jyothi VN. "Datura stramonium Leaves Mucilage Aided Buccoadhesive Films of Aceclofenac using 3^2 Factorial Design with Design-Expert Software". *Indian Journal of Pharmaceutical Education & Research*. Vol. 55, no. 2, pp. 396-404, 2021.
- [31] Elkady OA, Tadros MI, El-Laithy HM. "QbD approach for novel crosslinker-free ionotropic gelation of risedronate sodium–chitosan nebulizable microspheres: optimization and characterization". *AAPS PharmSciTech*. Vol. 21, no. 14, pp. 1-18, 2020.
- [32] Komati S, Swain S, Rao MEB, Jena BR, Unnam S, Dasi V. "QbD-based design and characterization of mucoadhesive microspheres of quetiapine fumarate with improved oral bioavailability and brain biodistribution potential". *Bulletin of Faculty of Pharmacy, Cairo University*. Vol. 56, no. 2, pp. 129-145, 2018.
- [33] Siraskar PR, Mishra DK. "In-vivo estimation of optimized floating microspheres design by QBD approach". *Research Journal of Pharmacy and Technology*. Vol. 16, no. 8, pp. 3697-3700, 2023.
- [34] Shanmugasundaram S. "QBD Approach for Design and Characterization of Pramlintide Microspheres for Controlled Drug Release". *Journal of Pharmaceutical Innovation*. Vol. 18, no. 4, pp. 2325-2347, 2023.
- [35] Bhavani J, Saloni S, Rajeshri D. "Formulation and evaluation of floating microspheres using factorial design". *Int J Recent Sci Res*. Vol. 10, no. 12, pp. 36363-36370, 2019.
- [36] Abdallah MH. "Box-behnken design for development and optimization of acetazolamide microspheres". *International Journal of Pharmaceutical Sciences and Research*. Vol. 5, no. 4, pp. 1228-1239, 2014.
- [37] Kyada C, Ranch K, Shah D. "Optimization of mucoadhesive microspheres of acyclovir by applying 3^2 full factorial design. Ratio". *Journal of Drug Delivery Science and Technology*. Vol. 24, no. 1, pp. 61-68, 2014.
- [38] Shinde ML, Shah VA, Ghodke DS, Shah RR. "Impact of formulation and process variable on the preparation of acyclovir microspheres by spray drying using factorial design". *Polymer*. Vol. 1, no. 3, pp. 353-357, 2010.
- [39] Hardenia A, Gupta AK. "Development and optimization of gastroretentive mucoadhesive microspheres using 3^3 sup 3^3 factorial design". *International Journal of Pharmaceutical Sciences and Research*. Vol. 7, no. 5, pp. 2020-2030, 2016.
- [40] Patel P, Dhake A. "Design and development of colon specific microspheres for chronotherapy of hypertension". *Journal of Pharmacy and Bioallied Sciences*. Vol. 4, no. 1, pp. 33-34, 2012.
- [41] Pande A, Nimbalkar U, Dhoka M, Sonawane P. "Floating Microspheres of Cefpodoxime Proxetil: Formulation and Optimization by Factorial Design". *IJRPC*. Vol. 1, no. 3, pp. 222-229, 2011.
- [42] Rai S, Ravikumar P. "Development and evaluation of microsphere based topical formulation using design of experiments". *Indian Journal of Pharmaceutical Sciences*. Vol. 78, no. 2, pp. 182-192, 2016.
- [43] Shazly GA, Tawfeek HM, Ibrahim MA, Auda SH, ElMahdy M. "Formulation and evaluation of fast dissolving tablets containing taste-masked microspheres of diclofenac sodium for sustained release". *Digest Journal of Nanomaterials & Biostructures (DJNB)*. Vol. 8, no. 3, pp. 1281-1293, 2013.
- [44] Panwar MMS. "Factorial design approach for optimization of floating microspheres of diltiazem hydrochloride". *Asian Journal of Pharmaceutics (AJP)*. Vol. 9, no. 3, pp. 206-212, 2015.
- [45] Thombre NA, Shaikh HA, Ahire ED. "Formulation Development and Evaluation of Colonspecific Esomeprazole Microspheres". *Biosciences Biotechnology Research Asia*. Vol. 19, no. 3, pp. 679-691, 2022.
- 46] Nagpal M, Maheshwari D, Rakha P, Dureja H, Goyal S, Dhingra G. "Formulation development and evaluation of alginate microspheres of ibuprofen". *Journal of Young Pharmacists*. Vol. 4, no. 1, pp. 13-
-
-

16, 2012.

[47] Firke SN, Siraskar PR, Nagore DH, Ghiware NB. "Formulation and Optimization of Floating Microspheres of Ivabradine Hydrochloride by 32 Factorial Design Approach". *IJPBS*. Vol. 9, no. 3, pp. 1025-1034, 2019.

[48] Patel KS, Patel MB. "Preparation and evaluation of chitosan microspheres containing nicorandil". *International journal of pharmaceutical investigation*. Vol. 4, no. 1, pp. 32-37, 2014.

[49] Lal C, Garg R, Gupta G. "Formulation and optimization by applying 32 full factorial design of mucoadhesive microspheres of nifedipine". *Asian J Pharm Clin Res*. Vol. 12, no. 6, pp. 321-327,

[50] Neelam SO, Meenakshi BH. "Formulation and evaluation of polymeric microspheres using box-Behnken design". *Asian J Pharm Clin Res*. Vol. 15, no. 10, pp. 47-55, 2022.

[51] Bhopte DK, Sagar R, Kori ML. "Fabrication, Optimization, and Characterization of Floating Microspheres of Quinapril Hydrochloride using Factorial Design Method". *Biomedical and Pharmacology Journal*. Vol. 15, no. 4, pp. 2011-2024, 2022.

Nanocarriers as Promising Novel Systems for Controlled Delivery of Diclofenac Sodium

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ABSTRACT

The formulation and evaluation of diclofenac sodium from liposomes, niosomes and nanoemulsion are analyzed. The release profiles of diclofenac sodium were almost similar in all the formulations. It is found that 85% of diclofenac sodium diffused out from the colloidal systems within 8hrs and practically all the drug was released within 12hrs. In addition to this controlled release, the similarity of the release profiles obtained for liposomes, niosomes and nanoemulsion signifies that the internal structure has little role in the release process. The drug released fast and completely from the carriers upon high dilution, but it is slowed down a little when they are not diluted. The maximum amount of diclofenac sodium was released from nano emulsion as well as liposomes after 12 hrs at 1 in 200 dilution where as in niosomes, it was found at 1 in 100 dilution. But surprisingly, the release was decreased upon further dilution. The higher the surfactant content, the higher the globule size of the nanoemulsion. The mean size of the systems was decreased upon increasing dilution. Among all the systems, the mean size of niosomes was decreased upon increasing the dilution up to 1:200. It was finally depicted that the dilution effect on the zeta potential of the systems shifted from negative to positive by adding polysorbate 80. The zeta potential of all the systems was significantly good indicating stable systems.

