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ESTABLISHMENT OF ACRONINE LOADED EUDRAGIT-E NANOCARRIER FOR CANCER THERAPY

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ABSTRACT:

Acronine is a natural alkaloid molecule with an acridine structure that have potent power to stop the progression of tumor/cancer cells but its therapeutic potential for clinical purpose is significantly hindered by its poor solubility due to its hydrophobic nature. The recent study is focus to establish a novel natural polymeric platform of Acronine loaded Eudragit-E nanoparticles (AENPs) using nanoprecipitation technique. The AENPs were experimentally characterized for encapsulation potential, size distribution, morphology of nanoformulation, zeta potential, drug release rate evaluations, and FTIR. The drug or Acronine release profile was evaluated for *in vitro* antioxidant and anticancer potential. Experimentally found that AENPs possess particle size within the range of 20 - 45 nm and having encapsulation efficiency of 56.37%. Furthermore, Eudragit-E at 2.5 mg/ml and Pluronic at 0.6% w/w were competent to produce isolated and free flowing nanoparticles. The *in vitro* anti-cancer assay of AENPs demonstrated inhibition of cancer cells to a greater extent and induced programmed cell death in Hela cell lines as compared to drug particles alone. These findings suggest the utilization of acronine entrapped Eudragit-E nanoparticles as advance drug delivery tool to combat cancer.
Keywords: Acronine, Polymeric Nanoplatforms, Particle size, Anti-cancer activity

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INTRODUCTION:

Cancer is still among the various life threatening disorders that debilitate health of the person with very high global death rate [1]. Since years, a lot of natural and synthetic bioactive molecules are being utilized in chemotherapy for treatment of cancer. However, conventional chemotherapeutic molecules often lead to the establishment of drug resistance and neoplastic growth [2]. Malignancy is a group of ailments involving uncontrollable anomalous cell development with the possibility to invade different areas of the body. High dosage of the drug can havoc healthy normal cells with noticeable side effects [3]. Therefore, in order to combat cancer main focus is on designing such anti-cancer drugs, which would have low IC50 value to kill cancer cell by employing nanoformulation [4].

Nanoparticles involved natural colloidal range carriers molecules with particle size below 200 nm are potent pharmacological vectors for drug delivery to combat cancer effectively [5-7].Acronine have significant natural potency to combat various diseases because of their alkaloid nature [8]. Acronine exhibits diverse biological curative properties such as anti-inflammatory potential [9], hepatoprotective in nature [10], antioxidant action [11], antibacterial nature, antiviral activity [12] and anti-tumor actions [13]. But, its nonpolar hydrophobic nature and low solubility profile limit its clinical potential and efficiency [14]. However, improving drug release kinetics pattern of Acronine can render it beneficial in drug delivery potentials [15].

Eudragit-E, a copolymer is frequently employed as tablet coat material in preparation of sustained drug release medications. Butylated methacrylate copolymer (E100) is basically cationic nature copolymer that has a ability to be easily absorbed in the abdomen [16]. This property helps to enhance bioavailability of nonpolar drugs [17].

In the current investigation, polymeric nanoformulation of Acronine was designed based on Eudragit-E polymeric structure which leads to improve the water solubility as well as the bioavailability of the drug molecules.

MATERIALS AND METHODS:

Materials

Eudragit-E was procured from Evonik Rohm GmbH & Co, Germany. Acronine was obtained from Sigma Aldrich, India, and Minimum Essential Eagle Medium, Foetal bovine serum (FBS), penicillin & streptomycin were obtained from Himedia Laboratories Pvt. Ltd. Mumbai, India. Cell line (Hela-Human cervical carcinoma cell line) was purchased from National Centre for Cell Science (NCCS), Pune. All chemicals used in the present research were of analytical reagent grade.

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Preparation of Acronine loaded Eudragit-E nanoparticles (AENPs)

The Acronine polymeric nanoparticles were synthesized by nanoprecipitation approach [18]. 80 mg of Acronine and 80 mg of Eudragit-E were mixed in 40 ml of the methanol/organic phase. The drug-methanol solvent mixture was added drop wise into 100 mL aqueous phase or hydrophilic polar phase (0.6 % Pluronic water based solution), and the resulting solution was kept under magnetic stirring at 600 rpm for 4 h. After achieving complete solubility of drug and Eudragit-E, organic (nonpolar) phase solutions were used. Methanol component of organic phase was completely evaporated and centrifuged at 9000 rpm at 4°C for 30 minutes. The supernatant was collected and evaluated by UV spectrophotometer for the unbound drug molecule. The AENPs formed were then separated, rinsed two times with water (double distilled) and lyophilized.

