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Formulation and evaluation of hydrogel based nano crystals of Quetiapine

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Key words: Hydrogel based nanocrystal, Quetiapine, Bioavailability, Zeta potential, Spectral analysis.

ISSN 0799-3757

http://caribjscitech.com/

Abstract:

The aim of this study was to prepare and characterize hydrogel based nanocrystals of Quetiapine to enhance the dissolution rate and oral bioavailability. Hydrogel based nanocrystal was prepared by the crosslink method using poloxamer 407 and sodium tripolyphosphate as a polymer. Prepared Hydrogel based nanocrystal was subjected to various evaluation parameters like drug content, solubility studies, pH, density, particle size analysis, viscosity, zeta potential, SEM, *In vitro* drug release studies. The FT-IR spectral analysis revealed that there was no interaction between the drug and excipients. Based on the results obtained for nanosuspension formulation, QTP24 formulation was successfully studied for hydrogel. The *in vitro* dissolution rate of Quetiapine was significantly increased by reducing the particle size.

1. Introduction:

Quetiapine is a 2-[2-(4-{2-thia-9-azatricyclo[9.4.0.0]pentadeca-1(15),3,5,7,9,11,13-heptaen-10-yl}piperazin-1-yl)ethoxy]ethan-1-ol belonging to the antipsychotic benzodiazepine class of drugs. Quetiapine is indicated for the treatment of schizophrenia as well as for the treatment of acute manic episodes associated with bipolar I disorder [1-3]. The antipsychotic effect of Quetiapine is thought by some to be mediated through antagonist activity at dopamine and serotonin receptors. Specifically the D_1 and D_2 dopamine, the alpha 1 adrenoreceptor and alpha 2 adrenoreceptor, and 5-HT_{1A} and 5-HT_2 serotonin receptor subtypes are antagonized. Quetiapine also has an antagonistic effect on the histamine H_1 receptor [4]. Quetiapine tablets are available in diffrent strengths equivalent to 50 mg, 100 mg, 150 mg, 200 mg, 300 mg and 400 mg of Quetiapine in the form of Quetiapine Fumarate as an active ingredient for oral administration as extended-release tablets [5]. Nevertheless the drug is extensively metabolized by the liver limiting its oral bioavailability [6]. Quetiapine fumarate is a weak acid with dissociation constant (pKa) 3.3 and 6.8 with moderate pH dependent solubility, 94.3 mg/ml to 2.37 mg/ml at pH values from 1 to 9 reported [7]. The elimination half-lives of quetiapine and N-desalkyl Quetiapine are approximately 7 and 12 hours respectively. Quetiapine is approximately 83% bound to plasma proteins [8].

Nanocrystals possess high entrapment efficiency, with enhanced solubility and drug bioavailabil**i**ty. The improvement in bioavailability by orally administered nanocrystalline API is attributed to an enhanced dissolution rate through the enlarged surface area and to an increased solubility [9]. Hydrogel is a transparent, viscoelastic and thermodynamically stable system consisting of a polar solvent and a polymer, where the polar solvent is the external phase [10].

Hydrogels are crosslinked polymer networks that absorb substantial amounts of aqueous solutions. Due to their high water content, these gels resemble natural living tissue more than any other type of synthetic biomaterial i.e. there is an increase in biocompatibility also the hydrophilic surface of hydrogel has a low interfacial free energy when in contact with body fluids, which results in a low tendency for proteins and cells to adhere to these surfaces [11-13]. The main endeavor of this project is to increase the solubility of Quetiapine, so as to increase the bioavailability of hydrogel based nanocrystal formulation.

2. Materials and methods:

2.1. Chemicals and reagents

The Quetapine (purity> 99.5%) was obtained from the Dr. Reddy's laboratories Ltd., (Hyderabad, India). Tween-80, PEG 200, 400 and 600, Transcutol, PVP K30 and Poloxamer-407 were purchased from LobaChem Ltd., (Mumbai, India). E.P. grade Sodium tripolyphosphate, Disodium Hydrogen Phosphate was procured from Molychem Lab Ltd., (Mumbai, India).

2.2 Preparation of hydrogel based nanocrystals

Quetiapine nanosuspension was prepared by the nanoprecipitation–ultrasonication method with different concentration of surfactant [PEG 600 (0.2, 0.4 and 0.6 ml)] and polymer stabilizer PVP-K30 (20, 40 and 60mg). PEG 600 and PVP-K30 were dissolved in distilled water (10 ml), mixed and labeled as mixture QTP1. This mixture was kept on a magnetic stirrer for 15 min to get a homogeneous mixture. Quetiapine (25 mg) was dissolved using defined volume of methanol (3ml). This solution was slowly added drop wise to mixture 1 with continuous stirring on a magnetic stirrer for specified time 30, 60 and 90 min. After the solutions were kept in an ultrasonicator for the 15 min [14]. Twenty seven formulations were prepared with different concentration of PVP-K30 and PEG 600 with variation in magnetic stirring time as indicated in Table 1.