Keywords Liposomes, Nanoemulsion, Niosomes, Release Studies, Zeta-potential

1. Introduction

Of the various nanosystems available, synthetic carriers such as liposomes have been used extensively^{1,2}. Liposomes are biodegradable, nontoxic, uni or multilamellar vesicles, formed from naturally occurring phospholipids which have the ability to entrap and retain a wide range of drugs either in the aqueous or lipid phase³⁻⁶. Liposomes can be administered by oral⁷, parenteral and transdermal routes^{8,9}. Their pharmacokinetic properties depend upon their size, surface charge, lipid composition, fluidity of the membrane, dose and route of administration¹⁰⁻¹¹. When administered intravenously, the multilamellar (MLV) liposomes have an elimination half life of 30 to 120 minutes, whereas small unilamellar (SUV) liposomes are eliminated exponentially with a half life of 80 minutes to 2 days. In the liver, irrespective of surface charge, MLV liposomes are predominantly internalized by kupffer cells, while cationic SUV liposomes are taken up by the hepatocytes, neutral and anionic SUV liposomes are distributed equally between kupffer cells and hepatocytes. Niosomes have been largely proposed as drug delivery systems¹². This class of vesicles appear are similar in terms of their physical properties to liposomes, being prepared in the same way and under a variety of conditions, forming unilamellar or multilamellar structures¹³. They may be regarded either as inexpensive alternatives of non-biological origin to liposomes. Particularly, these vesicles were introduced in reason of the higher chemical stability of the nonionic surfactants compared to that of phospholipids. Phospholipids are in fact subjected to stability problems due to the easy hydrolysis of esters bonds or to the possible peroxidation

of unsaturated bonds. Moreover, another disadvantage related to the nature of liposomes is referred to the unreliable reproducibility. This series of problems in the use of liposomes as drug carriers thought provoked the researchers the utilization of alternative sources to make vesicles and among them the use of non-ionic surfactants. An increasing number of non-ionic surfactants have been found to form vesicles able to entrap hydrophobic and hydrophilic solutes. Particularly, niosomes are prepared from glucosyl dialkyl ethers¹⁴, crown ethers, spans¹⁵ and polyoxyethylene alkyl ethers¹⁶. Nanoemulsion¹⁷ is preferred because of nanoscale size range of the dispersed droplets with a mean droplet size of about 250 nm. The physical stability of nanoemulsion can substantially be improved with the help of suitable. In addition, some studies have compared the performance of different nanoemulsified systems prepared with similar oils and surfactants for applications such as controlled drug release¹⁸ and or drug incorporation for protection¹⁹. The possible usefulness of NEs as nanocarriers is their ability to solubilize substantial amounts of hydrophilic/hydrophobic drug either at the innermost (oil or water) phase or at the o/w or w/o interfaces. They are biocompatible, biodegradable, physically stable, and relatively easy to produce on a large scale using proven technology²⁰. This allows efficient delivery of therapeutic agents to target sites in the body. Nanocarriers, in their various forms, have the possibility of providing endless opportunities in the area of drug delivery and therefore are increasingly being investigated to harness their potential²¹. The pharmaceutical colloidal carriers such as liposomes, niosomes and nanoemulsions are normally used to control the drug release in a desirable fashion. They improve the solubility of hydrophobic and hydrophilic compounds and render them suitable for different routes of administration²². Several reports prove that most of the NSAIDS are potential candidates for delivering through nanocarriers by various routes of administration. The following Table 1.0 lists some of the NSAIDS incorporated in nanocarriers. Diclofenac sodium (DS) is an analgesic and non-steroidal anti-inflammatory drug (NSAID). Its use is associated with fatal gastrointestinal side effects. It is documented that the solubility and bioavailability of DS has been attributed to the higher affinity of the drug molecules towards the lipids, oil and nonionic surfactants^{32,33}. Studies have shown that nanoemulsion, liposome or niosome can enhance transmembrane passage across the digestive tract and transdermal drug permeability, thus improving the bioavailability of the guest molecules³⁴⁻³⁶. The following Table 2.0 lists various nanocarriers developed for diclofenac sodium and thus demonstrates the versatile suitability for DS.

Table 1. NSAIDS and nanocarriers

SNO	NSAIDS	Nano carrier	Results	References
1	Celecoxib	NLC	Gel formulations of celecoxib prepared with NLC exhibited fasted drug input and sustained anti-inflammatory activity up to 24 h.	Joshi et al., 2008 ²³
2	Flufenamic acid	Poly(lactide-coglycolide) Nanoparticles	Nanoencapsulation of flufenamic acid has significantly increased drug transport and accumulation in the skin.	Luengo et al., 2006 ²⁴
3	Flurbiprofen	NLC	NLC formulation of flurbiprofen was led to the increase in drug permeation with respect to its conventional solution.	Gonzales-Mira et al., 2011 ²⁵
4	Flurbiprofen	SLN	SLN dispersion and gel formulation showed a sustained drug release over 24 h period.	Jain et al., 2005 ²⁶
5	Indomethacin	NLC	Prolonged in vivo anti-inflammatory activity of indomethacin was observed with NLC hydrogels compared to its aqueous solution and hydroalcoholic gel.	Ricci et al., 2005 ²⁷
6	Indomethacin	Nanocapsule	Transdermal delivery of indomethacin with polyn-butylcyanoacrylate nanocapsules was improved with respect to conventional gel formulation.	Miyazaki et al., 2003 ²⁸
7	Ketoprofen	SLN	Ketoprofen loaded SLN formulations showed a prolonged anti-inflammatory effect compared to its solution.	Puglia et al., 2008 ²⁹
8	Ketorolac	NLC	Topical application of the ketorolac HPBCD complex resulted in transdermal delivery of ketorolac whereas liposomal ketorolac dispersions promoted dermal localization of the drug.	Puglia et al., 2006 ³⁰
9	Nimesulide	Nanocapsule /nanoemulsion /nanospheres	Nimesulide-loaded nanocarriers formulated in hydrophilic gels exhibited good physicochemical properties for its dermal administration.	Alves et al., 2005 ³¹

Table 2. Diclofenac Sodium and nanocarriers

SNO	Nano carrier	Results	References
1	Microemulsion	Skin irritation study on human volunteers showed no visible reaction indicating the safety as a drug carrier for topical application.	Gulten Kantarc et al., 2007 ³⁷
2	Liposomes, Ethosomes and Transfersomes	Results revealed that both ethosome and transfersome formulations can act as drug reservoir in skin and extend the pharmacologic effects of Diclofenac sodium	Saeed Ghanbarzadeh et al., 2013 ³⁸
3	Microemulsion	Diclofenac permeability through Caco-2 monolayer cells increases when lecithin is embedded into the interface.	Aviram Spemath et al., 2007 ³⁹
4	Microemulsion	The transdermal fluxes were significantly higher than those obtained by application of the drug in aqueous solution	Amnon C. Sintov et al., 2006 ⁴⁰
5	Magnetic microspheres	Majority of injected dose was recovered from lungs, spleen and liver indicating that particle size is crucial to avoid the entrapment of microspheres in non-target organs.	Muniyandy Saravanan et al., 2004 ⁴¹
6	Normal microspheres	Both in the arthritic and normal contralateral knee joints radioactivity count ratios demonstrated less arthritic lesions.	M. tunċ ay et al., 2000 ⁴²
7	Nanocapsules	The percentage loading efficiency of DS was only 50± 60% and release at 8 h was only 60%.	A. R. kulkarni et al., 2000 ⁴³
8	Micelles	The thermodynamic parameter of standard free energy change indicates the greater ease of aggregation for the surfactant system with increasing concentration of Diclofenac Sodium.	S.K. Mehta et al., 2005 ⁴⁴

Therefore, the rational design of formulations takes advantage of any interactions taking place within the system. The main aim of the work is to explore the influence of nanosystems (Liposome, Niosome and Nanoemulsion) on the release characteristics and performance of DS and thereby to analyze the change in behavior of nanosystems in the presence of DS and thus to develop and compare nanoformulations as drug carriers for oral, parenteral and topical application and to demonstrate the release features of the formulations. In the present study, detailed physicochemical properties of selected nanoemulsion, liposome and niosome in the presence of DS for size, zeta potential and release studies were examined.

2. Materials and Methods

2.1. Preparation of Diclofenac Sodium Nanosystems

The composition of the tested liposomes, niosomes, and nanoemulsions formulae are reported in Table 3.0. Nanosystems containing diclofenac sodium were prepared by flash evaporation method^{45,46}. Briefly, diclofenac sodium, surfactants, cholesterol and lipids were accurately weighed into a long necked quick fit round bottom flask and dissolved in 10 ml dichloromethane and added to with 10 ml of phosphate buffered saline (PBS, pH 7.4). The organic solvent was slowly evaporated at 60°C under reduced pressure, using a rotary evaporator (Buchi R-110 Rotavapor, Switzerland) at 150 rpm. The nanosystems were left to mature overnight at 4°C. For sterility, all the above mentioned steps were done under aseptic conditions. All glassware was sterilized by autoclaving, phosphate buffered saline was passed through a 0.22 µm membrane filter, and the entire procedure was carried out in a laminar flow hood (Esco, Singapore).

