



CERTIFICATE

No. 170/CERT.X/ISPST/2020

THIS CERTIFICATE IS PROUDLY PRESENTED TO

Anisa Amalia, M.Farm.

AS POSTER PRESENTER

Fourth International Seminar on Pharmaceutical Science and Technology (4th ISPST 2020)
Bandung, October 27th - 28th, 2020



Ajeng Diantini
Prof. Dr. apt. Ajeng Diantini, M.Si

Dean Faculty of Pharmacy



Nyi Mekar Saptarini
Dr. apt. Nyi Mekar Saptarini, M.Si

Chairman of 4th ISPST 2020





PHYSICAL PROPERTIES AND RATE OF DIFFUSION TRANSETHOSOME CURCUMIN USING TWEEN 60 AND SPAN 60 AS SURFACTANT

Anisa Amalia*, Yudi Srifiana, Amalia Anwar

Faculty of Pharmacy and Science, Universitas Muhammadiyah Prof. DR. HAMKA



Background and Objectives

Curcumin is a yellow compound in turmeric (Curcuma longa Linn), and has activities such as antimicrobial, anti-inflammatory, antioxidant, and anticancer. Curcumin has a low solubility in water, is metabolized first by causing its bioavailability to decrease. This problem can be overcome by formulating curcumin into the form of a transethosome delivery system. Surfactant is one of the transethosome component that affect the physical properties and penetration of vesicles. In this study, a combination of two surfactants (Tween 60 and Span 60) was used to see the effect of using a combination of two surfactant on physical properties and penetration of curcumin.

Conclusion

Diffusion rate testing results show that the use of a surfactant combination can increase the diffusion rate of curcumin, where there is a significant differential between each formula ($p < 0.05$).

Reference

- Jantarat C. Bioavailability Enhancement Techniques of Herbal Medicine: A Case Example of Curcumin. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 5 Suppl 1:493-500.
- Shaji J, Bajaj R. Transethosomes: A New Prospect for Enhanced Transdermal Delivery. International Journal of Pharmaceutical Sciences and Research 2018; 9 Suppl 7:2681-5

Method and Result

This study used a combination of tween 60 and span 60 with a concentration ratio 0:5 (F1), 1:1 (F2), 2:1 (F3) and 1:2 (F4) was used to form transethosome of curcumin. The evaluations performed on the transethosome include: testing the distribution of particle size, zeta potential and entrapment efficiency in the system. Evaluation continued with the determination of the diffusion rate. The transethosome system formed has a particle size of 167.9 ± 4.7 nm - 396 ± 3.7 nm with a potential zeta value (-) 49.54 ± 1.77 mV - (-) 59.05 ± 0.95 mV, polydispersion index 0.0% - 57.1% and entrapment efficiency of 83.76% - 93.75%. The cumulative amount of curcumin diffused through the millipore membrane of F1, F2, F3, and F4 for 8 hours (Table 3). The results of the cumulative amount of curcumin showed that the surfactant combination provides better penetration ability compared to a single surfactant.

Table 1: Formulation of Transethosome Curcumin

No.	Material	Formula (%)			
		F1	F2	F3	F4
1	Curcumin	1	1	1	1
2	Lechitin	10	10	10	10
3	Tween 60	-	2.5	3.33	1.67
4	Span 60	5	2.5	1.67	3.33
5	Ethanol	30	30	30	30
6	Aquades ad	100	100	100	100

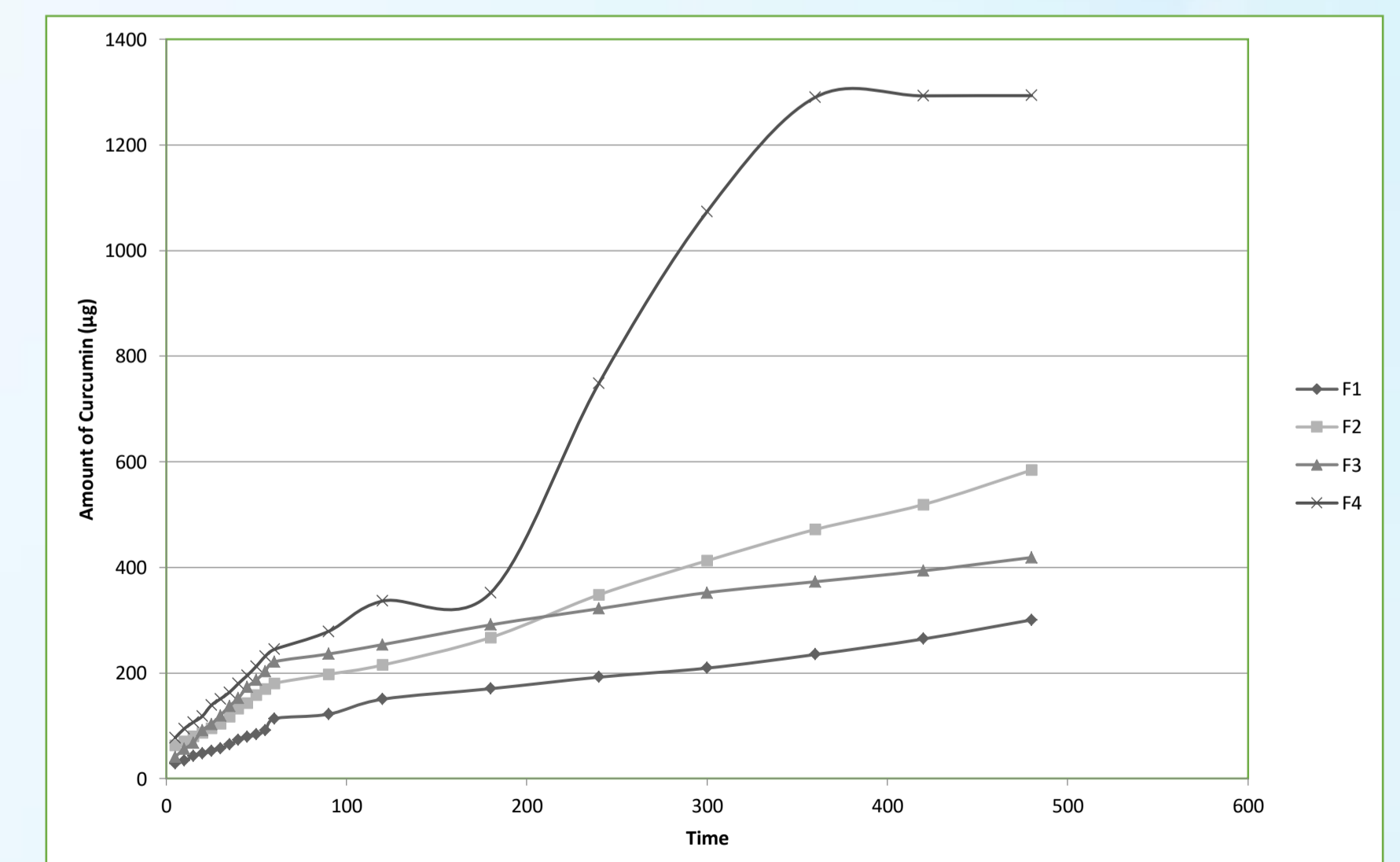


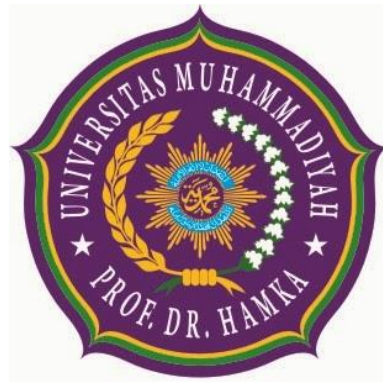
Fig 1: Profile of diffused cumulative amount of curcumin

LAPORAN KEGIATAN
THE 4th INTERNATIONAL SEMINAR ON
PHARMACEUTICAL SCIENCE AND TECHNOLOGY

Online Seminar
October 27 & 28, 2020

PESERTA:

- 1. Anisa Amalia, M.Farm**
- 2. apt. Nining, M.Si.**



PROGRAM STUDI FARMASI
FAKULTAS FARMASI DAN SAINS
UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA
JAKARTA

A. Surat tugas



UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA FAKULTAS FARMASI DAN SAINS

Islamic Center, Jl. Delima II/IV Klender, Jakarta Timur 13460 Telp. (021) 8611070, Fax. (021) 86603233
www.uhamka.ac.id, www.ffs.uhamka.ac.id, Email: ffs@uhamka.ac.id

SURAT TUGAS

NOMOR: 526 /F.03.01/2020

Pimpinan Fakultas Farmasi dan Sains, Universitas Muhammadiyah Prof. DR. Hamka dengan ini memberi tugas kepada:

- Nama : 1. apt. Nining, M.Si.
2. Anisa Amalia, M.Farm.
- Jabatan : Dosen FFS UHAMKA
- Alamat : Islamic Center Jl. Delima Raya II/ IV, Perumnas Klender – Jakarta Timur
- Tugas : Mengikuti Seminar Internasional "The 4th International Seminar On Pharmaceutical Science And Technology" Secara Daring
- Waktu : Selasa - Rabu, 27 - 28 Oktober 2020
- Penyelenggara : Farmasi UNPAD
- Lain-lain : Setelah melaksanakan tugas agar memberikan laporan kepada Dekan atau kepada yang memberi tugas.

Demikian surat tugas ini diberikan untuk dilaksanakan dengan sebaik-baiknya sebagai amanah dan ibadah kepada Allah Subhanahu Wata`ala

Jakarta, 09 Oktober 2020

Wakil Dekan II,

Dra/ Sri Nevi Gantini, M.Si.

Telah melaksanakan tugas:

1.

