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FORMULATION AND DEVELOPMENT OF LUTEIN LOADED NANOPARTICLES

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ABSTRACT

The present research was aimed at formulation and development of lutein loaded nanoparticles by nanoprecipitation method using ethyl cellulose as polymer. Lutein is present in retina of eye but humans are not able to synthesize it, therefore it has to be obtained through dietary lutein intake. Lutein is natural antioxidant and natural sense of sight enhancer. Lutein helps in age related macular degradation. Optimization of selected parameters including the type of solvent, stirring time, drug polymer ratio and centrifugation speed were performed to obtain polymeric nanoparticles with optimum attributes. The prepared nanoparticles were evaluated for surface morphology, particle size, polydispersity index, zeta potential, entrapment efficiency and in-vitro drug release. Burst release of drug was observed at simulated intestinal pH (pH 6.8). The development of lutein loaded nanoparticles would be beneficial for better absorption of lutein.

KEYWORDS: Lutein, antioxidant, ethyl cellulose, nanoprecipitation.

INTRODUCTION

Nanotechnology represents the design, production and application of materials at atomic, molecular and macromolecular scales, in order to produce new nano-sized materials. Pharmaceutical nanoparticles are defined as solid, submicron-sized (less than 100 nm in diameter) drug carrier that may or may not be biodegradable. The term nanoparticle is a combined name for both nanospheres and nanocapsules. Nanospheres are matrix system in which drug is uniformly dispersed, while nanocapsules are the system in which the drug is surrounded by a unique polymeric membrane.[1] The major goals in designing nanoparticles as drug delivery system are to control particle size, surface properties and release of

pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.[2]

Lutein is a part of the carotenoid family of pigments, more specifically they are xanthophyll hydroxyl carotenoids.[3] Lutein is poorly water soluble. Lutein is well known antioxidant, antifree radical used in nutraceutical industry with potential application in pharmaceutics as supportive antioxidant treatment. It is one of the bioactive food compounds found as lipophilic pigment in various vegetables.[4]

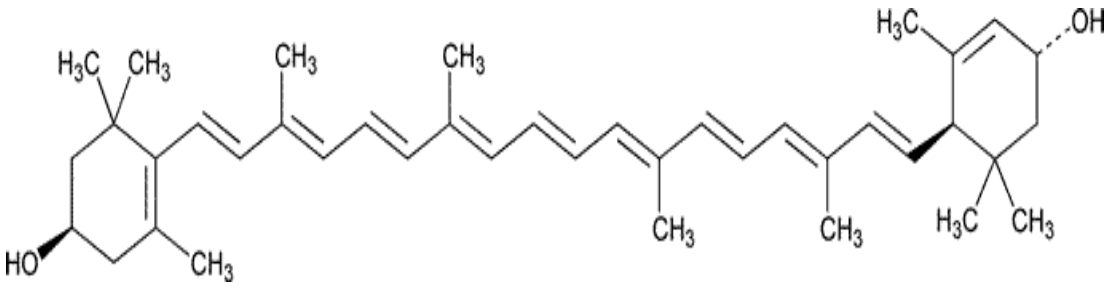

Figure 1: Chemical structure of Lutein.

It is well known as a yellowish pigment for improving food and beverage colour. In addition, lutein plays an important role in improving vision.[5] Age-related macular degeneration (AMD) is the leading cause of blindness in person's aged 65 years.[6] It affects the central region of the retina *(macula lutea)* at which visual acuity is the greatest. Macular pigment possibly acts as a blue light filter to protect the macular region against photo oxidation by light. In addition, macular pigment can scavenge free radicals.[3,7] The carotenoids lutein and zeaxanthin are the predominant pigments in this area. Some observational epidemiologic studies showed a reduced risk of AMD in subjects with a higher intake of lutein and zeaxanthin or higher plasma concentrations of lutein and zeaxanthin.[8,9] The present study therefore aimed at designing and developing nanoparticles loaded with lutein to facilitate its absorption in human body.