2.2. Size and Zeta Potential Measurements

The size and surface charge were determined by light scattering based on laser diffraction⁴⁷ using the Malvern Zeta sizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). The measurements were performed using a 45 mm focus objective and a beam length of 2.4 mm. All the nanosystems were diluted 1:200, 1:100, 1:50, 1:25 and 1:10 with phosphate buffer for size and zeta potential

measurements, respectively to observe the dilution effect. For this purpose, the samples were diluted to appropriate concentration. The ZP characterizes the surface charge of dispersed phase and thus gives information about repulsive forces between vesicles and droplets. Absolute higher values than 30mV usually indicate good stability of the system. Also, the size and ZP values were measured with time over 5 weeks during storage at room temperature.

2.3. Entrapment Efficiency

The entrapped diclofenac sodium in the nanosystems was separated from the unentrapped by centrifugation. At predetermined time intervals 0.25 mL of the dispersion was withdrawn and the aliquot was centrifuged (Eppendorf centrifuge, Eppendorf, Germany) at $14,000 \times g$ for 30 min, and the supernatant was analysed using UV spectrophotometer. All experiments were conducted in triplicate. The entrapment efficiency was calculated according to the following equation⁴⁸:

$$\text{Entrapment efficiency (\%)} = \left\{ \frac{\text{Amt of DFS entrapped}}{\text{Total amount of DFS}} \right\} \times 100$$

2.4. In-vitro Release of DFS from nanosystems

The release of DFS from nanosystems was determined using the membrane diffusion technique⁴⁹⁻⁵¹. An accurately measured amount of DFS nanosystems, equivalent to 1 mg drug or a volume of 0.5 mL of preparation was placed in a dialysis bag (3.8 cm in length). Dialysis tubing consisted of regenerated cellulose, a material chemically and physically treated to increase its resistance (MWCO 12,000-14,000 Da, 25-Å pore diameter, Himedia, India). Both ends were tied. The dialysis bag was suspended in 25 mL PBS at pH 7.4 and maintained at $37 \pm 0.5^\circ\text{C}$. The dispersion was rotated at 200 rpm on a magnetic stirrer (REMI, India). At predetermined time intervals of 0.5, 1, 2, 3, 4, 6, 8 and 12hrs, 1mL aliquots were sampled and replaced with 1 mL fresh pH 7.4 PBS, which was maintained at $37 \pm 0.5^\circ\text{C}$. Drug concentrations were quantified using UV spectrophotometer at 276nm, and all experiments were conducted in triplicate.

Table 3. Composition of diclofenac sodium nanosystems

Code	Composition
LIP001	LC+CH+SD+T8=1:1:2:3
NIO002	S6+CH+T8=2:3:1
NANOE003	O+T8+LC+P2=2:1:1

LC:Soya lecithin; CH:Cholesterol; T8:polysorbate 80; DFS:Diclofenac sodium; SD:sodium deoxycholate; S6:span 60; O:oil, P2:polysorbate 20

3. Results and Discussion

Size Analysis of nanosystems Table 4.0 shows the results of size measurement. As evident from table 1, the size of nanosystems was not much significantly different with negative zetapotential. The formation of stable liposomes can be attributed to the inclusion of charge inducer which increases the spacing between the adjacent layers due to repulsion forces, resulting in the formation of stable liposomes⁵². Furthermore, the charged lecithin electrostatically attracts the DS counterion, which may be expected to push phospholipids lead groups apart, hence increasing the size⁵³. The mechanism of niosome

formation⁵⁴ is from splicing of two monolayers of inverted micelles collapsing to each other and hence more easily curved, which lead to a decrease in the size. In most typical processes nanoemulsions are formed from an aqueous surfactant solution aggregating into micelles. In the course of the water dilution process of the condensed reverse micelles (concentrates), a bicontinuous phase, consisting of long domains, inverts into O/W droplets and eventually the structures turn into oil nano-droplets dispersed in a water continuous phase. The two major processes of nucleation and growth are inverted. The large domains of the O/W droplets (obtained from the bicontinuous phase) slowly shrink (by a chopping process and edge termination) but in parallel, many of the reformed droplets re-assemble (coalesce) into larger droplets above 60 wt% water content. These results are well correlated to the results obtained by de Campo et al., 2004⁵⁵. Our results are in good agreement with the results of Ko et al., 2003⁵⁶, who studied a nonionic O/W microemulsion system containing C18:1E10 as surfactant, MCT and retinyl palmitate (1:1 w/w) as the oil phase and water. They calculated the O/W droplet size from the diffusion coefficients obtained from PGSE-NMR measurements. They found that upon increasing the oil phase concentration at the expense of the water concentration (from 1 to 7 wt.%) while keeping the surfactant concentration fixed, the droplet size increased which correlates with our hypothesis that oil increases the size of the droplet. The average droplet size is not affected by the presence of PC molecules in the surfactant mixture Araya et al., 2005⁵⁷. The droplets in the empty system are larger than in the loaded ones, but the trend is similar. Thus, the presence of diclofenac sodium increases the restructuring effect of the droplets upon dilution, thus smaller droplets are detected. The results also suggest that diclofenac sodium is solubilized at the interface of the O/W droplets. The mean globule size depended on the concentration of polysorbate 80 and was strongly influenced by the presence of lecithin. The addition of PS increased the globule size. The higher the PS content, the higher the globule size of the nanoemulsion. As shown in Fig 1, the mean size of the systems was decreased upon increasing dilution. Among all the systems, the mean size of niosomes was least which was decreased to 204 nm upon increasing the dilution upto 1:200. Fig 2 depicts the dilution effect on the impact of charge i.e; negative zeta potential of the nanosystems. On similar lines with size, the zeta potential also decreased with increasing dilution as shown in Fig 2. The ZP changes followed the same pattern in all the nanosystems at higher dilutions. The stability assessments of the formulations were tested as change in results with respect to time. Zeta potential reduction was observed in all carrier systems with increase in dilution. Among these, niosomes had shown a significant decrease in negative zeta potential. The reduction in zeta potential of niosomes was possibly due to the change surfactant adsorption.

Table 4. Characterization of diclofenac sodium nanosystems

Code	Size (nm)	Zetapotential (mv)	Encapsulation (%)
LIP001	201±5.4	-14.3±0.3	75
NIO002	212±4.6	-9.32±0.2	82
NANOE003	204±3.5	-11.8±0.5	78

LC:Soya lecithin; CH:Cholesterol; T8:polysorbate 80; DFS:Diclofenac sodium; SD:sodium deoxycholate; S6:span 60; O:oil, P2:polysorbate 20