4th ISPST
International Seminar On
Pharmaceutical Science And
Technology

Nyi Mekar Saptarini
Chairwoman of 4th ISPST

B. Brosur informasi kegiatan

THE 4th INTERNATIONAL SEMINAR ON PHARMACEUTICAL SCIENCE AND TECHNOLOGY

» About

The 4th ISPST 2020 is a biennial seminar which a continuation of the 3rd ISPST which has been held in 2018. This seminar gives the latest finding in the area related to pharmaceutical science and technology, poster discussion about current research trends, and establish new collaborations. ISPST is a forum for students, researchers, educators, observers, and practitioners from university, research institutions, industry, and general public to exchange ideas and latest information in the field of pharmaceutical science, technology, and engineering.

» Theme

"Future Prospective In The Transformation of Pharmaceutical Research Into Applicable Industry"

Due to Covid-19 Pandemic, the conference will be conducted online (Webinar). Keynote and invited speakers will do live presentation.

Tuesday - Wednesday, October 27th - 28th, 2020

» Keynote speakers

- ✓ Prof. Tomoya Uehara (Chiba University, Japan)
Expertise on Radiopharmaceuticals.
- ✓ Dr. Hien Duong (The University of Sydney, Australia)
Expertise on Nanomaterial for Drug Delivery.

» Invited speakers

- ✓ Assoc. Prof. Pornchai Rojsittthisak, Ph.D. (Chulalongkorn University, Thailand) *
Expertise on Design, Chemical Kinetics and Pharmacokinetics.
- ✓ Mary Jho-Anne T. Corpuz, Ph.D. (Santo Tomas University, Phillipines)
Expertise on Cancer Chemopreventive Potentials of Natural Products in-vitro and in-vivo.
- ✓ Prof. Dr. Yahdiana Harahap, M.S., Apt. (University of Indonesia & President of Asian Federation for Pharmaceutical Science, Indonesia)
Expertise on Bioanalysis and DNA-Adduct Analysis.
- ✓ Dr. Nyi Mekar Saptarini M.Si., Apt. (Faculty of Pharmacy, Universitas Padjadjaran)
Expertise on Enzyme Isolation from Natural Resources
- ✓ Dr. Sriwidodo M.Si., Apt. (Faculty of Pharmacy, Universitas Padjadjaran)
Expertise on Protein Therapeutic hEGF Nanoparticle Drugs Delivery

* Under Confirmation

» Important dates

- October 27th - 28th, 2020 Seminar
- October 12th, 2020 Registration & payment closed
- July 10th, 2020 Abstract notification
- July 5th, 2020 Full paper submission deadline
- July 5th, 2020 Abstract deadline
- March 1st, 2020 Registration starts, Abstract submission

» Seminar fees

Category	Conference Fees
Student	IDR 250,000
Academic/professional	IDR 500,000
International	\$ 50

» Please transfer to

Bank BNI Perguruan Tinggi Bandung
 Account No. : 0440246374
 Account Name : Rina Fajri Nuwarda
 Swift Code (for international transfer) : BNINIDJAUPB

» Online registration

<http://farmasi.unpad.ac.id/ispst2020>

» Collaboration journals

- ✓ Journal of Advanced Pharmacy Education and Research (Q3 scopus indexed)
- ✓ Journal of Pharmacy and Bioallied Sciences (Q3 scopus indexed)
- ✓ Indian Journal of Pharmaceutical Sciences (Q3 Scopus Indexed)
- ✓ Drug Invention Today (Q4 scopus indexed)
- ✓ Indonesian Journal of Pharmaceutical Science and Technology (S2 sinta indexed)

» In Collaboration with :

C. Sertifikat poster presenter

14
SKP IAI

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[Signature]
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International Seminar on
Pharmaceutical Science
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Dr. apt. Nyi Mekar Saptarini, M.Si
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SKP IAI ACCORDING TO DECREE NO: SKP065/PP/IAI/VI/2020

14
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Pharmaceutical Science
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Dr. apt. Nyi Mekar Saptarini, M.Si
Chairman of 4th ISPST 2020



SKP IAI ACCORDING TO DECREE NO: SKP065/PP/IAI/VI/2020

D. Poster yang dipresentasikan



PHYSICAL PROPERTIES AND RATE OF DIFFUSION TRANSETHOSOME CURCUMIN USING TWEEN 60 AND SPAN 60 AS SURFACTANT

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No.	Material	Formula (%)			
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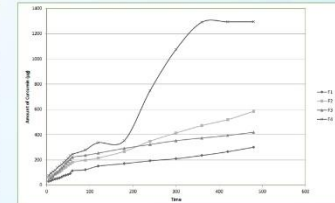



Fig 1: Profile of diffused cumulative amount of curcumin

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PREPARATION AND CHARACTERIZATION OF ENTERIC-COATED DELAYED-RELEASE MICROSPHERE OF PHYTOSOME LOADING ALLICIN-RICH EXTRACT

N. Nining*, Yudi Srifiana, Elly Malinda Fadlianty
Faculty of Pharmacy and Science, Universitas Muhammadiyah Prof. DR. HAMKA

Background and Objectives

Alliin, a natural organosulfur compound, is the main garlic ingredient, which has extensive pharmacological activities. Its unstable under acidic conditions due to alliinase's inactivation causes the need for preparations that delayed-release in the stomach to maximize alliin absorption. One of the new vesicular complexes is a phytosome made from the encapsulation of phospholipid and extracts into phytocostituents to improve bioavailability and enhance therapeutic benefits in oral and topical use. Phytosome technology is considered a suitable method for obtaining a better pharmacodynamic and pharmacokinetic profile for active constituents in extracts because the structure of the vesicles protects the herbs from being damaged in an acidic environment. The concentration of enteric coating polymers influences microparticles characteristics and their drug release in the gastrointestinal tract. Based on this, research carried out on increased Eudragit L30D-55 as an enteric-coated polymer on characterizing the microsphere alliin-rich extract phytosome (ARE-Ps) to protect it from gastric acid.

Conclusion

Based on these results, the polymer's increase in three microsphere formulation can affect characteristics and retain drug release under acidic conditions. The three formulations follow Korsmeyer-Peppas kinetics release with k values 12.71 ± 0.18 ; 17.93 ± 1.56 ; and 12.96 ± 1.27 , for F1, F2, and F3, respectively.

Method and Result

Optimized alliin-rich extract phytosome (ARE-Ps) has a size of 251.6 nm, polydispersity index 0.466, zeta potential 34.11, entrapment efficiency of 62.62 %, and specific gravity of 1,005 g/mL. Microsphere was made in three formulas with different molar ratios of ARE-Ps and Eudragit L30D-55 (1:1; 1:1.5 and 1:2) by spray dry. The surface topography of the three formulas shows an almost spherical shape with concave surfaces. The particle size of the microsphere ranges from 215 ± 6.27 nm to 548.8 ± 10.15 nm. Entrapment efficiency increases with an increasing number of polymers with a maximum value of 65.44 % at F3. The results dissolution test *in vitro* showed no drug release in acidic medium, and drug release occurred at a 7.4 pH medium. Drug release of three microsphere formulation followed the Korsmeyer-Peppas model with a k value of 12.7088 ± 0.1769 ; 17.9322 ± 1.5621 ; and 12.958 ± 1.2677 , respectively.

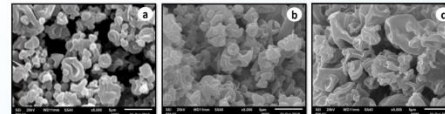


Fig. 1: Morphological structure of 5000x magnification microcapsules (a) ratio 1:1 (b) ratio 1:1.5 (c) ratio 1:2

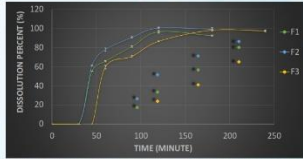


Fig. 2: Percent dissolution of the microsphere in a buffer medium pH of 7.4

Reference

- Guler E, Demir B, Guler B, Demirkol DO, Timur S. Biofunctionalized nanomaterials for targeting cancer cells. *Nanostructures for Cancer Therapy*. Elsevier Inc.; 2017. 51–86 p.
- Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Pol Pharm - Drug Res*. 2010;67(3):217–23.

<http://farmasi.unpad.ac.id/ispst2020>

E. Bukti pembayaran kegiatan

WEBINAR

**THE 4th INTERNATIONAL SEMINAR ON
PHARMACEUTICAL SCIENCE
AND TECHNOLOGY**

RECEIPT OF PAYMENT
(No: 0048/08/4thISPST-2020)

Received from : **ANISA AMALIA**
Institution : Universitas Muhammadiyah Prof. Dr. Hamka
For Payment of : Registration of 4th ISPST
As : Academic/Professional
Amount : IDR 500,000.00

Jatinangor, 1 August 2020
Treasurer,

Rina Fajri Nuwarda, M.Sc.


International Seminar on
Pharmaceutical Science
and Technology


<http://farmasi.unpad.ac.id/ispst2020>

WEBINAR

**THE 4th INTERNATIONAL SEMINAR ON
PHARMACEUTICAL SCIENCE
AND TECHNOLOGY**

RECEIPT OF PAYMENT
(No: 0047/08/4thISPST-2020)

Received from : **NINING**
Institution : Universitas Muhammadiyah Prof. Dr. Hamka
For Payment of : Registration of 4th ISPST
As : Academic/Professional
Amount : IDR 500,000.00

Jatinangor, 1 August 2020
Treasurer,

Rina Fajri Nuwarda, M.Sc.