MATERIALS AND METHOD

Lutein was kindly provided by Omniactive Health technologies Mumbai, India. Ethyl cellulose was purchased from Signet Chemical Corporation, Mumbai. Polyvinylpyrrolidone (PVP) was purchased from Power Pack Chem, Mumbai. Tween 80 was purchased from Mohini Organic Pvt. Ltd. Mumbai. All the other chemicals were of analytical grade and used as such.

Method

Preparation of lutein loaded nanoparticles

Lutein loaded nanoparticles were prepared by nanoprecipitation method / solvent displacement method.[10] This method is suitable for poorly water soluble drug as is lutein. Here, water miscible solvents like acetone and ethanol were used. Nanoprecipitation method involves two phases namely the organic phase and the aqueous phase. For the organic phase**,** acetone was used as water miscible solvent. Polymer and lutein were dissolved in acetone under stirring at 1000 rpm. For the aqueous phase**,** purified water was used. Tween 80 (surfactant) and PVP (stabilizer) were dissolved in purified water under stirring at 800 rpm. After preparing both the phases emulsification under homogenizer at 10240 rpm was done. The organic solution was added drop wise to aqueous phase under homogenizer at 10240 rpm. Nanoparticles were formed instantaneously by the rapid solvent diffusion. The solution was then centrifuged at 3000 rpm for 10 min for separation of nanoparticles. The separated nanoparticles were then dried in hot air oven at 45°C. Dry nanoparticles were collected for further analysis. The formulations along with ingredients are represented in table 1. Composition of all nine batches after performing DOE statics is given in table 2.

Table 1: Composition of prepared lutein loaded nanoparticles.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Lutein	6.66	15	7	5	5	15	15	15	5
Ethyl cellulose	26.66	7.5	15.75	20	20	7.5	7.5	7.5	20
PVP	10	10	15	20	20	10	20	20	10
Tween 80	5	5	7.5	5	10	10	5	10	10
Water	1000	1000	1000	1000	1000	1000	1000	1000	1000

*All the ingredients were in g except water in ml

Table 2: Composition of all nine batches after performing DOE statics.

Batch Run Order	Center PT	Blocks	Lutein: Polymer ratio (w/w)	Lutein: Co-polymer ratio (w/w)	Concentration of surfactant (%)	Homogenization speed (rpm)
1	1	1	4	1	0.5	10000
2	1	1	0.5	1	0.5	3000
3	0	1	2.25	1.5	0.75	6500
4	1	1	4	2	0.5	3000
5	1	1	4	2	1	10000
6	1	1	0.5	1	1	10000
7	1	1	0.5	2	0.5	10000
8	1	1	0.5	2	1	3000
9	1	1	4	1	1	3000

HPLC Analysis of Lutein

HPLC analysis of standard lutein was performed and the chromatogram is presented in figure 2.

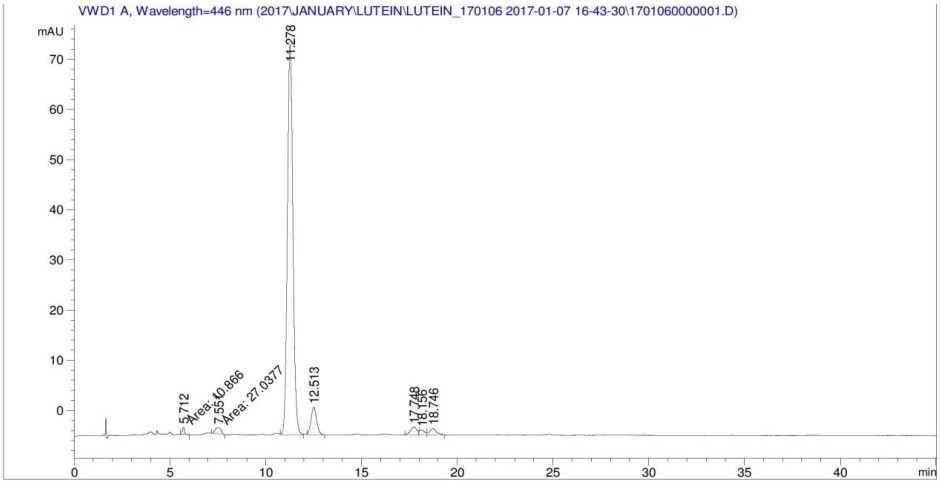

Figure 2: Chromatogram of standard lutein.

