DEVELOPMENT AND EVALUATION OF BIOACTIVE QUERCETIN FOR ACTIVE TARGETING IN SKIN CANCER USING METAL NANOPARTICLES

Shweta Gupta[1], Khomendra Kumar Sarwa[2*], Manmohan Singh Jangdey[3]

Abstract

The present study aimed to the preparation of bioactive Quercetin copper-conjugated bromelain nanoparticles in gel form for the treatment of skin cancer. The Quercetin nanoparticles have been prepared by using polyacrylamide and Tween 80 as surfactant. Synthesized nanoparticles were evaluated for drug entrapment efficiency and particle size and further proceed to prepare metallic nanoparticles. Quercetin copper nanoparticles have been prepared by dissolving copper nitrate in 50 ml deionized water heated to 60-70ºC in a conical flask. Copper nitrate solution was added dropwise in the Quercetin nanoparticle till the color changed under a magnetic stirring followed by centrifugation at 6000 rpm for 10 min. Pellet was separated from the supernatant, and washed with deionized water. Dried at room temperature and kept at -20ºC. In the next phase, the Quercetin copper nanoparticle was bioconjugated with bromelain and subsequently evaluated for entrapment efficiency, particle size, zeta potential, and XRD study. Bioconjugated Quercetin-loaded copper nanoparticles were dispersed in Carbopol 934 gel base and characterized by measuring pH, viscosity, gel strength, extrudability study, and spreadability. *In vitro,* release studies & skin permeation have been determined using Franz diffusion cell. The MTT assay has been analyzed against HaCaT (immortalized human keratinocytes) cell lines, to determine their anti-cancer potentials. The conjugated formulation was found to decrease cell viability and higher skin targeting efficacy in *in-vitro* studies. The metal-conjugated gel of Quercetin is an efficient and economic approach for the treatment of skin cancer.

Keywords: Quercetin, Copper nanoparticle, HaCaT cell line, Skin cancer, Bromelain.

[1*,3]Shri Rawatpura Sarkar College of Pharmacy, Shri Rawatpura Sarkar University, Dhaneli, Raipur, Chhattisgarh, India

[2*]Department of Pharmacy, Government Girls Polytechnic, Byron Bazar, Raipur, Chhattisgarh, India

[*]**Corresponding Author:-** Dr. Khomendra Kumar Sarwa

*Department of Pharmacy, Government Girl's Polytechnic Byron Bazar, Raipur, Chhattisgarh, India 492001

Email: khomendra.sarwa@gmail.com

DOI: - 10.48047/ecb/2023.12.si5a.014

INTRODUCTION

India along with the majority of the urban population as well as developed countries today is at war against several diseases like AIDS, cancer, malaria, tuberculosis, etc. The status of the treatment of these diseases is still an area to be explored and prevention of these diseases continues to be a challenge for society and mankind. Among them, cancer is the second cause of mortality in the universe and every year about 10 million new cases of cancer are arising. Cancer is a life-threatening disease, in which cells metastasize in hysterical manner and attack to surrounded tissues, extending to the whole body parts by the lymphatic system and blood [1,2].

Usually, cells are divided as per body requirements and die after a certain time of cell division. When cancer develops, the normal death process of cells gets interrupted and new cells are formed without the needs of the body. These superfluous cells grow unrealistically and form a solid mass of tissues (except blood cancer) called tumors[3][4].

Skin cancer, which is the most prevalent malignancy and accounts for one out of every three cancer diagnoses, has also experienced an increase in the local application of therapies (WHO 2020). According to the Centers for Disease Control and Prevention (CDC 2020), skin cancer is the most common type of cancer in the US, with one in five people getting it at some point in their lives (WHO 2020). Melanoma is a type of skin cancer, caused by ultraviolet radiation and sometimes genetic defects. It is initiated by melanocytes (pigment-producing) in the basal layer of the epidermis. Most melanomas are black or brown, but in some cases, they may be skin-colored, pink, red, purple, blue, or white. Melanoma grows mostly in the back area, legs, arms, and face. It may also find and grow in the soles of feet, the palm, fingernail beds, eyes, and internal organs such as intestines, etc.

At the initial stage, the treatment of melanoma becomes essential otherwise it spreads to other parts of the body and is very difficult to treat and even it may cause death. An abnormal mole also indicatesmelanoma or skin cancer, it is recognized by (ABCDEs) 'asymmetric' shape of mole (A), irregular 'border' on moles (B), bumpy 'color' of moles (C), 'diameter' of mole (D) and 'evolving; of the moles (E) means to change the size of the moles with time and it may bleed or cause itching. Melanoma is mainly three types: (a) Superficial spreading melanoma, which covers about 70% of case of melanoma and most commonly occur between the ages of 30-50. It developes mainly in the legs of the women and the back area of the men. (b) Nodular melanoma, it spreads more rapidly compared to others. Untreated superficial spreading melanoma may become nodular and invasive. (c) Lentigo melanoma arises in the face and develops slowly. It seems like a rough shape, with large or colored patches [5].

Quercetin is a plant flavonol belonging to the polyphenolic flavonoid family. It is present in a wide variety of fruits, vegetables, leaves, seeds, and grains; common foods having significant levels of it include capers, red onions, and kale (Micronutrient Information Center 2018; USDA Database 2011). It is a dietary flavonoid regarded as the foundation for many other flavonoids. It has many pharmacological properties, including cardioprotective, antioxidant, analgesic, antiviral, antidiabetic, anticancer, immunosuppressor, and antithyroid.

The anticancer activity was exhibited by several mechanisms, including the elimination of free radicals, interaction with estrogen receptors, proteins, and transcription factors, inhibition of cell proliferation, induction of apoptosis, and decreased expression of metalloproteinases 2 and 9 [6]. Although Quercetin has many therapeutic benefits, its use is restricted due to several drawbacks including poor aqueous solubility, poor permeability, hydrophobic nature and low bioavailability. Therefore, novel drug delivery systems such as nanoparticles can be created for Quercetin to enhance these properties [7].

Nanocarriers have been demonstrated to increase the solubility and bioavailability of poorly water insoluble drugs [8]. Nanoliposomes and superparamagnetic iron oxide nanoparticle systems, which are not only promising and effective for improving the aqueous solubility of Quercetin but also have the ability for controlled drug release and precise targeting which have been reported previously [9][10].

Nanovesicular carriers have been used to deliver drugs to target sites for cancer therapeutics [11]. These lipid nanocarriers are adsorbed into the skin surface, allowing lipid exchange between the outermost layers of the stratum corneum [11][12]. In the segment of nanocarriers, a metallic nanoparticle has great importance due to long-term stability and easily tailoring properties.

Especially copper nanoparticle are selectively uptaken by epithelial cells and provides site-specific delivery if attached to the ligands like bromelain. In this research work, bromelain conjugated Quercetin-loaded copper nanoparticle gel formulation was developed and evaluated against skin cancer cell lines as well as physically characterized onvarious physical parameters.

MATERIALS AND METHODS

Materials

Quercetin was purchased from Sigma Aldrich. Tween 80, [1-Ethyl-3,3-(dimethyl aminopropyl carbodiimide)] (EDC)], Phospholipids, Polyacrylamide, Copper nitrate, Isopropyl alcohol, PEG (Polyethylene glycol), PG (Propylene glycol), Carbopol, Triethanolamine were purchased from Himedia Pvt, Ltd. Mumbai, India. N-Hydroxysuccinimide (NHS) was supplied as a gift sample from Shivam Enterprises, Pune, Maharashtra (India). Purified water from Millipore ultrapure water system (Synergy UV water purifier system, India) was used throughout the study. All other chemicals used in the study were of analytical grade.