In vitro release profile of AENPs

The dialysis sac method was utilized to study the drug release profile. Acronine (8 mg) entrapped Eudragit-E nanoparticles were kept in dialysis sac and positioned in water (20ml) and then immersed in 25% C_2H_5OH solution phosphate buffer (0.1 M) saline having pH 7.4 and constantly stirred at 80 rpm at a steady temperature of 37 °C [19]. One ml sample was withdrawn and collected at regular intervals of 1, 2, 3, 6, 12, and 24 h and evaluated by UV spectrophotometerat 300 nm.

Characterization of synthesized AENPs

Zetasizer Nano ZS-90 was employed to compute the average size range of nanoparticles and their size distribution [20]. The unbound drug concentration in the supernatant obtained after centrifugation at 7500 rpm 4˚C for 40 minutes was analyzed by UV spectrophotometer thereafter percent encapsulation efficiency was calculated using following formula:

Percent Entrapment efficiency = (Total Drug-Unbound Drug/Total Drug) × 100.

The morphologyof optimized batch of AENPs was examined by transmission electron microscope (TEM-Hitachi-H-7501SSP/N-817-0520, Japan). The optimized nanosuspension was initially loaded on a copper grid, hot air-dried and 60,000 magnification factor and 80,000V accelerating voltage was employed to capture the TEM image [21].FTIR analysis of Acronine, Eudragit-E, and AENPs was analyzed by Fourier transform infrared spectrophotometer (FTIR Affinity-1, Shimadzu, Japan) in range of 4500–500 cm^{-1}.

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Antioxidant activity

The antioxidant potential of Acronine, Eudragit-E, and AENPs was evaluated by DPPH assay. 1,1-diphenyl-2-picrylhydrazyl (DPPH), a free radical, was dissolved in methanol (3.9 mg/100 ml) [21]. The pure Acronine, Eudragit-E, and AENPs were incubated along with DPPH for 30 min in dark condition to evaluate the inhibition of DPPH and the absorbance was recorded by the UV spectrophotometer at 517 nm in triplicate. Blank Eudragit-E NPs was employed as a negative control while pure Acronine was used as positive control.

$$\text{Percent antioxidant activity} = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

In-vitro assay for cytotoxic activity (MTT assay)

Cell lines (both normal and cancer) were raised in artificial media supplemented with inactivated FBS (10%), 80µl/ml streptomycin antibiotic and 80µl/ml penicillin and incubated at 37^0C (310K), in 5% CO_2 enviorment. After attaining 70-75% confluence, the cells were subcultured in 0.25% trypsin enzyme based solution under sterile conditions [23]. The seeding of cells was performed in 96 – well plates (5×10^3 per 100µl per well). The density or growth pattern was evaluated for each cell line on the basis of growth kinetics. The wells were treated, after 8 h incubation, with different concentration of AENPs (0.1-1000µg/ml) & acronine for three days (in triplicate). Subsequently, after three days of treatment, the medium was replaced by 3µl of MTT solution (5mg/ml) and incubated for 3 h. The percent of metabolically active cells were compared with untreated controls, on the basis of MTT assays. Formazan crystals dissolved in DMSO and its absorbance was calculated by microplate reader at 570 nm. The anticancer activity of AENPs was evaluated using pure Acronine as the standard by MTT assay against cell line Hela (Human cervical carcinoma cell line). The percentage cell growth inhibition (1) or percentage cytotoxicity (2) was calculated by following formula:

$$\textbf{\%viability} = (A_{Tr} - A_{Bl}) / (A_{Ct} - A_{Bl}) \times 100 \ldots\ldots\ldots (1)$$

Where A_{Tr} = Absorbance for treated cells (drug); A_{Bl} = Absorbance for blank

A_{Ct} = Absorbance for control (untreated)

%cytotoxicity = 100 – Percent cell survival (%) ……. (2)

RESULT AND DISCUSSION:

Optimization of the formulation variable

A nanoprecipitation approach was employed to prepare AENPs. During research outcomes demonstrate that both, Eudragit-E and pluronic influenced the size of particle as well as encapsulation efficiency of nanoparticles.