Hydrogel based nanocrystal was prepared by the crosslink method [15,16], by weighing and dissolving the required quantity of pluronic F-127, 1gm and sodium tripolyphosphate (STPP) 50 mg in distilled water to obtain a

concentration of 25 % (w/w). The calculated amount of lyophilized nanocrystal powder of Quetiapine nanosuspension was incorporated into the gel matrix at the ratio of 1:5 under constant stirring in an ice bath until a clear solution was obtained. The final formulation exhibited sol and gel characteristics in 4 and 35 ^{o}C, respectively.

Table 1: Formulation of Quetiapine Nano suspension by nano precipitation method

Sr. No.	Formulation code	QTP(mg)	PVP K30 (mg)	PEG600 (ml)	Magnetic stirring (min)	Ultra-sonication (min)	Methanol (ml)	D. W. (ml)
1	QTP 1	25	20	0.2	30	15	3	10
2	QTP 2	25	40	0.2	30	15	3	10
3	QTP 3	25	60	0.2	30	15	3	10
4	QTP 4	25	20	0.4	30	15	3	10
5	QTP 5	25	40	0.4	30	15	3	10
6	QTP 6	25	60	0.4	30	15	3	10
7	QTP 7	25	20	0.6	30	15	3	10
8	QTP 8	25	40	0.6	30	15	3	10
9	QTP 9	25	60	0.6	30	15	3	10
10	QTP 10	25	20	0.2	60	15	3	10
11	QTP 11	25	40	0.2	60	15	3	10
12	QTP 12	25	60	0.2	60	15	3	10
13	QTP 13	25	20	0.4	60	15	3	10
14	QTP 14	25	40	0.4	60	15	3	10
15	QTP 15	25	60	0.4	60	15	3	10
16	QTP 16	25	20	0.6	60	15	3	10
17	QTP 17	25	40	0.6	60	15	3	10
18	QTP 18	25	60	0.6	60	15	3	10
19	QTP 19	25	20	0.2	90	15	3	10
20	QTP 20	25	40	0.2	90	15	3	10
21	QTP 21	25	60	0.2	90	15	3	10
22	QTP 22	25	20	0.4	90	15	3	10
23	QTP 23	25	40	0.4	90	15	3	10
24	QTP 24	25	60	0.4	90	15	3	10
25	QTP 25	25	20	0.6	90	15	3	10
26	QTP 26	25	40	0.6	90	15	3	10
27	QTP 27	25	60	0.6	90	15	3	10

2.3 Characterization of Nanosuspension

2.3.1 Drug Content

About 1 ml of nanosuspension preparation was taken and diluted appropriately with water and the drug content of the samples was estimated by UV-Visible spectrophotometer at 249.8 nM. Drug content of all formulations was determined in triplicate.

2.3.2 Particle Size Analysis

The particle size of all the formulations was analyzed by Motic BA 210. Particle size analysis of all formulations was determined in triplicate.

2.3.3 Saturation solubility studies

Nanosuspension equivalent to 25 mg of Quetiapine was taken and separately introduced into 25 ml stoppered conical flask containing 10 ml distilled water. The flasks were sealed and placed in a rotary shaker for 24 hrs at 37°C and equilibrated for 2 days. The samples were collected after the specified time interval and it is filtered and diluted appropriately. The diluted samples were analyzed using a UV spectrophotometer (Shimadzu -1800) at 249.8 nM.

2.3.4 *In-vitro* Dissolution Study

The *In vitro* drug release study was performed for all the formulations and pure drug powder using USP type I dissolution apparatus under the following conditions.

Dissolution test parameters

- Dissolution medium : 900 ml of 0.1N HCl
- Rotation speed : 50 rpm
- Temperature : 37 ± 0.5ºC
- Sampling time : 5, 10, 15, 30, 45, 60 min

At predetermined time intervals aliquot samples (5ml) were collected and replaced with the same volume of fresh medium. The aliquot samples (5ml) were filtered through 0.45 µm membrane filter and the filtrate was diluted appropriately and was estimated using UV-Visible spectrophotometer at λ_{max} 212.3 nM.