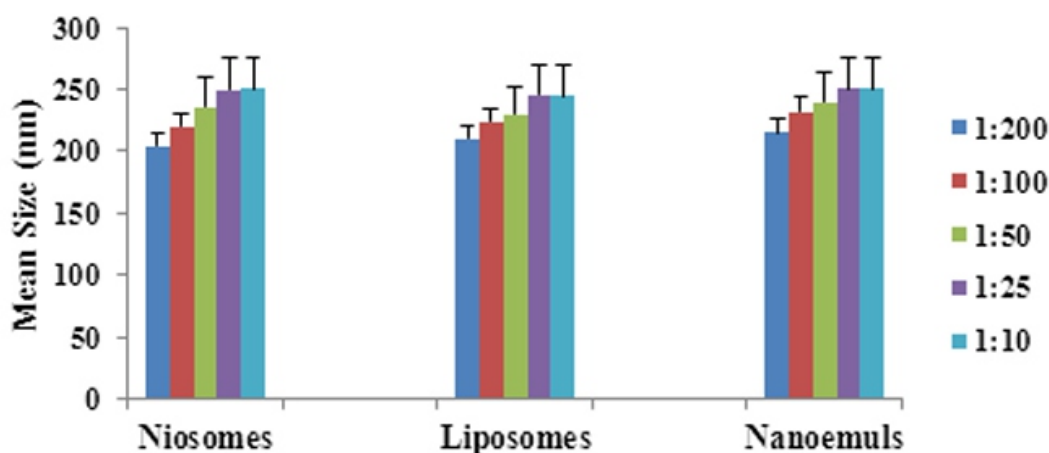


Figure 1. Dilution effect of liposomes, niosomes and nanoemulsion on size

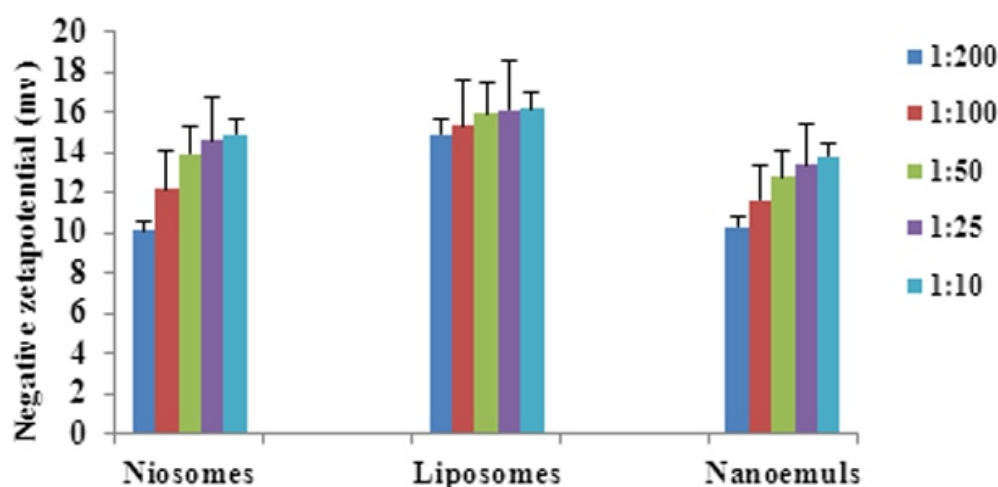


Figure 2.. Dilution effect of liposomes, niosomes and nanoemulsion on zeta potential

Entrapment efficiency

By inspection of Table 4.0, it is shown that diclofenac sodium encapsulation efficiency varied with the composition and type of nanosystems. The entrapment efficiencies for niosomes prepared were superior to those of nanoemulsion and liposomes. This may be explained by the fact that the vesicles obtained from Span 60 produce higher entrapment efficiencies as the length of alkyl chain is a crucial factor of permeability. Thus, long chain surfactants produce high entrapment⁵⁸. Additionally, the alkyl chain length influences the HLB value of the surfactant which in turn directly influences the drug entrapment efficiency⁵⁹. The lower the HLB of the surfactant the higher will be the drug entrapment efficiency and stability as in the case of niosomes prepared using Span 60 (HLB=4.6). Cholesterol is one of the common additives included in the formulation in order to prepare stable niosomes. Cholesterol is known to abolish the gel to lipid phase transition of niosome systems⁶⁰, which could be able to effectively prevent leakage of drug from niosomes⁶¹, notably the membrane permeability, encapsulation efficiency and bilayer rigidity⁶². Charged oil is often used to prevent globule aggregation and increase the stability of nnanoemulsion thus was found to have more entrapment than liposomes⁶³. Also, the concerning the effect of nanosystem on the encapsulation efficiency of diclofenac sodium, results showed that the EE% had higher values in the case of niosomes than in liposomes. This finding may be because niosomes were prepared at pH 7.4 and DFS has pH dependent solubility and it has minimum solubility at this pH. This will increase the unionized fraction of DFS, which is lipophilic in nature⁶⁴. Due to the fact that MLVs

contain multiple lamellae capable of loading a higher mass of lipophilic drug than ULVs⁶⁵, EE% had higher values in the case of niosomes than liposomes. Concerning the effect of type of surfactants on EE%, results showed that the EE% had higher values in the case of nanoemulsion than liposomes. This result may be because the encapsulation of DFS depends on the fluidity of the phospholipids bilayer⁶⁶. The increase in EE% by increasing P2 content occur because by increasing the P2 concentration, resulting in higher stability and reduced permeability of the oil⁶⁷ and hence greater drug retention⁶⁸. Decreasing EE% with increasing CH ratio above a certain limit may be due to the fact that increasing CH above certain concentration can disrupt the regular linear structure of liposomal membrane⁶⁹. Concerning the effect of dilution and effect of time on the entrapment of DFS in nanosystems, results showed increase in dilution decreased entrapment proportionately in all the nanosystems (Fig 3, 4 and 5) and with time decrease in entrapment (Fig 6, 7 and 8). This behavior could be due to increase in size of nanosystems, thus causing leakage of the drug.

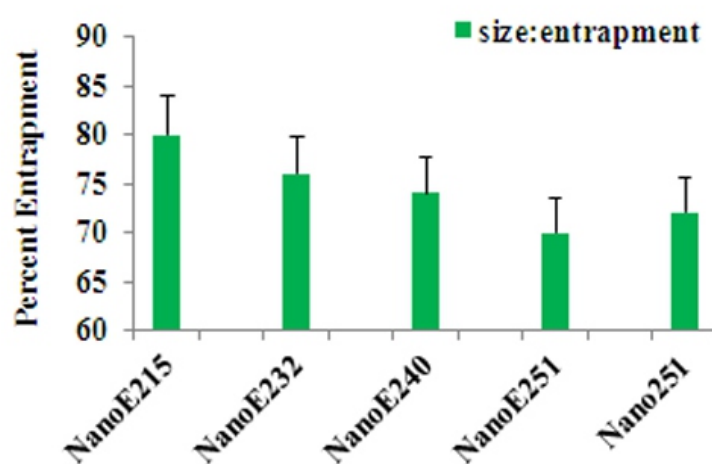


Figure 3. Dilution effect on entrapment of diclofenac sodium in nanoemulsions (1:200, 1:100, 1:50, 1:25, 1:10)