Characterization of prepared lutein loaded nanoparticles

Surface morphology

The size and morphological examination of the nanoparticles loaded with lutein were observed using a Philips 208 S electron microscope (Eindoven, Netherlands). The lutein-nanoparticles sample was diluted with ultrapure water (in a ratio of 1:50). One drop of the dispersion was deposited on a carbon film-covered copper grid and kept for 15 min to allow some of the particles to adhere to the carbon substrate. The sample was then examined and photographed.[11]

Particle size and polydispersity index (PDI)

The particle size parameters of lipid nanoparticles were given by the hydrodynamic diameters, zavg. and polydispersity index. Polydispersity index of prepared NLC dispersion were determined by the dynamic light scattering (DLS) technique (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, UK), at a scattering angle of 90˚ and 25˚C. Dispersions were analyzed after appropriate dilution with deionised water to an adequate scattering intensity prior to the measurement. The particle size analysis data were evaluated using intensity distribution. The average diameters were calculated based on three individual measurements.[11]

Zeta potential

The electrophoretic mobility (zeta potential, ζ) of the lutein-nanoparticles and their surface charge have been measured by combining laser Doppler velocimetry and phase analysis light scattering using a Zetasizer Nano ZS (Malvern, Instruments Ltd., Worcestershire, UK). Before zeta potential measurements, dispersions were analyzed after appropriate dilution with ultrapure water. The zeta potential results reported are the mean ± standard deviation of at least three determinations.[11]

Table 3: Stability behaviour of the particles against the zeta potential.

Zeta potential (mV)	Stability behaviour
0 to ±5	Rapid coagulation and flocculation
10 to ± 30	Incipient instability
30 to ± 40	Moderate stability
40 to ±60	Good stability
From ±61	Excellent stability

Drug encapsulation efficacy

Entrapment efficiency (EE) of lutein loaded nanoparticles was determined by centrifugation method with rotor at 10,000 rpm for 30 min. The supernatant was collected and the quantity of drug present in the supernatant was determined by the UV spectrophotometer at 446 nm. The entrapment efficiency was calculated using the following equation:

$$\text{Entrapment Efficiency (\%)} = \frac{W_{\text{initial drug}} - W_{\text{free}}}{W_{\text{initial drug}}} \times 100$$

Where, W initial drug is the total amount of lutein used in preparation of lutein loaded nanoparticles and W free drug is the amount of lutein detects in the supernatant, respectively.[12]

***In vitro* drug release**

In vitro drug release of lutein loaded nanoparticles were performed in pH changing dissolution media using USP dissolution (type 1) apparatus in 900 ml of the dissolution medium, stirred at 50 rpm at 37 ± 0.2°C. The in vitro release profile of various batches was performed using two different dissolution media to mimic the GI condition. The dissolution were performed at 1.2 pH condition for 2 hrs than the pH condition were changed to basic pH by adding phosphate buffer into it till 6.8 pH for two hrs. The dissolution test was performed

for 4 hrs. Sample (10 ml) was withdrawn after every 30 min and replaced by an equal volume of fresh dissolution medium to maintain sink conditions. The withdrawn samples were filtered and were further analyzed using HPLC. Table 4 shows *in-vitro* release profile of drug from ethyl cellulose coated lutein loaded nanoparticles.

Statistical analysis

The statistical analysis was performed using Minitab 17 version. All the tests were according to the design of experiments (DOE). Half factorial design was applied on experimental analysis by choosing various dependent and independent variables. All the experiments were performed in triplicate and the results were expressed as mean ±S.D.

