Original Article

Preparation and Characterization of Silymarin Nanocrystals and Phytosomes with Investigation of their Stability using Gamma Irradiation

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ABSTRACT

Introduction: In this study, Silymarin exhibits poor water solubility so, we developed drug delivery system. Different formulation strategies have been proposed for this problem. **Materials and Methods:** We were study the preparation of silymarin by dissolving it in different solvent (acetone,acetonitrile ,ethanol and methanol) to form nanocrystal and combination between lecithin and silymarin to prepare phytosome. And determined physicochemical characterization including dissolution, drug content in crystals or phytosome. On the other hand, the effect of gamma irradiation had been evaluated. Determination of crystal morphology was undertaken using SEM and TEM. Solid state was characterized by XRD, DSC and FT-IR. Particle size was determined using DLS and *in-vitro* drug release was evaluated for the prepared nanocrystals and phytosomes. **Results:** Indicated that the nanocrystal (NCy6) and phytosome (Phy1) significantly increased the solubility of silymarin by 17.12 and 35.59 %, respectively. The nanocrystals and phytosomeshavea small size (31.9 nm; Ncy6) and (186.7 nm; Phy1), also, XRD data showing semicrystalline state of (Ncy6) and amorphous nature of phytosomes. We noted that,the two dissolution formulations exhibited highest dissolution profile. Gamma radiation induced physical changes in the amorphous structure leading to semicrystalline and crystalline forms that, caused a decrease in drug solubility. We found that, nanocrystals and phytosomes could be considered as successful strategies for enhancing properties of Silymarin, and may be used as sustained release after radiation..

Key words: Silymarin, Nanocrystals, Phytosomes, Gamma irradiation, *in-vitro* dissolution.

INTRODUCTION

Owing to the importance of solubility and good bioavailability for oral drug delivery technology for oral drug,[1-2] formulation researches face a great challenge. Thus, several strategies have been proposed to improve solubility and drug release include complexion with cyclodextrin,[3] liposome,[4] polymeric nanoparticles[5] and micelles.[6]

Several manufactured nanoparticles mean particles with one dimension less than 100 nm[4-5] Bulk materials of the same composition, mostly due to the increased specific surface area and reactivity, which may lead to increased bioavailability, solubility and toxicity.[7] Drug nanocrystals can be defined as formula in crystals shape with a size in the nanometer range.[8] There are various possibilities to produce nanocrystals in the desired shape and size. Three main principles were reported for preparation of nanocrystals, namely milling, precipitation and homogenization methods, as well as a combination of both techniques.[9-11]

It was reported that phytosomes are cell-like structures "Phyto" means plant while "some" means cell like.[12-13] Phytosomes are advanced herbal products produced by binding individual component of herbal extract

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to phosphatidylcholine resulting in a product that is better absorbed and produces better results than the conventional herbal extracts.[13] The phospholipid molecular structure includes a water-soluble head and two fat-soluble tails.[14] Because of this dual solubility, the phospholipid acts as an effective emulsifier.[13] Therefore, phytosomes provide dramatically enhanced bioavailability for lipid soluble drugs,[7] and[15] explained by faster and improved absorption in the intestinal tract.[16]

Two types of radiation are commonly differentiated in the way they interact with normal chemical matter: ionizing and non-ionizing radiation.[17] The effects of radiation as a method of sterilization on the physicochemical properties of solid drugs have to be addressed in order to avoid solid state changes during storage and/or further processing of the drugs in the manufacturing pathway.[18-20]

Silymarin consists of a family of flavonoids (silybin, isosilybin, silychristin, silydianin and taxifoline) commonly found in the dried fruit of the milk thistle plant Silybum marianum.[21] Although silymarin has a role as an antioxidant and hepatoprotective it is also well known for its role as an anticancer agent.[16,21] Silymarin absorption rate levels vary between 20 and 50%.[2] Silybin (60 % of the silymarin extract) is slightly soluble in water and oil. It has poor permeation across the intestinal epithelial cells and minor gastrointestinal (GI) tract absorption in rats has been reported.[22] Several reasons have been attributed for this poor bioavailability, e.g., poor internal absorption,[21] degradation by gastric fluid,[16] or its poor solubility.[23-25]

This study addressed developing formulations of silymarin in order to improve its water solubility and enhance its oral bioavailability using two recent techniques. Nanocrystals and phytosomes's formulation were prepared, investigated for their chemical and physical properties. Also, the effect of gamma radiation on selected formulations was carried out to determine its sterility and stability.

MATERIALS AND METHODS

Materials

Silymarin was purchased from Arab Company for Pharmaceuticals and Medicinal plant (MEPACO- MED), Cairo, Egypt. Ethanol, acetone, acetonitrile and methanol HPLC Grade solvents that were purchased from Sigma-Aldrich Company.