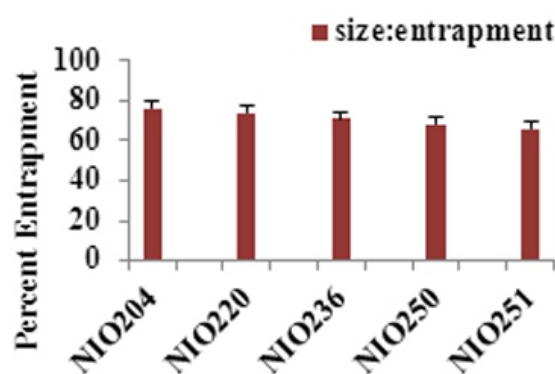


Figure 4. Dilution effect on entrapment of diclofenac sodium in niosomes

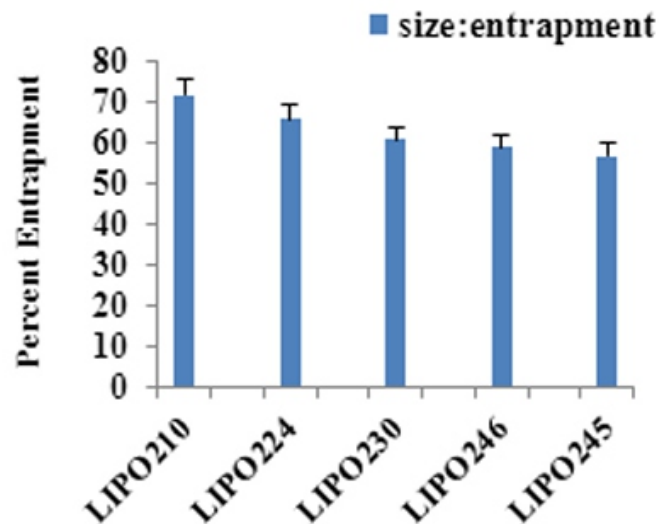


Figure 5. Dilution effect on entrapment of diclofenac sodium in liposo

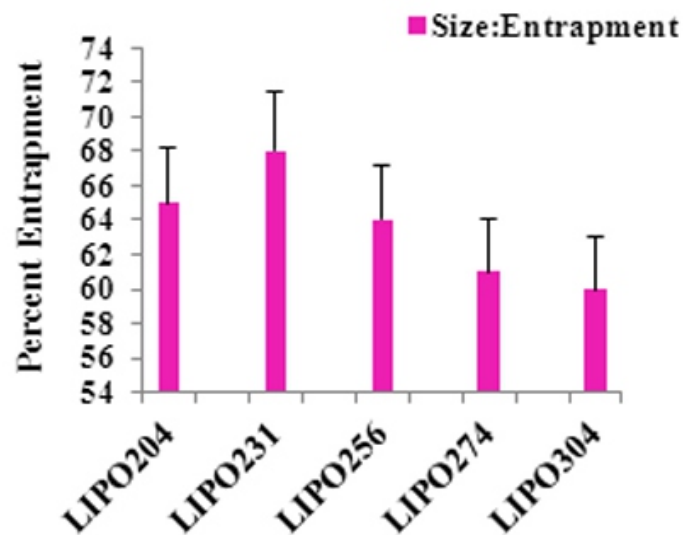


Figure 6. Effect of time on the entrapment of diclofenac sodium from liposomes (5 weeks, 4 weeks, 3 weeks, 2 weeks and 1 week)

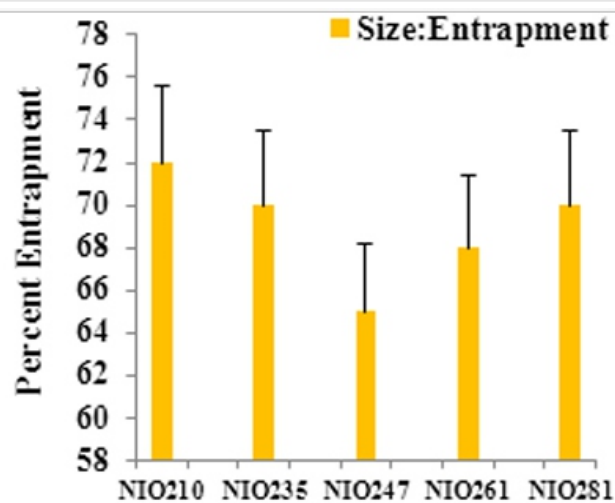


Figure 7. Effect of time on the entrapment of diclofenac sodium from niosomes (5 weeks, 4 weeks, 3 weeks, 2 weeks and 1 week)

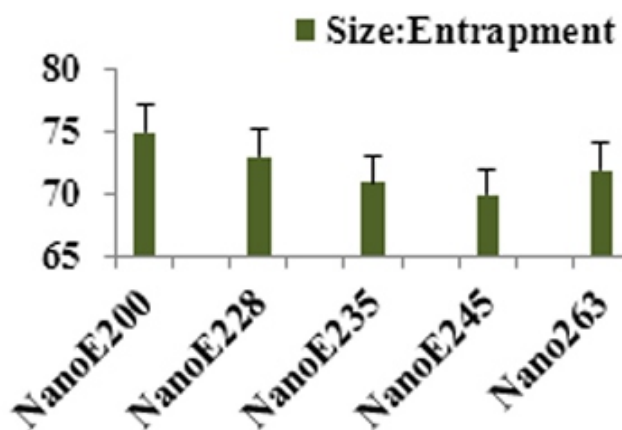


Figure 8. Effect of time on the entrapment of diclofenac sodium from nanoemulsions (5 weeks, 4 weeks, 3 weeks, 2 weeks and 1 week)

In Vitro Release Studies

Results of in-vitro studies on the release of diclofenac sodium nanosystems prepared using lipids, surfactants and cosolvents are shown in Figs. 9 respectively. The percentage of drug released with the effect of dilution and time from the nanosystems are shown in Fig 10 to 15 respectively. Nano formulations showed slower release rate than diclofenac sodium solution. Significant changes in release were observed upon changing the composition used in the nanosystems. By inspection of the data, it could be concluded and can be explained by the fact that niosomes exhibit an alkyl chain length-dependent release and the higher the chain length, the lower the release rate⁷⁰. It is clear that the release is retarded in presence of lecithin in liposome, span 60 in niosome and oil in nanoemulsion. This confirms that the components stabilize the structure of outer membrane and renders them less permeable⁷¹. From the results, it is obvious that the increase of cholesterol markedly reduced the efflux of the drug from vesicular preparations (Fig 9), which is in accordance with its membrane stabilizing ability⁷². Cholesterol is known to abolish the gel to liquid phase transition of niosome systems⁷³, resulting in vesicular that are less leaky⁷⁴. Therefore, the diffusion of DFS entrapped in the hydrophobic regions of the vesicles would be expected to occur over a prolonged period of time. On the contrary to previous results, the increase of cholesterol time slightly increased the efflux of the drug from nanosystems. The release profiles of DFS from nanosystems reveal that the presence of structured components in the nanosystems stabilizes the bilayers and decreases their permeability. However, contrary to previous results, increase in time gradually reduces the permeability. Therefore, it is to be noted that the in vitro release results are consistent with those of the entrapment efficiency. Thus, the comparative release data indicate that, by encapsulation of drug into nanosystems, it is possible to sustain and control the release of the drug for a longer duration⁷⁵. The invitro release of the systems was also affected with increase in time. Fig 13, 14 and 15 shows the changes in release profile with time. In Fig 13, 75% release was observed from niosomes after 1 week. But it was decreased to 59% after 5 weeks of time period. Similarly as shown in Fig 14 and 15 from liposomes and nano emulsion, the release was decreased from 65% to 60% and 75% to 70% respectively. Thus, the release profiles obtained were almost similar in all the formulations as shown in Fig 9. It is found that 85% of diclofenac sodium diffused out from the colloidal systems within 8hrs and practically all the drug was released within 12hrs. In addition to this controlled release, the similarity of the release profiles obtained for liposomes, niosomes and nanoemulsion evidences that the internal structure has little role in the release process.

Consequently, it can be established that the release of diclofenac sodium from the colloidal carriers is solely affected by the partition of the drug between the oily droplets/vesicles and the external aqueous medium. This release mechanism was also corroborated by the fact that the main factor controlling the release of diclofenac sodium is the volume of the aqueous medium. This was explained by the dilution effect as shown in Fig 10, 11 and 12. The drug releases fast and completely from the nanocarriers upon high dilution, but it is slowed down a little when they are not diluted. In Fig 10 and Fig 12, maximum amount of diclofenac sodium was released from nanoemulsion as well as liposome after 12 hrs at 1 in 200 dilution where as in niosomes (Fig 11) it was found at 1 in 100 dilution. But surprisingly, the release was decreased upon further dilution. The major mechanism for drug release was partitioning of drug from carrier as a result of changes in phase volume ratio. Fig 13, 14 and 15 states the effect of time on release profiles of diclofenac sodium with respect to size

