ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 8, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

QUANTIFICATION USING ULTRAVIOLET SPECTROSCOPY METHOD AND *IN VITRO* STABILITY STUDY OF NANOVESICULAR SYSTEM CONTAINING PHYTIC ACID

MALTI ARYA, KRISHNA P GUPTA, SHUBHINI A SARAF*

Department of Pharmaceutical Sciences, School of Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow – 226 025, Uttar Pradesh, India. Email: shubhini.saraf@gmail.com

Received: 12 April 2018, Revised and Accepted: 08 May 2018

ABSTRACT

Objective: The quantification of drug and stability of nanoparticulate delivery systems is one of the major apprehensions in biomedical applications. The present research work was attempted to quantify phytic acid by utilizing ultraviolet (UV) spectroscopy method and to evaluate the stability of nanovesicular (niosomes) system containing phytic acid.

Methods: Niosomes containing phytic acid were developed by thin-film hydration method. Nanoformulation was subjected to stability testing as per the International Council for Harmonisation (ICH) guidelines. The formulation was stored at 30°C±2°C and 65%±5% RH, samples were withdrawn at 15th, 30th, 60th, 90th, 120th, and 180th day of analysis and examined for the integrity of vesicular/particle size, polydispersity index, zeta potential, and percent encapsulation efficiency.

Results: Prepared nanoformulation displayed a straight line (y=mx+c) equation of y=−0.0309x+1.0413. Optimized batch of niosomes, which was prepared including dicetylphosphate showed zeta potential value of −36±0.36. Stability study showed that prepared niosomal formulation was stable up to 180 days at room temperature.

Conclusion: Findings of the current research work suggested that UV spectroscopy method can be effectively used for the quantification of phytic acid and niosomal formulation of phytic acid. The formulation was found to be stable as per the ICH guidelines for stability testing.

Keywords: Phytic acid, Niosomes, Quantification, Stability testing.

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INTRODUCTION

Phytic acid is a natural constituent present in almost all the cereals and legumes. It is extensively being studied to assess its effects on health and is shown to have chelation ability with minerals. It possesses various benefits on the human body such as lowering serum cholesterol, inhibitor for renal stone development, strong antioxidant, and antiangiogenic properties. It is confirmed by numerous researches that antioxidant scavenge free radicals, thus ultimately help in the cancer prevention. So far, molecular mechanism of action of phytic acid for chemoprevention is not very clear [1-3]. Previous *in vitro* and *in vivo* reports proved its anticancerous properties against prostate, colon, lung, metastatic, and mammary cancers [4].

Investigation and quantification are important elements in the formulation development of any drug. An appropriate method must exist for the quantification of the drug molecule in formulation for analysis of entrapment efficiency, dissolution studies, biological samples, etc. [5]. Ultraviolet (UV) spectroscopy is simplest and commonly used technique for such study. However, the UV region lies from 200 to 400 nm and visible region ranges from 400 to 800 nm. Phytic acid does not come under the UV region. Here comes the play of colorimetric analysis. Thus, phytic acid needs chromophores to be added for its quantification through UV spectroscopy.

Nanotechnology is meant for the development of nanomedicines that deliver drugs in a sustained or controlled mode and enhances residence time of drug [6]. Nanomedicine emerged as a new epoch with growth in the application of nanotechnology in the field of diagnosis and therapy. Nanoparticles are widely being used as pharmaceutical nanocarriers, and these include metallic, polymeric, vesicular, and lipidic nanoparticles [7,8]. Non-ionic surfactant vesicles (niosomes) are of intervening importance as a delivery system [9,10]. Niosomes are flexible carrier which is pertinent for systemic as well as topical applications [10]. These may be uni- or multi-lamellar spheroids in structures [11,12]. Niosomes are formed through self-assembly of non-ionic surfactant monomers which are proficient in entrapping a variety of drugs (both hydrophilic and lipophilic) [12,13]. They generally exhibit a longer storage time and may also act as targeting agents [7,14].

Drug delivery systems play a key role in therapy development. The extension of the pharmaceutical industry and dosage form development is well-recognized [15]. Development of pharmaceutical products for global use necessitates that the prepared dosage form must be stable for longer duration along with persistent pharmacological potential [16]. Nanotechnologies being actively involved in the area remain linked with the development of effectual and stable dosage form [17]. To test the stability of newly developed dosage form is performed to show the evidence how properties change with the influence of time, temperature, and humidity or how long product may be used, thus to establish shelf lives [18, 19].