Preparation of Silymarin Nanocrystals

Nanocrystal formulations of Silymarin were prepared according to a method reported in the literature (26-27) by adding 250 mg of silymarin to several organic solvents namely acetone, acetonitrile, ethanol and methanol followed by vigorous mixing for 30 min. The mixture was then added drop wise to distilled water with mixing at 1000 rpm for 30 min at temperature 30 ± 2°C.

The phase volume ratio between the organic and aqueous phases was varied according to values mentioned in Table 1. The final nanocrystals were obtained by evaporating the solution using either hot air oven or by vacuum rotary evaporator.[26-27]

Preparation of Silymarinphytosomes

Two methods were used for the preparation of silymarin-phytosomes; the mixing method and co-grinding

Table 1: Composition of Silymarin Nanocrystals' formulations.

Formula	Silymarin (mg)	Water (mL)	Org. Solvent (mL)	Vol. Ratio(v/v)	Evaporation	Evaporation temp.(°C)
Nc1	250	200	Acetone (4)	1:50	Hot air oven	a
Nc2	250	400	Acetonitrile (8)	1:50	Hot air oven	a
Nc3	250	350	Ethanol (7)	1:50	Hot air oven	a
Nc4	250	400	Methanol (8)	1:50	Hot air oven	a
Nc5	250	200	Acetone (4)	1:50	Rotary evaporation	b
Nc6	250	400	Acetonitrile (8)	1:50	Rotary evaporation	b
Nc7	250	350	Ethanol (7)	1:50	Rotary evaporation	b
Nc8	250	400	Methanol (8)	1:50	Rotary evaporation	b

*a=90°C and b=60°C

Table 2: Composition Of Silymarin phytosome Formulations.

Formula	Silymarin (Mg)	Lecithin (Mg)	Stoichiometry	Method	Evaporation	Evaporation Temp. (°C)
Ph1	482.4	750	1:1	Physical Mixing	Hot Air Oven	a
Ph2	482.4	750	1:1	Co-Grinding	Hot Air Oven	a
Ph3	482.4	750	1:1	Physical Mixing	Rot. Evap.	b
Ph4	482.4	750	1:1	Co Grinding	Rot. Evap.	b
Ph5	482.4	1125	1:1.5	Physical Mixing	Hot Air Oven	a
Ph6	482.4	1125	1:1.5	Co-Grinding	Hot Air Oven	a
Ph7	482.4	1125	1:1.5	Physical Mixing	Rot. Evap.	b
Ph8	482.4	1125	1:1.5	Co-Grinding	Rot. Evap.	b
Ph9	482.4	1500	1:2	Physical Mixing	Hot Air Oven	a
Ph10	482.4	1500	1:2	Co-Grinding	Hot Air Oven	a
Ph11	482.4	1500	1:2	Physical Mixing	Rot. Evap.	b
Ph12	482.4	1500	1:2	Co-Grinding	Rot. Evap.	b

*A=80°C And B=40°C.

method followed by evaporation either in hot air oven or under vacuum. In the first approach, phytosomes were prepared using 482.4 mg (1mM) Silymarin placed in 250 mL rounded flask and an equivalent amount 750 mg(1mM) of lecithin with varying molar ratios (1:1, 1:1.5 and 1:2). Then solvents were added in different ratio. The flask was stirred using magnetic stirrer for 30 minutes and placed under reflux at 40°C for 24 hrs. The remaining solvent was evaporated under vacuum then the precipitate was scratched and collected for further chemical and physical inspection.[7,28]

In the second approach, phytosomes were prepared by first placing 482.4 mg of Silymarin with the calculated amounts of lecithin (Table 2) in a porcelain mortar and pestle and co-grinding was continued for 30 min. The collected powder was then dissolved in a mixture of 90 mL anhydrous ethanol and 40 mL acetone. Stirring and reflux were then continued at 40°C for 24 hrs. The remaining solvent was evaporated under vacuum then the precipitate was scratched and collected for further chemical and physical inspection as mentioned above.[28-30]

Morphological characterizations

The morphology of the prepared formulations was examined using scanning electron microscope (JEOL-JSM-5400, JEOL Ltd. Tokyo, Japan).[7] Few particles of powder were precisely fixed to aluminum stubs using double- sided adhesive carbon discs and then were made electrically conductive by coating with gold sputter under vacuum (SPI-Module Sputter Coater, SPI Supplies Inc., USA). Transmission Electron Microscope (TEM-JEOL-JSM-5400 JEOL Ltd. Tokyo, Japan) was also used to detect particles morphology in suspension form. One drop of the resultant complex dispersions was placed onto a carbon-coated copper grid, leaving a thin liquid film. The air-dried films were then stained and viewed under the transmission electron microscope.[2,14]