—●— Liposomes —■— Niosomes —▲— Nanoemulsions

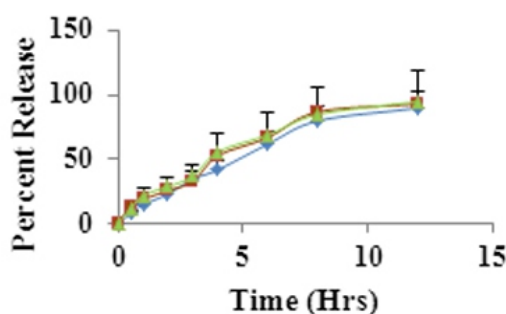


Figure 9. Release profiles of diclofenac sodium liposomes, niosomes and nanoemulsion in phosphate buffer

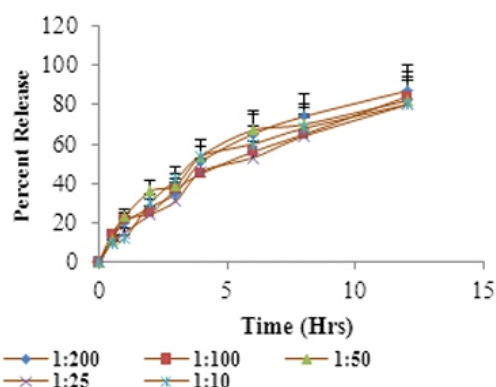


Figure 10. Dilution effect on release profile of diclofenac sodium from nanoemulsions

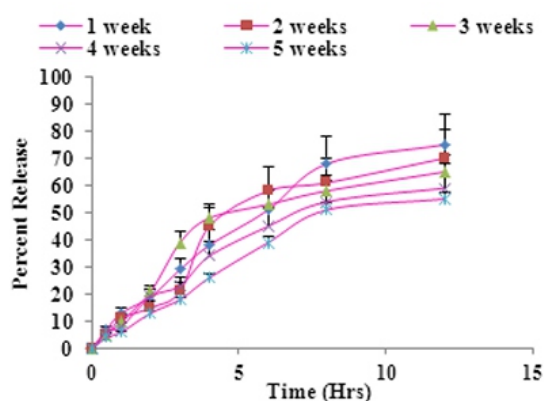


Figure 13. Effect of time on the release profile of diclofenac sodium from niosomes

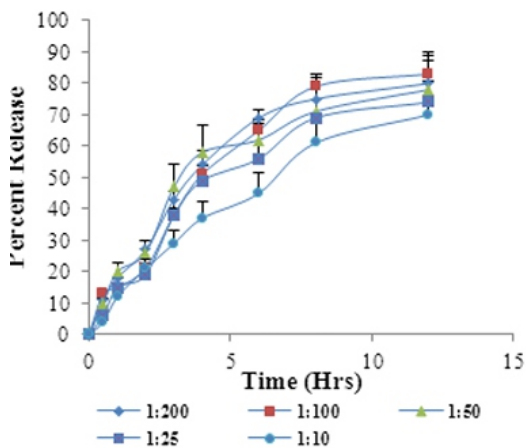


Figure 11. Dilution effect on release profile of diclofenac sodium from niosomes

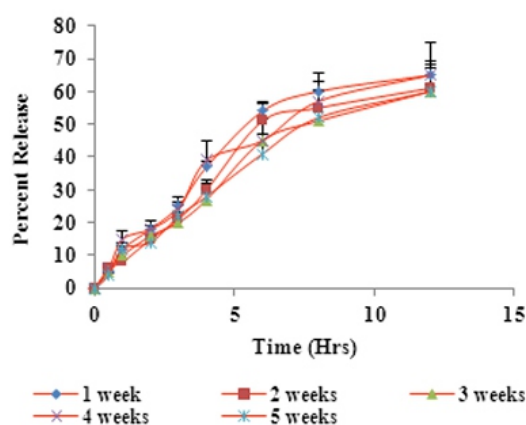


Figure 14. Effect of time on the release profile of diclofenac sodium from liposomes

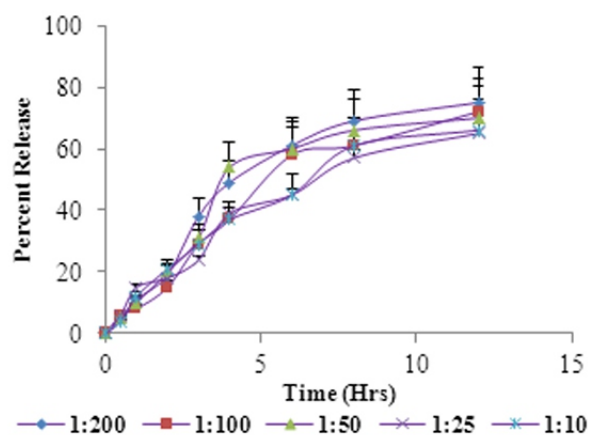


Figure 12. Dilution effect on release profile of diclofenac sodium from Liposomes

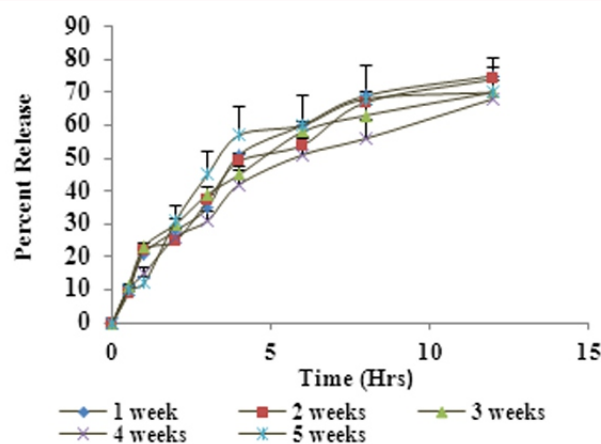


Figure 15. Effect of time on the release profile of diclofenac sodium from nanoemulsions

4. Conclusions

This work describes the development of nanosystems of diclofenac sodium. The interest of these diclofenac sodium loaded nanosystems was evidenced by the fact that they significantly controlled the release of diclofenac sodium. These nanosystems released their drug content rapidly upon dilution. The similar behavior of the three systems indicates that the colloidal nature of the systems is the key factor responsible for their positive behavior. In conclusion, the results of this study emphasize the potential of liposomes, niosomes and nanoemulsions as promising future nanosystems for diclofenac sodium. The results of this study show that cholesterol content, type of surfactant and the presence of charge altered the entrapment efficiency and release rate of DFS from nanosystems.

REFERENCES

- [1]Gregoriadis, G. (1976) *The carrier potential of liposomes in biology and medicine*. *N. Engl.J. Med.* 295,704-710.
- [2]Grimaldi, S., Lisi, A., Pozzi, D. a and Santoro, N.(1997) *Attempts to use liposomes and RBS ghosts as vectors in drug and antisense therapy of virus infection*. *Res. Virol.* 148, 177-180.
- [3]M. S. Mufamadi, V. Pillay, Y. E. Choonara et al., "A review on composite liposomal technologies for specialized drug delivery," *Journal of Drug Delivery*, pp. 2–19, 2011
- [4]Gregoriadis, G. (1989) *Liposomes as carriers of drugs. Observations on vesicle fate after injection and its control*. *Subcell. Biochem.* 14, 363-378.
- [5]Gregoriadis, G. and Florence, A.T. (1993) *Recent advances in drug target in*. *Trends. Biotechnol.* 11, 440-442.
- [6]Ranson, M., Howell, A., Cheeseman, S. and Margison, J. (1996) *Liposomal drug delivery*. *Cancer Treat. Rev.* 22, 365-379
- [7]Chen, H. and Langer, R. (1997) *Magnetically-responsive polymerized liposomes as potential oral delivery vehicles*. *Pharm. Res.* 14, 537-540.
- [8]Senior, J. (1987) *Fate and behavior of liposomes in vivo: A review of controlling factors*. *Crit. Rev. Ther. Drug Carrier Syst.* 3, 123-193.
- [9]Gregoriadis, G. (1989) *Liposomes as carries of drugs. Observations on vesicle fate after injection and its control*. *Subcell. Biochem.* 14,363-378.
- [10]Hwang, C. (1987) *Liposome pharmacokinetics*. In: *Liposomes: From Biophysics to Therapeutics*, (ed.) M. Ostro, New York: Marcel Dekker, pp. 109-156.

