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Original Article

Nanodelivery of herbal drug (via) biocompatabile polymer on MCF-7 cell line

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ABSTRACT

The development of new delivery systems for the controlled release of drugs is one of the most innovative fields of research in pharmaceutical sciences. The objective of this work is to prepare and characterize chitosan loaded gallic acid nanoparticales. The organoleptic propertity of the drug were complys with USP standard. The solubility studies were performed. The FTIR studies were carried out for drug with polymer in different ratio which shows there was no chemical interaction between the drug and polymer. The drug content and entrapment efficiency of the nanoparticale was increase as the concentration of polymer increase. The F4 formulation shows more entrapment efficacy compared to other formulations nearly 70.87% and the drug content was found to be 86.7%. The particle size and the surface morphology results revealed that Gallic acid nanoparticles (GNPs) were smoothed spheroidal with a size ranging from 67.2 nm-100.5nm. The in-vitro dissolution studies were carried out for all the Formulation. Formulation F4 shows 97.67% of drug release at the end of 24 hr. The results suggest that chitosan polymer based nanoparticulate formulations are potential means to achieve release of Gallic acid for the prolonged period of time for effective therapy. The results showed that this method is reproducible easily.

Key words: Gallic acid, nanoparticle, chitosan, ionization-gelation method, MCF-7 cell line

INTRODUCTION

Nanotechnology is simply known as nanotech deals with the study of controlling of matter on anatomic size 100nm or smaller in at least one dimension. It involves in the improving the materials or devices in Nano scale. [1] Nanoparticles are the novel drug delivery system that defined as the particulate dispersion or solid particulate which have the size in the range of 100nm. In nanoparticle matrix the drug is entrapped, dissolved, encapsulated or attached. [2]Mostly the nanoparticles are made from the biocompatible and biodegradable material such as polymers which may be natural (gelatin, albumin) or synthetic (poly lactides, poly alkyl cyano acrylates) or solid lipids. [3]

Nanoparticle being a drug delivery system which may reduce the toxicity and also low dose is enough to achieve required therapeutic effect in brain. Chitosan loaded Gallic acid produce a sustained release of drug, this may minimize the dosing frequency and may reduce the toxicity which is a side effect of conventional formulation. The Chitosan as a carrier play an important role in transport the drug across the blood brain barrier which may be effective in producing the therapeutic effect. On this view the present work is to formulate and characterized Gallic acid loaded nanoparticles for annticancer delivery. **Gallic acid** is a trihydroxybenzoic acid, a type of phenolic acid, a type of organic acid, also known as 3,4,5-trihydroxybenzoic acid, found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants. The chemical formula is $C_6H_2(OH)_3COOH$. Gallic acid is found both free and as part of hydrolyzable tannins. The Gallic acid groups are usually bonded to form dimers such as ellagic acid. Hydrolysable tannins break down on hydrolysis to give Gallic acid and glucose or ellagic acid and glucose, known as gallotannins and ellagitannins respectively.

MATERIALS AND METHODS

Gallic acid was obtained from Sigma, chitosan and tripoly phosphate was obtained from Otto biochemika, acetic acid, sodium hydroxide and potassium dihydrogen phosphate were obtained from SISCO research laboratories, India. All equipments for the synthesis and evaluation were

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carried out in the Vels University and Malvern laboratories.

Preparation of nanoparticles containing gallic acid: A Chitosan nanoparticle was prepared by ionotropic gelation process. Chitosan solution (0.1% w/v) as prepared by dissolving 100 mg of chitosan in 100ml of 1% v/v acetic acid.TPP (tripolyphosphate)) solution of 0.1% was prepared by dissolving 100mg of TPP in 100ml of deionized water. gallic acid was added to the TPP solution. The chitosan solution was then stirred at1500rpm for 30min in an ultrasonicator (vibronics) and add TPP solution containing Gallic acid drop by drop (10ml) and kept stirring for 1hr on magnetic stirring. Nanoparticles were obtained upon the addition of a TPP aqueous solution to a chitosan solution [5].The NP suspension is then centrifuged at 15,000 rpm for 10 min using high-speed centrifuge (Sigma). Discard the sediment and the preserve the supernatant. The formation of nanoparticles results in interaction between the negative groups of TPP and the positively charged amino groups of chitosan.