Evaluation of average particle size

Average particle size and size distribution of the prepared formulations were determined by the dynamic light scattering (DLS) technique (PSS-NICOMP 380-ZLS, USA). Before measurements, the samples were diluted to 10 times its original volume with de-ionized water. Samples of 250 µL suspensions were transferred to a disposable low volume cuvette. Samples were left to equilibrate to a temperature of 25°C for 2 minutes. Then the analysis was performed using five measurements in 12 runs of 10 seconds each.[2,7,26]

Differential scanning calorimetry (DSC)

Samples of 5 mg of the individual formulations (nanocrystals and phytosome) and the drug were filled into tightly sealed aluminum flat- bottomed pans and heated in DSC-60 instrument (Shimadzu, Japan) in an atmosphere of nitrogen to eliminate the oxidative and paralytic effects. The range of heating temperatures was set between 0 – 300°C which exceeds the melting points of all used materials and the heating rate was kept at 5°C/min.[7,31] The obtained thermo grams were evaluated for any interactions or possible changes in the thermal behavior of the samples.

Fourier Transform -Infrared Spectroscopy (FT-IR)

Samples of Silymarin pure and the prepared nanoparticles were diluted with potassium bromide in the ratio of 1:10 and after drying at room temperature they

were compressed to form discs. The discs were later subjected to FT-IR spectroscopy measurements using a JASCO FT-IR -3600 spectrometers (Easton, USA). Data was collected at a resolution of 4 cm^{-1} in a wave number region of 400 cm^{-1} to 4000 cm^{-1}.[14,7]

X-ray Diffraction (XRD)

Pure silymarin and samples of the prepared nanocrystals and phytosomes, were examined using Shimadzu XRD -6000 (X-Ray Diffract meter SERIAL NO. Q30344700643CZ, Tokyo, JAPAN). The measurements were carried out using Cu as an anode material and operated at a voltage of 25 KV with a current of 40 mA. The samples were analyzed in the 2 Theta angle range of 4 to 50 degrees and a scanning speed of 1.2degree/min.[26-27]

Solubility, drug content and *in-vitro* dissolution studies

Powder samples of the prepared formulations equivalent to 5 mg Silymarin were taken and then dissolved in 50 mL solvent system composed of methanol-water (75:25). Samples of 0.1 mL were then placed in 10 mL volumetric flasks and completed to 10 mL using the previous system. Spectrophotometric analysis was carried out at 288 nm. The concentration of Silymarin in each formulation was determined after the construction of a calibration curve of Silymarin in the same each formulation was calculated.

The solubility of Silymarin from the prepared nanocrystals and phytosomes formulations were determined using UV–Visible spectroscopy (JASCO- model V- 560, Japan).[14] Samples from each formulation equivalent to 5 mg Silymarin were dissolved in 50 mL methanol-water (75:25) then serial dilutions of 0.1, 0.2, 0.3, 0.4 and 0.5 were completed to 10 mL from the solvent system composed of methanol-water (75:25). The samples were measured at 288 nm for comparing differences in the solubility between the prepared nanocrystals and phytosomes to that of pure Silymarin.[32]

The *in-vitro* release studies were carried out on nanocrystals and phytosomes using a dissolution test analyzer (RC-3B, Tianjin Instrument Company, China). The powder (containing 5mg formula) was added to 250 mL of dissolution medium (0.1N HCL) and the instrument was operated at a paddle speed of 100 rpm at 37°C.[33] At definite time intervals, 5mL dispersion samples were withdrawn out and the fresh dissolution medium was placed to maintain constant volume (250 ml). The samples were centrifuged, filtered and measured spectrophotometry at 288 nm to determine the percentage of silymarin dissolved.

Sterility testing after exposing to gamma radiation

Each of NC6 and PH1 formulation was separately filled into 2 ml ampoules (under air). The ampoules in small boxes were sterilized by Cobalt-60 as a source of gamma radiation at ambient temperature using Indian Gamma Cell. At National Center for Radiation Research and Technology (NCRRT) Cairo, Egypt. (Dose rate =1678.98 Gy/h), at the time of experiments.

In this test, two gamma radiation doses were attempted for sterilization, viz., 15 kGy and 25 kGy. Two formulation ampoules were exposed to Gamma radiation, while another two ampoules were left non-irradiated as control. The irradiated samples were examined underusing SEM, XRD and FT-IR to determine the solid state stability of the formulations after irradiation.[18-20]

For sterility testing, the total plate count (TPC) determination was performed according to a reported method.[34-35] In this method, 250 mg Silymarin powder was added to 25 mL of sterilized saline solution, (1:10 dilution). The mixture was homogenized and then gradually diluted to 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. A sample of 1 mL was poured into a sterile Petri dish then 15-20 mL of nutrient agar medium was added, shaken gently until evenly mixed and left until gelled. The Petri dish was reversed and incubated at 30°C for 48 h. The number of bacteria was calculated by multiplying average number of colonies by the dilution factor.

