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Preparation & Characterization of Phytosome of *Morinda citrifolia* Extract

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Abstract The aim of the present investigation is to prepare & evaluate the Phytosome of *Morinda citrifolia* extract. The leaves of plant were air-dried until dryness at room temperature and under shade. The dried leaves were then powdered to a fine grade by using laboratory scale mill. Further it was sequentially extracted successively with ethanol using soxhlet apparatus. The stock solution of 1000µg/ml was prepared by dissolving approximately100mg of pure *Morinda citrifolia* extract in 100ml of pH 7.4 phosphate buffers. From the stock solution, 10ml was taken and was further diluted to 100ml with the buffer solution. To prepare the phytosomes of *Morinda citrifolia* extract, drug extract and soya lecithin at molar ratio of 1:1, 1:2 ,1:3 , 1:4 , 1:5 , 1:6 and 1:7 were taken in the flask of vacuum rotary evaporator. 100 mg of *Morinda citrifolia* phytosomal complex were centrifuged at 2000rpm for 30 min using a Remi centrifuge to separate phytosomes from un entrapped drug. The skin was mounted on the receptor compartment with the stratum corneum facing towards the donor compartment. The receptor compartment was filled with 15 ml of pH 7.4 phosphate buffer maintained at 37˚C and was constantly stirred a magnetic stirrer. The λmax of *Morinda citrifolia* Extract was determined by scanning the prepared solution in the wavelength range of 200-400 nm. The maximum wavelength was found to be 279nm. The entrapment efficiency was calculated from the absorbance obtained from the supernatant solution. The formulation F1 showed highest release entrapment efficiency of 89.87% indicating the optimum amount of lipid required for the formation of phytosomes. The drug content of *Morinda citrifolia* extract in the complexes was found to be in the range of 88.43% - 78.08% indicating the presence of an acceptable amount of drug in the formulations. It was observed that the formulation showed an optimum release of 92.57% over a period of 12 hours. In conclusion, the application of phytosomal formulation as topical pharmaceutical agent and cosmetics with improved safety and efficacy results in proper utilization of herbal drugs and cost-effective pharmaceutical product.

Keywords Preparation, Characterization, Phytosome, *Morinda citrifolia* extract, Optical microscopy

Introduction

Herbal medicine is one of the oldest and most universal system of health care system. The advancement in the field of herbal drug delivery started recently with the aim to manage human diseases efficiently. World Health Organization (WHO) estimates that 80% of the world populations presently use herbal medicine for primary health care. Every nation is seeking healthcare beyond the traditional boundaries of modern medicine; turning to self-medication in the form of herbal remedies [1]. They are absolutely natural and safe form of curing illness form occurring repeatedly. They help in curing the ailment and are also known to prevent the illness from occurring

repeatedly. Herbal medicines may have long curing periods, but they eradicate the illness from it and prevent any future episodes of the same [2].

Despite criticism of herbal medicine among mainstream medical professionals , it is wise to remember that many common drugs we use today were derived from plant based sources .Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies , including opium , aspirin , digitalis and quinine . According to World HealthOrganization (WHO) approximately 25% of modern drugs used have been derived from plants [3].

Phytosomes are vesicular drug delivery systems which incorporate plant extracts or water solublephytoconstituents into phospholipids to produce lipid compatible molecular complexes. They provide better absorption and bioavailability than the conventional herbal extracts. The aim of the present study was to prepare and evaluate topical phytosomal gel with an objective to increase its bioavailability and therapeutic efficacy.

Materials and Methods

Preparation of Plant Extract [4]

The leaves of plant were air-dried until dryness at room temperature and under shade. The dried leaves were then powdered to a fine grade by using laboratory scale mill. Further it was sequentially extracted successively with ethanol using soxhlet apparatus. The solvent was removed and concentrated in a rotary evaporator and water bath. The dried extracts were stored in refrigerator for further studies.

Determination of λ_{max}

The stock solution of 1000µg/ml was prepared by dissolving approximately100mg of pure *Morinda citrifolia* extract in 100ml of pH 7.4 phosphate buffer. From the stock solution, 10ml was taken and was further diluted to 100ml with the buffer solution. The prepared solution was then scanned in a wavelength range of 200-400nm, to find the maximum absorbance. The maximum wavelength was found to be 279nm and was used for further studies.