EVALUATION STUDIES

Preformulation studies:

Organoleptic properties: Color, odor, taste, and appearance play an important role in the identification of the sample and hence they were recorded in a descriptive terminology.

Solubility studies: The solubility of drug and polymer was carried out in various solvents such as distilled water, buffer solutions and organic solvents. The resulting solutions were filtered and analyzed for dissolved drug by measuring absorbance at 270 nm.

Compatability studies: Fourier Transform InfraRed Spectroscopy(FTIR) studies*:* The fourier transform infrared analysis was conducted for the structure characterization. FTIR spectra of Gallic acid, pure polymer and formulated nanoparticles were recorded. FTIR spectra were recorded on Bomem FTIR MB II Spectrophotometer. Test samples were mixed with KBr, pressed into a pellet and scanned from 400 to 4000 cm^{-1}.

Characterization of nanoparticles: The optimized nanoparticles containing Gallic acid were characterized by studying various physico-chemical properties.

Particle size[6]: Nanoparticle size was determined using Photon Correlation Spectroscopy (PCS). All samples were diluted with ultra-purified water and the analysis was performed at a scattering angle of 90° and at a temperature of 25ºC. The mean diameter for each sample and mean hydrodynamic diameter was generated by cumulative analysis in triplicate.

Zeta potential: Nanoparticles were characterized with Zeta potential using a Zeta Sizer. The measurements were performed using an aqueous dip cell in an automatic mode by placing diluted samples in the capillary measurement cell and cell position is adjusted.

Surface morphology*:* The surface morphology of the particles was studied using Surface Electron Spectroscopy set at 200 kV by placing an air dried nanoparticle suspension on copper electron microscopy grids.

Drug content: The total drug amount in nanosuspension was determined spectrophotomertically. A 0.50-ml aliquot of nanosuspension was evaporated to dryness under reduced pressure at 35°c. the residue was dissolved in water and filtered with a 0.45µm filter, and Gallic acid content was assayed spectrophotomertically at 270nm.

Drug entrapment efficiency*:* The entrapment efficiency is also known as Association Efficiency. The drug loaded nanoparticles are centrifuged at a high speed of 3500-4000 rpm for 30 min and the supernatant is assayed for non-bound drug concentration by UV spectrophotometer. Entrapment efficiency was calculated as follows:

In- vitro release studies: In-vitro diffusion studies (drug release studies) were performed by using diffusion apparatus. A semi-permeable membrane was supported on a ring of diffusion cell and the sample was kept on a membrane in such a way backing layer was phased towards donar compartment. The glass beaker was filled with 100ml of phosphate buffer of (ph:6.8) at a temp 37° c sample of 1ml was withdrawn at regular intervals from glass beaker for analysis.1ml of phosphate buffer was replaced immediately after sampling to maintain volume equal to 100ml.The absorbance of sampling was measured at 270nm by using uv spectrophotometer [7].

CYTOTOXICITY STUDIES –MTT ASSAY: MTT (3- 4, 5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazoliumbromide were cleaved by mitochondria dehydrogenase in viable cells there by yield a measurable purple product Formosan. This Formosan production is proportionate to the viable cell number and inversely proportional to the degree of cytotoxicity.

Preparation of medium: L6 Rat skeletal muscle Cells were cultured in Dulbecco's Modified eagle

Medium (DMEM) at 37°C in a humidified atmosphere of 5% CO2 in air. The tissue culture bottle was observed for growth [8]. Then TPVG was added over the cells of a medium and incubate at 37ºC for 5 minutes for Disaggregation and the cells become individual and its present as suspension. Take one ml of the suspension and pour it into96 wellplates then incubate for 2 days.

Assay procedure: After incubation, remove the medium from the wells for MTT assay. And add 200µl of MTT concentration of (5mg/ml and incubate for 6-7hrs in 5% CO2 incubator. After incubation 1ml of DMSO was added in each well and mix by pipette and leave for 45seconds and it shows the purple color formation. The suspension is transferred in to the cuvette of spectrophotometer and O.D values are read at 270nm and % of cell viability was calculated using the formula. The result was shown in Table 6 and Figure 4.
Graph was plotted using the % of cell viability at Y-axis and concentration of the sample in X-axis.

(OD of sample/OD of cell control) × 100 = % cell viability

RESULT AND DISCUSSION

Standard plot of G**allic acid: A standard plot of Gallic acid was plotted for concentration of 10, 20, 30,40,50µg/ml with the absorbance measured at 270nm . Calibration equation for the standard graph was found to be slope=0.0284 and was used in all calculations.