Determination of the number of mold and yeast was also performed according to the method mentioned above.[36] However, in this method, 15-20 ml of potato dextrose agar medium was poured onto 1 mL sample and the sterile Petri dish was shaken gently until evenly mixed, gelled and incubated at 20-24°C for 5 days. The numbers of mold and yeast were calculated by multiplying the average number of colonies by the dilution factor.

RESULTS AND DISCUSSION

Particle size and morphological characterizations

The morphology of the nanocrystals and phytosome formulations are demonstrated from SEM and TEM

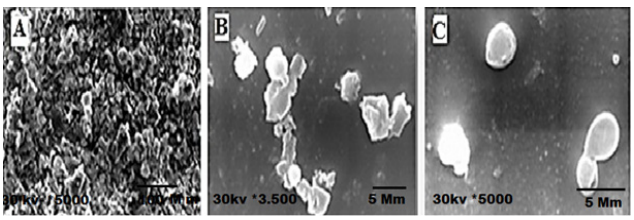

Figure 1: (SEM) of Silymarin powder (A)Nanocrystals(B) and Phytosomes (C)

Table 3 The particle size distribution of silymarin Nanocrystals and phytosomes determined by DLS.

Formula	Particle size distribution	Formula	Particle size distribution
Ncy1	11.9	Phy3	88.0
Ncy2	11.7	Phy4	151.0
Ncy3	11.2	Phy5	159.1
Ncy4	49.4	Phy6	104.4
Ncy5	31.7	Phy7	123.3
Ncy6	31.9	Phy8	252.6
Ncy7	95.6	Phy9	752.0
Ncy8	99.5	Phy10	891.7
Phy1	186.7	Phy11	891.1
Phy2	71.3	Phy12	904.0

micrographs in Figure 1 and 2. It was found that Silymarin nanocrystals prepared from acetone, acetonitrile and ethanol were smaller in size than those prepared from methanol and more uniform in shape , which is in line with its different solubility in these liquids.[36-37] The average particle size (nm) of nanocrystals and phytosomes measured using DLS are demonstrated in Table 3, Figure 3.

Differential scanning calorimetry (DSC)

The DSC thermo grams of the bulk silymarin, Nano crystal and phytosome formulations are illustrated in Figure 4. The bulk silymarin showed a sharp melting endotherm at 159°C (Figure 4). The DSC melting temperature of silymarin is similar to that reported in the literature (158°C) that suggest high content of silybinin in the bulk powder of silymarin. The Nano crystal formulation showed broad endotherms representing semi crystalline phases where the initial melting endotherm was observed around 85-90°C that may represent evaporation of remaining solvents (Ncy2 and Ncy6 in Figure 5). The second main melting endotherm was observed at 150-152°C that is slightly lower than the melting peak reported for silymarin in the literature that may due to plasticizing effect of remaining solvent and effects of size reduction to the Nanorange.[30]

The melting endotherm of lecithin demonstrated a melting peak at 124°C (Figure 5). The melting endotherms observed for phytosomes were short and broad which represent amorphous composites. The degree of broadness of the endotherm, increased with the increased proportion of lecithin (Molar ratio) in the mixture as demonstrated by Phy1 (1:1), Phy6 (1:1.5) and Phy11 (1:2) of Silymarin to lecithin. It was also found that no marked differences in DSC were observed for phytosomes prepared by physical mixing or co-grinding.

Fourier Transform Infra-Red Spectroscopy (FT-IR)

The FT-IR spectra of the samples are shown in Figure 6. Silymarin showed characteristic peaks for –OH, -CH stretching at 2900 to 3400 cm^{-1}, C-C stretching and C=O stretching in the region 1000-1700 cm^{-1} which was retained in all nanocrystal formulations (Fig. 6 A,B,C and D).

Phytosomes and physical mixtures showed FT- IR pattern similar to that of lecithin (Fig. 6) with complete disappearance of the characteristic peaks of Silymarin between 1000 and 1700 cm^{-1} suggesting formation of new composite. In the FT-IR spectrum of PH11(Fig 6), a new peak appeared at 2999 cm^{-1} which may indicate H-bonding formation between the C=O groups of lecithin and the free OH groups of Silymarin and disappearance of peaks at1705 cm^{-1} (C- O stretching) indicating also formation of a new coamorphous phase.

