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ORIGINAL ARTICLE

Formulation and characterization of curcumin nanoparticles prepared from eudragit L-100 and poly vinyl alcohol as polymer

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Abstract

The purpose of the study is to prepare eudragit-S100 nanoparticles loaded with curcumin by using solvent evaporation method and using tween 80 as surfactant. Previously formulated solution drug in IPA was used as dispersed phase and with 2% PVA solution 1% Tween 80 is dispersion medium and permitted for continuous stirring at least 3hr resulting in nanoparticle formation. Numerous parameters like rate of stirring, polymer concentration relative to drug and quantity of organic solvent were optimized. For optimizing the drug concentration, organic solvent and polymer, eight formulations were prepared by changing the solvent and polymer concentration. A comparison of the obtained results were made. On comparing the formulated preparations F-4 (1:4) was showing particles better entrapment efficiency (95.68%). *In-vitro*, studies of drug release were accomplished for a period of 12hrs and the released drug from the formulation was 46.02%. The results showed that as the ratio of polymer increases up-to fourth fold, encapsulation efficiency is enhanced and the rate of release is also sustained, but further increase in polymer show agglomeration of nanoparticles.

Keywords

Curcumin
Eudragit
In-vitro
Nanoparticles
Polyvinyl Alcohol

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Introduction

In recent era, nanotechnology has become more preferred area of research because special systems like nanoparticles are being used as physical approach to improve and change the pharmacokinetic and pharmacodynamics properties of various drug molecules (Nagavarma et al., 2012). Nanomaterials can made through the congregation of nanometer scale units and they offer stimulating perspectives as novel materials of which electronic, magnetic, optical, mechanical transport and thermodynamic properties can be controlled by the selection of composition and sizes of building block units. It have novel properties and functions because of their nano (small) and intermediate size (S Darvesh et al., 2012). Nanoparticles are small enough to confine their electrons and produce quantum effects so they also possess unexpected optical properties. An important physical property of nanoparticles is the ability to form suspensions. This is possible if the interaction of the particle surface with the solvent is strong enough to overcome density differences. In bulk materials this interactions usually result in a material either sinking or floating in a liquid.

The extract from natural South Asian spice turmeric, the main component curcumin, have various pharmacological properties such as antioxidant, anticancer, anti-microbial, anti-HIV and anti-inflammatory (Khatri et al., 2008) but the curcumin use

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is limited owing to its poor water solubility, which is around 0.021 mg/ml (Huang et al., 2007). One simple method for enhancing the curcumin bioavailability is to enhance local concentration of drug by increasing surface area of particle by decreasing powder size to nanosize. Nanoparticles are frequently prepared by two methods, bottom-down or top-down methods. Although in industry, top-down methods are extensively used, bottom-up methods are preferable in lab-scale for low energy, flexibility in regulating particle morphology and low contamination.(Huang et al., 2007). Curcumin is a yellow compound having a molecular weight of 368.37 g/mol (Thorat & Dalvi, 2012). The practical availability of curcumin may be limited due to its poor bioavailability attributed to poor water solubilty, proficient first pass metabolism, poor absorption from gastrointestinal tract and swift elimination (Mahmoudi et al., 2013). Two main approaches are being employed to increase the bioavailability of curcumin. One strategy is based on the chemical modification of the curcumin molecule into water soluble derivatives. Curcumin possesses two reactive terminal hydroxyl groups (Wang et al., 2012). Curcumin has ability to attach with different types of proteins and inhibit the activity of different kinases. In this way, it is considered as an anti-proliferative, anti-invasive, and antiangiogenic agent (Zhang et al., 2008).

It is documented from the last several years that anti-inflammatory drugs, NSAIDs, are related with numerous side effects (Thüne et al., 2011). Consequently, there is an amassed demand for harmless and more effective anti-inflammatory agents. Curcumin has been stated as one of the utmost auspicious candidates of natural origin anti-inflammatory agents, with nearly no stated side effects (Aggarwal et al., 2009).