Preformulation studies: All the organoleptic characters of Gallic acid were studied and it was found that all the characters comply with USP standards. The result was shown in Table 2.

Solubility: The drug was found to be very soluble in water, pH6.8 phosphate buffer, 1M HCL and pH4.5 acetate buffer.

Compatibility studies: (FTIR): The FTIR spectrum of Gallic acid, polymer, and the prepared nanoparticles were studied to detect the compatibility studies. The peaks in the IR spectrum of Gallic acid were compared with that of the prepared nanoparticles. The distinct peaks at 1722.67 shows a C=O stretching, and peaks at 1646, 1591 and 1502 shows at N-H bending. From the above observations we can conclude that, there is no incompatibility between the pure drug and polymer. The result was shown in Figure 1.

Measurement of particle size of Nanoparticles: The particle sizes of prepared nanoparticles were measured from the microphotograph of 100 particles. The particle size ranged from 200.7 to 1317 nm for various batches. The result was shown in Table 3.

Drug content and entrapment efficiency: The total drug amount in nanosuspension was determined spectrophotomertically. A 0.50-ml aliquot of nanosuspension was evaporated to dryness under reduced pressure at 35°c. the residue was dissolved in water and filtered with a 0.45µm filter, and gallic acid content was assayed spectrophotomertically at 270nm, The result was shown in Table 4.

In vitro release studies: In vitro release studies In vitro diffusion studies (drug release studies) were performed by using diffusion apparatus .A semi permeable membrane was supported on a ring of diffusion cell and the sample was kept on a membrane in such a way backing layer was placed towards donor compartment. The formulation F1 shows 78.9% of drug release at the end of 24 hr The formulation F2 shows 80.42% of drug release at the end of 24 hr. The formulation F3 shows 84.06% of drug release at the end of 24 hr The formulation F4 shows 97.9% of drug release at the end of 24 hr compared to other formulation.

The rate of release was based on concentration of a polymer, if the concentration of polymer increase the rate of release of a drug was slow. Based on the solubility nature of an polymer the drug will get release slowly in a desire amount at the particular site of action there by fluctuation of drug was avoided and the plasma concentration of an drug was maintained there by the therapeutic action of an drug was more at the site of action there by the side effects were avoided. The formulation F5 and F6 shows 90 % of drug release within 10 hrs. There by the sustained action of an drug at the targeted site was not obtained. The result was shown in Figure 2.

Kinetic modelling: The release kinetics was studied by fitting the values in zero order, first order, Higuchi equation and Korsmeyer Peppas equation. The equation of best fit was determined. The values showed that the release follows first order (R2 = 0.9929) and Korsmeyer peppas equation and does not follow zero order and Higuchi equation. The “n” value is 0.78 hence the drug release follows anomalous Non-Fickian Diffusion. The result was shown in table no.5

In-vitro cytotoxicity study: The Cell viability study was determined by MTT assay using MCF 7 cell line .In the study the drug Gallic acid has significantly reduced the cell viability in a dose-dependent manner.

In the present study the cells were treated with different concentrations of drug like 1000, 500,250, 125, 62.5, 31.2, 15.6, and 7.8µg/ml. From the above concentration 62.5µg/ml shown 51.92% of cell viability, which means the drug has shown the activity based on the concentration. The result was shown in table 6 figure no.4

Conclusion:

The F4 formulation with the Gallic acid concentration of 30mg provided the highest entrapment efficiency of 71.87% when compared to that of other formulations and the highest extent of release (97.67%at 24th hr) suggesting the possibility to achieve a therapeutic dose and would be capable of reducing the frequency of administration and the dose-dependent side effects associated with the repeated administration of conventional dosage form. The cell line studies had shown the percentage of cell viability was depending on concentration of drug. According to the data obtained, this chitosan-based nanotechnology opens new and interesting perspectives for anti-cancer activity.