X ray Diffraction (XRD)

The solid states of the drug and the prepared nanocrystals as well as the phytosomes Silymarin demonstrated sharp diffraction lines in the 2 Theta region 5-20 indicating its crystalline state (Figure 7), most nanocrystals retained their crystalline structure (Ncy2 and Ncy4) while Ncy6 (Figure 7) showed too short diffraction lines at the previous region which indicates a semicrystalline state. In Figure 7; lecithin demonstrated a very short diffraction pattern indicating its amorphous nature and also the prepared phytosomes (Figure 7) showed a characteristic amorphous halo in the 2 Theta regions 5-20 which indicated complete dissolution of the crystalline silymarin into the amorphous lecithin forming a completely amorphous composite.

Solubility, drug content and *in-vitro* dissolution

The results of water solubility, content of Silymarin in each Nano crystal and phytosome formulation are summarized in Table 4. The Nano crystal formulations showed higher percentage of drug content compared to phytosomes which might be due to partial loss of the drug during physical mixing and/or co-grinding and also to different relative solubility of the drug and lecithin in the used solvents. The Nano crystal Ncy6 demonstrated more than 8 folds increase in solubility (17.10 %) compared to pure bulk Silymarin (2.10 %). This formula was prepared using fast evaporation of

acetonitrile solution using rotary evaporation at 60°C, while Nano crystal Ncy1 (solubility 15.2 %) was prepared from acetone using evaporation in hot air oven at 90°C.

Phytosomes enhanced the solubility of pure drug more than nanocrystals, where formula PH-1 (molar ratio 1:1) demonstrated more than 13 folds increase in solubility of Silymarin. When the molar ratio of drug to lecithin was increased to 1:2, the solubility was increased to 35.6 % with more than 17.5 folds as demonstrated by PH-11. It was also noticed that phytosomes prepared using physical mixing resulted in higher Percentage solubility than those prepared by co-grinding. The results of solubility of phytosomes which converted the crystalline Silymarin to the amorphous form are consistent with the data reported in the literature.[38-42] It is well known that the amorphous materials do not have long-range order of molecules such as the crystal lattice found in crystalline materials.[42] The amorphous state also has higher internal energy, larger free volume, and greater molecular mobility and thus their solubility is much higher.[43] However, the amorphous structure is metastable with respect to crystalline state, and has a tendency to be spontaneously converted into a crystalline state of lower energy.[42] The presence of lecithin in high proportion with Silymarin might help retardation of the changes in the amorphous state by the intermolecular H-bonding and coamorphous composite formation.

The results of *in-vitro* dissolution are shown in Figure 8. As the *in-vitro* dissolution of poorly water-soluble drugs, especially in the stander biopharmaceutical classification system (BCS) class II drugs, is proportional to the oral absorption, evaluation of *in-vitro* dissolution was conducted to predict the *in-vivo* performance. In addition, dissolution under non-sink conditions is a better way to evaluate the quality of a drug formulation.[31-32] Thus, testing the *in-vitro* dissolution behavior of nanocrystals, phytosomes compared to Silymarin bulk powder and a marketed Silymarin tablet was conducted under non-sink conditions.

As shown in Figure 8 it was found that Nano crystal NC6 and phytosome Phy 1 exhibited much faster and higher dissolution profiles than Silymarin powder and the reference formulation. The maximum release of

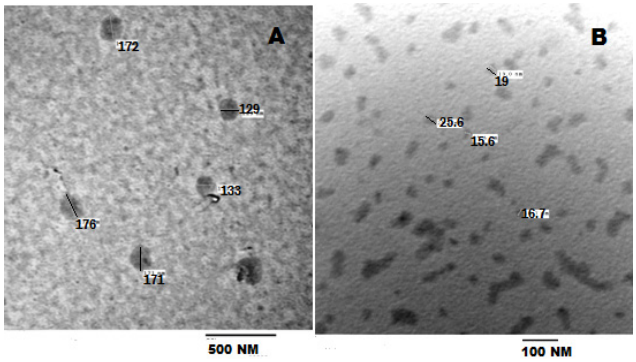

Figure 2: (TEM) Transmission electron micrographs (A) Nanocrystal Ncy6 (B) Phytosome Phy1

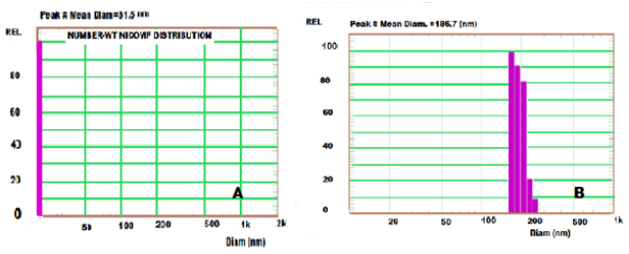

Figure 3: grah from DLS show (A)Nanocrystal Ncy6mean diameter=31.5 nm, (B)Phytosome Phy1mean diameter= 196.7nm.