Determination of Standard Curve

The serially diluted stock solution was obtained in the range of 2- 10µg/ml by taking 0.2, 0.4,0.6, 0.8 and 1 ml from the stock solution, into 100ml volumetric flask. The final solution is made by using phosphate buffer of pH 7.4. The serially diluted solutions were measured in a UV spectrometer at 279nm of the drug.

Formulation of Phytosomes [5]

To prepare the phytosomes of *Morinda citrifolia* extract, drug extract and soya lecithin at molarratio of 1:1, 1:2,1:3, 1:4, 1:5, 1:6 and 1:7 were taken in the flask of vacuum rotary evaporator. Dichloromethane were added in the flask. The mixture was shaken at a temperature not exceeding 40˚C for 2 hours. The resultant solution was evaporated by increasing temperature up to 60˚C and by using vacuum pump in vacuum rotary evaporator. Ethanol was added to the flask with continuous stirring. The phytosomes was precipitated and ethanol was evaporated under vacuum to remove the traces of solvent. The dried residues were gathered and placed desiccators over night, than crushed in the mortar and sieved through 80 mesh then subjected to further characterization [6].

Table 1: Formulation table of *Morinda citrifolia* phytosome

Ingredients	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**	**F_6**	**F_7**
Morinda citrifolia:Soyalecithin	1:1	1:2	1:3	1:4	1:5	1:6	1:7
Dichloromethane(ml)	20	20	20	20	20	20	20
Ethanol (ml)	5	5	5	5	5	5	5

Evaluation of Phytosomal Complex

1. Microscopic view [7]

Optical microscopy was used for characterization of the complex. The complex was suspended in buffer and a drop was placed on a slide and covered with a cover slip. Microscopic view of the complex was observed at a magnification of 45X.

2. Percentage Practical Yield [8]

Percentage practical yield was calculated to know about percent yield or efficiency of any method, thus its help in selection of appropriate method of production.

3. Entrapment efficiency [9]

100 mg of *Morinda citrifolia* phytosomal complex were centrifuged at 2000rpm for 30 min using a Remi centrifuge to separate phytosomes from un entrapped drug. Concentration of the free drug as the supernatant was determined by measuring absorbance at 279nm using UV-Visible spectrophotometer. The percentage drug entrapment was calculated by using the formula,

4. Drug content [10]

Phytosomes equivalent to 10 mg of drug was accurately weighed and taken into a 100 ml volumetric flask. The contents of the flask was dissolved in small quantity of ethanol and sonicated for 30 minute. Volume was adjusted to 100 ml with ethanol. Contents of the flask were filtered and drug content was determined spectrophotometrically using UV spectrophotometer after appropriate dilutions.

5. Solubility Determination [10]

To determine the change in solubility due to complexation, the apparent solubility of drug extract and phytosomal complex was determined by adding an excess amount of drug and phytosomes to 6 ml distilled water, 7.4 pH phosphate buffer and n-octanol in screw capped vials. The vials were then shaken at 25ºC for 24 hr in a water bath. After equilibrium had been attained, the saturated solutions obtained were centrifuged to remove the excess drug (15 min, 1000 rpm). The supernatant was filtered immediately and rapidly and diluted suitably with same solvent to prevent crystallization. The filtered and diluted solutions were then analyzed spectrophotometrically at 279 nm.

6. *In-vitro* Drug Diffusion Study Through Egg Membrane[11]

Preparation of egg membrane: From local department store egg was purchased. The egg yolk was separated carefully by means of hole on the surface of the egg. After that the egg shell was immersed in 0.1N HCl for 2 hours with constant stirring followed by the complete separation of egg membrane. The membrane was washed with phosphate buffer of pH 7.4 and further used for the experimental work.

7. *Ex-vivo* Skin Permeation Study [10] (optimized batch)

The skin was mounted on the receptor compartment with the stratum corneum facing towards the donor compartment. The receptor compartment was filled with 15 ml of pH 7.4 phosphate buffer maintained at 37˚C and was constantly stirred a magnetic stirrer. 1g of phytosome were placed on the skin in the donor compartment. At each sampling interval, samples were withdrawn for a period of 10 hours and were replaced by equal volumes of fresh receptor fluid to maintain sink condition. Withdrawn samples were analyzed spectrophotometrically at 279nm.

