Instruments, Malvern, UK). To determine the particle size, a dilute suspension of nanoparticles (100 µg/ml) was prepared in Milli-Q water, sonicated on an ice-bath for 30 seconds and subjected to particle size measurement.

2.4 SEM Studies

The surface morphology of nanoparticles was characterized by SEM (Zeiss EVO 18 Special Edition, Germany). The nanoparticles were sputtered (Quorum Technologies, Q150T ES) with gold to make them conductive.

2.5 FT-IR Spectral Studies

The chemical integrity of the drug and the polymer matrix was investigated using FT-IR spectra (Perkin-Elmer, California, USA).

2.6 *In vitro* Release Kinetics Study

In vitro release of EGCG was carried out by dissolving nanoparticles in PBS (pH 7.4) at 37°C [7].

2.7 Cytotoxicity Study

The cytotoxicity studies on K562 cells were performed with different concentrations of free drug and nanoparticles formulations containing different ranges of concentrations of drug by MTT based colorimetric assay [8].

3. Results and Discussion

EGCG may be responsible for most of the anticancer activity of tea [9]. Its anticancer properties can be enhanced by successful nanoformulations and efficient drug delivery mechanisms [10]. EGCG loaded PLGA polymer nanoparticles were prepared and DLS analysis revealed that the formulated nanoparticles had an average diameter of 300.8 ± 2.6 nm (Figure 2).

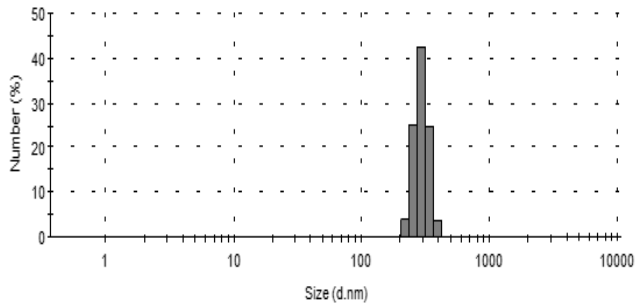

Figure 2: Hydrodynamic size measurement by dynamic light scattering

SEM image identified the surface morphology of drug-loaded nanoparticles to be spherical with smooth exterior (Figure 3).

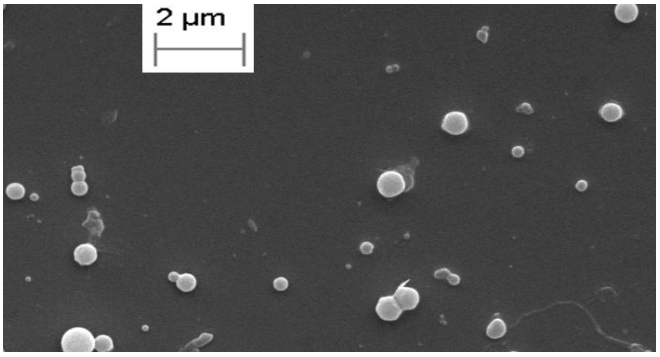

Figure 3: Scanning electron micrograph of EGCG loaded PLGA nanoparticles.

Information on the nature of the molecular interaction within the solid matrix of the nanoparticles was obtained using FT-IR. The spectra for free PLGA nanoparticles showed the characteristic bands of the polymer, –CH, $–CH_2$, $–CH_3$ stretching (2850–3000 cm^{-1}), carbonyl –C=O stretching (1700–1800 cm^{-1}), C–O stretching (1050–1250 cm^{-1}) [11], [12]. FT-IR spectra for EGCG exhibited the characteristic bands corresponding to –OH stretching (3600–3200 cm^{-1}), C=C stretching (1600–1400 cm^{-1}), C–O stretching of the oxygen in the ring (1272 cm^{-1}). These findings are in agreement with previous studies [13], [14]. In spectra of EGCG loaded nanoparticles, the intensity of –CH, $–CH_2$, $–CH_3$ stretching, and C=O stretching deceased compared to void PLGA.