International Seminar on
Pharmaceutical Science
and Technology


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F. Submisi *full paper*

Email address	anisa.amalia@uhamka.ac.id
Your Name	Anisa Amalia
Preference of Participation	Poster Presenter
Article Tittle	Physical Properties and Rate of Diffusion Transethosome Curcumin using Combination of Tween 60 and Span 60 as Surfactant
To be published in the scientific journal	Yes
Preferred journal	IJPS
Please upload your full paper file in doc/docx format	File 1

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Your Name	apt. Nining, M.Si.
Preference of Participation	Poster Presenter
Article Tittle	Preparation and Characterization of Enteric-Coated Delayed-Release Microsphere of Phytosome Loading Allicin-Rich Extract
To be published in the scientific journal	Yes
Preferred journal	IJPS
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**Physical Properties and Rate of Diffusion Transethosome
Curcumin using a Combination of Tween 60 and Span 60 as
Surfactant**

ANISA AMALIA*, YUDI SRIFIANA, AMALIA ANWAR

Faculty Pharmacy and Sains, Universitas Muhammadiyah Prof. DR. HAMKA,

East Jakarta 13460, Indonesia

**Running title: Tween 60 and Span 60 as Surfactant in Transethosome
Curcumin**

Jl. Delima II/IV, Malaka Sari, Jakarta Timur 13460
E-Mail: anisa.amalia@uhamka.ac.id

Curcumin penetration can be increased by formulating it into the transethosome system. Surfactant is one of the transethosome component that affect the physical properties and penetration of vesicles. In this study, a combination of two surfactants was used to see the effect of using a combination of two surfactant on physical properties and penetration of curcumin. This study used a combination of tween 60 and span 60 with a concentration ratio 0:5 (F1), 1:1 (F2), 2:1 (F3) and 1:2 (F4). An evaluation included testing the distribution of particle size, zeta potential and entrapment efficiency in the system. Evaluation continued with the determination of the diffusion rate in vitro. The transethosome system formed has a particle size of 167.9 ± 4.7 nm - 396 ± 3.7 nm with a potential zeta value (-) 49.54 ± 1.77 mV - (-) 59.05 ± 0.95 mV, polydispersion index 0.0% - 57.1% and entrapment efficiency of 83.76% - 93.75%. Diffusion rate testing results show that the use of a surfactant combination can increase the diffusion rate of curcumin, where there is a significant differential between each formula ($p < 0.05$).

Keywords: Curcumin, transethosome, surfactant, physical properties, diffusion rate.

Curcumin is a yellow compound in turmeric (*Curcuma longa* Linn), and has activities such as antimicrobial, anti-inflammatory, antioxidant, and anticancer. Curcumin has a low solubility in water, is metabolized first by causing its bioavailability to decrease^[1]. This problem can be overcome by formulating curcumin into the form of a transethosome delivery system.

Transethosome is a lipid vesicle originating from transfersome and ethosome which is characterized by the presence of high concentrations of surfactants and ethanol. Surfactant is a component that affects transethosome penetration. Surfactants have role in elasticity and penetration in the transethosome system^[2-3].

Previous research showed that the transethosome system can increase voriconazole penetration and has a higher penetration rate than the liposome and ethosome system^[4]. Shen *et al.* have been doing research on technology vesicles transethosome using a single surfactant tween 60 and span 60^[5]. The study showed transethosomes can increase the rate of penetration, but produce vesicles are unstable. Effect of increasing the penetration rate of drug

using the transethosome system is also seen in the results of research conducted by Grag *et al.*, in that study, the surfactant used was span 80 with a concentration of 77.64 % and produced a stable vesicle system^[6]. However, the use of surfactants with high concentrations can cause an irritating effect on the skin, so it needs a combination of surfactants to reduce irritation to the skin and was expected to increase the rate of penetration of vesicles. Surfactants that has the ability to enter the phospholipid bilayer is tween 60 and span 60. Tween 60 and span 60 are nonionic surfactants that can maintain a balance between hydrophilic and lipophilic groups, non-toxic, and when combined have low potential to cause hypersensitivity reactions^[7]. Therefore, it is necessary to research the effect of using tween 60 and span 60 as surfactants on the physical properties and diffusion rate of curcumin transethosome.

MATERIALS AND METHODS

Curcumin (Gift from Insular Multi Natural, Indonesia), lecithin (Purchased from Sunshine, Indonesia), Tween 60 (Purchased from Clorogen, Indonesia), Span 60 (Purchased from Clorogen, Indonesia), millipore membrane (Purchased from Asian, Indonesia), ethanol, sodium hydroxide, potassium dihydrogen phosphate (Purchased from Merck, Indonesia).

Formulation and production of curcumin transethosome

This research is an experimental study that begins with the stage of making transethosome curcumin with the various concentrations of surfactant (tween 60 and span 60), and continues to observe the physical properties of the transethosome system. Formula of transethosome curcumin can be seen in Table 1.

Transethosome curcumin was made using the cold method. Lecithin was dispersed with ethanol at 30° C (lipid phase). Surfactants and curcumin are added into the lipid phase and homogenized with a magnetic stirrer at 750 rpm for 5 minutes to form a colloidal system. Then add water to the colloidal system little by little in a constant flow, stirring for 5 minutes to form a suspension of transethosome curcumin vesicle. The size of the vesicles was reduced by sonication for 15 minutes^[3,8].

Evaluation of Transethosome Containing Curcumin

Determination of Particle Size, Polidispersity Index and Zeta Potensial

Determination of particle size distribution, polydispersity index value, and potential zeta value were measured using a particle size analyzer (Delsa Nano Beckman Coulter, USA)^[3,9].

Determination of entrapment efficiency of curcumin

Determination of the efficiency of curcumin entrapment was done by centrifugation method at a speed of 4000 rpm for 2 hours. Sediment was dissolved with ethanol, taken 10.0 ml then put into a 100.0 ml measuring flask. Ethanol is added to the measuring flask boundary line. The solution was measured with a UV/Vis spectrophotometer (Shimadzu, Japan) at a wavelength of 425.5 nm. The concentration of absorbed curcumin obtained was converted to a unit of weight and the percent efficiency calculation is calculated using the formula^[10]:

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of entrapment curcumin}}{\text{Total curcumin}} \times 100\%$$

Transethosome Diffusion Test

The diffusion test was carried out using a milipore membrane that is placed between two parts of the diffusion cell. The receptor compartment was

conditioned at 37°C by flowing water of the same temperature in a water bath. The receptor compartment was filled with phosphate buffer pH 7.4 and ethanol as much as 5 % to 150 ml then as much as 1 ml of the test sample is applied to the membrane surface. Samples were taken as much as 5 ml at interval times for 8 hours. Every sampling was replaced by phosphate buffer pH 7.4 with addition of ethanol 5 % as much as 5 ml. Absorption was measured at a wavelength of 421.0 nm. The absorbance value obtained was entered into the linear regression equation to find the diffused percent. Then a curve is made between percent diffused with time^[11].

Analysis of Diffusion Rate Kinetics Model

Equation line regression linear for each model of the kinetics of the rate of diffusion is made by: Zero order kinetics, first order kinetics, Higuchi model and Korsmayer-peppas model^[12-13].

RESULTS AND DISCUSSION

The particle size distribution and potential zeta value are the most important physical properties of the transetosome. Transetosome is a lipid-based delivery system that is included in the nanoparticle delivery system. The nanoparticle delivery system is a delivery system which has a particle size of 1 - 1000 nm^[14]. The results obtained from this test can be seen in Table 2. The Table 2 showed the average transetosome curcumin particle size of each formula is still included in the nanometer criteria (1-1000 nm). This result proves the formula and conditions for making transetosome curcumin can produce vesicles with nanometer size with polydispersion percent values 0.0-57.1. The polydispersion index value is a parameter that shows the particle size distribution of the

dispersion system. The smaller the polydispersion index value, the resulting dispersion system the more homogeneous the particle size of the system^[15].

Zeta potential is an important parameter for characterizing nano particles, aimed at predicting the stability of colloid solutions. Nanoparticles with potential zeta values above (+/-) 30 mV have been shown to be stable because surface charges prevent particle aggregation^[15,16]. The potential values obtained from each of these formulas are -52.54 ± 0.93 mV; -49.54 ± 1.77 mV; -50.10 ± 0.52 mV; -59.05 ± 0.95 mV. This results showed, each of the transethosome formula is stable because the values of zeta potential obtained above the value of -30 mV, so that the possibility of smaller particle aggregation.

The entrapment efficiency value of curcumin can be seen in Fig 1. The results showed the efficiency of curcumin entrapment was still above 80% so it can be concluded that transethosome can entrap curcumin quite well. The entrapment efficiency value also shows the use of a combination of surfactants can increase the entrapment efficiency, where the entrapment efficiency of F2 with a ratio of Tween and Span 1:1 (F2) was the system with the highest absorption efficiency. This results are in accordance with the results of research conducted by Lv *et al.*, where the results obtained indicate that the combination of tween - span nonionic surfactants will increase the efficiency of entrapment^[17].

The cumulative amount of curcumin diffused through the millipore membrane of F1, F2, F3, and F4 for 8 hours (Table 3). The results of the cumulative amount of curcumin showed that the surfactant combination provides better penetration ability compared to a single surfactant. Based on these results, the highest amount of diffused curcumin was at F4 with a ratio of tween 60 and span 60 (2:1). This

can be influenced by particle size. The particle size of F4 is 167.9 ± 4.7 nm. The smaller the particle size, the greater the surface area, causing rapid drug release^[18]. Based on the cumulative amount of diffused curcumin, it could be calculated the diffused curcumin cumulative persentation of each formula was 23.02 ± 2.12 %, 33.11 ± 2.4 %, 24.85 ± 0.5 %, 93.77 ± 0.29 %.