2.4 Characterization of Hydrogel based Nanocrystal

2.4.1 Drug Content

About 1 ml Hydrogel Based Nanocrystal preparation was taken and diluted appropriately with water and the drug content of the samples was estimated by UV-Visible spectrophotometer at 249.8 nM. Drug content of all the formulations was determined in triplicate.

2.4.2 Particle Size Analysis

The particle size of all the formulations was analyzed by Motic BA 210. Particle size analysis of all formulations was determined in triplicate.

2.4.3 Saturation solubility studies

Hydrogel Based Nanocrystal equivalent to 25 mg of Quetiapine were taken and separately introduced into 25 ml stoppered conical flask containing 10 ml distilled water. The flasks were sealed and placed in a rotary shaker for 24 hrs at 37°C and equilibrated for 2 days. The diluted samples were analyzed using a UV spectrophotometer (Shimadzu -1800) at 249.8 nM.

2.4.4 *In-vitro* Dissolution Study

The *In vitro* drug release study was performed for all the formulations and pure drug powder using USP type I dissolution apparatus under the following conditions.

Dissolution test parameters

- Dissolution medium : 900 ml of 0.1N HCl
- Rotation speed : 50 rpm
- Temperature : 37 ± 0.5ºC
- Sampling time : 5, 10, 15, 30, 45, 60 min

At predetermined time intervals aliquot samples (5ml) were collected and replaced with the same volume of fresh medium. The aliquot samples (5ml) were filtered through0.45 µm membrane filter and the filtrate was diluted appropriately and was estimated using UV-Visible spectrophotometer at λ_{max} 212.3 nM.

2.4.5 Particle Size Analysis

Average particle size of formulations was determined by Malvern Zeta Sizer ZS (Nano series ZS 90 UK) using water as dispersion medium. The sample was scanned 100 times for determination of particle size.

2.4.6 Zeta Potential

The zeta potential of the hydrogel based nanocrystal was measured by using an additional electrode in the instrument used for particle size analysis (Malvern Zetasizer). For Zeta Potential determination samples of formulations were diluted with water and placed in the electrophoretic cell. Each sample was measured three times at 25ºC and average values were employed for measuring the response. Refractive index and dielectric constant of dispersant were 1.33 and 78.5 respectively.

2.4.7 Scanning Electron Microscopy (SEM)

SEM was used to verify the uniformity of particle shape and size. Hydrogel Based Nanocrystal was suspended in distilled water and later dropped into a silicon grid and dried at room temperature. The formulation was vacuum coated with gold for 3 min. The surface morphology of the samples was observed under a scanning electron microscope (JEOL/ JSM-6390LP, Japan) operated at 15-keV pulse at different resolutions.

2.4.8 Stability study

Stability studies for nanosuspension were conducted at two different storage conditions, viz. room temperature and refrigerated conditions (2-8°C) for 3 months. A batch of hydrogel based nanocrystal was used for each storage condition. At periodic time intervals, the samples were withdrawn and analyzed for particle size and drug content.

3. Results and Discussion:

3.1 Optimization of process parameters

The optimization of nanosuspension formulations was done on the basis of process parameters like stirring rate, stirring time and ultrasound intensity. Ultrasonic application provided homogeneous and stable nanosuspension system of Quetiapine. Quetiapine nanocrystal formulation was successfully prepared by nanoprecipitation technique. The formulation was optimized using a combination of Polyethylene Glycol-600 as a surfactant and PVPK30 as a polymer stabilizer. Twenty seven formulations were subjected to density, viscosity, pH, % Drug content, Particle Size and solubility studies. Out of that formulation QTP24 was showing good and satisfactory results for density-0.9978, viscosity-1.3150, pH-5.52, % Drug content-99.23, particle size-457 nM and solubility 27.28 mg/ml respectively. So formulation QTP 24 was selected for further study.

3.2 Characterization of nanosuspension

3.2.1 Particle size

The particle size analysis of all the formulations was done by using Motic BA 210. It was observed that the particle size of all the formulations ranges between 411- 591nM. The QTP 24 showed less particle size (457nM), so it was used for further analysis.

3.2.2 Saturation solubility

Saturation solubility of prepared nanosuspensions by nanoprecipitation technique was found to be in the range of 24.07-27.28 mg/ml. Saturation solubility of pure drug was found to be 3.29±0.055 µg/ml. This increase in saturation solubility of formulation was due to particle size reduction and subsequent increase in surface area. The increase in saturation solubility will increase in bioavailability and dissolution rate. The formulation QTP24 showed the higher solubility than the other formulations.