Chemicals for cell lines and culture

The HaCaT cell lines were obtained from the National Center for Cell Science (NCC), Pune, India.3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide (MTT), DMSO (Dimethyl sulfoxide) was purchased from Sigma Aldrich Ltd.(India), and the cells were incubated in a carbon dioxide incubator.

Methods

Preparation of Quercetin nanoparticles and Quercetin copper nanoparticles

An ethanolic solution containing Quercetin (1.2 mg/ml) i.e.,12mg Quercetin in 10ml ethanol, Polyacrylamide (1gm in 100ml distilled water), and Tween 80 (1gm in 100ml distilled water) was dissolvated by stirring at 200 rpm and 30 ±1°C using distilled water [13].

The extent of dissolvation was controlled by absorbance measurements at 257 nm. The resultant turbid dispersion was again stirred vigorously at 500 rpm for 10 min and finally dried at 30°C by applying it as a thin film on glass plates.

Quercetin nanoparticles were obtained and stored for further study in a dry place. Composition of the nanoparticles is reported in Table 1.

Quercetin copper nanoparticles were prepared by taking about 10 mg of copper nitrate dissolved in 50 ml of deionized water heated to 60-70ºC in a conical flask [14]. Copper nitrate solution was kept in a burette and added dropwise in the Quercetin nanoparticle till the colour changed under a magnetic stirrer at 800 rpm for 30 min. The solution was then centrifuged at 6000 rpm for 10 min. Pellet was separated from the supernatant, and washed with deionized water. Dried at room temperature and kept at -20ºC.

Bioconjugation of Quercetin copper nanoparticle with bromelain

Quercetin copper nanoparticles [($1 mgml^{-1}$) i.e., 1mg of Quercetin copper nanoparticles in 1ml of ethanol] were suspended in phosphate buffer saline pH 5.5 and incubated in the dark with [1-Ethyl-3,3-(dimethyl aminopropyl carbodiimide) (EDC) (1mL of 0.1 M) i.e., 1.5524gm] for 30 min, at room temperature[15]. Immediately, the sample was mixed with N-hydroxysuccinimide (NHS) [(1mL of 0.11 M) i.e., 1.26599gm] for 6 h, at room temperature incubated in dark. The sample was then washed several times with Millipore water and filtered. To the filtrate, bromelain was added at a concentration of 100µg ml^{-1} (10mg in 100mL solvent) in phosphate buffer saline, under the condition of overnight stirring. The filtrate was again centrifuged for 20 min at 12,000 rpm. Excess linking reagents and soluble by-products were removed by washing thrice with 1ml of phosphate buffer saline. Finally, the bromelain conjugated Quercetin copper nanoparticle was lyophilized further to get the dry sample.

Evaluation of Quercetin copper nanoparticle with bromelain

Entrapment efficiency (%) determination

Entrapment efficiency (EE) of Quercetin copper nanoparticles with bromelain was determined by measuring the concentration of unentrapped drug in an aqueous medium by centrifugation method [16] [17].

The nanoparticles were centrifuged in a high-speed cooling centrifuge (C-24, Remi) using Nanosep centrifuge tubes with ultrafilter having molecular weight cutoff 100 KD (Pall life sciences, India) at 5,000 rpm for 15 min at 4ºC, and the supernatant was separated.

The amount of drug in the supernatant was determined using a UV-VIS spectrophotometer (Labindia 3000^{+}) after appropriate dilution. The

percentage entrapment efficiency was calculated by using the following formula:

Entrapment efficiency (%) = Mass of a drug in nanoparticle / Mass of a drug in the formulation X 100

Measurement of mean particle size

The mean size of the nanoparticles was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer (Malvern Instruments) at a scattering angle of 90°. A sample (0.5mg) of nanoparticles suspended in 5ml of distilled water was used for the measurement [18] [19].

Preparation of conjugated nanoparticle-based gel

Dispersing the 1gm of the Carbopol 934 in a sufficient quantity of Millipore distilled water [20]. After complete dispersion, the Carbopol 934 solution was kept in the dark for 24 h for complete swelling. Afterward, bromelain conjugated Quercetin copper nanoparticle was gradually added to the viscous solution of Carbopol 934 with magnetic stirring. The pH values were subsequently regulated to 5–6. Then other ingredients like isopropyl alcohol [(5% wt/wt) i.e., 5gm isopropyl alcohol in 100gm solution], PEG-400[(5% wt/wt) i.e., 5gm of PEG-400 in 100gm of solution], PG [(5% wt/wt) i.e., 5gm of PG in 100gm solution] and triethanolamine (0.5gm) were added to obtain a homogeneous dispersion of gel.

Characterization of conjugated nanoparticle-based gel

pH measurements

The pH of selected optimized formulations was determined with the help of a digital pH meter (Eutech Eco Testr Ph1) [21]. Before each measurement of pH, the pH meter should be calibrated with the help of a buffer solution of pH 4, pH 7, and pH 9.2. After calibration, the electrode was dipped into the gel, and pH of the selected formulation was measured and readings shown on display were noted.

Determination of gel strength

The method by which the properties of the polymeric system may be conveniently determined is the texture profile (TA-XT2 Texture analyzer). The experiment was done by placing the gel in a standard beaker below the probe. An analytical probe was then immersed into the sample [22]. The Texture Analyzer was set to the 'gelling strength test' mode or compression mode with a test speed of 1.0 mm/s. An acquisition rate of 50 points per second and a trigger force of 5gm were selected. An aluminum probe of 7.6 cm diameter was used for all the samples.

The study was carried out at room temperature. The force required to penetrate the gel was measured as gel strength in terms of gm.

Drug content

An accurately weighed amount of gel formulation equivalent to 10mg Quercetin of prepared gel formulation was taken in a beaker and added 10ml of methanol [23]. This solution was mixed thoroughly and filtered using a 0.45µm membrane filter. Then 0.1mL of filtered solution was taken in a 10mL capacity volumetric flask and the volume was made up to 10mL with methanol, this solution was analyzed using UV-VIS Spectroscopy.

Extrudability study

Extrudability was based on the quantity of the gel extruded from the collapsible tube on the application of a certain load. More the quantity of gel extruded shows better extrudability. It was determined by applying the weight on the gel-filled collapsible tube and recording the weight on which gel was extruded from the tube.

Spreadability

Spreadability of the formulation is necessary to provide a sufficient dose available to absorb from the skin to get a good therapeutic response. An apparatus in which a slide fixed on a wooded block and upper slide has movable and one end of the movable slide is tied with a weight pan. To determine spreadability, placing 2-5gm of gel between two slides and gradually weight was increased by adding it to the weight pan, and the time required by the top plate to cover 10 cm upon adding 80gm of weight was noted. Good spreadability shows lesser time to spread.

In vitro drug release study

The *in-vitro* diffusion study was carried out by using a Franz diffusion cell. The egg membrane was taken as a semi-permeable membrane for diffusion [24]. The Franz diffusion cell has a receptor compartment with an effective volume of approximately 60mL and an effective surface area of permeation of 3.14 cm^2. The egg membrane was mounted between the donor and the receptor compartment. A 2 cm^2 size patch was taken and weighed then placed on one side of the

membrane facing the donor compartment. The receptor medium was phosphate buffer pH 7.4.

The receptor compartment was surrounded by a water jacket to maintain the temperature at 32 ± 0.5°C using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by Teflon coated magnetic bead which was placed in the diffusion cell.