The results of the transethosome diffusion test curcumin can then be analyzed to determine the mechanism of drug release from the transethosome system (Table 3 dan Fig 2). The mechanism of drug release can be known by calculating the linearity value of several drug release equations, such as zero-order kinetics (Fig 3), first-order kinetics (Fig 4), Higuchi (Fig 5) and Korsmeyer-Peppas (Fig 6)^[13, 19, 20]. The results of the analysis of drug release mechanisms can be seen in Table 4. Based on the highest coefficient (R) in the table shows that the diffusion rate kinetics in F1 and F3 follow the Higuchi kinetics model. Drug release that follows Higuchi kinetics model illustrates drug release that is affected by time^[19, 20]. The release kinetics of F2 follow zero order kinetics with a value of $R = 0.9942$. The kinetics of zero-order release describe the release of drugs slowly and always constant over time. Increased drug concentration is directly proportional to time. The kinetics of F4 release followed the Kormeyer-Peppas kinetics that was $R = 0.9872$ with an n value of 0.1484. The value of n obtained is lower than that set by Ritger and Peppas which is <0.45 . The low value of n is likely due to the large size and erosion distribution^[19]. The value of n obtained can be determined that the process occurs through the mechanism of Fickian diffusion^[13, 20]. In Fickian diffusion, the rate of release is independent of the drug concentration^[18].

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TABLE 1: FORMULATION OF TRANSETHOSOME CURCUMIN

No.	Material	Formula (%)			
		F1	F2	F3	F4
1	Curcumin	1	1	1	1
2	Lechitin	10	10	10	10
3	Tween 60	-	2.5	3.33	1.67
4	Span 60	5	2.5	1.67	3.33
5	Ethanol	30	30	30	30
6	Aquades ad	100	100	100	100

TABLE 2: TEST RESULTS OF THE PARTICLE SIZE, POLYDIPERSION INDEX AND ZETA POTENSIAL OF TRANSETHOSOME CURCUMIN

Formula	Test result		
	Particle Size (nm) ^a	Polydispersion (%) ^b	Zeta potential (mV) ^c
F1	350.4 ± 6.7	0.0	-52.54 ± 0.93
F2	263.4 ± 2.4	57.1	-49.54 ± 1.77
F3	396.0 ± 3.7	0.0	-50.10 ± 0.52
F4	167.9 ± 4.7	57.1	-59.05 ± 0.95

TABLE 3 : CUMULATIVE RELEASE OF CURCUMIN

Time (minutes)	Cumulative release of Curcumin (μg)			
	F1 ^a	F2 ^b	F3 ^c	F4 ^d
5	28.68 \pm 5.58	62.45 \pm 7.60	41.40 \pm 1.44	77.37 \pm 3.72
10	33.85 \pm 3.61	70.95 \pm 1.72	55.90 \pm 5.94	94.45 \pm 9.40
15	42.55 \pm 0.35	80.00 \pm 8.58	67.97 \pm 1.05	106.70 \pm 7.90
20	47.95 \pm 2.19	86.97 \pm 9.93	91.47 \pm 1.79	117.95 \pm 1.93
25	52.00 \pm 2.26	94.83 \pm 1.25	103.10 \pm 1.61	138.70 \pm 6.93
30	57.55 \pm 0.49	103.80 \pm 1.14	119.43 \pm 1.51	150.80 \pm 7.07
35	64.50 \pm 3.25	117.40 \pm 1.62	137.03 \pm 2.57	163.15 \pm 7.28
40	72.95 \pm 5.16	132.17 \pm 1.97	152.25 \pm 2.89	179.75 \pm 1.91
45	79.00 \pm 7.21	143.00 \pm 1.50	173.67 \pm 3.20	195.40 \pm 5.52
50	83.85 \pm 7.42	158.00 \pm 8.37	186.60 \pm 3.30	212.70 \pm 7.64
55	91.45 \pm 4.03	169.80 \pm 1.11	203.30 \pm 3.08	231.75 \pm 0.48
60	113.65 \pm 2.86	180.57 \pm 1,22	221.20 \pm 2.74	244.70 \pm 1.27
90	122.05 \pm 2.24	197.63 \pm 1.41	236.07 \pm 3.06	278.80 \pm 3.11
120	150.45 \pm 5.58	215.13 \pm 1.71	253.80 \pm 2.35	336.30 \pm 4.24
180	170.40 \pm 5.52	267.33 \pm 2.60	291.27 \pm 9.15	351.95 \pm 2.47
240	192.20 \pm 1,51	348.17 \pm 7.23	321.83 \pm 1.65	748.50 \pm 0.42
300	209.30 \pm 1.38	412.77 \pm 1.11	352.27 \pm 1.20	1037.60 \pm 7.05
360	235.10 \pm 1.66	471.87 \pm 7.66	372.93 \pm 9.88	1290.15 \pm 1.48
420	264.6 \pm 5.37	519.10 \pm 7.33	393.67 \pm 1.11	1293.05 \pm 0.21
480	300.65 \pm 2.79	584.33 \pm 4.28	418.83 \pm 8.7	1293.45 \pm 0.21

TABLE 4: CURCUMIN TRANSETHOSOME DIFFUSION RATE KINETICS

		Diffusion Rate Kinetics			
Formula	Parameter	Zero	First Order	Higuchi	Korsmeyer-
		Order	one	(\sqrt{t} , $Q_t /$	Peppas
		(t , $Q_t /$	(t , $\ln Q_t /$	Q_0)	($\ln t$, $\ln Q_t / Q_0$)
		Q_0)	Q_0)		
F1	R	0.9776	0.8837	0.9948	0.6698
	k	0.5383	0.0042	13.3708	37.9701
	n	-	-	-	0.1111
F2	R	0.9942	0.9295	0.9902	0.9802
	k	1.0682	0.0043	25.9698	88.5617
	n	-	-	-	0.1066
F3	R	0.9256	0.7887	0.9944	0.8858
	k	0.8309	0.0036	12.9101	67.6806
	n	-	-	-	0.0977
F4	R	0.9676	0.9443	0.9532	0.9872
	k	2.7019	0.0057	13.3708	66.1947
	n	-	-	-	0.1484