In spite of the established efficacy of curcumin, it seems that its deprived systemic bioavailability after oral dosing negotiations the potential for therapeutic uses. The chief reasons contributing to the low bioavailability of curcumin embrace poor absorption and swift systemic elimination (Strimpakos & Sharma, 2008). Curcumin is hydrophobic in nature with very low water solubility. Its water solubility and partition coefficient was determined to be 0.6 and 3.2 µg/ml, respectively (Yinsong et al., 2007), (Roa et al., 2009). "Nanocurcumin" is another currently developed formulation for curcumin. This formulation is based on the principle that curcumin is captured in cross-linked polymeric particle with a hydrophilic shell and hydrophobic core. The group established the product efficacy on pancreatic cancer cells and NFkB by demonstrating the inhibition of these cells and having similar effect as free curcumin on inflammatory cytokines (Bisht et al., 2007).

EUDRAGIT® L 100 are anionic copolymers based on methacrylic acid and methyl methacrylate**,** the basis of sustained release polymer such as Poly (meth) acrylates for pharmaceutical applications, and is known worldwide in the pharmaceutical industry under the trade name EUDRAGIT®. The flexibility to combine and interact with the different polymers enables to achieve the desired drug release profile by releasing the drug at the target place and if necessary, over a desired period of time.

Poly vinyl alcohol is a white to cream color granular material which is tasteless and odorless. It is soluble in water, sparingly soluble in ethanol and insoluble in organic solvents. Its use in pharmaceutical industry is very important i.e. as coating and binding agent, thereby acting to mask the taste and facilitate swallowing of the formulation (Lee et al., 2012).

Materials and Methods

Materials: Curcumin used in formulations was taken from Uni-Chem^R Chemical Reagents. Eudragit L 100 used as polymer for sustain release effect taken from Fisher Scintific^R. Iso Propyl Alcohol **(**IPA) is used as organic solvent and act as external phase, taken from a Fine Chemical Limited China. Distilled water was used as a solvent to dissolve poly vinyl alcohol to formulate the external phase of the system in which the internal phase was dispersed. Poly vinyl alcohol is a polymer used as a plasticizer, was obtained from Fine Chemical Limited, China. It formed the external phase or aqueous solution, dissolved in during the formulation process. Potassium dehydrogenate phosphate was used to formulate USP 6.8 pH phosphate buffer as per dissolution media for curcumin nanoparticles. Potassium hydroxide was used a conjugate of strong base in the constitution of the USP 6.8 pH phosphate buffer as a dissolution media for curcumin nanoparticles were obtained from Sigma Aldrich^R.

Method of preparation

a. Preparation of PVA solution: PVA solution preparation was carried out by dissolving 2g of polyvinyl alcohol in distilled water. Dissolution was carried on hot plate magnetic stirrer.

b. Preparation of 6.8 pH phosphate buffer:

- Preparation of solution A: 40.86g of potassium phosphate was dissolved in 1500 ml of distilled water.
- Preparation of solution B: 5.6g of potassium hydroxide was dissolved in 700 ml of distilled water.

250ml was taken from solution A and 114.5 ml from solution B in volumetric flask and fill it with distilled water up to 1000ml.Then pH was checked using pH meter and adjusted accordingly (USP).

Preparation of curcumin nanoparticles: Solvent evaporation method is adapted for the preparation of curcumin nanoparticles. Eudragit and curcumin were

accurately weighed and dissolved in ethanol. At 80^0C solution of PVA 2% is prepared. Both the solutions were sonicated .Organic solution is added drop wise to aqueous solution during propeller mixing. After 2 hours of propeller mixing particles were separated through filtration. Then washed with distilled water (Galindo-Rodriguez et al., 2004)

Rheological studies

Tapped density: The tapped density of the formulated nanoparticles is found by pouring the weighed amount of powder in the volumetric cylinder and tapping it for 100 times on the even surface from height of 2 cm the volume on the volumetric cylinder is noted then by applying the formula in given equation:

$$\text{tapped density} = \frac{\text{mass}}{\text{volume}}$$

Bulk density: Bulk density is determined for weighed amount of formulated nanoparticles by pouring it in the volumetric cylinder. The powder volume is noted without tapping while density is calculated by using formula in given:

$$\text{Bulk density} = \frac{\text{Mass}}{\text{volume}}$$

Hausner's ratio: Hausner's ratio is calculated as a ratio of tapped to the bulk density as given in the equation (Lee & Hsu, 2007).