Phytic acid has momentous prospective to act as an antiangiogenic agent. Instead of having a great potential, it is grossly underutilized due to a flaw of rapid absorption and excretion from the body as it forms insoluble chelates when it comes in contact with biological ions. It is thus required to develop some novel drug delivery systems of phytic acid that can reduce its flaw, thereby increasing its anticancer effect. For the development of a novel delivery system, it is required to quantify it effectively through proper method for precise characterization. Hence, the endeavor of the current work was to quantify phytic acid and develop and characterize a niosomal delivery system of it.

METHODS

Materials

Phytic acid and ammonium iron (III) sulfate.12 H_2O were purchased from Sigma Co. (St. Louis, MO). 2,2'-bipyridine was procured from Abcam. Thioglycolic acid was purchased from Merck-millipore. All other chemicals were obtained from local commercial source and were of analytical grade.

Quantitative estimation of drug through UV-spectrophotometer

Quantitative estimation of phytic acid was performed as reported by Haug and Lantzsch [20]. Drug stock solution (100 µg/ml) was prepared in distilled water, named as the solution (i). Ferric solution was prepared separately by dissolving 0.2 g ammonium iron (III) sulfate.12 H_2O in 100 ml 2 N HCl and volumes were made up to 1000 ml with distilled water, solution named as the solution (ii). Solution (iii) was prepared by dissolving 10 g 2,2'-bipyridine and 10 ml thioglycolic acid in distilled water and volume make up to 1000 ml. 0.5 ml of drug solution was pipetted (3–30 µg/ml) into a test tube, 1 ml of solution (ii) added to it and covered with parafilm. Tubes were heated in a boiling water bath for 30 min, by taking care for the first 5 min that the tubes remain well covered. After that, tubes were cooled in ice water for 15 min and allowed to adjust to room temperature. Once the tubes had reached room temperature, the content of tubes was mixed and centrifuged for 30 min at 3000 g. 1 ml of the supernatant was transferred to another test tube and 1.5 ml of solution (iii) was added. Solutions were scanned from a range of 400–600 nm, and the observed absorbance maxima value was utilized for further evaluations.

Table 1: Composition of the different batches of formulation of phytic acid loaded niosomes

Batches	Cholesterol:surfactant ratio (molar ratio)	Sonication time (min)	DCP (µM)
1(NIO1)	1:1	1	0
2(NIO2)	1:2.5	1	0
3(NIO3)	1:1	2	0
4(NIO4)	1:2.5	2	0
5(NIO5)	1:1	1	5
6(NIO6)	1:2.5	1	5
7(NIO7)	1:1	2	5
8(NIO8)	1:2.5	2	5
9(NIO9)	1:1.25	0.5	2.5

Table 2: Zeta potential values of phytic acid niosomes

Batches	Zeta potential
1(NIO1)	−6±0.21
2(NIO2)	−7±0.13
3(NIO3)	−6±0.11
4(NIO4)	−8±0.14
5(NIO5)	−31±0.23
6(NIO6)	−36±0.36
7(NIO7)	−30±0.23
8(NIO8)	−29±0.31
9(NIO9)	−18±0.39

Values are given as mean±SD and experiments were performed in triplicate for each batch, i.e., n=3. SD: Standard deviation

Preparation of phytic acid containing niosomes

Niosomes were prepared through thin-film hydration method and dispersed in a suitable suspension base. Detailed preparation and characterization of the formulation are reported elsewhere [21,22]. Briefly, niosomes were prepared using Span 80-Chol-dicetylphosphate (DCP) dispersion. An appropriate quantity of cholesterol, surfactant, and stabilizing agent in variable molar ratios (Table 1) was dissolved in 10 ml of chloroform in a round-bottomed glass flask. The chloroform was allowed to evaporate at 45°C under reduced pressure using a rotary evaporator (IKA® Rotavapor, Bengaluru, India). After solvent evaporation, the flask was kept overnight to remove residual solvent, resulting in the formation of a thin film. The thin film was hydrated with 5 ml of aqueous drug solution by rotating at 45°C and 120 revolutions per minute (rpm). This dispersion was sonicated at 80% energy for 1 min by utilizing probe-type sonicator (Labsonic®-M, Sartorius stedim) and kept for 24 h at 4°C for maturation.