3.2.3 *In-vitro* drug dissolution study

The dissolution study was performed in order to find the percentage of drug release in different formulations. The *in-vitro* dissolution study of nanosuspension and standard Quetiapine were carried out using the USP type-II dissolution test apparatus using Hydrochloric acid 0.1 N solution as a dissolution medium at 37 ± 2 °C with 50 RPM rotating speed. A sample of 5 ml was withdrawn at regular time interval of 5, 10, 15, 30, 45, and 60 min respectively (Fig. 1) and filtered using 0.45µm filter paper. An equal volume of the respective dissolution medium was added to maintain the sink condition. Drug content of the sample was analyzed using UV-spectrophotometer at 212.3 nM. All measurements were done in triplicate from three independent samples. The formulation QTP24

showed maximum drug release 99.93% in 45 min. *In vitro* drug release study of pure drug was found to be 85.71% in 60 min as indicated in Table 2.

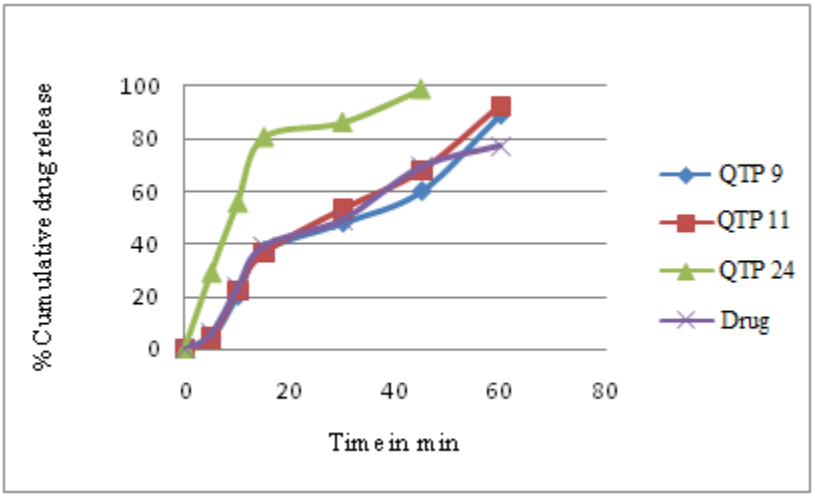

Figure 1: *In- vitro* dissolution study of Nanosuspension of QTP9, QTP11 QTP24 and Drug.

Table 2: *In- vitro* dissolution study of Nanosuspensions of QTP 9, QTP 11, QTP 24 and standard Drug.

Sr. No.	% Cumulative drug release				
	Time in min	QTP9	QTP11	QTP24	Std QTP
1	0	0	0	0	0
2	5	4.12±1.57	4.95±0.85	29.07±1.7	4.9±3.2
3	10	18.72±2.1	17.61±2.1	55.76±4.4	16.60±1.8
4	15	45.17±1.6	43.01±0.90	80.85±0.17	22.65±0.92
5	30	66.08±0.36	62.35±1.6	86.51±0.62	42.50±1.02
6	45	89.13±0.74	92.39±0.90	99.93±0.61	65.23±1.6
7	60	94.2±4.4	94.28±1.9	–	85.71±1.3

3.3 Characterization of hydrogel based nanocrystals

The prepared hydrogel was subjected for preliminary analysis like pH, density, viscosity, particle size, drug content, *in vitro* dissolution and solubility study. The results are summarized in Table 3.

Table 3: Evaluation parameter of hydrogel based Nanocrystal

Sr. No.	Parameter	Observation
1	pH	6.02
2	Density (gm/ml)	1.022
3	Viscosity (cps)	34-96
4	Particle Size (SEM)nM	240
5	Drug Content (%)	99.25
6	Dissolution (%)	99.94
7	Solubility(mg/ml)	27.30
8	Swelling index (%)	97.30

3.3.1 Particle size and Size distribution

The particle size of the formulation (QTP24) was found to be 103.0 nM. as shown in Fig. 2. Polydispersity index (PDI) is the measure of size distribution and generally varies from 0.0 to 1.0. The closer the PDI value to zero, the more homogenous is the nanosuspension. The PDI of the selected nanosuspension was found to be 0.128 closer to zero showing homogeneity of nanosuspension.