During each sampling interval, samples were withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The sample withdrawn was analyzed by a UV-VIS Spectrophotometer.

Release kinetics from the Quercetin copper nanoparticle conjugated bromelain gel formulation was compared to different kinetic models. The models studied were: Zero order rate kinetics, first-order rate kinetics, and Higuchi's kinetics.

In this kinetic behavior, the graph is plotted between the cumulative percent release concerning time. In Korsmeyer-Peppas exponential kinetics, the graph is between the log of cumulative percent drug release concerning time [25].

MTT Assay

MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] is a pale-yellow substrate that is cleaved by living cells to yield a dark blue formazan product.

This process requires active mitochondria, and even freshly dead cells do not cleave a significant amount of MTT. Thus, the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods.

The assay was performed using the standard operating procedures. Briefly, the compounds were dissolved in DMSO (Dimethyl sulfoxide) and serially diluted with a complete medium to get the concentrations a range of test concentrations. DMSO concentration was kept < 0.1% in all the samples, HaCaT cells maintained in appropriate conditions were seeded in 96 well plates and treated with different concentrations of the test samples and incubated at 37 °C, 5% CO_2 for 96 h.

MTT reagent was added to the wells and incubated for 4 h; the dark blue formazan product formed by the cells was dissolved in DMSO under a safety cabinet and read at 550 nm. Percentage inhibitions were calculated and plotted with the concentrations used to calculate the IC_{50} values [26].

RESULTS AND DISCUSSION

The mean size of the nanoparticles was determined by photo correlation spectroscopy (PCS) on a submicron particle size analyzer (Malvern particle size analyzer) at a scattering angle of 90°. A sample (0.5mg) of the nanoparticles suspended in 5ml of distilled water was used for the measurement.

The results of measurement of the mean particle size of formulations of nanoparticles were found to be 85.65 to 124.02 nm.

The drug entrapment of all formulations was determined spectrophotometrically. The drug entrapment efficacies of different formulations were in the range of 67.85 to 74.65% w/w. Results demonstrated that an increase in the concentration of polymer increased the entrapment of the drug. The drug entrapment efficiency was found more than 65% in all the formulations (**Table 1**) which is considered acceptable.

Table 1: Results of particle size and entrapment efficiency

Code	Polyacrylamide	Tween 80	Stirring Speed	Particle Size (nm)	Entrapment Efficiency (%)
QCNF1	0.5 gm	1.5 gm	500	123.65	74.65
QCNF2	1 gm	1 gm	500	120.47	67.85
QCNF3	1 gm	2 gm	500	85.65	73.35
QCNF4	1.5 gm	1.5 gm	500	124.02	71.95

QCN : QUERCETIN COPPER NANOPARTICLE

Response surface plots for particle size

The response surface diagrams are known to facilitate an understanding of the contribution of the variables and their interactions (**Figures 1, 2, 3, and 4**). The Contour graph of particle size is shown in **Figures 5 and 6**.

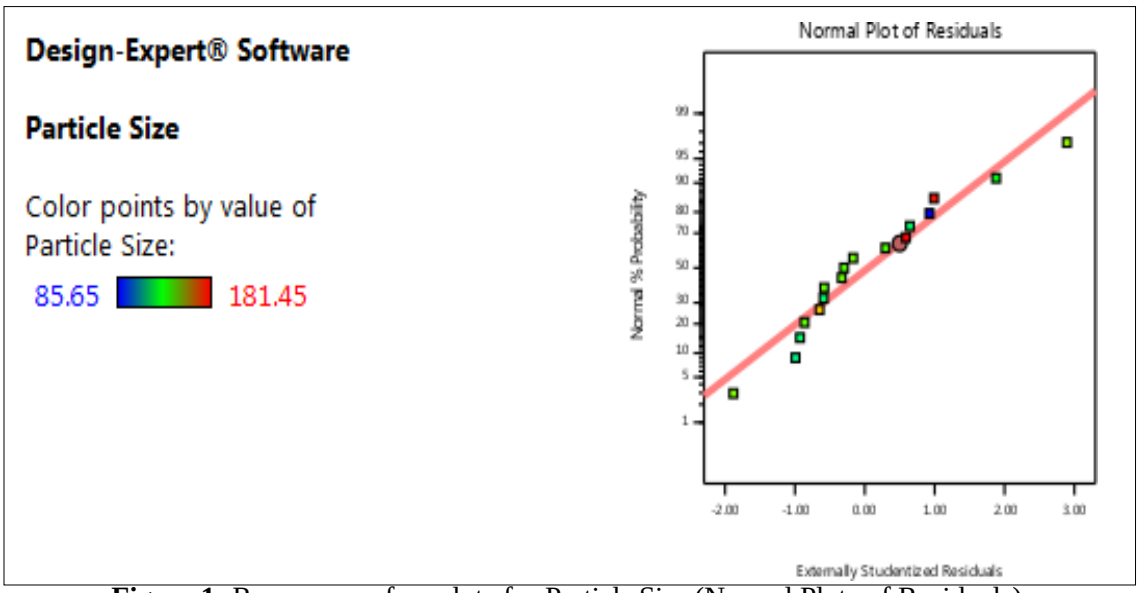

Figure 1: Response surface plots for Particle Size (Normal Plots of Residuals)

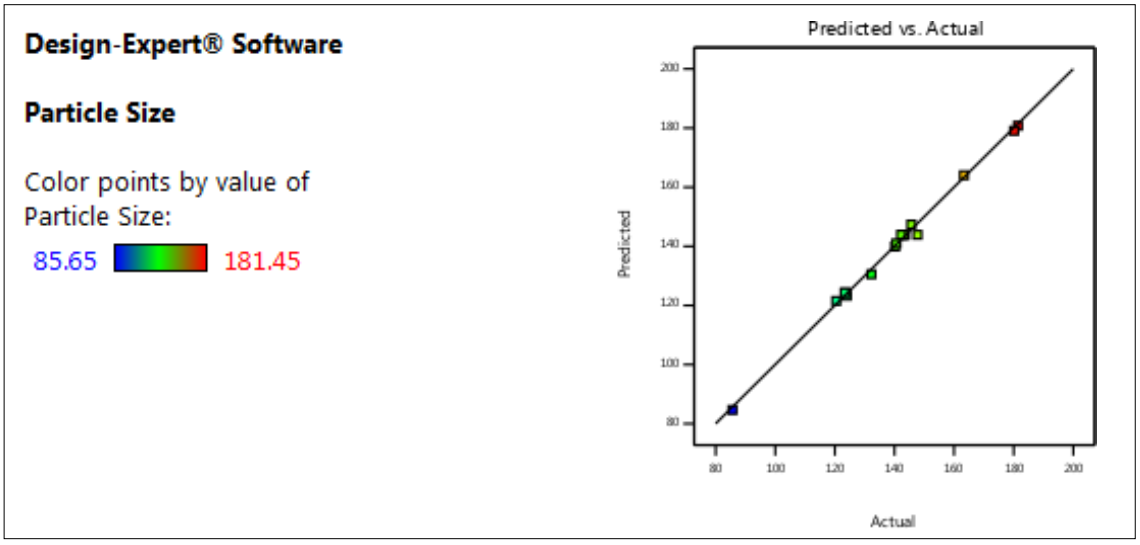

Figure 2: Response surface plots for Particle Size (Predicted vs. Actual)