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The minimum particle size (140-213 nm) with greatest entrapment efficiency (51-58%), Eudragit-E 2 mg/ml and pluronic 0.6% (Fig, 1). Eudragit-E natural polymer has already been employed for encapsulating various therapeutic molecules. Increase in Eudragit-E concentration enhances entrapment efficiency to greater extent. As we find at low concentration of pluronic Eudragit-E, the encapsulation efficiency was less. The reason could be the low polarity of both Acronine and Eudragit-E, thus establishing a strong attraction between Acronine and Eudragit-E. Pluronic at (0.6%w/w) concentration was most favorable to increase entrapment efficiency [24].

Particle size and Zeta potential

AENPs were analyzed for particle size and zeta potential measurements. The prepared nanoparticles size ranged from 418 to 140 nm (Fig 1). AENPs had a zeta potential value of -45.5 mV (Fig 2), signifying relative stability of prepared nanoformulation.

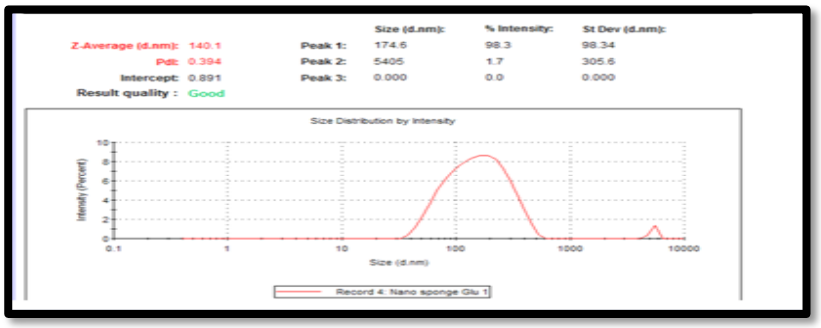

Figure 1. PSA image of AENPs

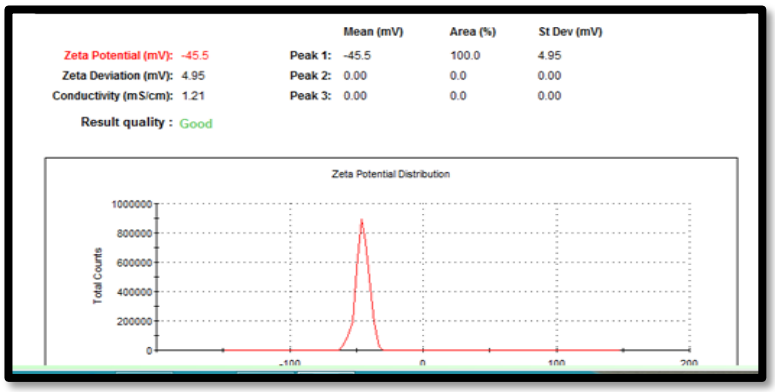

Figure 2. Zeta potential of AENPs

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In vitro release profile of AENPs

The rate controlled the discharge of drug from nanoparticles polymeric matrix shields it from rapid metabolism and degradation [25]. The *in-vitro* drug release data suggested that 84.63% of the pure Acronine was released within 3 hours. But a sustained release was observed in AENPs with 39.61% and 78.23% Acronine released after 3 h and 24 h respectively. Drug release profile of AENPs demonstrated a sustained release of Acronine with the passage of time, because of their hydrophobic (nonpolar) nature of Acronine. This is due to a cage like thick and strong walled dense matrix structure formed by Eudragit-E that made a cover around the Acronine particles, which assures its sustained release **(**Fig 3).