$$\text{Hausner}'\text{s ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Carr's index: Carr's index is the found as the function of density which tells us about the powder's flow properties. It is calculated as percentage by following formula(Trivedi et al., 2014).

$$\text{Carr}'\text{sindex} = \frac{\text{Tapped density}}{\text{Bulk density}} \times 100$$

Flow ability in relation to Carr's index

Carr's Index	Flowability
5-12	Free flowing
12-16	Good flow
18-21	Fair
23-35	Poor
33-38	Very poor
>40	Extremely poor

Angle of repose: The prepared formulation of nanoparticles after drying is poured through the funnel placed in a tripod stand on the smooth surface. The nanoparticles after flowing through the funnel form heap on the surface. The diameter and height of the heap is measured. Angle of repose is thus can be determined by putting values in the formula given in the equation (Nunthanid et al., 2004).

$$\theta = \tan^{-1}\frac{h}{r}$$

Range for flow properties in relation to angle of repose

Flow properties	Angle of repose
Excellent	25-30
Good	31-35
Fair	36-40
Passable	41-45
Poor	46-55
Very poor	56-65
Very very poor	>66

***In vitro* Dissolution studies:** The prepared formulations of the nanoparticles were passed through the dissolution process to estimate the release time of the drug from the formulated formulations and also the release profile studies were done. In this process, all formulation containing curcumin were subjected to dissolution using phosphate buffer of pH 6.8 by way of dissolution media for 12hrs.

900 ml of dissolution medium was used for nanoparticles of all eight formulations. Apparatus conditions for these studies were set constant to 50 revolutions per minutes and 37^0C±0.5^0C temperature. The amount of formulation equivalent to 400 mg dose for curcumin was wrapped in the cellophane membrane and submerged in 900 ml of dissolution media i.e. 6.8 pH phosphate buffer for curcumin. The sample is taken first after 30 min, then 1hr, 2hr, 3hr and so on up to 12hr. When the sample is taken the volume in the dissolution flask is refilled by the equal amount of dissolution medium removed. The samples are then passed through spectrophotometric analysis. The absorption shown at 425nm for curcumin, during UV spectroscopy for each formulation shows the amount of drug released at various intervals which can be estimated by following formula given in equation(Sailaja et al., 2014).

$$\%\ \text{release of drug} = \frac{\text{absorption of sample}}{\text{absorption of standard}} \times 100$$

Statistical analysis: All the experiments were performed in triplicate (n=3). Release models were applied on dissolution studies.

Results and Discussion

Rheology: Curcumin nanoparticles were synthesized and their rheological studies were conducted for all formulations. Studies are including bulk density, Hausner's ratio, tapped density, angle of repose and Carr's index. A comparative analysis was done on the basis of variation in the polymer concentration among the formulations. The micromeritic study of curcumin

nanoparticles indicated that as the polymer concentration increased, the flow properties of the nanoparticles changes. F1, F2, F3 and F4 has shown excellent flow properties that means polymer and drug both shows excellent compatibity in first four ratios.All the formulations had acceptable drug content illustrating the uniform drug distribution within the patch while the variations among formulations were quite negligible. This declares the drug homogeneity by solvent evaporation method. Further, it assures the suitability of the rheological properties of the casting solution. The addition of Tween-80 enhances the suppleness of eudragit molecules that's why more permeable to water molecule (Aillon et al., 2009). It marks both the patterns of drug release and mechanical properties. Hence minor moisture content is mandatory to preserve the stability, reduce brittleness, reduce susceptibility to microbial contamination and to prevent bulkiness (Ammar et al., 2009).

The carr's index showed that F 1, F 2, F 3 and F 4 had free flowing properties. Also the angle of repose data had shown to have the excellent flow properties of the formulated nano particles. Results were given in Table 1.