Table 4: Drug content and solubility (%) of silymarin from bulk powder, nanocrystals and phytosomes.

Formula	Drug content (%)	Solubility (%)	Formula	Drug content (%)	Solubility (%)
Silymarin	100.00	2.10	-	-	-
NCy1	87.40	15.20	Phy3	77.80	6.88
NCy2	93.60	9.00	Phy4	47.40	24.58
NCy3	92.50	14.20	Phy5	46.60	11.54
NCy4	80.40	10.80	Phy6	33.00	10.26
NCy5	87.40	11.50	Phy7	46.60	8.33
NCy6	93.60	17.10	Phy8	33.00	8.98
NCy7	92.50	12.70	Phy9	40.00	7.90
NCy8	80.40	10.60	Phy10	35.00	17.39
Phy1	77.80	27.00	Phy11	40.00	35.59
Phy2	47.4	13.8	Phy12	35.0	22.14

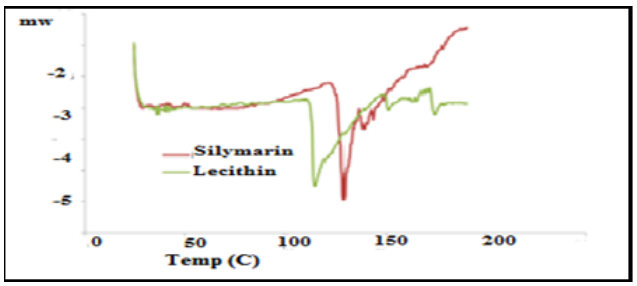

Figure 4: DSC graph of silymarin and lecithin

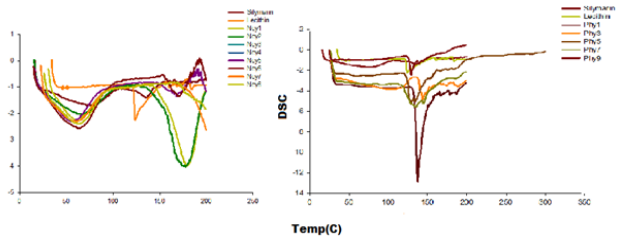

Figure 5: DSC graph (A)Nano crystal formula (B)phytosome formula.

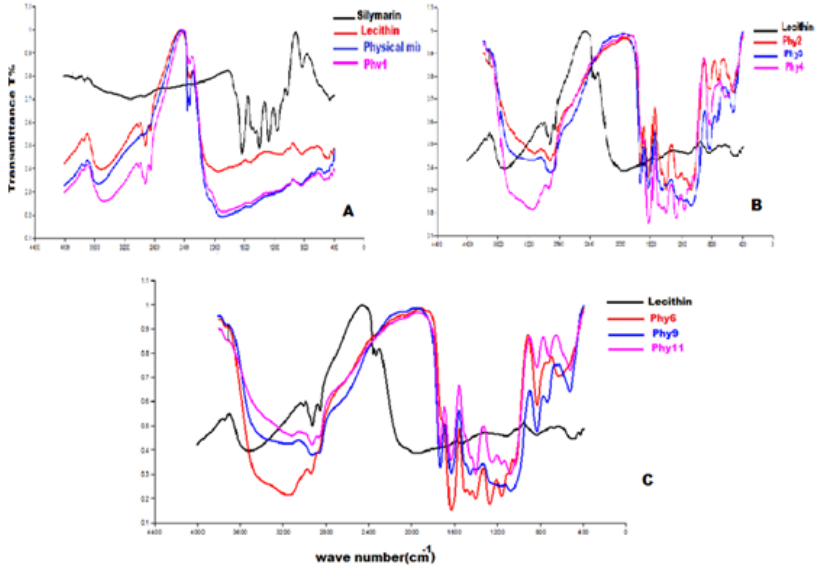

Figure 6: FT- IR spectra of (A) Silymarin; Lecithin; physical mixture and phytosme (Phy1),(B) spectra of phytosme (Phy2) ,(phy3) and (ph4) and (C) spectra of phytosme (Phy6) ,(phy9) and (ph11).

Table 5: Evaluation of solubility of Silymarin from NCy6 and Phy11 before and after gamma irradiation.