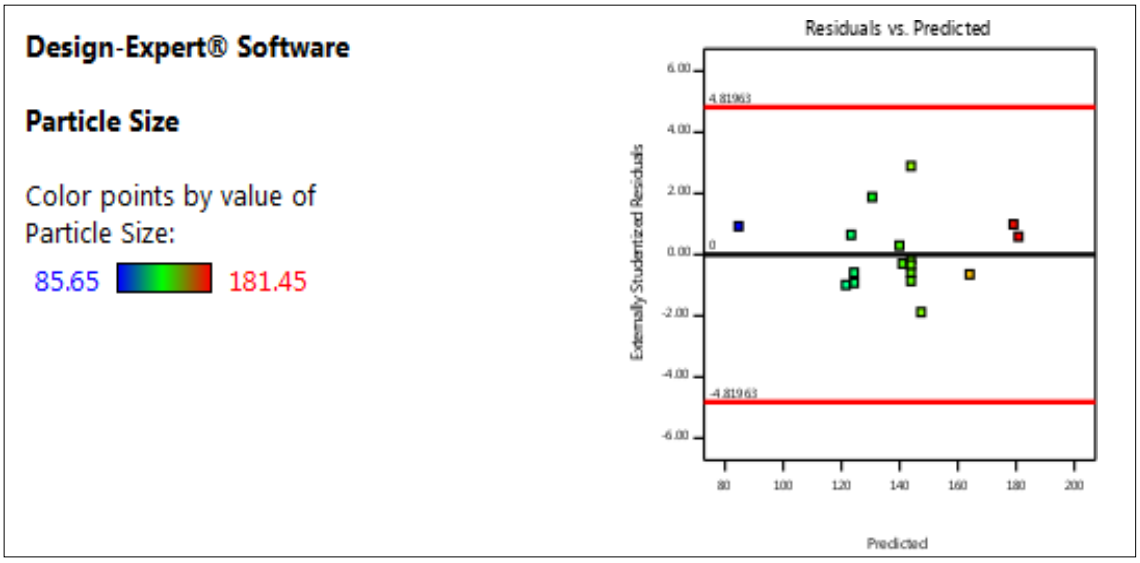

Figure 3: Response surface plots for Particle Size (Residual vs. Predicted)

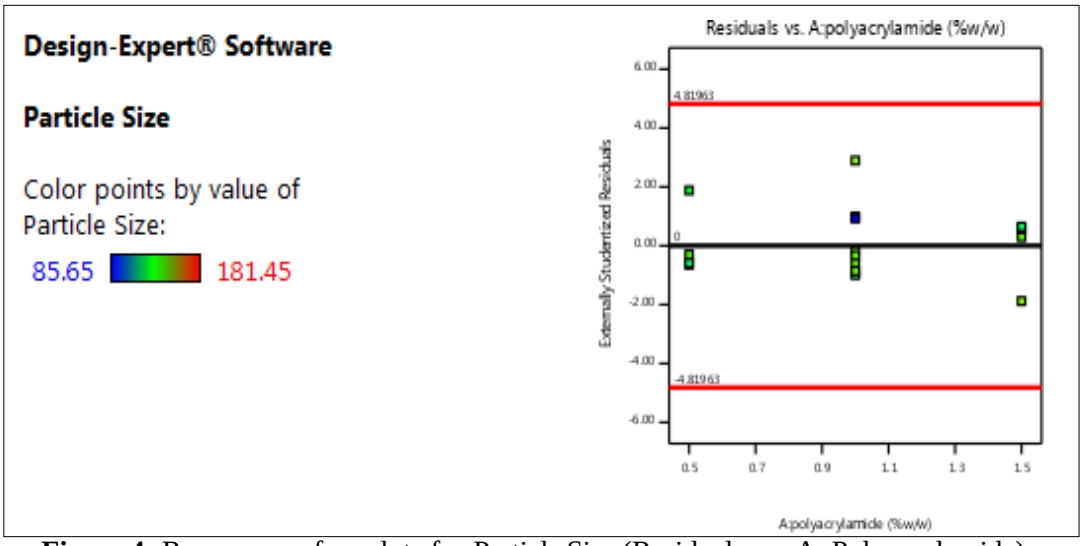

Figure 4: Response surface plots for Particle Size (Residuals vs. A: Polyacrylamide)

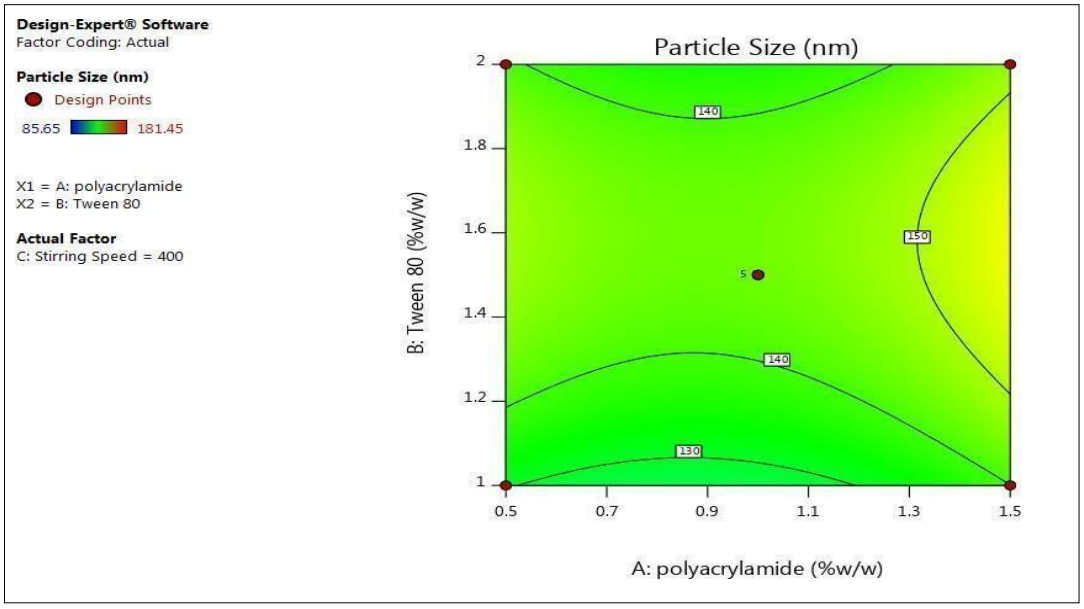

Figure 5: Contour graph plots for Particle Size (Polyacrylamide vs. Tween 80)

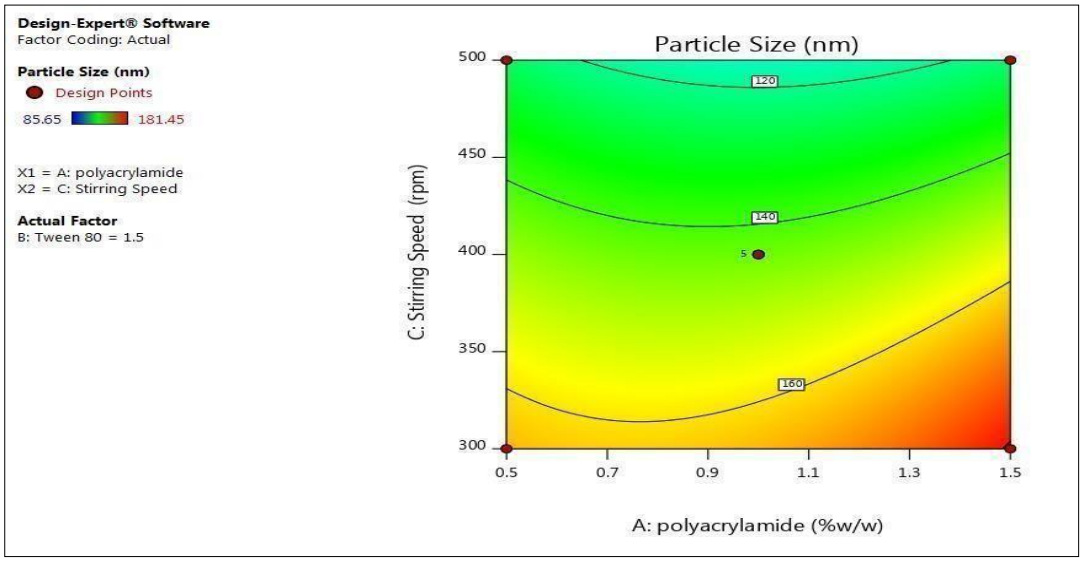

Figure 6: Contour graph plots for Particle Size (Polyacrylamide vs. Stirring speed**)**