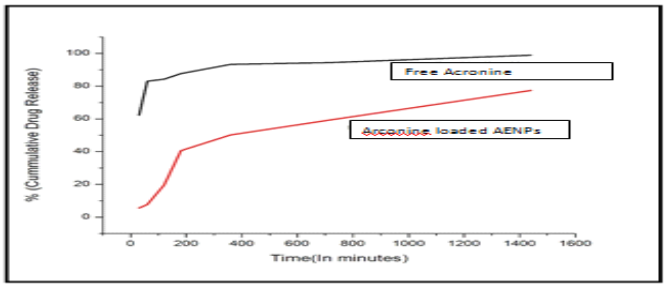

Figure 3. In vitro drug release of Acronine and AENPs

Percentage encapsulation efficiency

The encapsulation efficiency depends upon the nature of the method employed, the extent polarity of the molecule, the molecular nature of encapsulating materials and media for the synthesis of nanoparticles. The percentage of encapsulation efficiency was 61.22% respectively for Acronine. Acronine is hydrophobic in nature [26]. Due to the low dielectric constant of Eudragit-E, that creates a strong interaction between Eudragit-E and Acronine that resulted in increased encapsulation efficiency of the drug in Eudragit-E.

Morphological characterization of AENPs by TEM

The size and dimension of nanoparticles alters release rate, solubility rate and dissolution rate of a molecule/drug [27]. Nanoparticles migrate to different body parts, according to their shape and size [28]. The AENPs were found to be segregated, nearly spherical with a particle size range of 18 - 35 nm (Fig 4). A variation was recorded in the nanoparticles size estimated by PSA and TEM. This is because PSA principle works on the ionic environment of the particle, whereas TEM computes the particle dimension in the isolated atmosphere [29].

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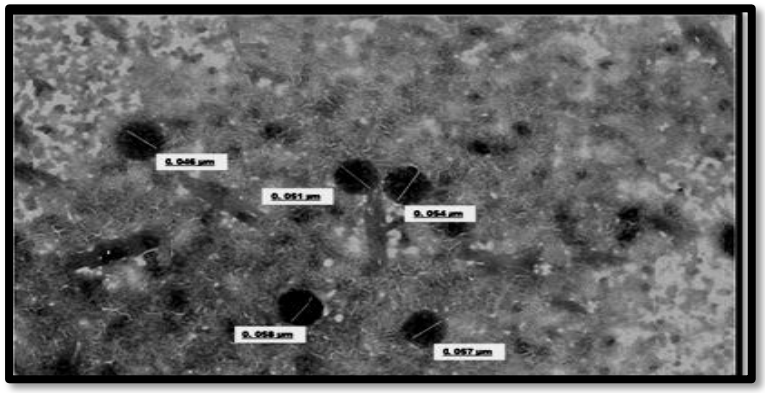

Figure 4: TEM image of AENPs

FTIR Analysis of Drug Samples

The FTIR spectroscopy was used to infer Acronine-Eudragit-E interaction studies [30] as well as to confirm the loading of Acronine in AENPs [29]. FTIR spectrum of Acronine shows absorption bands at 3321.85 cm $^{-1}$ for -OH represent intermolecular H- bonding and 2867.23 cm $^{-1}$ & 2268.06 cm $^{-1}$ showing stretching bond for the terminal $-CH_3$ groups (Fig 8 B). FTIR spectrum of Eudragit-E is represented in Figure 5. Variation was found among wave numbers 1274 cm^{-1} and 1154 cm^{-1} in AENPs due to the formation of weak intermolecular bonds such as dipole-dipole interaction, and weak Van der Waals (depends upon molecular mass). Eudragit-E and Acronine reveal characteristic peaks in FTIR spectrum. Although peak intensity was decreased, bands were not shifted, signifying there is no chemical bond among Acronine and Eudragit-E. Figure 8C represented the FTIR spectrum of the physical combination of Acronine and Eudragit-E (AENPs).