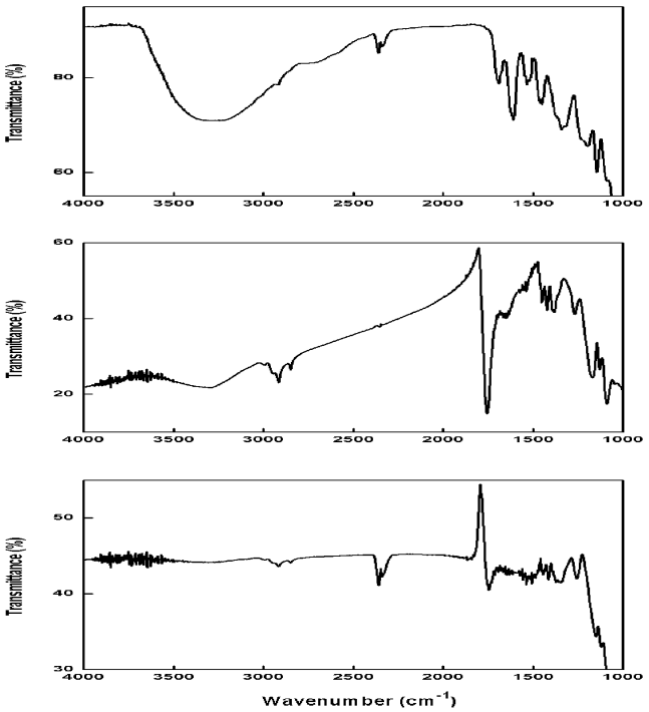

Figure 4: FT-IR spectra of EGCG, void PLGA nanoparticles, and EGCG loaded PLGA nanoparticles. (from top to bottom)

There is also a shift in C=O stretching peak position from 1802 cm^{-1} in void PLGA to 1794 cm^{-1} in loaded PLGA suggesting active participation of carbonyl group in the interaction. FT-IR measurement evidenced the encapsulation of EGCG within the polymer nanoparticles (Figure 4).

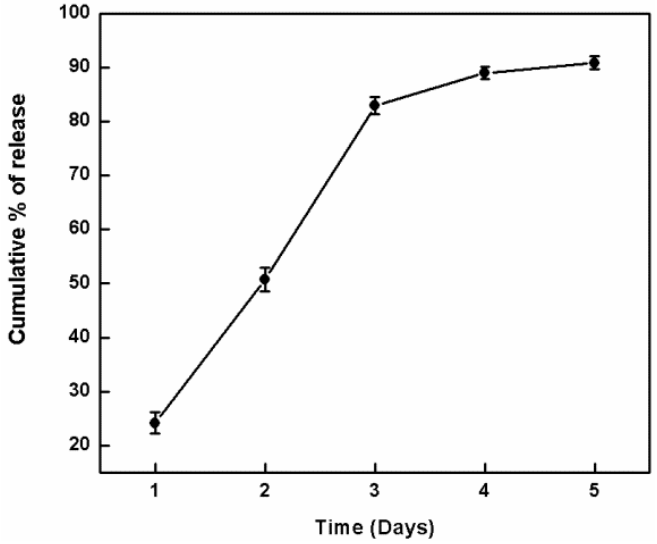

Figure 5: *In vitro* release of EGCG from nanoparticles.

The *in vitro* release of EGCG from PLGA nanoparticles is shown in Figure 5. Around 80% of EGCG is released in first three days, followed by very slow release upto five days and maximum of 90%. Gradual release of drug for longer periods time was achieved by this encapsulation and attempts to increase release time is under process. However, initial burst release was followed by a slower sustained release of EGCG present inside the core of nanoparticles [7], [15].

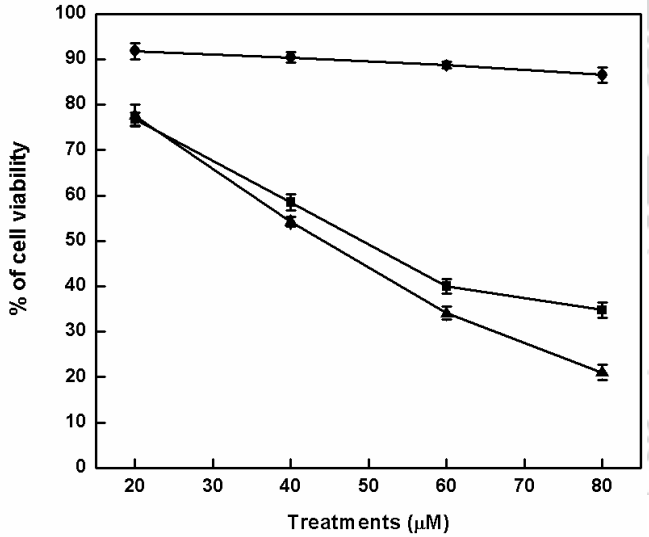

Figure 6: Dose dependent cytotoxicity of different concentration of void PLGA nanoparticles, EGCG, and EGCG loaded PLGA nanoparticles (from top to bottom)

To investigate the therapeutic efficiency of these formulations, K562 cells were treated with different concentrations of nanoparticles for 24 hours, and cell proliferation was measured by MTT assay. It can be seen from Figure 6 that nanoparticles exhibited significantly higher cytotoxicity compared to EGCG in solution. This observation indicates that EGCG loaded nanoparticles enhances the *in vitro* cancer cell death.

4. Conclusion

A novel biodegradable nanoparticle system has been developed for controlled delivery of EGCG, which is more effective in successful killing of cancer cells than EGCG alone. However, more work in this direction is required to promote this formulation as cancer therapeutic nanomedicine. In general, our results have important implications for the design and fabrication of polymeric nanoparticle delivery systems for bioactive compounds with beneficial effects on human health.

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