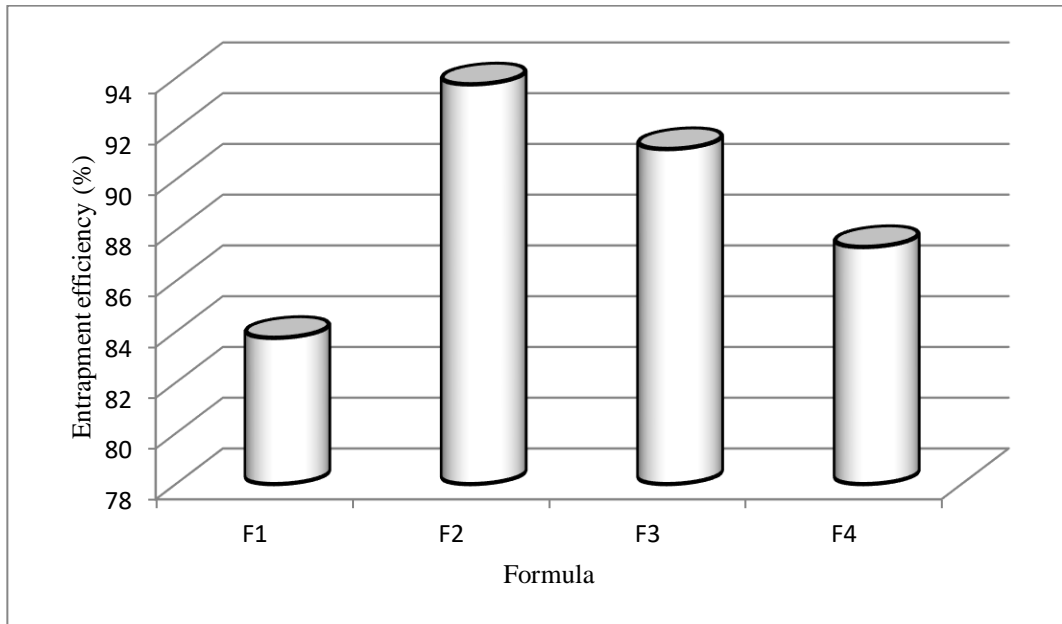


Fig. 1: Percentage Value of Entrapment Efficiency of Curcumin

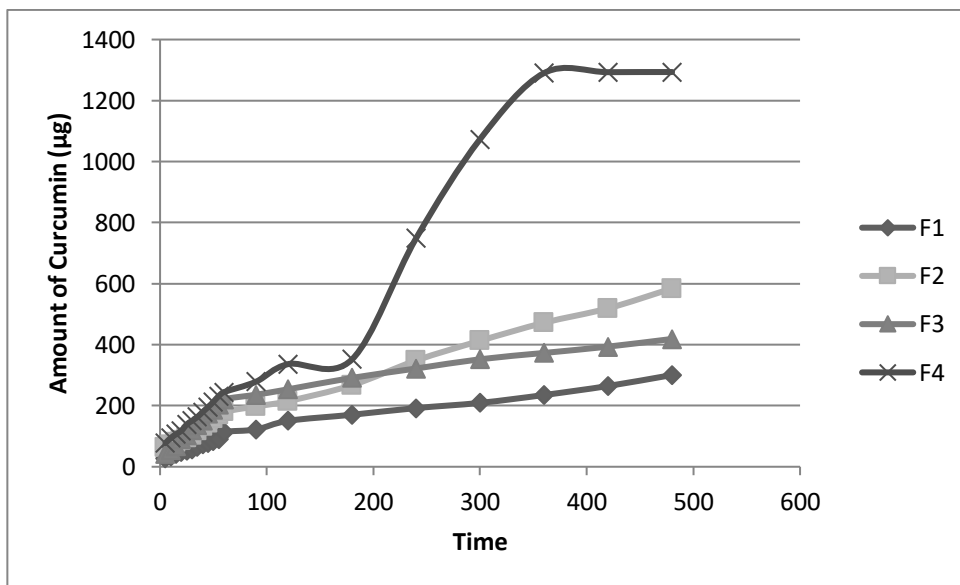


Fig 2: Profile of diffused cumulative amount of curcumin

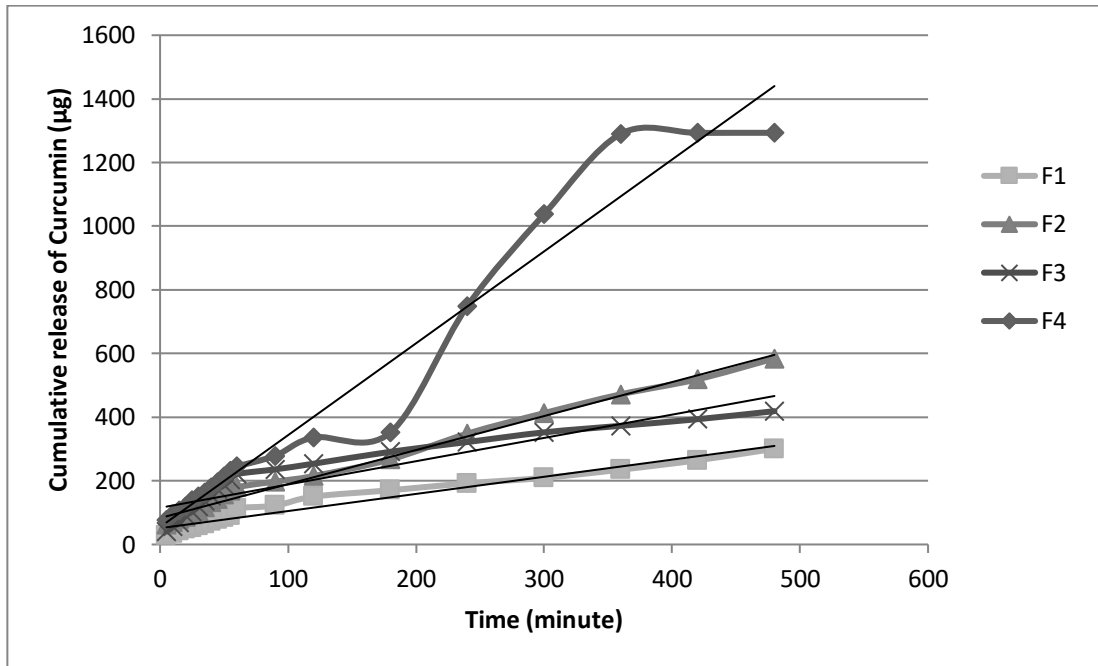


Fig 3: Drug release kinetic (Zero-order)

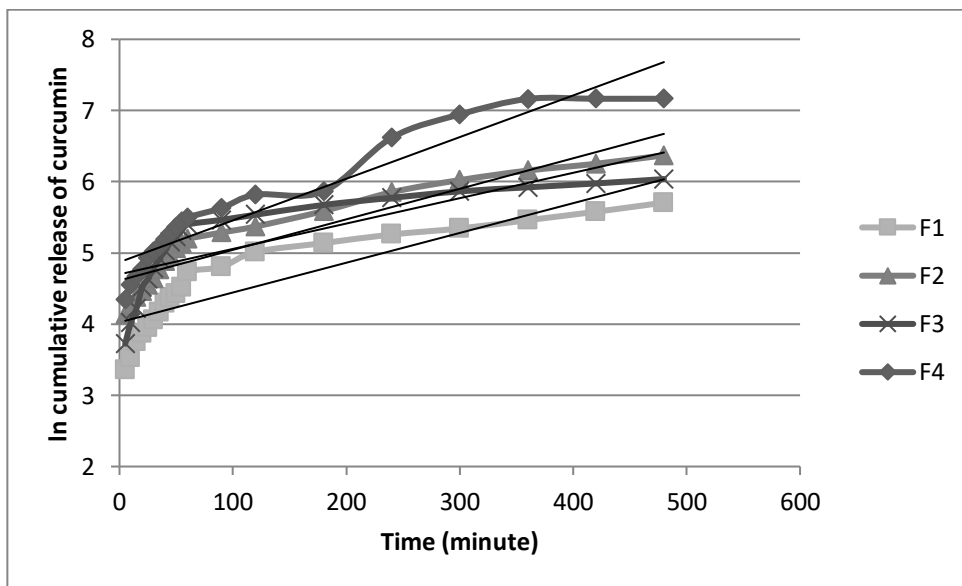


Fig 4: Drug release kinetic (First-order)

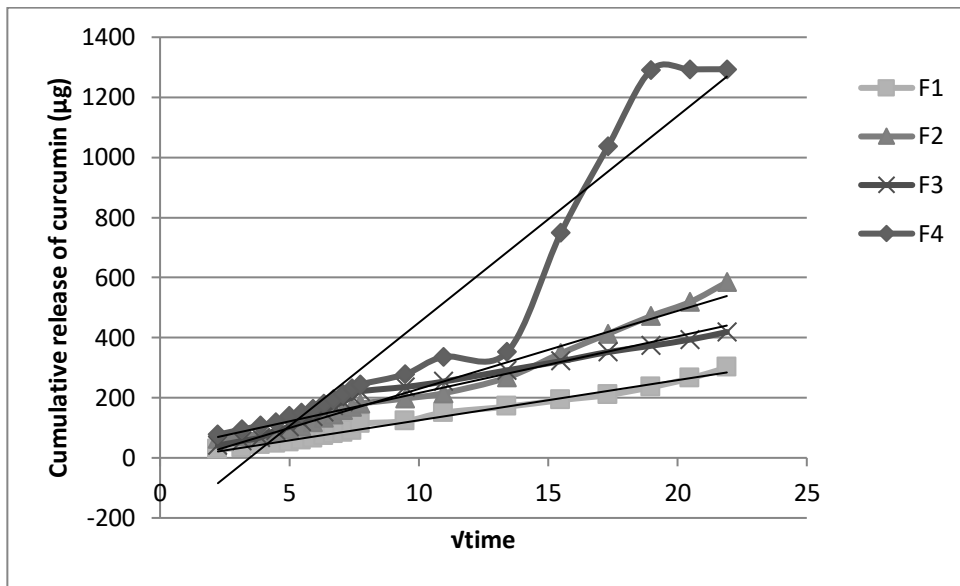


Fig 5: Drug release kinetic (Higuchi Model)

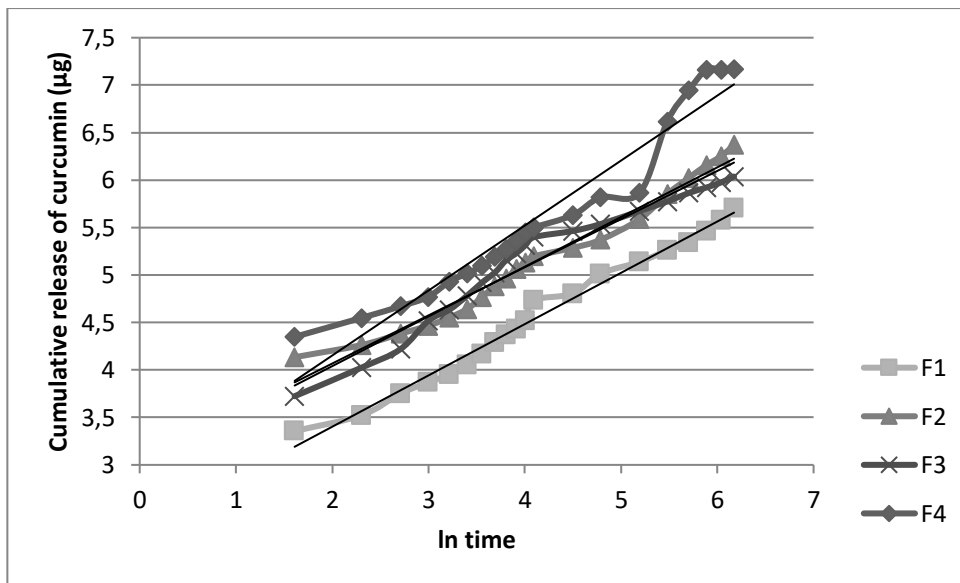


Fig 6: Drug release kinetic (Korsmeyer-Peppas Model)

Legends of Tables and Figures

TABLE 1: FORMULATION OF TRANSETHOSOME CURCUMIN

Each batch contains 3 transethosome in each formulas, values are in % w/w

TABLE 2: TEST RESULTS OF THE PARTICLE SIZE, POLYDIPERSION INDEX AND ZETA POTENSIAL OF TRANSETHOSOME CURCUMIN

a: Avg \pm SD, n=3; b: Avg \pm SD, n=3; c: Avg \pm SD, n=3

TABLE 3 : CUMULATIVE RELEASE OF CURCUMIN

a: Avg \pm SD, n=3; b: Avg \pm SD, n=3; c: Avg \pm SD, n=3; d: Avg \pm SD, n=3

TABLE 4: CURCUMIN TRANSETHOSOME DIFFUSION RATE KINETICS

Fig. 1: Percentage Value of Entrapment Efficiency of Curcumin

Avg of entrapment efficiency, n=3

Fig 2: Profile of diffused cumulative amount of curcumin

F1 (-◆-); F2 (-■-); F3 (-▲-); F4 (-×-)

Fig 3: Drug release kinetic (Zero-order)

F1 (-■-); F2 (-▲-); F3 (-×-); F4 (-◆-)

Fig 4: Drug release kinetic (First-order)

F1 (-■-); F2 (-▲-); F3 (-×-); F4 (-◆-)

Fig 5: Drug release kinetic (Higuchi Model)

F1 (-■-); F2 (-▲-); F3 (-×-); F4 (-◆-)

Fig 6: Drug release kinetic (Korsmeyer-Peppas Model)

F1 (-■-); F2 (-▲-); F3 (-×-); F4 (-◆-)