- [11]Gregoriadis, G. (1988) *Fate of injected liposomes; Observations on entrapped solute retention, vesicle clearance and tissue distribution in vivo.* In: *Liposomes as Drug Carriers: Recent Trends and progress*, (ed.). G. Gregoriadis, John Wiley and Sons Ltd. New York: Marcel Dekker, 3-18
- [12]Uchegbu, I.F., Vyas, S.P., 1998. Non-ionic surfactant based vesicles (niosomes) in drug delivery. *Int. J. Pharm.* 172, 33-70.
- [13]Uchegbu, I.F., Florence, A.T., 1995. Non-ionic surfactant vesicles (niosomes): physical and pharmaceutical chemistry. *Adv. Colloid Interface Sci.* 58, 1-55.
- [14]van Hal, D., Bouwstra, J.A., Junginger, H.E., 1992. Preparation and characterization of new dermal dosage form for antipsoriatic drug dithranol, based on non-ionic surfactant vesicles. *Eur. J. Pharm. Biopharm.* 38, 47s.
- [15]Echoyen, L.E., Hernandez, J.C., Kaifer, A.E., Gokel, G.W., Echoyen, L., 1988. Aggregates of steroidal lariat ethers: the first example of non-ionic liposomes (niosomes) formed from neutral crown ethers compounds. *J. Chem. Soc., Chem. Comm.* 8, 836-837.
- [16]Hofland, H.E.J., Bouwstra, J.A., Poncet, M., Bodde, H.E., Spies, F., Verhoef, J.C., Junginger, H.E., 1991. Interactions of non-ionic surfactant vesicles with cultured keratinocytes and human skin in vitro: a survey of toxicological aspects and ultrastructural changes in stratum corneum. *J. Control Release* 16, 155-168.
- [17]Tadros, T.F., Izquierdo, P., Esquena, J., Solans, C., 2004. Formation and stability of nano-emulsions. *Adv. Colloid Interface Sci.* 108-109, 303-318.
- [18]Gallarate, M., Carlotti, M.E., Trotta, M., Bovo, S., 1999. On the stability of ascorbic acid in emulsified systems for topical and cosmetic use. *Int. J. Pharm.* 188, 233-241.
- [19]Er Áfnofas, I., Csoka, I., Csany, E., Orosz, K., Makai, M., 1998. *Proceedings of 2nd World Meeting APGI/APV, Paris*, 805-806.
- [20]Fukushima, S., Kishimoto, S., Takeuchi, Y., Fukushima, M. 2000. Preparation and evaluation of o/w type emulsions containing antitumor prostaglandin. *Adv. Drug Deliv. Rev.* 45, 65-75.
- [21]Asiyanbola B, Soboyejo W. For the surgeon: an introduction to nanotechnology. *J Surg Educ* 2008; 65:155-61
- [22]Torchilin P. Multifunctional nanocarriers. *Advanced Drug Delivery Reviews*, 2006, 58:1532- 1555
- [23]Joshi, M. & Patravale, V. (2008). Nanostructured lipid carrier based gel of celecoxib. *Int J Pharm*, 346:124-132.
- [24]Luengo J.; Weiss, B., Schneider, M., Ehlers, A. & Stracke, F. et al. (2006). Influence of nanoencapsulation on human skin transport of flufenamic acid. *Skin Pharmacol Physiol*, Vol. 19(4):190-197.
- [25]Gonzalez-Mira E.; Nikolic, S., Garcia, ML., Egea, MA. & Souto EB., et al. (2011). Potential use of nanostructured lipid carriers for dermal delivery of flurbiprofen. *J Pharm Sci*, Vol. 100, 242-251.
- [26]Jain, SK.; Chourasia, MK., Masuriha, R., Soni, V. & Jain, A. et al. (2005). Solid lipid nanoparticles bearing flurbiprofen for transdermal delivery. *Drug Deliv*, Vol. 12, No. 4, 207-215.
- [27]Ricci, M.; Puglia, C., Bonina, F., Di Giovanni, C., Giovagnoli, S & Rossi, C. (2005). Evaluation of indomethacin percutaneous absorption from nanostructured lipid carriers (NLC): in vitro and in vivo studies. *J Pharm Sci*, Vol. 94, No. 5, pp. 1149-1159.
- [28]Miyazaki S.; Takahashi, A., Kubo, W., Bachynsky, J. & Löbenberg, R. (2003). Poly nbutylcyanoacrylate (PNBCA) nanocapsules as a carrier for NSAIDs: in vitro release and in vivo skin penetration. *J Pharm Pharm Sci*, Vol. 6, No. 2, pp. 238-245.
- [29]Puglia, C.; Blasi, P., Rizza, L., Schoubben, A., Bonina, F. et al. (2008). Lipid nanoparticles for prolonged dermal delivery: an in vitro and in vivo investigation. *Int J Pharm*, Vol. 357, No. 1-2, 295-304.
- [30]Puglia C.; Filosa, R., Peduto, A., de Caprariis, P. & Rizza, L.

et al. (2006). Evaluation of alternative strategies to optimize ketorolac transdermal delivery. *AAPS PharmSciTech*, Vol. 7, No. 3, pp. E1.

[31]Alves, PM.; Pohlmann, AR. & Guterres, SS. (2005). Semisolid dermal formulations containing nimesulide-loaded nanocapsules, nanospheres or nanoemulsion: development and rheological characterization. *Pharmazie*, Vol. 60, No.12, pp. 900-904.

[32]P.P. Tirumalasetty, J.G. Eley, Permeability enhancing effects of the alkylglycoside, octylglucoside, on insulin permeation across epithelial membrane in vitro, *J. Pharm. Pharm. Sci.* 9 (1) (2006) 32–39.

[33]J. Risbo, K. Jorgensen, M.M. Sperotto, O.G. Mouritsen, Phase behavior and permeability properties of phospholipid bilayers containing a short chain phospholipid permeability enhancer, *Biochim. Biophys. Acta- Biomembr.* 1329 (1) (1997) 85–96.

[34]M. Kirjavainen, A. Urtti, J. Mönkkönen, J. Hirvonen, Influence of lipids on the mannitol flux during transdermal iontophoresis in vitro, *Eur. J. Pharma. Sci.* 10 (2) (2000) 97102.

[35] D.Z. Liu, E.L. LeCluyse, D.R. Thakker, Dodecylphosphocholine-mediated enhancement of paracellular permeability and cytotoxicity in Caco-2 cell monolayers, *J. Pharm. Sci.* 88 (11) (1999) 1161–1168.

[36]D.Z. Liu, S.L. Morris-Natschke, L.S. Kucera, K.S. Ishaq, D.R. Thakker, Structure-activity relationships for enhancement of paracellular permeability by 2-alkoxy-3-alkylamidopropylphosphocholines across Caco-2 cell monolayers, *J. Pharm. Sci.* 88 (11) (1999) 1169–1174.