Evaluation of conjugated Quercetin copper nanoparticle with bromelain

Results of Entrapment Efficiency

In the experiment we prepared four different formulations of Quercetin copper nanoparticles with bromelain. The quantity of bromelain bound to the Quercetin-loaded copper nanoparticle was determined and the amount of bromelain was estimated. The percentage conjugation efficiency of Quercetin copper nanoparticle gel containing bromelain for QCNBF1, QCNBF2, QCNBF3, and QCNBF4 was calculated as 68.85%±0.32%, 76.65%±0.25%, 72.23%±0.20% and 69.85%± 0.14%, respectively (**Table 2**). The quantity of bromelain entrapped within Quercetin copper nanoparticle gel was determined by ultracentrifugation.

Table 2: Entrapment efficiency(%) QCNBF1 to QCNBF4

Formulation Code	Particle size (nm)	Zeta potential (Mv)	Entrapment efficiency* (%)
QCNBF1	98.85	-30.25	68.85±0.32
QCNBF2	83.32	-39.85	76.65±0.25
QCNBF3	102.52	-28.85	72.23±0.20
QCNBF4	110.23	-34.45	69.85±0.14

Average of three determinations

R*esults of particle size*

The mean size of the nanoparticles for QCNBF1, QCNBF2, QCNBF3, and QCNBF4 formulations was determined by Photo Correlation Spectroscopy (PCS), and the result of the study is shown in **Table2**.

Results of Zeta potential

The Zeta potential of bromelain conjugated Quercetin copper nanoparticle was found negative, which is a property beneficial for interaction with the dermal tissues and induced repulsive interaction between the vesicles which prevents aggregation of nanoparticles in the formulation also. The Zeta potential of bromelain conjugated Quercetin copper nanoparticle was -30.25 mV, -39.85 mV, -28.85 mV, and -34.45 mV, respectively. The Zeta potential of all four formulations is reported in **Table 2** and graphically shown in **Figures 7, 8, 9, and 10**.