Zeta potential measurement

Zeta potential of the niosomal formulation was measured using Malvern Zetasizer Nano ZS. It was determined by the electrophoretic mobility of niosome suspension in U-type tube, temperature set during the procedure was 25°C. Zeta potential was determined through the principle of dynamic light scattering [23].

Stability study of phytic acid containing niosomes

Prepared formulation was examined for stability testing according to the International Council for Harmonisation guidelines. The rationale of stability testing is to substantiate how the properties and entrapped drug amount of formulation vary with time under the effect of temperature and humidity [24]. Optimization was done previously through various characterization parameters. The optimized batch of the niosomal suspension was used for stability testing. Stability study of the niosomal formulation was carried out by estimating the ability of the prepared system to retain the physical properties. The formulation was kept for analysis at room temperature condition, namely 30°C±2°C/60%±5% RH. Samples were withdrawn at 15th, 30th, 60th, 90th, 120th, and 180th day and were examined for particle size, polydispersity index, zeta potential by Malvern Zetasizer Nano ZS, particle and zeta analyzer, and percent encapsulation efficiency through spectrophotometrically. Estimation procedure of these parameters reported elsewhere [22].

RESULTS AND DISCUSSION

Quantitative estimation of drug through UV-spectrophotometer

UV-spectrophotometric method was successfully used for the quantification of phytic acid. Processed phytic acid solutions were scanned from 400 to 600 nm, as shown in Fig. 1. Scanning showed absorbance maxima at 520 nm, result was found in accordance with the previous report [20]. Thus, all the absorbances were measured at 520 nm against distilled water. A representative calibration curve was obtained which was found linear till the concentration of 30 µg/ ml. Suggested concentration range of analysis is 3–30 µg/ml. Straight line (y=mx+c) equation was found to be "y=−0.0309x+1.0413" (Fig. 2), which was utilized for all further characterizations. Negative value suggests lower the absorbance higher will be the concentration of drug present in the solution and vice versa.

Table 3: Effect of storage on particle size, PDI, zeta potential, and percent encapsulation efficiency, where n=3

Interval in days	Particle size (nm±SD)	PDI (values±SD)	Zeta potential (values±SD)	Encapsulation efficiency (%±SD)
0 day	965.5±65.2	0.386±0.08	−36±0.28	82.9±2.6
15th day	966.3±71.2	0.312±0.02	−36±0.31	81.2±3.8
30th day	972.3±63.2	0.319±0.03	−35±0.42	81.3±3.6
60th day	979.0±76.3	0.421±0.08	−35±0.52	80.0±4.8
90th day	983.5±87.4	0.376±0.07	−34±0.35	78.3±2.8
120th day	988.0±75.7	0.436±0.07	−32±0.38	78.1±4.3
180th day	990.3±82.3	0.348±0.08	−32±0.56	76.0±3.6

Values have been reported as mean±SD, experiments were performed in triplicate for each parameter, i.e., n=3. SD: Standard deviation, PDI: Polydispersity index

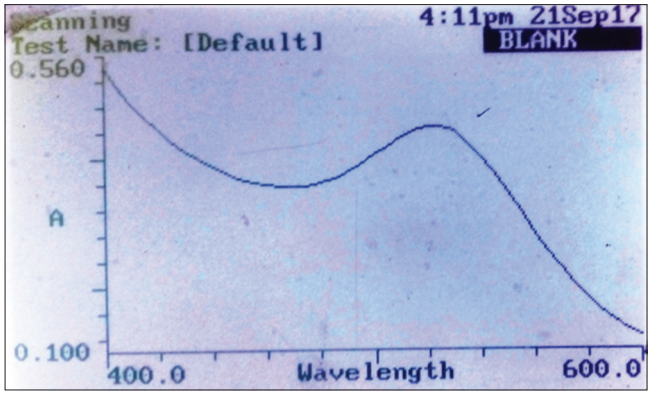

Fig. 1: Ultraviolet spectrum of phytic acid displaying it absorption maxima near 520 nm