[37]Gulten Kantarcı, Isik Ozguney, H. Yeşim Karasulu, Sevgi Arzik, and Tamer Guneri, *AAPS PharmSciTech* (2007); 8 (4): E1-E7

[38]Saeed Ghanbarzadeh, Sanam Arami. Enhanced Transdermal Delivery of Diclofenac Sodium via Conventional Liposomes, Ethosomes, and Transfersomes *BioMed Research International* (2013); 7: 1-8

[39]Aviram Spornath, Abraham Aserin, Lior Ziserman, Dganit Danino, Nissim Garti, Phosphatidylcholine embedded microemulsions: Physical properties and improved Caco-2 cell permeability *Journal of Controlled Release* 119 (2007) 279–290

[40]Amnon C. Sintov, Shafir Botner *International Journal of Pharmaceutics* 311 (2006) 55–62

[41]Muniyandy Saravanan, Kesavan Bhaskar, Gomathinayagam Maharajan, Kalathil Sadasivan Pillai *International Journal of Pharmaceutics* 283 (2004) 71–82

[42]M. tuncë ay, S. cë alisë, H. S. kasë, M. T. ercan, I. peksoy and A. A. hincal *J. microencapsulation*, 2000, vol. 17, No. 2, 145-155

[43]A. R. kulkarni, K. S. soppimath and T. M. aminabhavi *J. microencapsulation*, 2000, vol. 17, NO. 4, 449-458

[44]S.K. Mehta, Neeru Bala, Shweta Sharma *Colloids and Surfaces A: Physicochem. Eng. Aspects* 268 (2005) 90–98

[45]A.J. Baillie, A.T. Florence, L.R. Hume, G.T. Muirhead, A. Rogerson. The preparation and properties of niosomes non-ionic surfactant vesicles. *J. Pharm. Pharmacol.* 37:863868(1985).

[46]R. Agarwal, O.P. Katare, S.P. Vyas. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. *Int. J. Pharm.* 228:43–52 (2001).

[47]P. Arunothayanun, M.S. Bernard, D.Q.M. Craig, I.F. Uchegbu, A.T. Florence. The effect of processing variables on the physical characteristics of non-ionic surfactant vesicles (niosomes) formed from a hexadecyl diglycerol ether. *Int. J. Pharm.* 201:7–14 (2000).

[48] K. Ruckmani, B. Jayakar, S.K. Ghosal. Nonionic surfactant vesicles (niosomes) of cytarabine hydrochloride for effective treatment of leukemia: encapsulation, storage and in vitro release. *Drug Dev. Ind. Pharm.* 26:217–222 (2000).

-
-
- [49] O.N. El-Gazayerly, and A.H. Hikal. Preparation and evaluation of acetazolamide liposomes as an ocular delivery system. *Int. J. Pharm.* 158:121–127 (1997).
- [50] D. Rambhau. Release studies on niosomes containing fatty alcohols as bilayer stabilizers instead of cholesterol. *J. Interface Sci.* 251:360–365 (2002).
- [51] M. Glavas-Dodov, K. Goracinova, K. Mladenovska, and E. Fredro- Kumbaradzi. Release profile of lidocaine HCl from topical liposomal gel formulation. *Int. J. Pharm.* 242:381384 (2002).
- [52] Rania MH, Samar M, Nahed DM, Ahmed SG. Liposomes as an ocular delivery system for acetazolamide: in vitro and in vivo studies. *AAPS PharmSciTech.* 2007;8(1):1–12.
- [53] Gruner SM. Materials properties of liposomal bilayers. In: Ostro MJ, editor. *Liposomes from biophysics to therapeutics*. New York: Marcel Dekker; 1987. p. 1–38.
- [54] U. Olsson, K. Nakamura, H. Kunieda, Normal and reverse vesicles with nonionic surfactant: solvent diffusion and permeability *Langmuir* 12 (1996) 3045–3054.
- [55] L. de Campo, A. Yaghmur, N. Garti, M.E. Leser, B. Folmer, O. Glatter, Five-component food-grade microemulsions: structural characterization by SANS, *J. Coll. Interface. Sci.* 274 (1) (2004) 251–267.
- [56] C.J. Ko, Y.J. Ko, D.M. Kim, H.J. Park, Solution properties and PGSENMR self-diffusion study of C18:1E10/oil/water system, *Colloid Surf. A* 216 (1–3) (2003) 55–63.
- [57] H. Araya, M. Tomita, M. Hayashi, The novel formulation design of O/W microemulsion for improving the gastrointestinal absorption of poorly water soluble compounds, *Int. J. Pharm.* 305 (1–2) (2005) 61–74.
- [58] Y. Hao, F. Zhao, N. Li, Y. Yang, and K. Li. Studies on a high encapsulation of colchicine by a niosome system. *Int. J. Pharm.* 224:73–80 (2002).
- [59] R.A. RajaNaresh, G.K. Pillai, N. Udupa, and G. Chandrashekar. Anti-inflammatory activity of niosome encapsulated diclofenac sodium in arthritic rats. *Indian. J. Pharmacol.* 26:46–48 (1994).
- [60] C. Cable. An examination of the effects of surface modifications on the physicochemical and biological properties of non-ionic surfactant vesicles. PhD Thesis. University of Strathclyde, Glasgow, UK (1989).
- [61] A. Rogerson, J. Cummings, and A.T. Florence. Adriamycin loaded niosomes-drug entrapment, stability and release. *J. Microencap.* 4:321–328 (1987).
- [62] I.F. Uchegbu, and S.P. Vyas. Non ionic surfactant based vesicles (niosomes) in drug delivery. *Int. J. Pharm.* 172:3370 (1998)
- [63] E. Gianasi, F. Cociancich, I.F. Uchegbu, A.T. Florence, and R. Duncan. Pharmaceutical and biological characterization of a doxorubicin polymer conjugate (PK1) entrapped in sorbitan monostearate Span 60 niosomes. *Int. J. Pharm.* 148:139–148 (1997).
- [64] Claerhout I, Kestelyn Ph, Meire F, Remon J, Decaestecker T, Van Bocxlaer J. Corneal deposits after the topical use of ofloxacin in two children with verna keratoconjunctivitis. *Br J Ophthalmol.* 2003;87(5):646. doi:10.1136/bjo.87.5.646
- [65] Morilla MJ, Benavides P, Lopez MO, Bakas L, Romero EL. Development and in vitro characterization of a benzimidazole liposomal formulation. *Int J Pharm.* 2002;249:89–99. doi:10.1016/S0378-5173(02)00453-2.
- [66] Puglisi G, Fresta M, Mazzone G, Furneri P, Tempera G. Formulation parameters of fluoroquinolone-loaded liposomes and in vitro antimicrobial activity. *Int J Pharm.* 1995;118:6576. doi:10.1016/0378-5173(94)00340-B.
- [67] Perugini P, Pavanetto F. Liposomes boronophenylalanine for boron neutron capture therapy. *J Microencapsul.* 1998;15:473–83. doi:10.3109/026520498090006874
- [68] New RRC. *Liposomes: a practical approach*. Oxford: Oxford University Press; 1990
-
-

-
- [69]Gulati M, Grover M, Singh M, Singh S. Study of azathioprine encapsulation into liposomes. *J Microencapsul.* 1998;15:485-94. doi:10.3109/02652049809006875.
- [70]G.N. Devaraj, S.R. Parakh, R. Devraj, S.S. Apte, B.R. Rao, and D. Rambhau. Release studies on niosomes containing fatty alcohols as bilayer stabilizers instead of cholesterol. *J. Colloid Interface Sci.* 251:360–365 (2002).
- [71]Y. Hao, F. Zhao, N. Li, Y. Yang, and K. Li. Studies on a high encapsulation of colchicine by a niosome system. *Int. J. Pharm.* 224:73–80 (2002).
- [72]G.V. Betageri, and D.L. Parsons. Drug encapsulation and release from multilamellar and unilamellar liposomes. *Int. J. Pharm.* 81:235–241 (1992).
- [73]C. Cable. An examination of the effects of surface modifications on the physicochemical and biological properties of non-ionic surfactant vesicles. PhD Thesis. University of Strathclyde, Glasgow, UK (1989)
- [74]A. Rogerson, J. Cummings, and A.T. Florence. Adriamycin loaded niosomes-drug entrapment, stability and release. *J. Microencap.* 4:321–328 (1987).
- [75]K. Ruckmani, B. Jayakar, and S.K. Ghosal. Nonionic surfactant vesicles (niosomes) of cytarabine hydrochloride for effective treatment of leukemia: encapsulation, storage and in vitro release. *Drug Dev. Ind. Pharm.* 26:217–222 (2000)

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