RESULTS AND DISCUSSION

Characterization of lutein loaded nanoparticles

Surface morphology

The TEM image of prepared lutein loaded nanoparticles is depicted in figure 3. The structure revealed spherical shape of the nanoparticles with a uniform distribution. Similar reports were obtained of spherical shaped gelatin nanoparticles by nanoprecipitaion method.[13] The image exhibited particles average particle size 490 nm. The particles size results as revealed by the TEM image were fairly in compliance with those obtained by Malvern zetasizer.

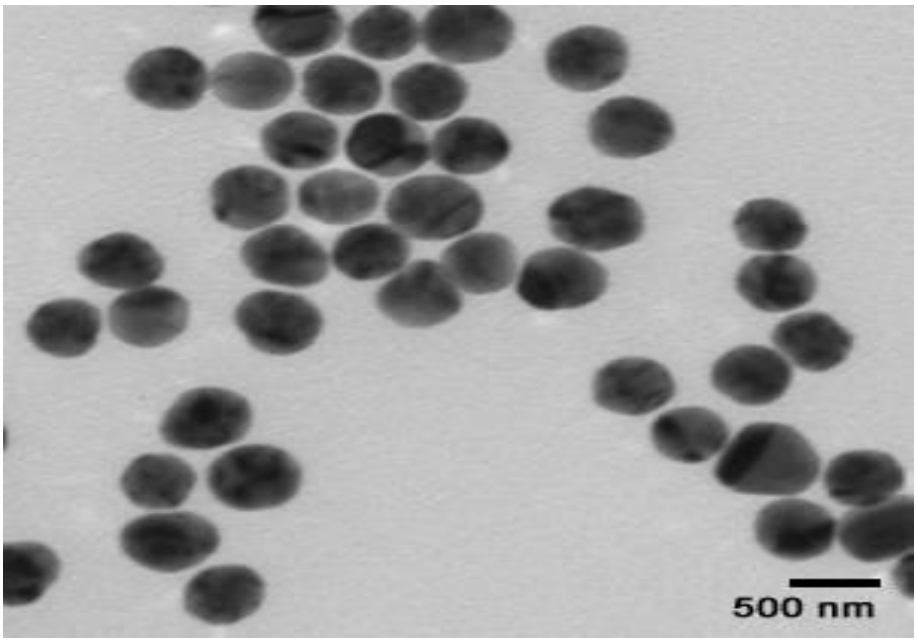

Figure 3: TEM image showing the morphological structure of lutein loaded nanoparticles.

Particle size and polydispersity index (PDI)

The particle size and PDI of lutein NPs were 490±3 nm, 0.039±0.03 respectively. Ali Hany S.M. et. al. prepared hydrocortisone nanosuspension by nanoprecipitaion technique and got nanoparticles in the range of 450 nm.[14]

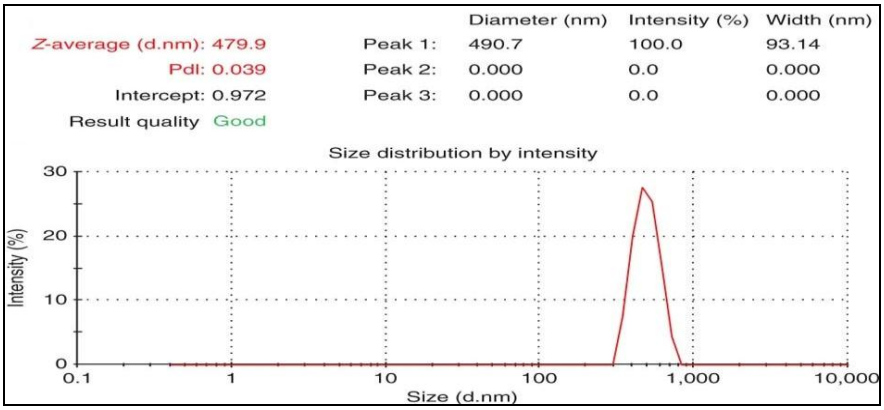

Figure 4: Particle size of prepared lutein loaded nanoparticles.