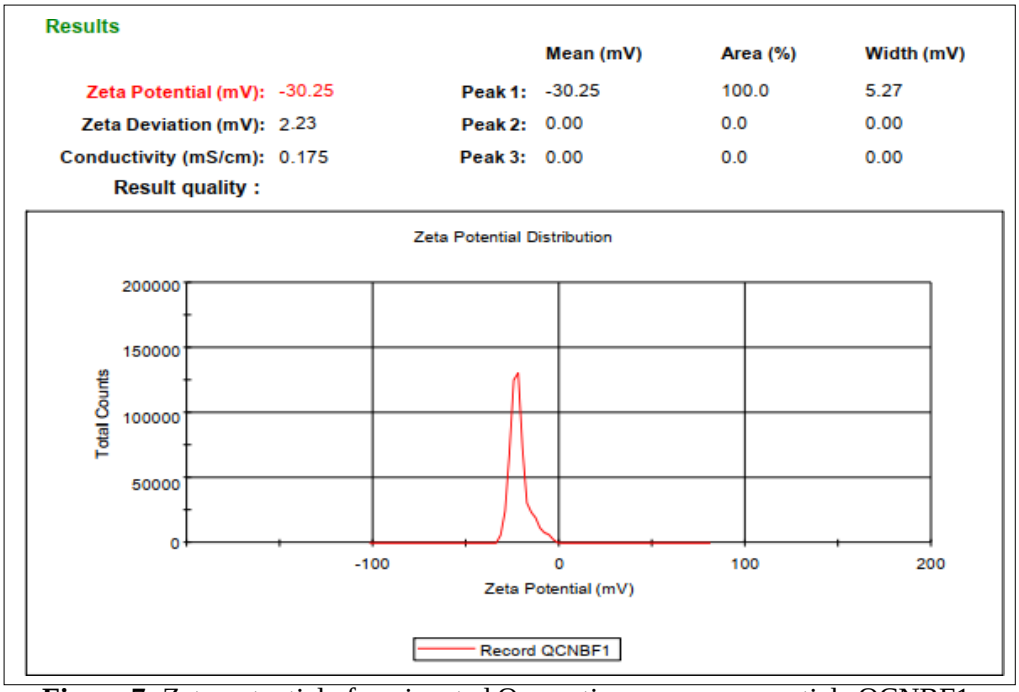

Figure 7: Zeta potential of conjugated Quercetin copper nanoparticle QCNBF1

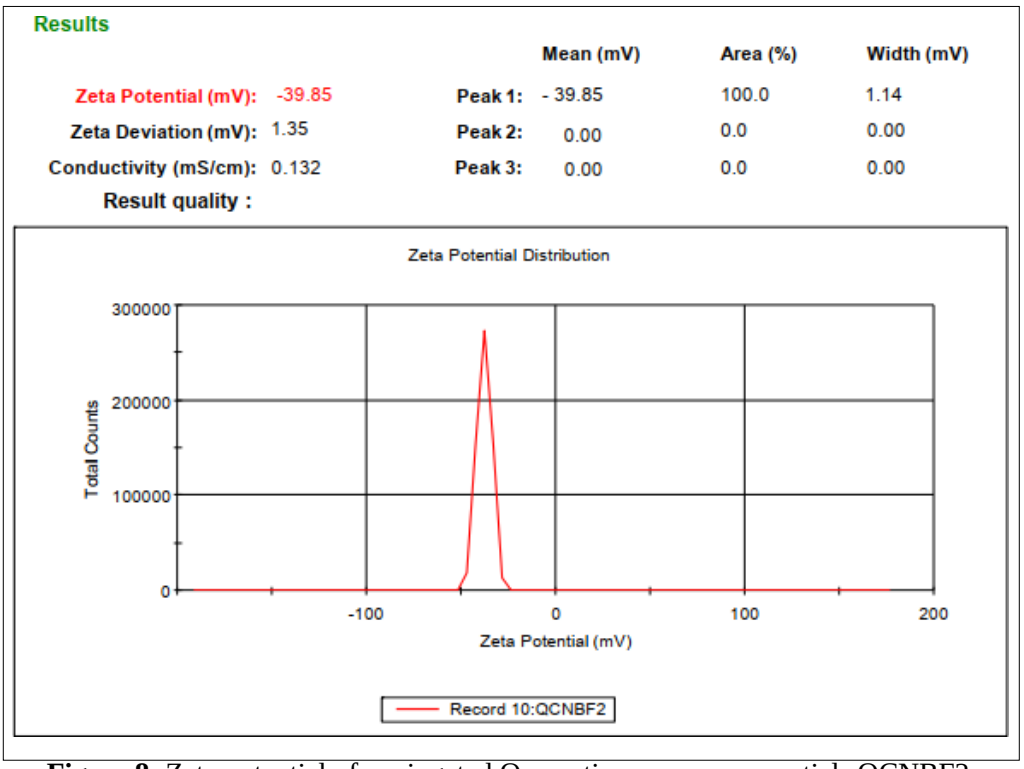

Figure 8: Zeta potential of conjugated Quercetin copper nanoparticle QCNBF2

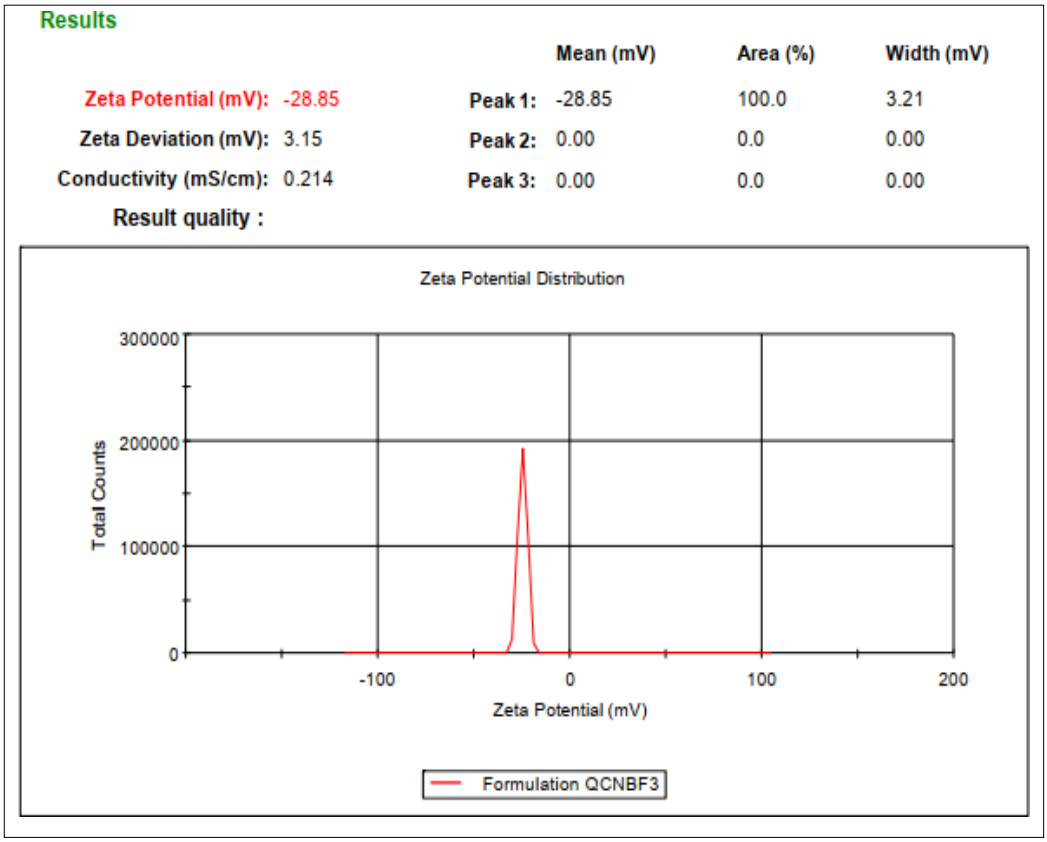

Figure 9: Zeta potential of conjugated Quercetin copper nanoparticle QCNBF3

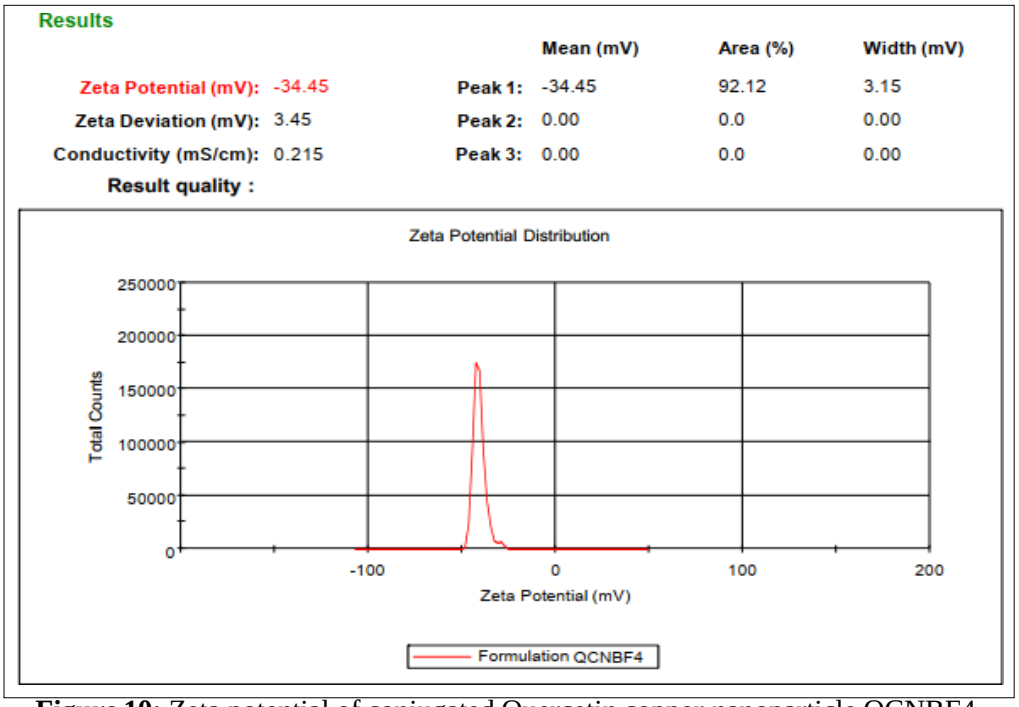

Figure 10: Zeta potential of conjugated Quercetin copper nanoparticle QCNBF4

XRD Study of* QCNBF2 *Formulation

The maximum entrapment efficiency, least particle size, and maximum Zeta potential value were found in formulation QCNBF2. On the basis of this finding the formulation, QCNBF2 was evaluated for XRD and further incorporated into the gel base.

The X-ray diffraction study of QCNBF2 formulation, indicated the existence of sharp diffraction peak at 2h values of 18.2˚, 28.5˚, 57.6˚, and 69˚ (**Figure 11**). This data indicates good conjugation of bromelain into Quercetin copper nanoparticles.