Irradiation dose	Formula	Mass (mg)	Theoretical content (mg)	Theoretical concentration (mcg/mL)	Practical concentration (mcg/mL)	% dissolved
Un-irradiated	NCy6	5.00	4.68	9.36	1.60	17.12
15 kGy	NCy6	5.00	4.68	9.36	1.19	12.70
25 kGy	NCy6	5.00	4.68	9.36	0.68	7.31
Un-irradiated	Phy1	4.90	1.96	3.92	1.39	35.59
15 kGy	Phy1	4.90	1.96	3.92	1.15	29.33
25 kGy	Phy1	4.90	1.96	3.92	0.95	24.23

Silymarin from the phytosome Phy1 was 1.8 after 90 min followed by Nano crystal Ncy6 2 which resulted in 2 fold release compared to bulk powder and respectively.

Sterilization using gamma radiation

The results of sterility of irradiated Nano crystal NC6 and phytosome Phy11 showed that there was no microbial bio burden in 25 kGy irradiated groups, indicating that the samples were sterilized. On the other hand, microbial bio burden were founded in the two positive control groups (non-irradiated).

The number of colonies was found to be 23×10^2cfu and 11.31×10^2cfu grown before and after gamma irradiation with 15 kGy for the Nano crystals group and

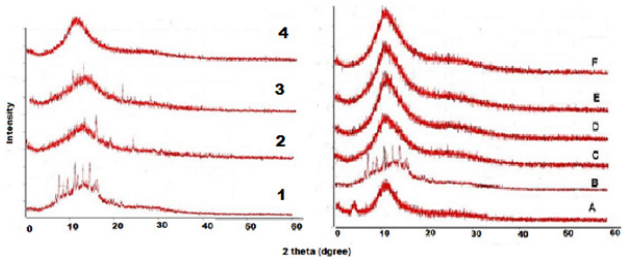

Figure 7: XRD spectra of 1) Silymarin; 2) nanocrystals Ncy2; 3) nanocrystal Ncy4 and 4) nanocrystal Ncy6. A) Lecithin; B) Silymarin and phytosomes: C) Phy-1; D) Phy-3; E) Phy-6 and F) Phy-11

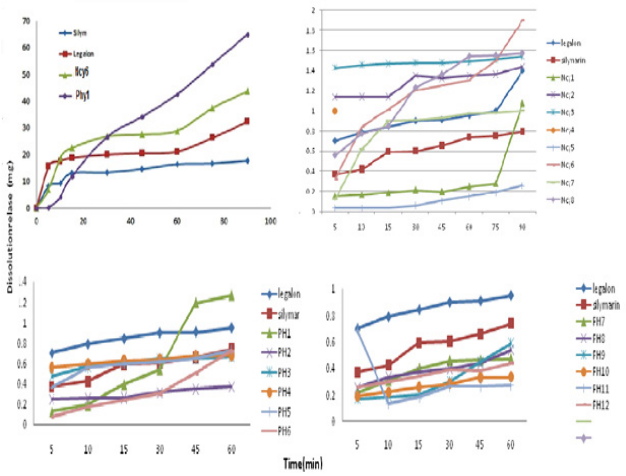

Figure 8: *In-vitro* dissolution graph

43×10^2cfu and 31×10^2cfu for phytosomes, respectively with no fungus contamination detected in all samples. Whilst no growth was observed at dose 25 kGy that is the dose known to stop viability of such microorganisms. These results indicate that the irradiation dose of 25 kGy is the optimum sterilization dose for Nano crystal and phytosome formulations, and it is the sufficient dose for complete radiation sterilization of the product.

Effects of radiation on the solid state

The XRD of the prepared formulation was repeated after irradiation and the results indicated that the semi-crystalline shape of Nanocrystal (Ncy6) changed to highly crystalline after both 15 kGyand 25 kGy (Figure 9).

For the phytosome (Phy11) the solid state also changed from completely amorphous to partially crystalline (Figure 10) that could affect the solubility parameter.

The FT-IR results obtained after irradiation also indicated dramatic changes in in the solid state IR pattern of NCy6 that showed elongation of the peaks between 1000 cm^{-1} and 1700 cm^{-1} and disappearance of the broad peaks in the region 2500-3000 cm^{-1} (Figure 9). For phytosomes there was no difference between the

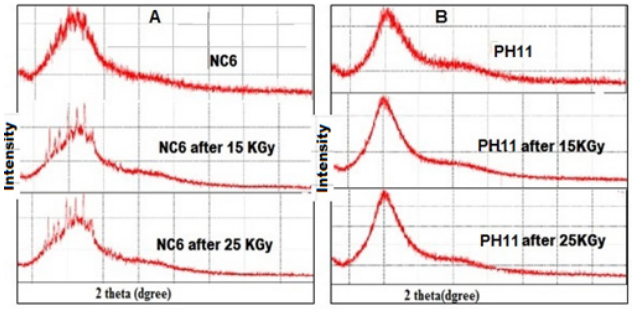

Figure 9: XRDPatterns of NCy6 (A) and Phy1 (B) formulations, before and after exposure to15 and 25 K Gry of gamma radiation.