Zeta potential

Zeta potential of prepared lutein loaded nanoparticles were -23.9 ± 5.60 mV. This is significant number for zeta potential. The zeta potential of the nanoparticles was negative with value of about -24 which showed good stability of nanoparticles.[15]

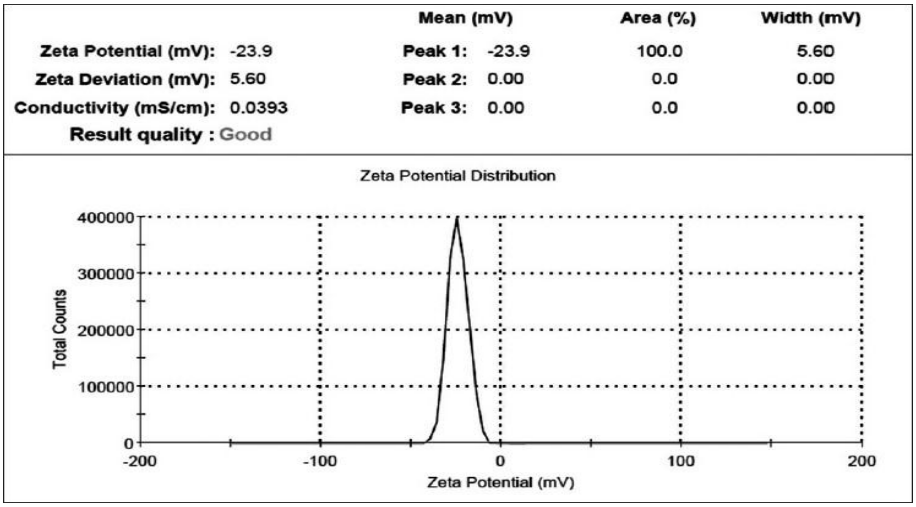

Figure 5: Zeta potential of prepared lutein loaded nanoparticles.

Drug encapsulation efficiency

Entrapment efficiency of lutein loaded nanoparticles was studied and was found to be in the range from 84.14 to 92.51.

In vitro drug release

Upon analysis of all nine formulations, F9 was showing higher solubility than other after dissolution in pH changing media from acidic to basic pH (till 6.8). Release percent of F3 was 88.05 and F2 was 92.51. Table 4 showed the percent release of different batches and figure 6 showed dissolution profile of all nine formulations. While F2 batch have great dissolution profile but its percentage release in acidic medium is more than 10 percent. But F3 batch great dissolution profile and in acidic medium release was less than 10 percent, it must be known that it may be possible because of the drug and polymer ratio and the surfactant has a great impact on formulation as seen in table 3 and formulation table 1.

Table 4: Release of all nine formulation batches of nanoparticles.

Time (min)	pH	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9
0	1.2	0	0	0	0	0	0	0	0	0
30		1.5	2.2	1.05	1.05	1.11	2.45	5.34	3.96	0.87
60		3.25	8.25	2.3	1.22	2.5	8.9	9.8	8	1.2
90		5.57	14.38	5.29	2.3	4.8	12.08	17.34	10.05	3.68
120		7.25	17	8.59	13.34	5.63	15.59	19.6	21.28	8.76
180	6.8	49	65	62.25	42.45	49	65.24	64.45	65.58	38.65
240		65	92.51	88.05	63.56	69.27	90.22	89.21	91.43	59.14