8. Scanning Electron Microcopy (SEM) Analysis [12]

To detect the surface morphology of the phytosome, SEM of complex was performed by Scanning Electron Microscope JSM 6390 (JEOL, Japan) at STIC, Cochin University, Ernakulam. The powder samples of phytosomes was sprinkled onto the tape. The aluminum stubs were placed in the vacuum chamber of scanning electron microscope. The sample was observed for morphological characterization using secondary electron detector attached to scanning electron microscopy.

Formulation of Gels of Phytosome Complex [13]

Preparation of gel: Gel bases were prepared by separately dispersing Carbopol 934 in distilled water with constant stirring at a moderate speed using mechanical shaker. The pH of all the formulations was adjusted to 5.5 - 6.5 using triethanolamine

Incorporation of Phytosomal complex into the gel: The solution of phytosome complex was prepared in 0.1 ml of ethanol in another beaker and was added to the Carbopol base. Different formulations were prepared using varying concentration of gelling agent. Prepared gels were stored in suitable containers at room temperature for further studies.

Table 6: Formulation of Gels of Phytosome Complex

Ingredients	F1	F2	F3	F4	F5	F6
Carbopol 934	1%	1.5%	2%	2.5%	3%	3.5%
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s
Propyl paraben	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Ethanol	1%	1%	1%	1%	1%	1%
Distilled Water	q.s	q.s	q.s	q.s	q.s	q.s

Evaluation of Gels of Phytosome Complex

Homogeneity [14]

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Measurement of pH [15]

The pH of the phytosome gels were measured with the help of digital pH meter. 0.5 g of phytosome gel was dissolved in 50 ml of distilled water and stored for two hrs. The measurement of pH of each formulation was determined.

Drug content [16]

1 g of the prepared gel was mixed with 100ml of suitable solvent. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and absorbance was measured at 279 nm.

Rheological study [16]

The measurements of viscosity of prepared gels were carried out with Brookfield Viscometer (spindle type S–96). The readings of each formulation were taken.

Spreadability [16]

On a glass plate of 10×5cm, 350mg emulgel was taken and another plate of same sized was dropped from a distance of 5cm. After 1 minute the diameter of the circle spread was measured.

Extrudability [16]

In the present study, extrudability was determined by measuring the weight (in grams) requiredto extrude at least 0.5cm gel from lacquered aluminum collapsible tube in 10 sec.

***In-vitro* drug release study [17]**

The *in-vitro* rug release studies were carried out using a modified Franz diffusion (FD) cell. The formulation was applied on egg membrane which was placed between donor and receptor compartment of the FD cell. Phosphate buffer pH 5.5 was used as diffusion media. The temperature of the cell was maintained at 37˚C. The whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. One ml of aliquots were withdrawn from the diffusion medium at specific time interval for 12 hours and same quantity of fresh, pre-warmed diffusion medium was replaced for the amount withdrawn. The samples withdrawn were analyzed spectrophotometrically at 279 nm and the cumulative % drug release was calculated.

Results and Discussion

Calibration Curve of *Morinda citrifolia* Extract

The λ_{max} of *Morinda citrifolia* Extract was determined by scanning the prepared solution in the wavelength range of 200-400 nm. The maximum wavelength was found to be 279nm. The calibration curve of *Morinda citrifolia* extract was constructed by dissolving the drug in pH 7.4 phosphate buffer. The linearity of the curve was found in the concentration range of 2-10µg/ml.A regression coefficient (R^2) value of 0.9989 was obtained.

Table 7: Calibration curve data of *Morinda citrifolia* extract

Concentration (µg/ml)	Absorbance
2	0.17
4	0.31
6	0.49
8	0.65
10	0.79

Evaluation of Phytosomal Complex

Optical Microscopy

Optical microscopy was performed by viewing the formulations under microscope. It was observed that the preparations showed vesicle formation. The vesicles formed were found to beof uniform size and shape.