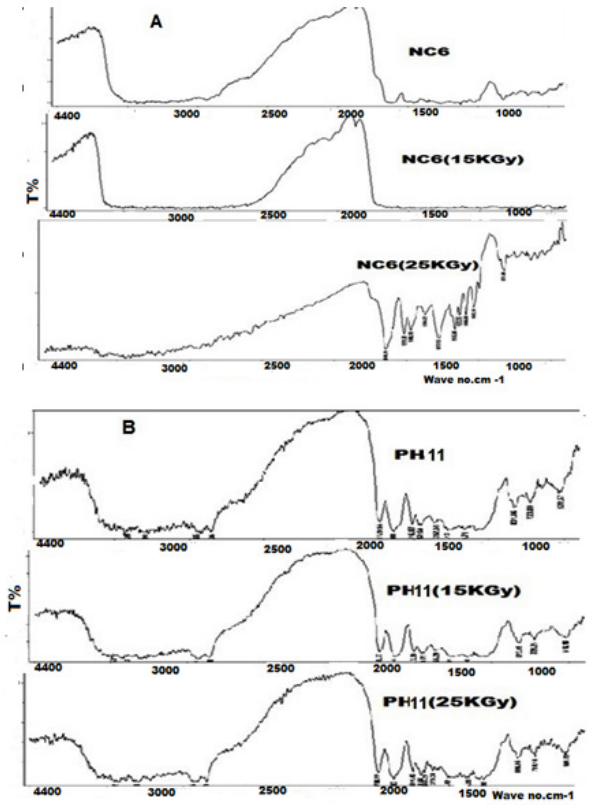

Figure 10: FT-IR of NCy6 (A) and Phy11 (B) formulations, before and after exposure to15 kGy and 25 kGy of gamma radiation

FT-IR patterns obtained for Phy11 before and after irradiation (Figure 10).

Effects of radiation on solubility

The amount of Silymarin dissolved before and after gamma irradiation was evaluated to determine the effects of radiation on solid state and as consequence on solubility. The results in Table 5 showed that the solubility of nanocrystal NCy6 decreased by 25.82 % and 57.30% after gamma irradiation with 15 kGy and 25

kGy, respectively. However, the solubility of phytosome Phy11 was also decreased only by 17.60 % and 31.92 % after irradiation with 15 kGy and 25 kGy, respectively. These results may suggest that the phytosome formulations managed to withstand the irradiation sterilization with 25 kGy more than nanocrystals with lesser effects on solubility of Silymarin

CONCLUSION

Nanocrystals and phytosomes were shown to be successful in enhancing the physicochemical properties of silymarin. The DSC, IR and XRD data also confirmed retaining the crystalline state of nanocrystals and the amorphous state of phytosomes. The increased solubility could be employed in oral and/or parenteral dosage forms to increase the bioavailability of Silymarin. The increased dissolution rate compared to marketed products is another proof of success for such promising new formulations. The process of sterilization using gamma radiation should be used carefully with semi crystalline or amorphous drug formulations to enable control of undesirable effects on solubility due to changes in the solid state. Therefore, nanocrystals and phytosomes could be considered valuable alternative approaches for overcoming problems of low solubility and bioavailability of Silymarin.

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CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

ABBREVIATION

DLS: Dynamic light scattering; **FT-IR:** Fourier Trans- form Infrared; **XRD:** X ray Diffraction; **DSC:** Differential scanning calorimetry; **TEM:** Transmission electron micrographs; **SEM:** Scanning electron micrographs.

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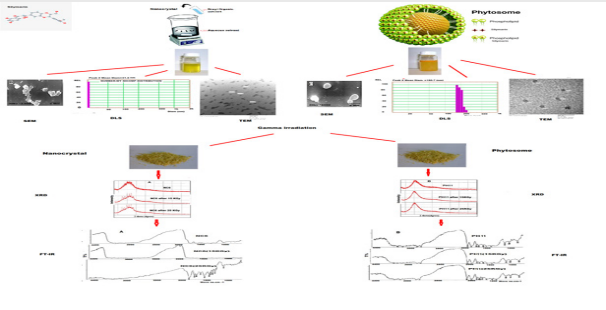
PICTORIAL ABSTRACT



SUMMARY

- Preparation of Silymarin as nanoparticle formula.
- Determination of physicochemical characterization of formula.
- Determination of the best soluble and efficient formula.
- Study the effect of gamma radiation on nanoformula.

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