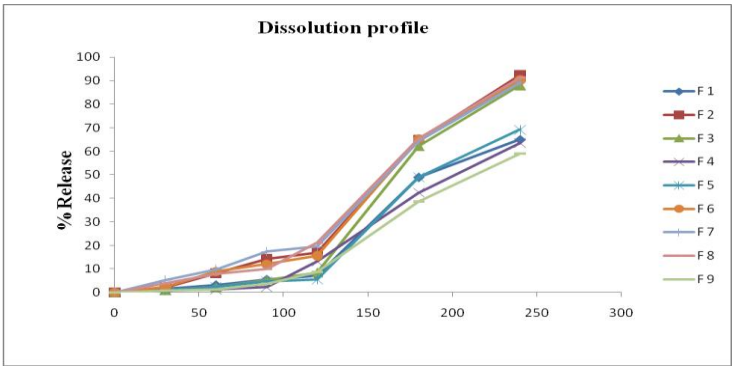

Figure 6: In-vitro release profile of lutein loaded nanoparticles.

Statistical analysis

The statistical analysis was performed using Minitab 17 version. All the tests were according to the design of experiments (DOE). Half factorial design was applied on experimental analysis by choosing various dependent and independent variables. Dependent variables were yield, assay and entrapment efficiency. Independent variables were concentration of polymer, concentration of surfactant. Figure 7 and 8 depicts the surface plot and contour plot between assay, lutein: polymer ratio and Lutein: stabilizer ratio, respectively.

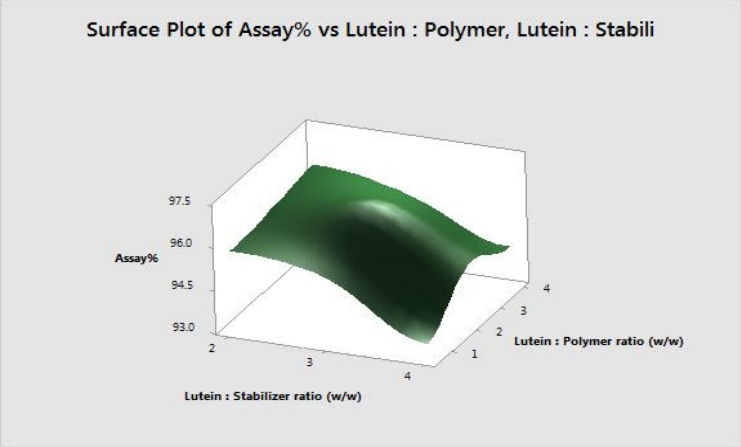

Figure 7: Surface plot between assay, lutein:polymer ratio and Lutein:stabilizer ratio.

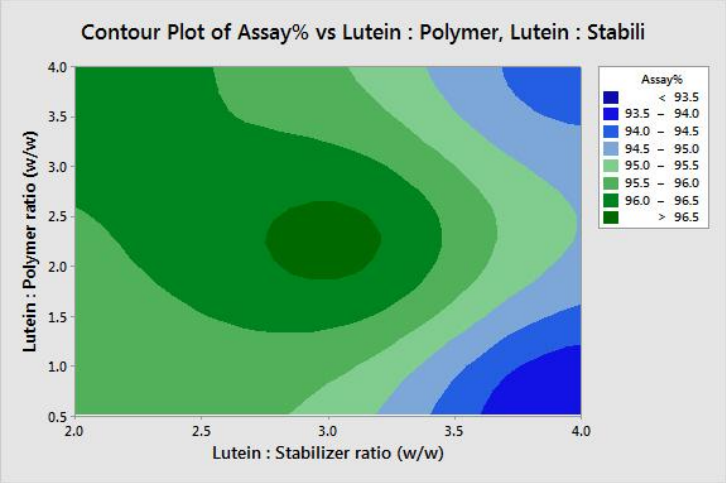

Figure 8: Contour plot between assay, lutein: polymer ratio and Lutein: stabilizer ratio.

CONCLUSION

The lutein loaded nanoparticles were successfully developed. The prepared formulation released the drug at intestinal pH of 6.8 where they could be better absorbed. Owing to its nano size, it seem to be a promising approach for age related macular degradation (AMD).

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