Percentage drug content and encapsulation efficiency: Percentage drug content and encapsulation efficiency in prepared nanoparticles was estimated by taking the weighed sample of nanoparticles and dissolving them in their relevant buffers i.e. 6.8 pH phosphate buffer and sonicated them for 4 hours at 30ºC. The solution was then filtered using filters and observed for percentage drug content of curcumin was observed at 425nm. The following formula was applied for the calculation of % drug contents (Carstensen et al., 1991).

$$\text{Percentage drug content} = M_{act}/M_{ms} \times 100$$

$$\text{encapsulation efficiency} = \frac{M_{act}}{M_{the}} \times 100$$

Where,

M_{act}= actual amount of curcumin in nanoparticles

M_{ms}=weighed amount of nanoparticles

M_{the}= theoretical amount of curcumin Nanoparticles

The prepared nanoparticles were evaluated for drug content and it was found that the nanoparticles prepared in 1:1, 1:2, 1:3 showed higher drug content i.e. 96.6% than that of F-5 and F-6 i.e. 78.9 % and 74.3% respectively. The nanoparticles were also evaluated for Encapsulation efficiency and it was found that nanoparticles prepared in first four ratios showed higher encapsulation efficiency i.e. 96.67% than that of F-7 and F-8 i.e. 72.19 % and 65.9% respectively (Sailaja et al., 2014).

Calibration curve: The general method for determining the concentration of drug in unknown sample is by calibration curve in comparison with the set of standards. Different dilutions (2, 4, 6, 8, 12, 16 µg/ml) of the drug were prepared in the buffer solution of the relative drugs and their absorption spectrums were observed. The peak values of absorption were observed for relative concentrations on Tween 80 UV-visible spectrophotometer (PG instruments Pvt. Ltd.) and the graph was plotted. Calibration curve of curcumin was obtained from the absorption data of different dilutions. Serial dilutions were formed by constituting the standard solution of 1mg/ml of curcumin in 6.8 pH phosphate buffer. 1ml of curcumin 1mg/ml was diluted up to 100 ml to form 10µg/ml solution and then 1, 2, 3, and so on up to 9 ml were taken from this stock and diluted up to 10ml to achieve the concentration of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 12 µg/ml and 16 µg/ml and their absorption spectrum was observed for these relative concentrations of dilutions as given in table and each time peak appeared to be at 425nm. The graph given in figure showed a coefficient of correlation (R^2) to be 0.983 (Yadav & Sawant, 2010).

***In vitro* dissolution studies:** The *In vitro* drug release studies shows that drug release from the particles prepared by F1, F2 and F3 were sustained for 12hrs with percentage drug release of 88.58%, 88.89% and 96.11% respectively. In this study attempts have been made to formulate eudragit L-100 nanoparticles loaded with curcumin by using solvent evaporation technique. In order to obtain best formulation, eight formulations were synthesized by changing the concentration of drug and polymer. In formulations 1, 2 and 3 the concentration of drug and polymer were maintained 1:1, 1:2 and 1:3 respectively. The effect of polymer concentration on nanoparticle size, shape, stability, encapsulation efficiency, loading capacity and drug release was studied and compared. The obtained particles are found to be in nanoscale in first seven formulations.

The encapsulation efficiency was found to be more in Formulation 3 i.e. 96.67%. This was mainly because of the higher polymer concentration. Increased polymer concentration is supporting maximum entrapment of the drug. *In vitro* drug release studies were performed for all the eight formulations. In all the formulations the drug release was constant upto 12 hrs. The percentage release of drug within a period of 12 hrs was found to be 88.58%, 88.89 and 96.11% respectively. In formulation 1 the drug and polymer were taken at equal concentration. So 88% of drug has been released. When the polymer concentration was increased from F1 to F2, drug release percentage was increased. By further increasing the drug concentration from F-4 to F-8 the drug release was decreased. From the results it was perceived that by increasing the polymer concentration the drug release was decreasing (Sailaja et al., 2014).