Table1: Formulation of Gallic acid nanoparticles

S.NO	Ingredients	F1	F2	F3	F4	F5	F6
1	Gallic acid	40mg	40mg	40mg	40mg	40mg	40mg
2	Chitosan	0.4%	0.5%	0.3%	0.2%	0.25%	0.35%
3	Tripoly phosphate	40ml	40ml	40ml	40ml	40ml	40ml
4	0.1% Acetic acid solution	100ml	100ml	100ml	100ml	100ml	100ml

Table 2: Organoleptic properties

Properties	**Results**
Description	Crystalline
Taste	Slightly bitter
Odour	Odourless
Colour	White to off white crystalline solid

Table 3: Particle size of Gallic acid NP formulations

Formulation	Average particle size (nm)
F1	67.2
F2	77.92
F3	100.8
F4	100.2
F5	82.97
F6	100.5

Table 4: Entrapment efficiency of Gallic acid nanoparticle formulations

Formulation	Average entrapment efficiency
F1	57.1%
F2	65.26%
F3	68.14%
F4	70.87%
F5	62.41%
F6	59.28%

Table 5:*In-vitro* release kinetics of optimized formulation

S.No	Release kinetics	R 2
1	zero order	0.7893
2	First order	0.9929
3	Higuchi equation	0.8905
4	Korsmeyer Peppas equation	0.9534

Table 6: In vitro assay for Cytotoxicity activity

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	**1000**	**Neat**	**0.03**	**5.76**
2	**500**	**1:1**	**0.10**	**19.23**
3	**250**	**1:2**	**0.18**	**34.61**
4	**125**	**1:4**	**0.22**	**42.3**
5	**62.5**	**1:8**	**0.27**	**51.92**
6	**31.2**	**1:16**	**0.34**	**65.38**
7	**15.6**	**1:32**	**0.40**	**76.92**
8	**7.8**	**1:64**	**0.47**	**90.38**
9	**Cell control**	**-**	**0.52**	**100**