Percentage Practical Yield

Table 8: Results of Percentage Practical Yield

Formulation	Percentage Practical Yield
F1	91.33
F2	88.52
F3	86.85
F4	86.06
F5	85.47
F6	83.88
F7	81.77

% Practical Yield of different formulations was shown in table. F1 have higher % Practical yield of 91.34% than other formulations.

Entrapment Efficiency

Table 9: Results of Entrapment Efficiency

Formulation	Percentage Entrapment Efficiency
F1	89.81
F2	86.94
F3	84.72
F4	79.66
F5	75.11
F6	71.57
F7	67.42

The entrapment efficiency was calculated from the absorbance obtained from the supernatant solution. The formulation F1 showed highest release entrapment efficiency of 89.87% indicating the optimum amount of lipid required for the formation of phytosomes. With further increase in the lipid concentration, the entrapment efficiency decreased indicating that the lipid concentration did not help in entrapping the drug into the matrix.

Drug Content

Table 10: Results of Drug Content

Formulation	Drug Content (%W/W)
F1	88.11
F2	86.56
F3	86.17
F4	84.14
F5	83.42
F6	80.74
F7	78.77

The drug content of *Morinda citrifolia* extract in the complexes was found to be in the range of 88.43% - 78.08% indicating the presence of an acceptable amount of drug in the formulations. The percentage of drug loading decreased with an increase in the concentration of lipid. The formulation F_1 showed the maximum drug content of 88.43%.

Solubility Determination

The solubility of the *Morinda citrifolia* phytosomes was found to be much higher than the pure drug extract. The increase in solubility of drug extract in the complex can be explained by the solubilization theory resulted from the formation of micelle in the medium and also by the amorphous nature of the complex. These amphiphilic surfactants (phospholipids) may increase the solubility of the drug extract by their wetting and dispersion properties. The formulation F_1 exhibited the highest degree of solubility.

Table 11: Solubility profile in different media

Formulation	Solubility inWater (mg/ml)	Solubility in pH7.4 PhosphateBuffer (mg/ml)	Solubility inn-Octanol (mg/ml)
Drug Extract	0.143	0.197	0.231
F1	0.789	5.273	5.976
F2	0.781	5.151	5.640
F3	0.652	4.837	5.284
F4	0.694	3.950	4.569
F5	0.528	3.752	4.191
F6	0.573	3.864	4.237
F7	0.617	4.356	4.587

The phytosomes of *Morinda citrifolia* showed better diffusion profile than the pure drug extract. Unlike the free drug extract (which showed a total of only 54.16% drug release at the end of the 10 hour), all the formulations showed the percentage cumulative drug release in the range of 65.31 – 91.23%. The formulation F_1 with drug extract: soya lecithin ratio of 1:1 showed the maximum release of 91.23% at the 10^{th} hour. The diffusion of drug particles from its dosageform is a complex operation influenced by a number of factors like the particle size, crystal habit, surface area, surface energies and wettability. Wetting and dispersion properties of phospholipids (an amphiphilic surfactant) increased the solubility of the drug and hence improved the diffusion profile of the complex.

***Ex-vivo* Skin Permeation Study**

Optimized formulation F1 was further subjected to the following studies:

The drug permeation of the formulation F1 through the abdominal skin of chicken was carried using Franz diffusion cell and the results are reported in table no:12. The permeation profile was plotted between% cumulative drug permeated v/s time. It was observed that the formulation showed an optimum release of 92.57% over a period of 12 hours.