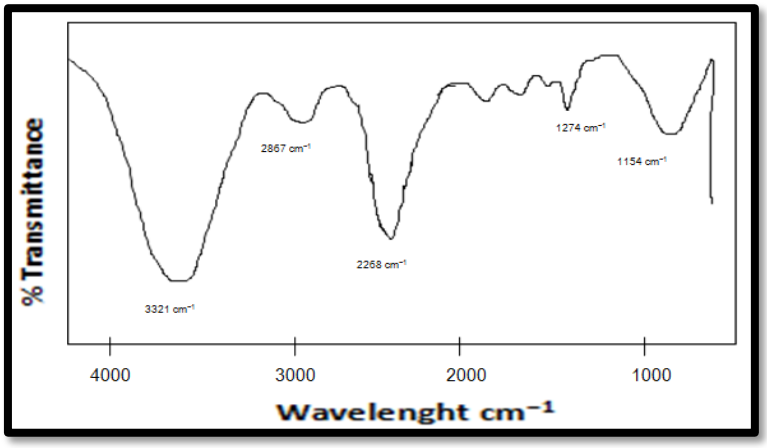

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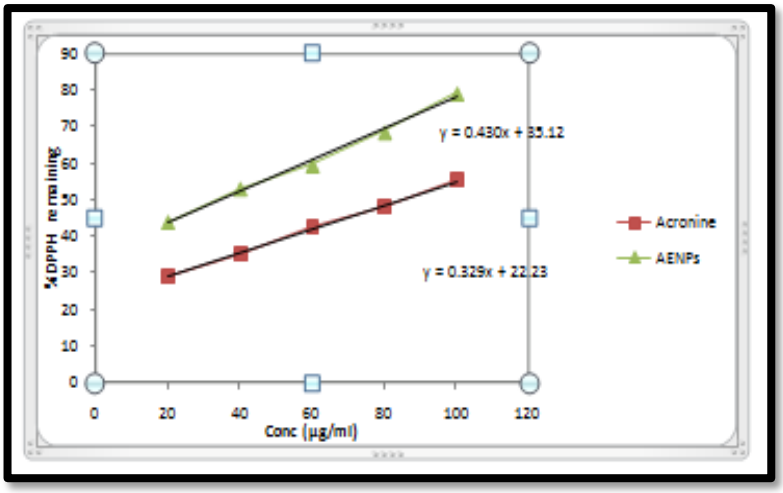
Figure 5. The FTIR spectra of AENPs

Anti-oxidant activity

The DPPH analysis is frequently used to find out the antioxidant activity of encapsulated molecules [31]. 1,1-diphenyl-2-picrylhydrazyl is a stable free radical having spare electrons delocalized over the whole molecule, giving a deep violet coloration. It shows an absorption band near 517 nm [32]. The disappearance of violet color is observed when a DPPH solution is uniformly mixed with a molecule that can donate (oxidizing nature) a hydrogen atom. The Acronine is a well known antioxidant molecule. The solution of Acronine was incubated with DPPH (a hydrogen atom donor) and change of violet color to pale yellow was seen. Hence, absorbance band was reduced. The AENPs exhibited lower inhibition of DPPH compared to the pure Acronine, due to the delayed release of loaded Acronine particles in them. The encapsulation of Acronine by Eudragit-E might due to size in nanometric scale which expose more surface area resulting in enhanced antioxidant activity as compared to the pure drug (Fig 6).



Figure; 6 Antioxidant activity of pure Acronine and AENPs

Anti-cancerous activity

The Acronine and AENPs were screened for anticancer potent actionagainst Hela cells lines through MTT assay and the outcomes are depicted in Table 1. The larger surface area of nanoformulations is mainly responsible for the better cytotoxic efficacy [33, 34]. The nano-sized nanoparticles can have potential to enter deep inside the cancer cells more efficiently [35-37]. The anti-cancer potential of Acronine is already evaluated in the various findings [38], taken as a positive control displayed linear cytotoxic response against Hela cell lines with respect to

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concentration. Mitochondrial dehydrogenase enzyme exists in viable cells. It cleaves tetrazolium ring structure of MTT dye (Pale yellow in color) [39] forming dark purple crystals of formazan (impermeable to cell membranes) that accumulate in the cells [40-42]. The AENPs killed the Hela cells more effectively than the free Acronine alone (Fig 7, 8). IC50values (µg/ml) of synthesized nanoformulations along with pure drug Acronine are given in Table 1. These results demonstrate that AENPs exhibit potent anticancer effect with IC50 of 7.2 µg/ml, which was more pronounced than pure Acronine particles with IC50 of 32.4 µg/ml against Hela cell lines.