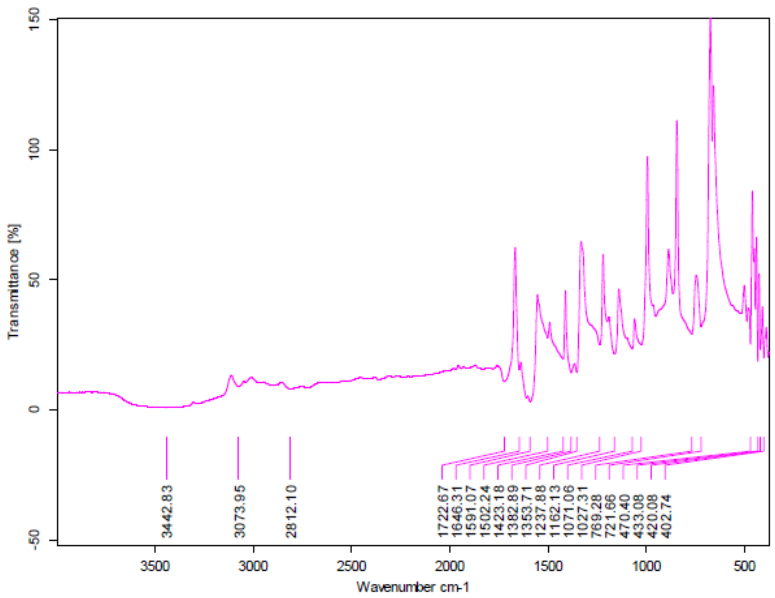

Fig 1: FTIR spectrum of Gallic acid and Chitosin

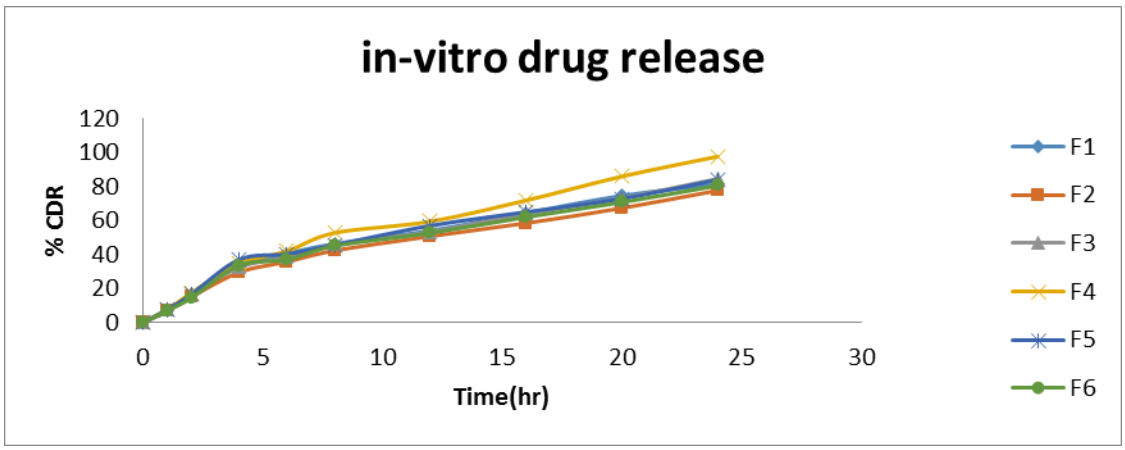

Fig 2:*In-vitro* release of Gallic acid nanoparticales

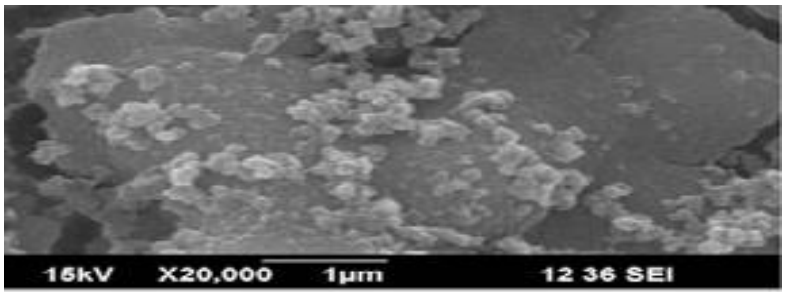

Fig 3: SEM of Gallic acid nanoparticle

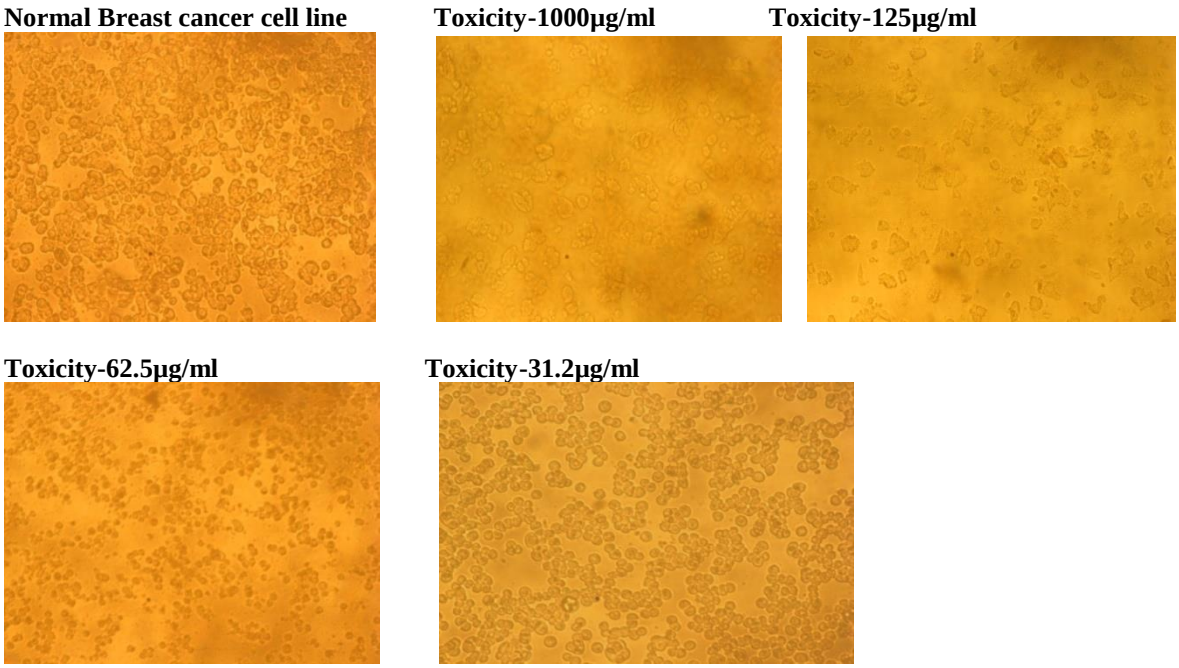

Fig 4: Cytotoxicity effect of optimized formulation on MCF 7 Cell line

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