Preparation and Characterization of Enteric-Coated Delayed-Release Microsphere of Phytosome Loading Allicin-Rich Extract

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Allicin, a natural organosulfur compound, is the main garlic ingredient, which has extensive pharmacological activities. Its unstable under acidic conditions due to alliinase's inactivation causes the need for preparations that delayed-release in the stomach to maximize allicin absorption. This study aimed to prepare and characterize the enteric-coated microsphere of phytosome loading allicin-rich extract to protect it from gastric acid. Optimized allicin-rich extract phytosome (ArE-Ps) has a size of 251.6 nm, polydispersity index 0.466, zeta potential 34.11, entrapment efficiency of 62.62%, and specific gravity of 1,005g/mL. Microsphere was made in three formulas with different molar ratios of ArE-Ps and Eudragit L30D-55 (1:1; 1:1.5 and 1:2) by spray dry. The surface topography of the three formulas shows an almost spherical shape with concave surfaces. The particle size of the microsphere ranges from 215 ± 6.27 nm to 548.8 ± 10.15 nm. Entrapment efficiency increases with an increasing number of polymers with a maximum value of 65.44% at F3. The results dissolution test in vitro showed no drug release in acidic medium, and drug release occurred at a 7.4 pH medium. Drug release of three microsphere formulation followed the Korsmeyer-Peppas model with a k value of 12.7088 ± 0.1769 ; $17.9322 \pm 1,5621$; and $12,958 \pm 1,2677$; respectively. Based on these results, the polymer's increase in three microsphere formulation can affect characteristics and retain drug release under acidic conditions.

Keywords: enteric-coated, delayed-release, phytosome, allicin-rich extract, characterization

Garlic (*Allium sativum* L.) is a bulbous plant that is easy to grow at temperatures and tropical conditions globally, including Indonesia. Generally, garlic used as a flavoring agent and seasonings (1). This plant has many organosulfur compounds such as allicin, volatile compounds (2). That reported having extensive pharmacological activities (3) such as antimicrobial (4), antihypertensive, nephroprotective, cardioprotective, antioxidant (5), anti-carcinogenic, antidiabetic, and cytochrome activity (2). Allicin is known to be unstable and results from the action of the alliinase enzyme in alliin.

Formulations containing allicin are complicated because of their instability. Conventional formulations to form allicin in vivo also have a big challenge because alliinase destroyed by gastric acid (6).

One of the new vesicular complexes is a phytosome made from the encapsulation of phospholipid and extracts into phytoconstituents to improve bioavailability and enhance therapeutic benefits in oral and topical use. Phytosome technology is considered a suitable method for obtaining a better pharmacodynamic and pharmacokinetic profile for active constituents in extracts because the structure of the vesicles protects the herbs from being damaged in an acidic environment. Increased penetration into the biomembrane can be achieved with lipid-containing vesicle complexes (7). The research conducted by Almajdoub shows that the phosphatidylcholine that forms the phytosome experiences instability in gastric fluid pH simulation (8). Another study stated that the enzyme alliinase in garlic extract, which converts alliin compounds into allicin deactivates at the pH of gastric acid. That causes the reduction of allicin compounds to 99% in the product (3). Based on this, the phytosomes system was made into an enteric-coated preparation, Eudragit L30 D55. Enteric-coated preparations prevent the drug's release in the stomach and release at a more suitable place, the intestine (9,10).

Polymeric Eudragit is a series of acrylic and methacrylate polymers available in various forms of ions. Eudragit L30D-55, with a molecular weight of 320,000 g/mol (11), is an anionic copolymer containing free carboxyl groups and esters in a ratio of 1: 1. The carboxyl group ionized in an aqueous medium at a pH of 5.5 and above makes the polymer resistant to the stomach's acidic environment but dissolves at intestinal pH (12). Spray dryer performed enteric coating—this method widely used to make microparticles, such as microspheres, with polymers containing hydrophilic, lipophilic, and macromolecular drugs (13). The main advantages are that the process is only one step, easy to control and improve, and possibly free from organic solvents (13). This study

expected that the formed microparticles could be protected from the acidic environment and released in the intestinal environment.

The concentration of enteric coating polymers influences microparticles' characteristics and their drug release in the gastrointestinal tract. A study conducted by Pyar and Peh in drug release tests in vitro showed that the concentration of L30D-55 Eudragit above 7.5% was able to hold active substances in the stomach environment (14). Based on this, research carried out on increased Eudragit L30D-55 as an enteric-coated polymer on characterizing the microsphere allicin-rich extract phytosome (ArE-Ps) to protect it from gastric acid.

MATERIALS AND METHODS:

The materials used include an allicin-rich extract (Lansida), soy lecithin (Lansida), S-allyl 2-propane-1-sulfinothioate (Sigma Aldrich), Eudragit L30D-55 (Evonik). All other reagents used analytical grade.

Preparation of phytosome:

A-4.5gram of ArE and soy lecithin weighed, respectively. Then soy lecithin diluted with dichloromethane, while ArE diluted with 96% ethanol. Both solutions mixed in a round-bottom flask. The solvent evaporated using a rotary evaporator (EYELA) at 30°C with 125 rpm. Then, a thin layer formed stored in a refrigerator for up to 24 hours at 7°C and hydrated with a phosphate buffer solution pH 5.5. Then, suspension sonicated for 60 minutes.

Evaluation of phytosome:

Phytosome evaluated to characterize the vesicle produced. Evaluations include entrapment efficiency, particle morphology, particle size, index polydispersity, potential zeta, and specific gravity.

Preparation of phytosome-loaded microsphere:

Phytosome-loaded microsphere formulations can see in Table 1. The polymer amount is proportional to the weight of solid phytosome in 1:1; 1:1.5; and 1:2 for F1, F2,

and F3, respectively. Eudragit L30D-55 was mixing with triethyl citrate and aqua dest to make a polymer solution. The dispersion of polymer and phytosome suspension dried with 150°C as inlet temperature and 70°C as outlet temperatures to obtain microsphere by spray dryer (BUCHI 190).

Entrapment efficiency determination:

The entrapment efficiency determined by breaking 200mg microcapsule by dissolved in phosphate buffer pH 7.4, then centrifuged at 10,000 rpm for 10min—1mL supernatant taken to measure allicin levels, which are not absorbed in the phytosome vesicles. Furthermore, the volume is sufficient with a mixed solvent phosphate pH 6.8 and 95% ethanol (2:8), until 10mL. A-0.5 mL is taken and diluted into a 10 mL volumetric flask, and absorbance is measured using a UV-Vis spectrophotometer (1601-SHIMADZU). Measurement of absorbed-allicin in the phytosome, centrifuge precipitate diluted 0.5mL dichloromethane, and vortexed. Then, it diluted in a 10mL volumetric flask, and the absorbance is measured. The percentage of absorbed-allicin determined using the formula, $\%EE=(M_1-M_2)/M_1 \times 100$, where M_1 is the total allicin in the microcapsule, and M_2 is allicin concentration in supernatant.

Morphology of microspheres:

Morphology of microsphere evaluated using scanning electron microscopy (SEM JEOL JSM-6510LA). The microsphere is attached to the holder then inserted into a vacuum evaporator. At a specific vacuum level, the holder is incandescent so that gold vapor will coat the material attached to the holder. The holder is then inserted into the SEM device and then examined

Particle size distribution:

Particle size distribution and zeta potential were measured using particle size analyzer (Delsa Max Pro). Some 200mg of microcapsules dispersed in aqua pro injection in a ratio of 1:19. Then it is directly inserted into particle size analyzer, and the particle

size distribution curve is determined. Zeta potential and polydispersity index measurements also performed.

***In vitro* drug release studies:**

Procedure on this study refers to Anwar et al. (15). The allicin releases from microcapsule carried out in two types of mediums, hydrochloric acid pH 1.2 and phosphate buffer pH 7.4. Drug release testing carried out on a glass beaker placed on a magnetic stirrer at $37^{\circ}\pm 0.5^{\circ}\text{C}$ and 100rpm, the medium used was 100mL. Some microcapsules inserted into the cellophane membrane and dipped in the dissolution medium. The liquid sample is taken as much as 10mL at a particular minute. On the acidic medium, samplings carried out at minutes of 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 420. While on the pH 7.4 medium, samplings carried out at minutes of 5, 10, 15, 30, 45, 60, 90, 120, and 180. Every 10mL sample liquid taken, then 10mL of the medium solution was added to the dissolution flask, in triplicate. Then allicin levels determined by UV-Vis spectrophotometer.

Release kinetic models: (16,17)

Mechanism of releasing allicin from three microspheres studied from the calculation with four different kinetic models was performed based on experimental data. Model 1 given by the Ritger-Peppas and Korsmeyer Peppas equations, $f_1 = M_i/M_{\infty} = K \cdot t^n$, where f_1 is the amount of drug released, M_{∞} and M_i is the amount of drug at the equilibrium state and over time t , respectively; K is the constant of incorporation of structural modifications and geometrical characteristics of the system, and n is the exponent of release in the function of time t . For the spherical delivery system, when $n=0.43$, the drug release mechanism is the Fickian diffusion. When $n=0.85$, the drug release mechanism is ultimately Case II transport. When $0.43 > n > 0.85$, anomalous transport observed.

Model 2 is the Higuchi model. Although this model is frequently used to describe the drug transport mechanism of thin-film hydrogels, this model can also be used to

analyze spherical hydrogel systems' transport mechanism —the equation, $f_i = K_H \sqrt{t}$, where K_H is the release constant of Higuchi.

Model 3 is based on the zero-order drug delivery and expressed when $n=1$ in Korsmeyer Peppas equation, $f_i = K_0 \cdot t$, where $f_i = 1 - (W_i/W_0)$ represents the fraction of active agent dissolved during the time t , and K_0 is a constant of the apparent velocity of dissolution.