Sample Code	Hela	
	IC50	pIC50
Acronine	32.4	-1.515
AENPs	7.2	-0.8573

Table 1: IC50 values of AENPs along with pure drug acronine

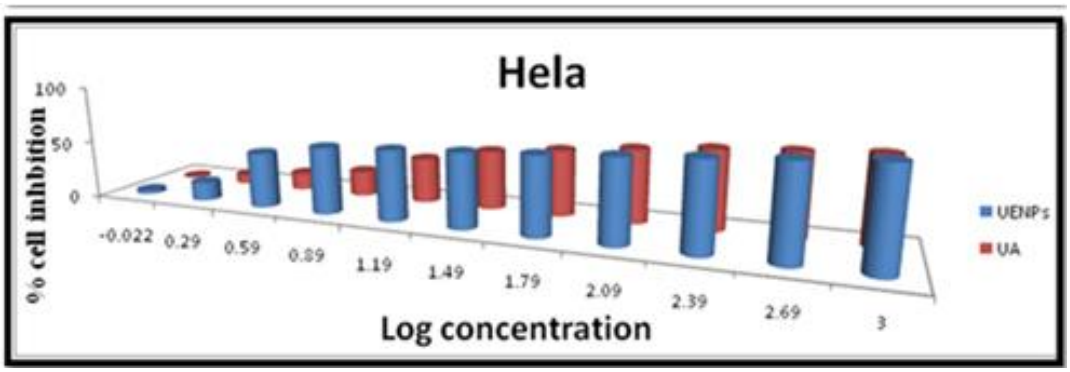

Figure. 7 MTT assay on Hela cell line

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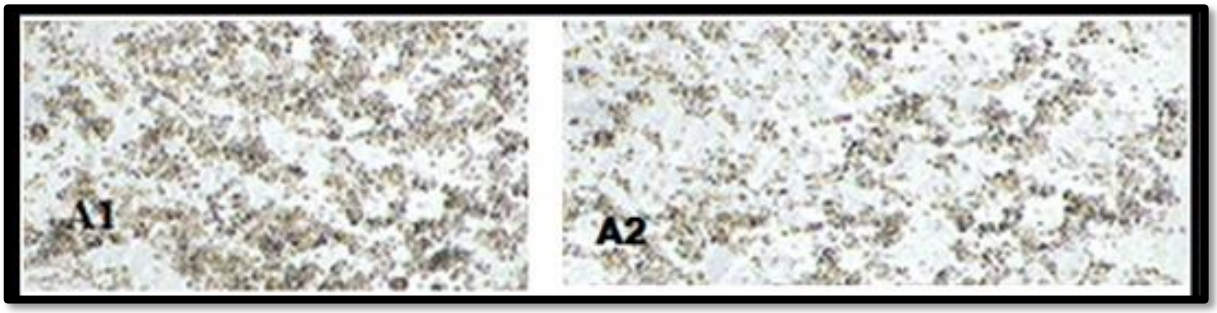

Figure 8 Optical microscope images of Cytotoxic effect of Acronine (A1), and AENPs (A2), on towards Hela cell lines after 24 h

CONCLUSIONS:

Nanotechnology has emerging technology to develop formulation that combats various diseases. A lot of novel nanoformulations are market zed these days to fight cancer cells. The present research demonstrates the preparation and evaluation (both qualitatively and quantitatively) of *in vitro* release rate, *in vitro* antioxidant potential and anticancer actions of polymeric nanoformulation of acronine loaded Eudragit-E nanoparticles. Eudragit-E polymer was used as nanocarrier due to it's highly safe action, biocompatible-biodegradable in nature and generally recognized as safe (GRAS) status by the Food and Drug Administration for human consumption and applications. The variations in nanoformulation size and drug entrapment efficiency were experimentally alters by changes in polymer concentration. Acronine is an anti tumour molecule, having ability to establish novel nanoformulation exhibit powerful excipients in order to enhance its water solubility, bioavailability and therapeutic action. The polar phase in this nanoformulation contains an high density aqueous solution with surfactant molecule present in it. The surfactant molecules aid penetration of acronine into nanoparticles so as to produce polymeric nanoplatform to efficient drug delivery. This, novel polymeric nanocarrier of acronine loaded Eudragit-E nanoparticles might serve as promising future to fight various cancer cells and holds a lot of potent power for preclinical and clinical trials in near future.

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