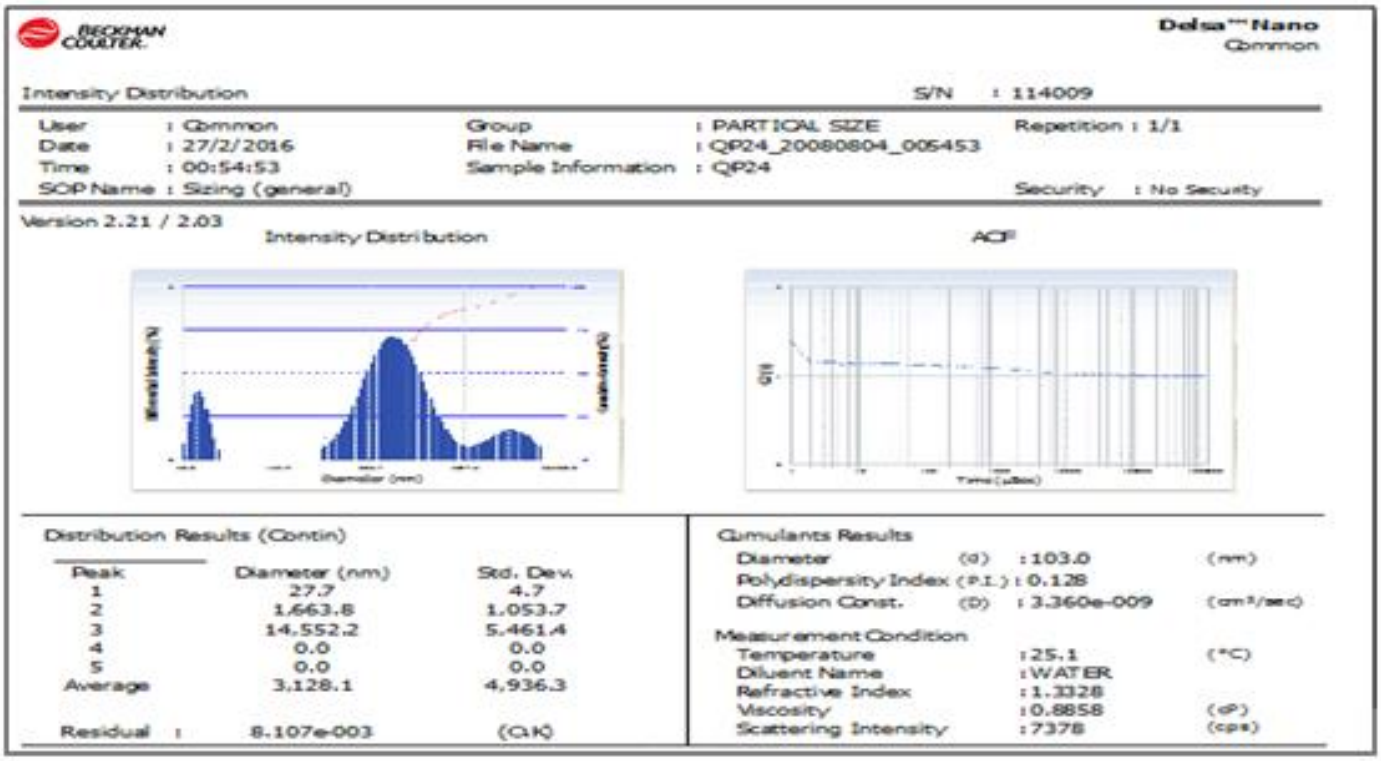

Figure 2: Particle size and zeta potential analysis of QTP 24 formulation

3.3.2 Zeta potential

Zeta potential is an important parameter to analyze the long-term stability of nanoparticles. Generally, higher zeta potential values, both (+) or (−), indicate long-term stability because of electrostatic repulsion between particles with the same charges, which avoids aggregation. The zeta potential was determined by Malvern zeta sizer. The zeta

potential of formulation (QTP 24) was found to be -25.2 mV which indicates that the formulation is stable. The mobility distribution graph is shown in Fig. 3.