Model 4 represent first-order drug delivery, and expressed by the following equation, $\log Q_1 = \log Q_0 + (k_1 \cdot t / 2.303)$, where Q_1 is the amount of active agent released on time t , Q_0 is the initial amount of drug dissolved, and K_1 is the first-order constant.

Statistical analysis:

One-way Analysis of Variance (ANOVA) analyzed dissolution rate data with a confidence level of 95% ($\alpha = 0.05$) to determine any differences in all formulas. Then, further test by Tukey HSD to determine significant differences in each formula.

RESULT AND DISCUSSION

Before use, ArE preformulated by various evaluations parameter presented in Table 2. Organoleptic observation gives a physical form, color, smell, and taste of the extract. The water content and acid-insoluble ash of ArE was a meet requirement. While total ash content showed exceed form requirement. These results are the same as those obtained by Phan et al. (18), total-ash value of 3.5-3.7% by gravimetric method, wherein mineral content associated with raw garlic condition used and planting grounds. Allicin (S-Allyl-2-propene-1-sulfinothioate) is a volatile compound; allicin in extracts identified using gas chromatography with a mass spectrometer detector. The chromatogram (fig. 1) showed a similar peak between extract sample and standard allicin at a retention time of 7.72 min. Allicin calibration curve equation is $y = -0.0497 + 0.0520x$ with a correlation coefficient (R) of 0.9981 by spectrophotometric. Based on the curve, allicin concentration in extract was 11.29%; it becomes a reference in preparing phytosome.

Optimal conditions for preparing phytosomes are using 4.5g ArE, 4.5g soy lecithin rotated with a temperature of 30°C and speed of 125 rpm. Soy lecithin is a phospholipids source with a higher proportion of around 76% phosphatidylcholine with a high content of polyunsaturated fatty acids, such as linoleic acid, about 70%, linolenic acid and oleic acid (19). Based on TEM result in fig. 2, phytosome vesicles have a round shape with varying sizes. This shape formed after the choline head of molecule binds with phytoconstituents, and the fat-soluble part of phosphatidylcholine then encloses the material bound to choline. It produces small cells such as micelles in the water environment. The vesicle size from TEM is not far from the PSA around 200nm.

Microsphere, made in three formulas with different molar ratios of ArE-Ps and Eudragit L30D-55 (1:1; 1:1.5 and 1:2) by spray dry, produce different sizes. Based on the result, the microsphere size was 548.8 nm, 215.0 nm, and 335.3 nm, respectively, for F1, F2, and F3. Particle size from spray drying can be influence by the size of the nozzle, viscosity of the polymer solution, dispersion of the active substance in the polymer solution, and surface tension (20). There are significant differences in the three formulas in the particle size values from the results of data analysis with differences in the concentration of Eudragit L30D-55 polymers. Microsphere of F1 have zeta potential below -30mV to allow agglomeration between particles to make the particle size larger. Nanoparticles with zeta potential below - 30 mV and above +30 mV have good suspension stability because the surface charge⁵ prevents aggregation between particles (15). Zeta potential values F1, F2, and F3 respectively; -28.88 ± 0.81 ; -32.15 ± 1.29 ; and -30.20 ± 0.61 . The results show that only F2 and F3 can prevent aggregation between particles because they have a negative potential zeta value below -30 mV.

The morphological structure presented in fig. 3, microsphere appears as an irregular shape with many deflections, no uniform sizes, and no porous surface. Nijdam and Langrish estimate that giving a high temperature causes water to evaporate faster and gives a non-uniform structure (21). During the high-temperature drying process, the

evaporation of water solvent molecules in the droplet occurs so rapidly that it causes "emptiness" in the droplet whose surface has formed a film layer and results in the form of rounded particles that deflate (22).

The in vitro dissolution profile of microsphere from all formulations in acidic medium (pH 1.2) and phosphate buffer medium (pH 7.4) shown in fig. 4. In acidic medium for 480 minutes, all formulation shows no allicin release, marked with the absence of absorbance in the sample taken. It proves that Eudragit from all three formulations can retain the release of allicin in acidic medium. The microsphere of F1 and F2 in alkaline pH 7.4, began to release allicin at 45 min by $55.23 \pm 0.00\%$ and $61.26 \pm 0.66\%$. At the same time, F3 microcapsules release allicin at 60 min by $59.50 \pm 1.85\%$. The F3 microsphere retains allicin release longer because it contains more polymers than F1 and F2. Release of allicin caused by salt formation between the polymer Eudragit with alkali at pH 7.4 so the polymer layer can dissolve. The microsphere of F1 and F2 dissolve entirely in the 120 min, while F3 in the 180 min. The different rates of dissolving active substances released from the microsphere influenced by the thickness of the coating wall and surface pores. Less amount of constituent polymers, thinner coating walls allow the release of active substances faster in phosphate buffer pH 7.4.

Furthermore, dissolution percent of microsphere from all formulation analyzed by fitting them to four model drug releases kinetics equations such as kinetics of zero-order, first-order, Higuchi and Korsmeyer-Peppas. From each equation, we get the drug release constant (k), correlation coefficient (r), and exponent dissolution of Peppas (n).

Based on Table 6, allicin release from the microsphere follows the Korsmeyer-Peppas kinetics. The Korsmeyer-Peppas equation explains the mechanism of drug release from preparations based on the Fickian model mechanism is the same as Higuchi. In the Korsmeyer-Peppas equation, the release mechanism depends on the value 'n' when $n = 0.43$ the Fickian diffusion governs the drug release mechanism when $0.43 < n < 0.85$ it is

anomalous (non-Fickian) transport, and when $n = 0.85$ it is case II transport (16). The n values of all formulation <0.45 indicate the release mechanism follows the Fickian model. According to Fickian, the dissolution rate of solid form determined by the dissolution rate of a thin layer of a solution formed around the solid. The drug dissolved in a saturated solution diffuses into the solvent from high concentration to an area of low drug concentration.

Data on the dissolution rate analyzed statistically to see the differences between the three formulas. The normality test resulted in sig values of 0.247; 0.429; dan 0.075 (sig >0.05); then, the homogeneity test resulted in sig values of 0.056 (sig >0.05); it concluded the data typically distributed and homogeneous. Furthermore, a one-way ANOVA test resulted in sig values of 0.002 (sig <0.05); it concluded a significant difference in the dissolution rate of microsphere between all formulations.

Based on these results, the polymer's increase in three microsphere formulation can affect characteristics and retain drug release under acidic conditions. The three formulations follow Korsmeyer-Peppas kinetics release with k values 12.71 ± 0.18 ; 17.93 ± 1.56 ; and 12.96 ± 1.27 , for F1, F2, and F3, respectively.

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TABLE 1: FORMULA OF ArE-Ps MICROSPHERE

Materials (Units)	Concentration		
	F1	F2	F3
Phytosome (%)	50	50	50
Eudragit L30 D55 (%)	15	22.5	30
Triethyl citrate (%)	0.9	1.125	1.35
Aqua dest (mL)	ad 100	ad 100	ad 100

TABLE 2: CHARACTERISTICS OF ArE

Parameters (Units)	Result	Requirement
Organoleptic		
Form		Sticky
Odor	Sticky	Strong aromatic
Color	Strong aromatic	Brown
Taste	Brown	Bitter
Water content (%)	Bitter	No more 12
Total ash content	1.21	No more 2.7
(%)	3.43	No more 0.7
Acid-insoluble ash	0.15	
(%)		

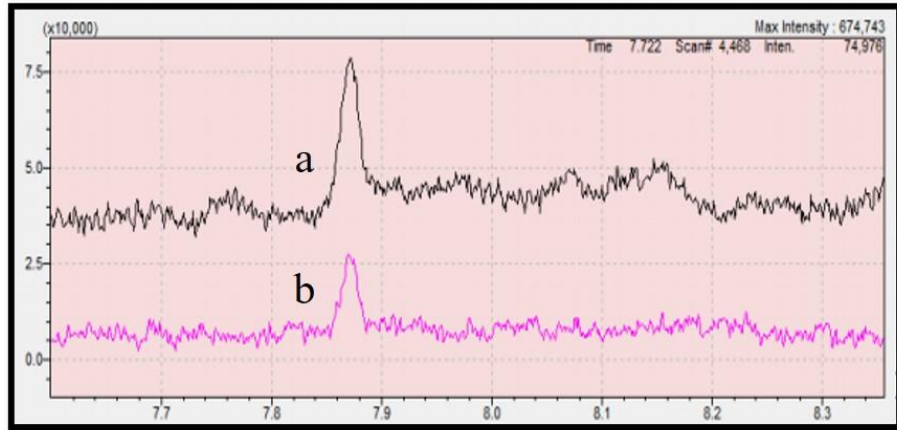


Fig. 1: Chromatogram of S-Allyl-2-propene-1-sulfinothioate from ArE (a) and standard allicin (b)

TABLE 3: CHARACTERIZATION OF ArE-Ps OPTIMUM FORMULATION

Parameters (Units)	Result
Entrapment efficiency (%)	62.62
D_{mean} (nm)	251.605
Polydispersity index (PDI)	0.466
Zeta potential	34.1088
Specific density (g/mL)	1.0051