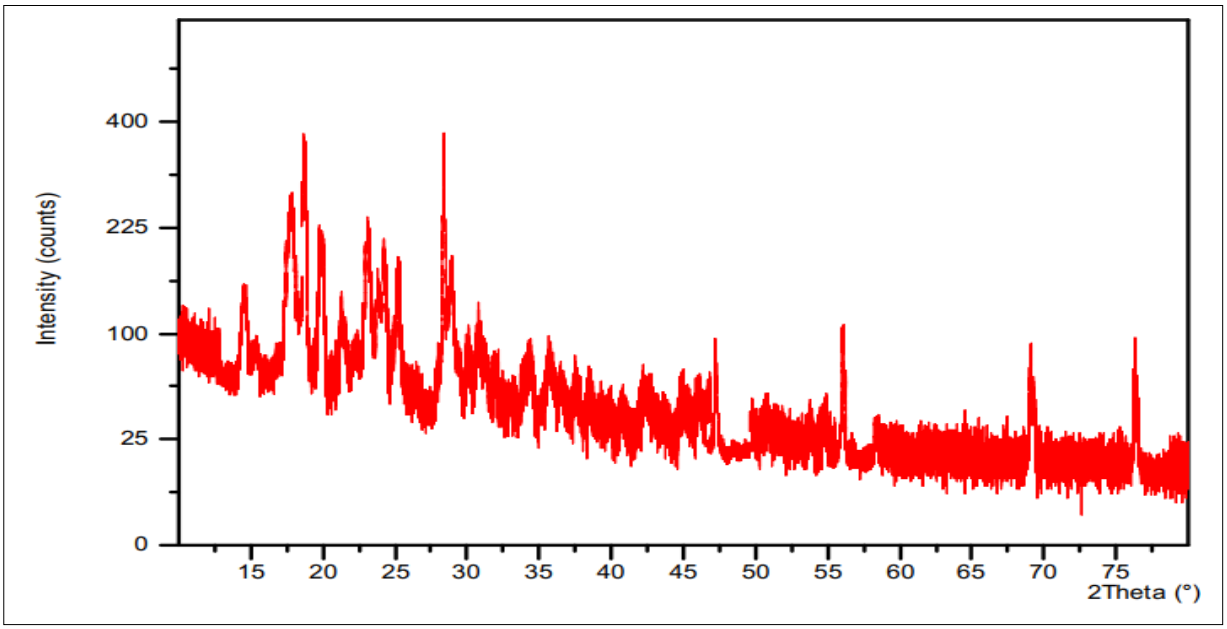

Figure 11: XRD Graph of QCNBF2 formulation

Results of gel formulation

Out of four formulations, QCNBGF4 was excluded from the further study due to its larger particle size. The rest of the formulations were subjected to pH, viscosity, gel strength, drug content, extrudability, and spreadability testing. The summary of the results is presented in **Table 3**.

Table 3: Results of three gel formulation						
Code	**pH**	**Viscosity (cp)**	**Gel strength (g)**	**Drug content (%)**	**Extrudability (g)**	**Spreadability (g/cm)**
QCNBGF1	6.72±0.02	2565±15	6±1	98.85±0.45	160±5	8.65±0.25
QCNBGF2	6.85±0.01	2450±10	8±1	99.45±0.25	155±8	7.32±0.30
QCNBGF3	7.12±0.03	1990±14	9±1	97.81±0.20	175±3	6.15±.20

Average of three determinations (N=3)

In comparison to all formulations, formulation QCNBGF2 showed maximum drug content and desired pH comparison to all formulations, selected as an optimized formulation for *in vitro* drug release study.

In-vitro drug release study of *gel formulation*

The *in-vitro* release studies of the drug from the bromelain conjugated Quercetin-loaded copper nanoparticle (QCNBGF2) gel was investigated *in vitro* throughout 24 h using an egg membrane and followed a biphasic release. The finding of our results of the percentage cumulative drug release curve of QCNBGF2 had the highest amount of drug release, almost 98.42%, observed after 24 h. The results of the drug release are reported in **Table 4** and **Figures 12, 13, and 14.**

Table 4: Results of particle size and entrapment efficiency						
Time (h)	**Square Root of Time(h)$^{1/2}$**	**Log Time**	**Cumulative* % Drug Release**	**Log Cumulative % Drug Release**	**Cumulative % Drug Remaining**	**Log Cumulative % Drug Remaining**
0.5	0.707	-0.301	13.36	1.051	88.75	1.948
1	1.000	0.000	19.95	1.368	76.68	1.885
2	1.414	0.301	26.60	1.472	70.35	1.847
3	1.732	0.477	38.85	1.552	64.35	1.809
4	2.000	0.602	46.65	1.669	53.35	1.727
6	2.449	0.778	59.98	1.778	40.02	1.602
8	2.828	0.903	68.85	1.817	34.35	1.536
10	3.162	1.000	79.98	1.933	14.35	1.157
12	3.464	1.079	88.85	1.965	7.68	0.885
24	4.899	1.380	98.42	1.986	3.15	0.498