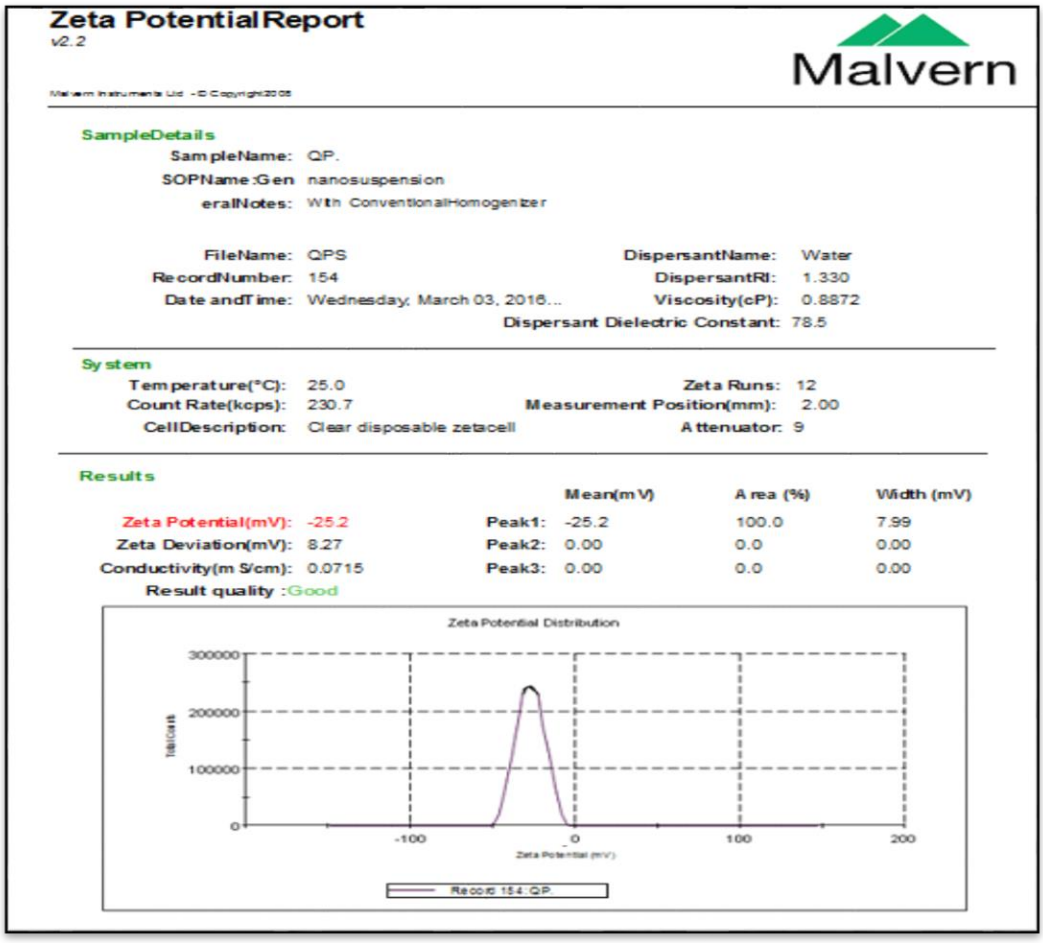

Figure 3: Zeta potential of batch formulation QTP24.

3.3.3 Surface morphology

The morphology of nanoparticles was determined by Scanning electron microscopy (SEM). The morphology of precipitated drug particles in the suspension after air drying followed by oven-drying is shown in Fig. 4. The drug particles were oval in shape and the size ranges from 240.00 to 184.38nM. The particles were discrete and uniform in size and there was no sign of agglomerations.