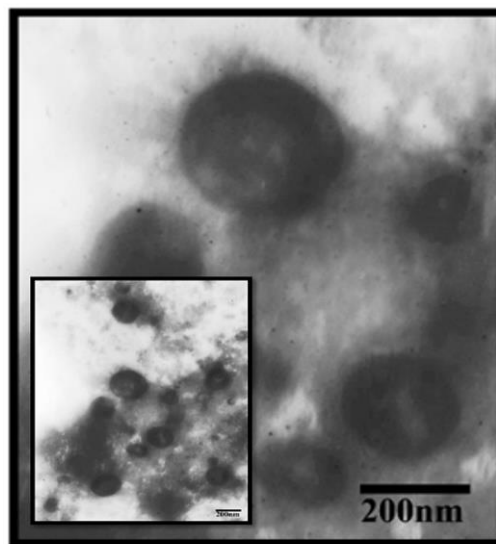


Fig. 2: Morphology of ArE-Ps using TEM

TABLE 4: MICROSPHERE EVALUATION RESULTS

Ratio (ArE- Ps:Eud)	Result			
	Entrapment Efficiency (%)	D _{mean} (nm)	Zeta potential	Polydispersity index
1:1	58,93	548,8 ±10,15	-28,88 ±0,81	0
1:1,5	62,31	215,0 ±6,27	-32,15 ±1,29	0,571
1:2	65,44	335,3 ±11,06	-30,20 ±0,61	0,571

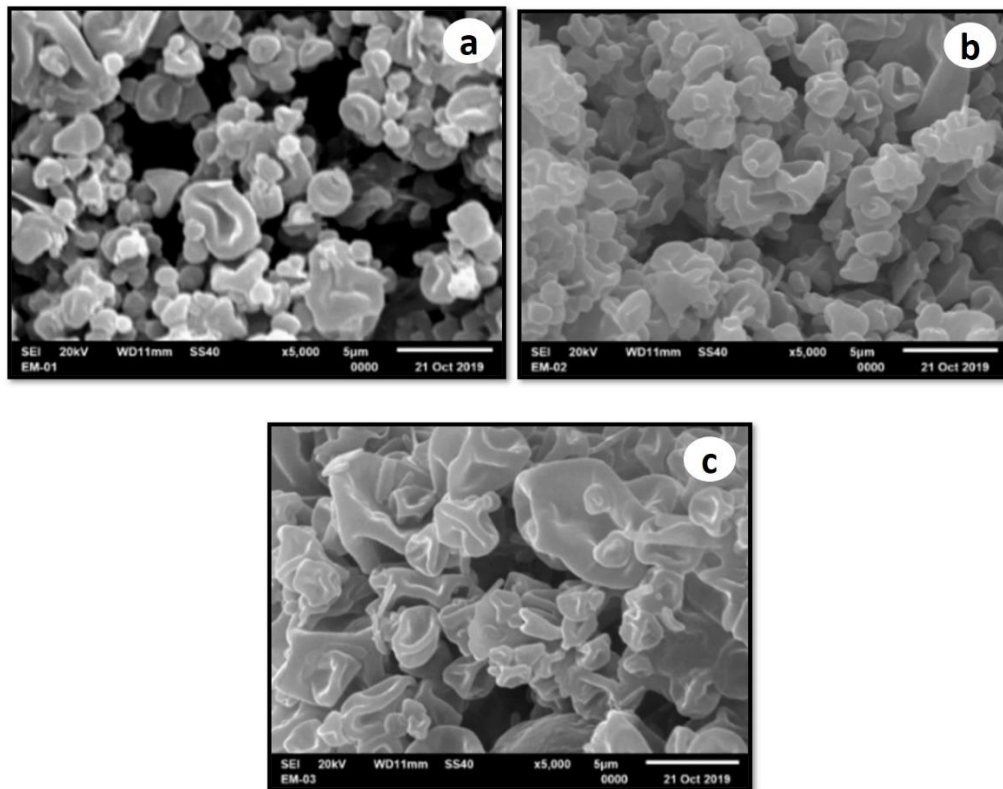


Fig. 3: Morphological structure of 5000x magnification microcapsules (a) ratio 1:1 (b) ratio 1:1.5 (c) ratio 1:2

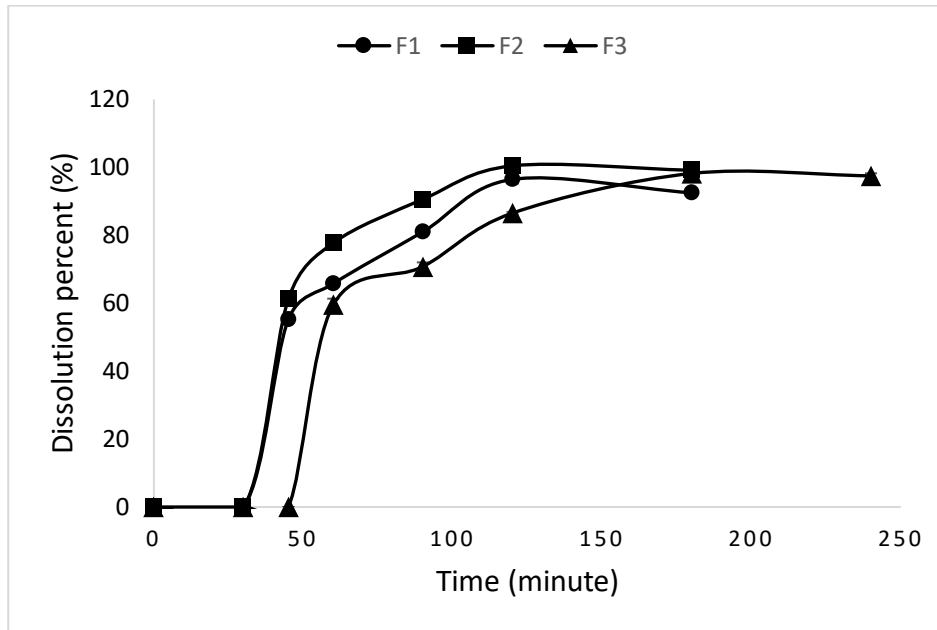
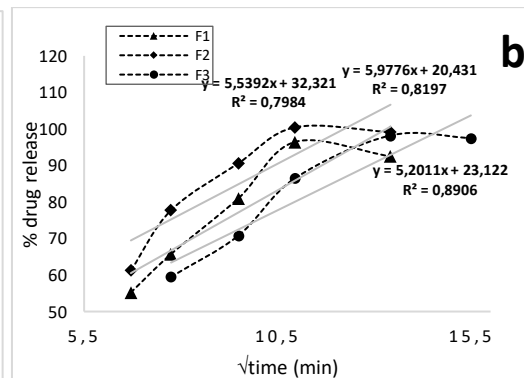
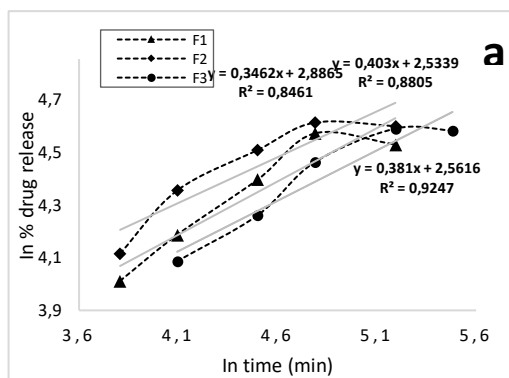


Fig. 4: Percent dissolution of the microsphere in a buffer medium pH of 7.4

TABLE 6: KINETICS OF ALLICIN RELEASE IN MICROSPHERE

Model	Formulation	Parameter		
		R ²	K	n
Korsmeyer-Peppas	F1	0.8805	12.60	0.40
	F2	0.8461	17.93	0.35
	F3	0.9247	12.96	0.38



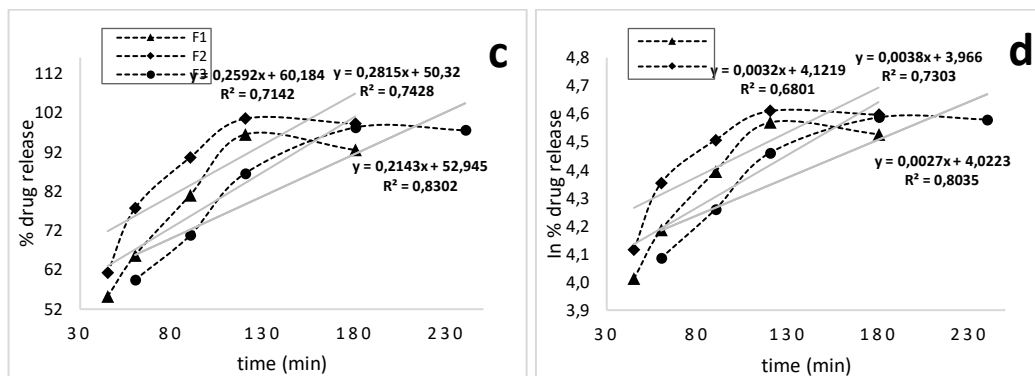


Fig. 5: Drug release kinetics plots for all formulations (a) Korsmeyer-Peppas (b) Higuchi (c) Zero-order (d) First-order

Table and figure titles and legends:

TABLE 1: ArE-Ps MICROSPHERE FORMULATION

ArE-Ps is a phytosome of allicin-rich extracts.

TABLE 2: ArE CHARACTERIZATION RESULT

ArE is an allicin-rich extract, this characterization as a pre-formulation test.

TABLE 3: OPTIMUM FORMULATION OF ArE-Ps CHARACTERIZATION RESULT

ArE-Ps is phytosome of allicin-rich extract, and optimum formulation comes from optimization by CCD-RSM.

TABLE 4: MICROSPHERE EVALUATION RESULTS

ArE-Ps: Eud is a ratio of Eudragit L30D-55 polymer amount is proportional to the weight of solid allicin-rich extract of phytosome.

TABLE 6: KINETICS OF ALLICIN RELEASE IN MICROSPHERE

Values calculated from the average value of 3 measurements.

Fig. 1: Chromatogram of S-Allyl-2-propene-1-sulfinothioate from ArE (a) and standard allicin (b)

Fig. 2: Morphology of ArE-Ps using TEM

Phytosome on the small image enlarged to a large image.

Fig. 3: Morphological structure of 5000x magnification microcapsules (a) ratio 1:1 (b) ratio 1:1.5 (c) ratio 1:2

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