Average of the three readings

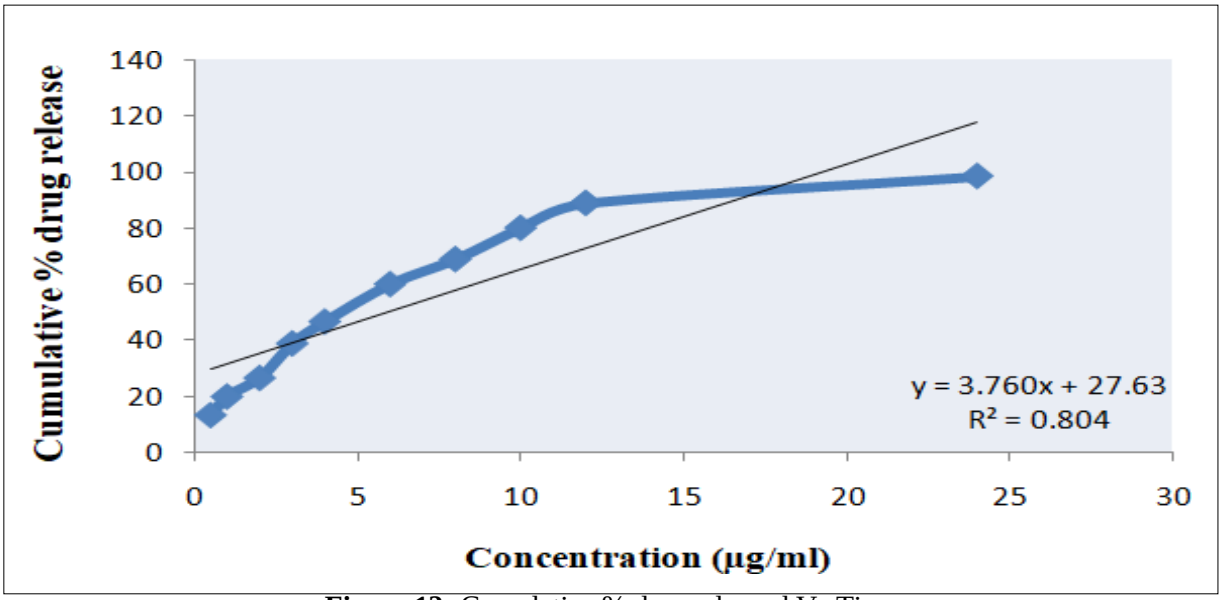

Figure 12: Cumulative % drug released Vs Time

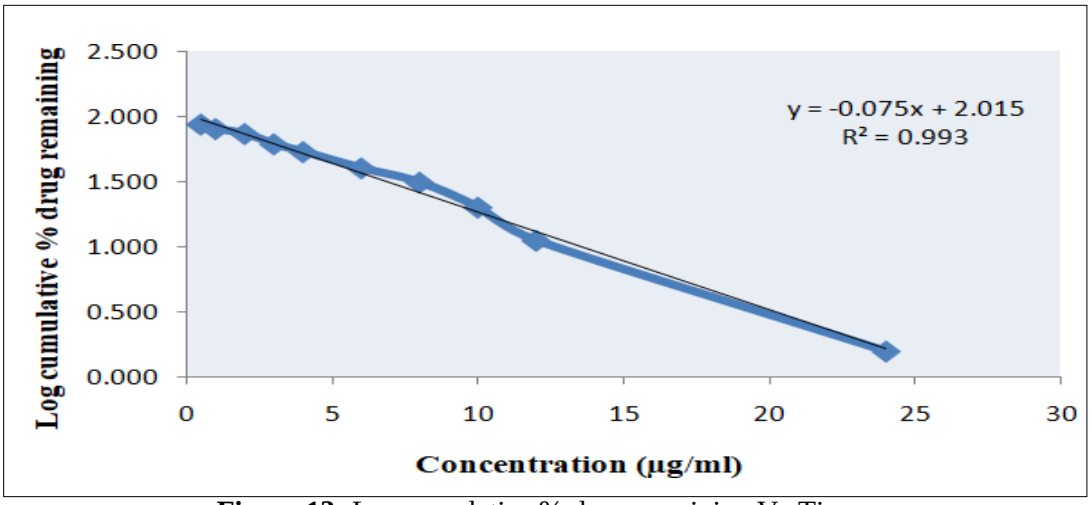

Figure 13: Log cumulative % drug remaining Vs Time

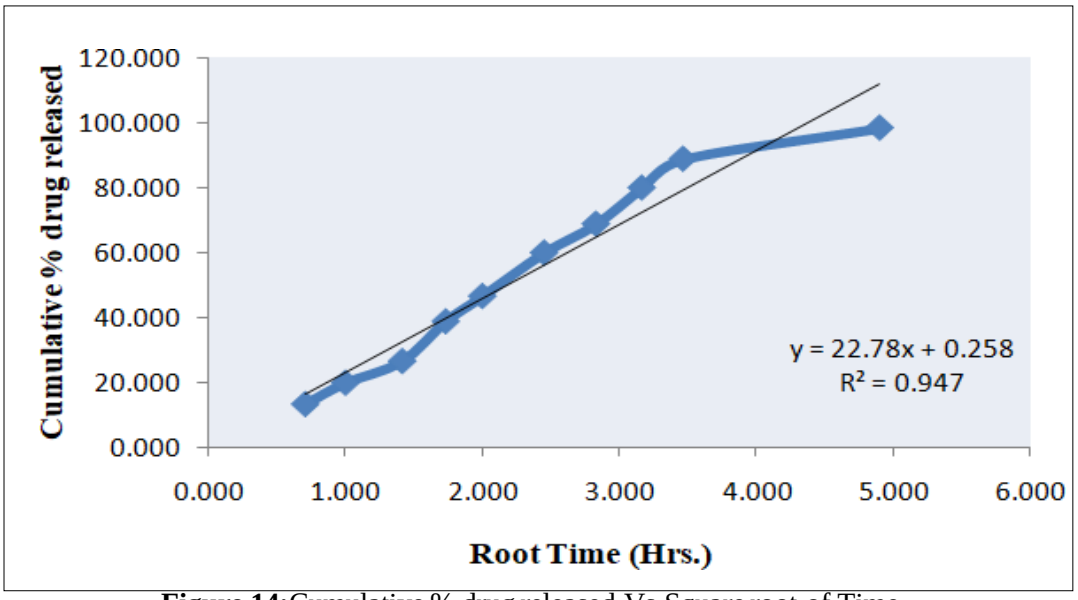

Figure 14: Cumulative % drug released Vs Square root of Time

MTT assay

Anticancer activities of bromelain conjugated Quercetin copper nanoparticles gel (QCNBGF2) were determined against selected HaCaT human (immortalized human keratinocytes) skin cancer cell line by the MTT method. The results of anticancer activity of Quercetin, Quercetin copper nanoparticle, and conjugated Quercetin copper nanoparticle with bromelain are expressed in terms of 50% inhibition concentration (IC_{50}µg/ml) values and are shown in **Table 5**. The dose-dependent activity is presented in **Figures 15,16 and 17**. The results showed that Quercetin copper nanoparticle conjugated with bromelain showed potent anticancer activity in a dose-dependent manner.

Table 5: *In vitro* anticancer (IC_{50}) of nanoparticle against skin cancer cell line (HaCaT)

Concentration	Q IC_{50}(µg/ml)	QCN IC_{50}(µg/ml)	QCNB IC_{50}(µg/ml)
10	62.31	68.54	76.51
1	46.15	48.52	52.31
0.1	32.14	28.61	35.67
0.01	6.16	6.67	12.94
0.001	2.37	1.22	8.84

Q: Quercetin, QCN: Quercetin Copper Nanoparticle, QCNB: Quercetin Copper Nanoparticle conjugated with Bromelain

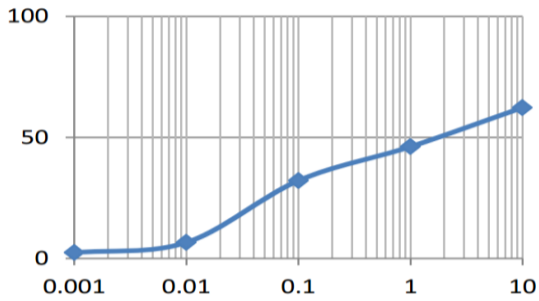

Figure 15: Cytotoxicity of Quercetin in HaCaT cells

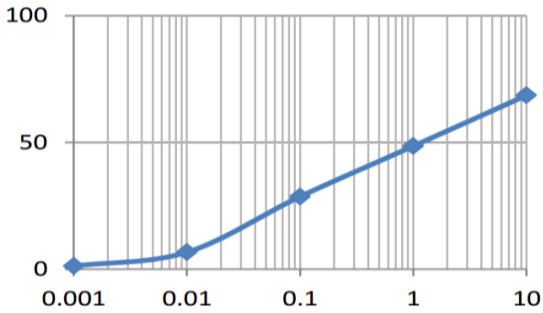

Figure 16: Cytotoxicity of Quercetin Copper Nanoparticle in HaCaT cells

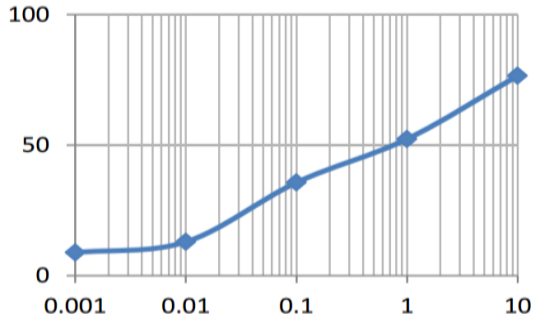

Figure 17: Cytotoxicity of Quercetin Copper Nanoparticle Conjugated with Bromelain in HaCaT cells

CONCLUSION

In this work, we have formulated Quercetin-loaded copper nanoparticles by ensuing nano-precipitation technique followed by conjugation with ligand viz bromelain and evaluated it as a novel targeted drug delivery system for targeting skin cancer. Encapsulated Quercetin in the form of a polymeric copper nanoparticle increased solubility and stability in the aqueous as well as in the physiologic medium. The present investigation found that an increase in the concentration of polymer increased the entrapment of the drug, also the concentration of polymer in the drug/polymer ratio performs a pivotal function in determining the nanoparticle size and stability. The results have supported the idea that bromelain conjugated Quercetin copper nanoparticle has great potential in the drug delivery ability and is suitable for enhancing the targeting approach of the bioflavonoid drug Quercetin. Novel nano-technological materials seem to be excellent cytostatic delivery methods that can target cancerous cells, reducing side effects, boosting therapy effectiveness, and lengthening patient survival for those with skin cancer. So, a promising field that will undoubtedly enhance skin cancer treatment for patients by enhancing either the quality of life or survival of affected patients is presented by new pharmaceuticals combined with enhanced delivery technologies.

Abbriviation List

MTT: [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)]

XRD: X-RAY Diffraction

QCNBG: Quercetin Copper Nanoparticle conjugated with Bromelain Gel

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