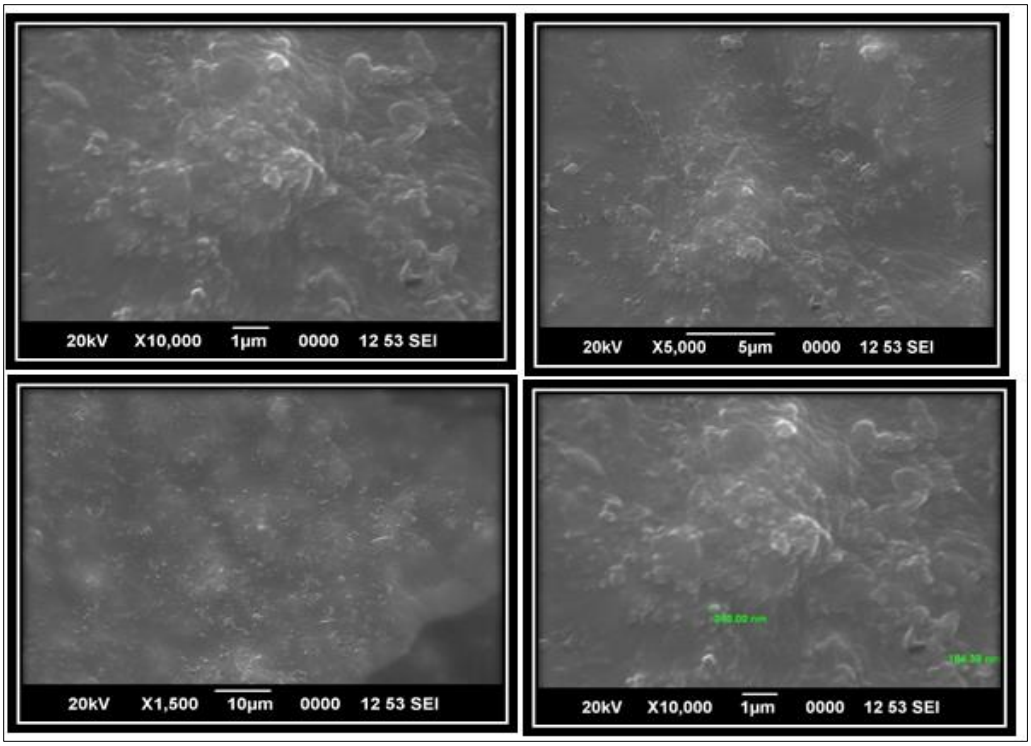

Figure 4: SEM photomicrograph of QTP24 formulation

3.3.4 Stability Study

Table 4: Stability study analysis of hydrogel based nanocrystal

Sr. No.	Time (Day)	Stability data of hydrogel based nanocrystal			
		Particle Size (nM)		Drug Content (%)	
		Room Temp. (25°C)	Refrigerator (2-4°C)	Room Temp. (25°C)	Refrigerator (2-4°C)
1	Initial	459±1.03	459 ±1.03	98.12 ±0.02	98.12 ±0.02
2	10	567±1.12	463 ±1.05	97.13 ±0.14	97.12 ±0.15
3	20	580 ±1.15	467.5±1.15	99.18 ±0.45	99.14 ±0.81
4	30	598 ±1.17	472.3 ±1.17	99.93 ±0.75	98.14 ±0.19
5	45	625±2.05	476 ±1.87	97.01 ±0.12	97.05 ±0.16
6	60	635 ±2.2	481±1.19	99.06 ±0.27	99.08 ±0.56
7	90	640 ±2.25	485 ±1.19	99.18 ±0.45	99.19 ±0.14

Mean ±SD, n=3

The results of the stability studies are shown in Table 4. In case of formulation stored at room temperature, the particle size was increased from 459 to 640nM in 90 days which was measured by Motic digital microscope. However, under refrigerated storage conditions, there was a nominal increase in particle size from 459 to 485 nM indicating better stability under these conditions. The results showed that the temperature had an influence on aggregation of nanoparticles and at room temperature; aggregation was higher compared to refrigerator condition for liquid nanosuspension. So, it can be stated that higher temperature results in increase in particle size. Another reason might be the Ostwald ripening resulting from fluctuations in room temperature. The result of the chemical stability of the formulation during different storage conditions is shown in Table 4. It could be observed that there was no significant change in the drug content of the formulation, under any of the two storage conditions viz. room temperature or refrigerated conditions. Thus the formulation was chemically stable at both the storage conditions.

3.3.5 Drug -excipients compatibility study by FT-IR

The Drug-excipients compatibility study was performed by recording the FT-IR spectrum of Quetiapine-Excipient Physical mixture and Quetiapine hydrogel based Nanocrystal Formulation (Fig. 5 and 6).