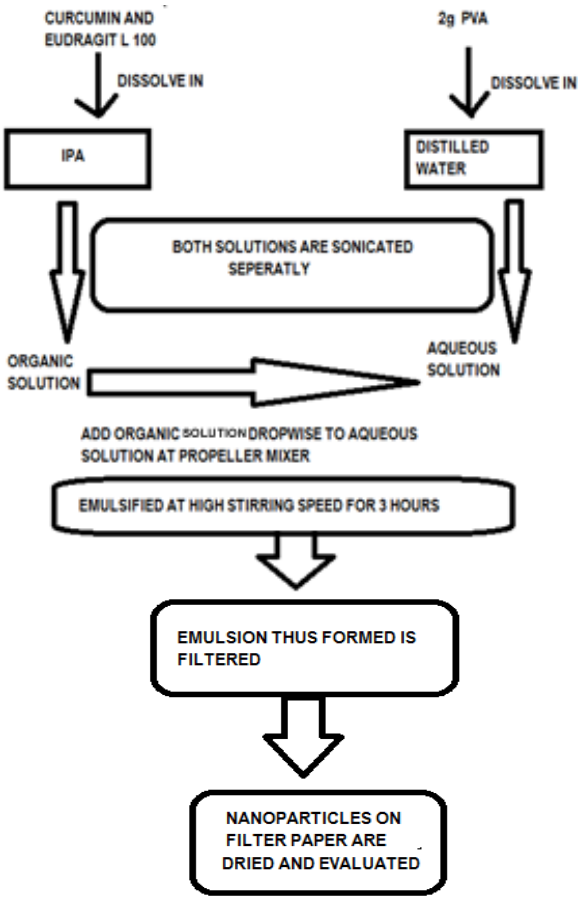

Figure 1: Schematic representation of nanoparticles containing curcumin and Eudragit L-100 as polymer.

Table 1: Micromeritics of nanoparticles containing curcumin and Eudragit L-100 as polymer.

Formulation	Bulk density	Tapped density	Hausner's ratio	Carr's index	Angle of repose
F1	0.27	0.31	1.148	12.9	28.07
F2	0.26	0.329	1.23	18.5	22.83
F3	0.30	0.340	1.133	11.7	26.5
F4	0.51	0.62	1.21	16.7	22.75
F5	0.48	0.52	1.08	7.6	24.77
F6	0.52	0.58	1.15	10.3	25.11
F7	0.50	0.55	0.80	0.9	27.80
F8	0.56	0.50	1.12	10.7	24.50

Table 2: Drug content and Encapsulation efficiency (%) of nanoparticles containing curcumin and Eudragit L-100 as polymer.

Formulation	Drug content (%)	Encapsulation efficiency (%)
F1	96.6	84.8
F2	94.2	82.3
F3	95.2	96.67
F4	81.3	81.8
F5	78.9	72.19
F6	74.3	65.9
F7	62.8	52.4
F8	65.6	39.9

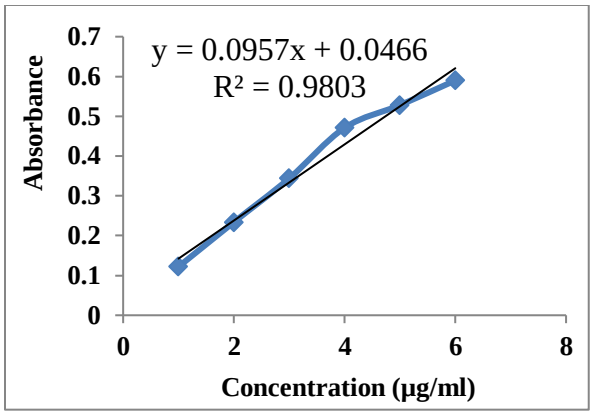

Figure 2: Calibration Curve for Curcumin.

Table 3: Percentage yield of nanoparticles containing curcumin and Eudragit L-100 as polymer.

Formulation	Percentage yield (%)
F1	95
F2	85
F3	72
F4	81
F5	45.5
F6	60.6
F7	76.8
F8	83.6

Conclusion: It can be concluded from the recent study that by increasing fourth fold increase in polymer ratio, encapsulation efficiency and release rate are enhanced but agglomeration is observed after further increase in polymer.

Conflict of interest: All authors declare no conflict of interest.

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