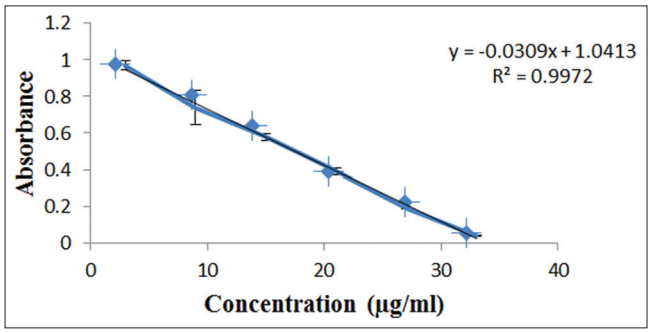

Fig. 2: Standard curve of phytic acid utilizing ultraviolet method

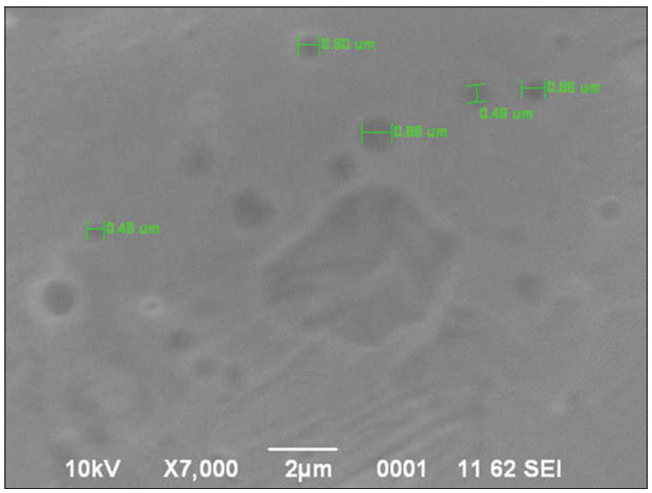

Fig. 3: Scanning electron microscope image showing spherical phytic acid niosomes

Experiments were performed in triplicate, i.e., n=3.

Preparation and characterization of phytic acid containing niosomes

Niosomes were prepared using thin-film hydration method and were dispersed in a base which was suitable for topical application. Extensive characterization (*in vitro* and *in vivo*) of prepared niosomal formulations was performed which have been reported elsewhere [22]. Prepared niosomes, when analyzed through scanning electron microscope, were found to be spherical, as shown in Fig. 3, resembling characteristic of noisome vesicles [7].

Zeta potential measurement

Zeta potential values for prepared niosomes are presented in Table 2. Zeta potential is related to the magnitude of the electrical charge present at the particle surface, and it indicates the degree of repulsion between adjacent particles. Either negative or positive, high zeta potential confers stability by resisting aggregation [23]. Negative value of the particle may be due to the presence of negative charge inducing agent, DCP. Batches which did not contain DCP showed lower values and those which contained DCP displayed higher values of zeta potential. This observation was in-line with characteristic property of DCP, i.e., inducing charge on the surface of the prepared vesicles to impart stability [25,26].

Stability study of phytic acid containing niosomes

Optimized formulation was kept for stability testing at above-mentioned condition. Results of stability study showed that the prepared niosomal formulation was stable up to 180 days at room temperature (Table 3). All the parameters were found stable during the analysis. This may be attributed to the presence of DCP which has the property to induce charge on the surface of the vesicles and would not allow its aggregation, thereby enhancing its stability [25,26]. No significant degradation was observed on storage at the condition of 30°C±2°C/60%±5%. Thus, shelf life of prepared formulation is estimated to be high.

CONCLUSION

In the present study, the findings revealed that UV spectroscopy method can be efficiently utilized for the quantification of phytic acid. DCP may be utilized as a charge inducing and stabilizing agent for the preparation of niosomes. Niosomal formulation of phytic acid was successfully prepared using DCP and through thin-film hydration method. Thus, prepared system was stable up to 180 days, i.e., it can be stored at room temperature for sufficient period.

AUTHOR'S CONTRIBUTION

M.A. performed all the bench work. K.P.G. and S.A.S. helped in conceptualizing and overall guidance of the work.

CONFLICTS OF INTEREST

All authors have no conflicts of interest to declare.

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