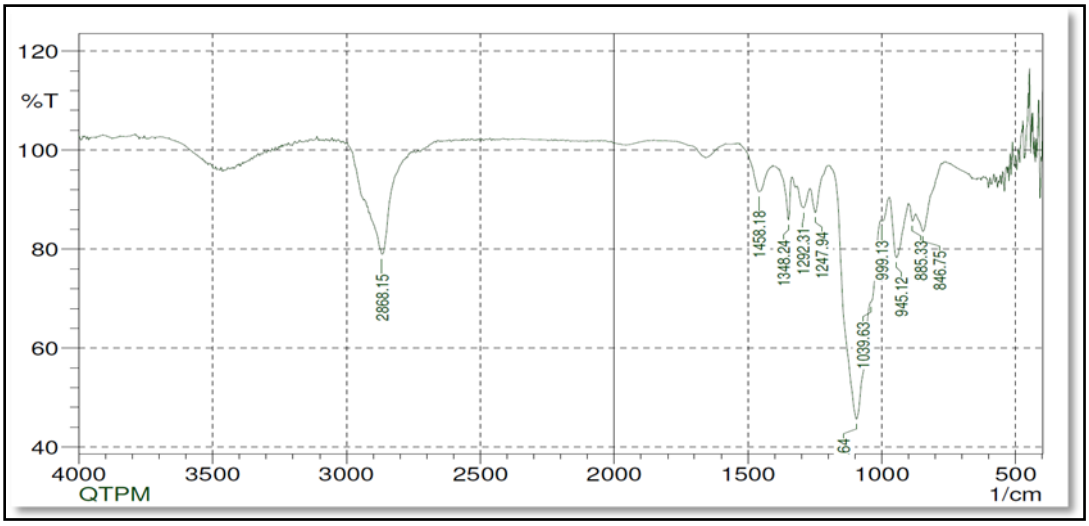

Figure 5: FT-IR spectra of Quetiapin -Excipient Physical mixture

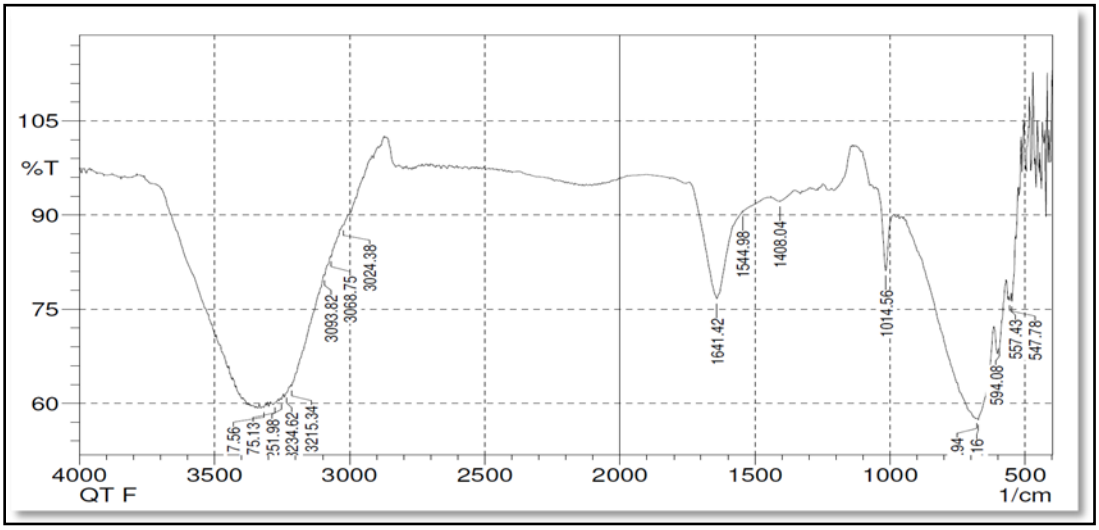

Figure 6: FT-IR Spectra of Quetiapine hydrogel based Nanocrystal Formulation

FT-IR study revealed that there was no appearance of new peaks and disappearance of existing peaks, which indicate that there is no interaction between the Quetiapine, PEG 600, PVP K30, Poloxamer 407 and STPP (Table 5).

Table 5 Comparison of FT-IR Peak of Physical Mixture and Quetiapine Nanosuspension

Observed peaks in Physical mixture (cm^{-1})	**Observed peaks in formulation (cm^{-1})**	**Reported peaks (cm^{-1})**	**Functional Group**	**Interaction**
3415	3317	3400-3200	OH	No
1093	1014	1300-1000	C-O-C	No
2868	3024	3000-2850	CH str. of $-CH_2$	No
1247	1260	1350-1000	C-N	No
1660	1641	1690-1640	C=N	No
3045	3068	3150-3050	Ar-CH str.	No
1458	1544	1465	CH def.	No
685	678	680	C-S	No
1348	1408	1450-1375	CH def. of $-CH_3$	No
1620	1641	1600-1700	C=O	No
1292	1310	1320-1140	P=O	No
999	960	1025-900	P-O-P	No

4. Conclusion:

Nanoparticle drug delivery was a promising approach developed for the Quetiapine Hydrogel based nanocrystal. Nanotechnology can be used to solve the problems associated with these conventional approaches for solubility and bioavailability enhancement. During this investigation, nanocrystals based hydrogel was prepared by most adaptable crosslink method. The nanosuspension was prepared by nanoprecipitation-ultrasonication method and subsequently evaluated by particle size, density, viscosity, drug content and saturation solubility and *In vitro* drug dissolution study, in that formulation QTP24 showed, density 0.9978, viscosity 1.3150, pH 5.52, % Drug content 99.23, particle size 457 nM and solubility 27.28 mg/ml respectively so it was used for preparation of hydrogel. The hydrogel showed optimum swelling at pH 6.02 with higher swelling index 97.30 and particle size 240 nM. The long term stability of the formulation was checked by zeta potential analysis. The SEM analysis showed particle size in the range of 240.00 to 184.38 Nm. So, hydrogel based nanocrystals can be promising alternative to current delivery systems aiming to improve the bioavailability of drugs with low solubility.

Acknowledgment:

Authors are heartily thankful to honorable Prashant Gadakh Patil, President, Mula Education Society, Sonai, India. The authors also express gratitude to the management and the Principal Dr. V. K. Deshmukh, M.E.S.'S. College of pharmacy Sonai, India for providing amenities to carry out this work.

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