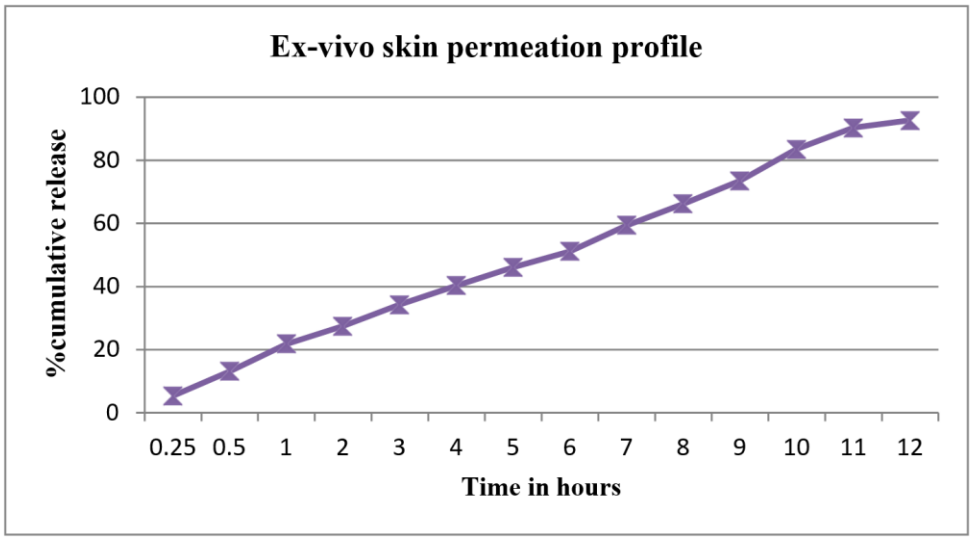

Figure 1: Drug Permeation Profile of F1

Scanning Electron Microcopy (SEM) Analysis

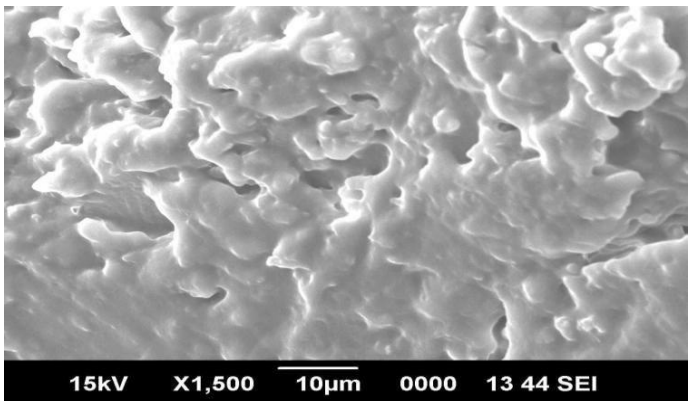

Figure 2: *SEM image of Morinda citrifolia Phytosome (F1)*

The surface morphology of the formulated phytosome (optimized formulation) were confirmed by scanning electron microscopy. The vesicles are spherical in shape and smooth in nature. The purpose of the stability testing is to provide evidence on how the quality of a drug substance or drug varies with time under the influence of variety of environmental factors like temperature, humidity and light and to establish a test period for the drug substance or a shelf life for the drug and recommended storage conditions. Here the gel are packed in collapsible aluminum tubes and were loaded at accelerated condition at 40 ± 2°C/ RH 70 ± 5% RH in a stability chamber. Samples were withdrawn at initial 40 ± 2°C/ RH 70 ± 5% and days and evaluated for homogeneity, drug content, pH, spreadability, extrudability, viscosity and in-vitro diffusion profile. The results showed that the storage at these conditions had no effect on those parameters.

Conclusion

The complexation of phytoconstituents and phospholipids makes the phytoconstituents more stable in the complex form due to liopophilic nature and offering the herbal drugs with sufficient lipid penetrability, higher concentration, sustained therapeutic levels and increased cosmetic value. In conclusion, the application of phytosomal formulation as topical pharmaceutical agent and cosmetics with improved safety and efficacy results in proper utilization of herbal drugs and cost effective pharmaceutical product.

References

[1]. Vennila Srinivasahan, Brindha Durairaj: Antimicrobial activities of Hydroethanolic Extract of *Morinda citrifolia* Fruit, *Int. J. Current Microbiol. App. Sci.* (2014); 3(9): 26-33.

[2]. Diogo Matias, Luis Roque, Maria de Fatima Simoes, Ana Diaz-Lanza, Patricia Rijo, Catarina P. Reis: *Plectranthus madagascariensis* Phytosomes: Formulation Optimization, *J Biomedical and Biopharmaceutical Research*. 2015; (12) 2: 223-231.

[3]. Rowe RC, Sheskey PJ. Handbook of pharmaceutical excipients, 5th edition. Landon: Pharmaceutical Press; 2006;111-115,466-470, 474-475.

[4]. S. L. Kakad, S.S. Pise, A. J. Dhembar: Evaluation of phytochemical, antibacterial, antifungal activities of leaf extracts of *Morinda citrifolia* (Linn), *Pelagia Research Library*. 2015;6(4):9-12.

[5]. Parul A. Ittadwar, Prashant K. Puranik: Novel Umbelliferone Phytosomes: Development and Optimization Usin Experimental Design Approach and Evaluation of Photo- Protective and Antioxidant Activity, *IJPPS*.2016;9(1)219-227.

[6]. Sandeep Arora, Arvind Sharma and Parneet Kaur: Preparation and Characterization of Phytosomal-Phospholipid Complex of *P. Amarus* and its Tablet Formulation, *J PharmaTech Research*: 2013;1:1-18.

[7]. Mairim Russo Serafini, Rodrigo Correia Santos, Adriana Gibara Guimaraes, Joao Paulo Almeida dos Santos, Alan Diego da Conceica Santos, Izabel Almeida Alves, Daniel Pens Gelain, Paulo Cesar de Lima Nogueira, Lucindo Joses Quintans-Junior, Leonardo Rigoldi Bonjardim, Adriano Antunes de Souza Araujo: Morinda citrifolia Linn Leaf Extract Possesses Antioxidant Activities and Reduces Nociceptive Behavior and Leukocyte Migration, *J Med Food.*2011;14(10):1159-1166.

[8]. Hiroshi Okamoto: *Morinda Citrifolia* (Noni) in the Treatment of Psoriasis, *The Open General and Internal Med J.* 2012;*5:*1-2.

[9]. Alisha Pereira, Rashmi Mallya: Formulation and evaluation of a photoprotectant cream containing *Phyllanthus emblica* extract phospholipid complex, *J Pharmacognosy and Phytochemistry*. 2015; 4(2):232-240.

[10]. Jadhav S.J, Gorde Vikas.D, Gahandule M.B, Dr. Gadhave M.V, Gaikwad D.D: Formulation Development of Hepato-Protective *Butea monosperma*-Phytosome, *IJIPSR*. 2016;4(6):657-670.

[11]. Ahmed N. Allam, Ibrahim A. Komeil, Ossama Y. Abdallah: Curcumin phytosomal soft gel formulation: Development, optimization and physicochemical characterization, *Acta Pharm.* 2015; 65: 285–297.

[12]. Malay K Das, Bhupen Kalita: Design and Evaluation of Phyto-Phospholipid Complexes (Phytosomes) of Rutin for Transdermal Application, *J Applied Pharma Sci*. 2014;4 (10): 051-057.

[13]. Asija Sangeeta, Gopal Garg, Rajesh Asija, Chirag Patel: Formulation and Evaluation of*Prosopis Cineraria* Druce Phytosomes, *Deccan J. Pharmaceutics &Cosmetoloy.* 2012; 3(3):1-12.

[14]. Ashwini S Dhase, Shweta S Saboo: Preparation and Evaluation of Phytosomes Containing Methanolic Extract of Leaves of Aegle Marmelos (Bael), *Int.J. PharmTech Res*. 2015;8(6):231-240.

[15]. K. Rajashekar, P. J. Prasuna Sundari, Dr. Prathima Srinivas: Development of a Topical Phytosomal Gel of Woodfordia Fruticosa, *WJPPS*. 2015;4(11):919-931. Keerthi B, Prasuna Sundari Pingali, Dr. Prathima Srinivas: Formulation and Evaluation of Capsules of Ashwagandha Phytosomes, *Int. J. Pharm. Sci. Rev. Res*. 2014; 29(2):138- 141.

[16]. Rudra Pratap Singh and Ramakant Narke: Preparation and Evaluation of Phytosome of Lawsone, *IJPSR.* 2015;6(12):5217-5225.

[17]. G. Valli, M. Murugalakshmi: Isolation, Preliminary Phytochemical and AntibacterialActivity Studies of the Constituents Present in Ethanol Extract of Manjanathi Fruits, *International Journal of Innovative Research in Science, Engineering and Technology*. 2014;3(3):9940-9944.