Chapter 4

Oral administration of orcinol glucoside loaded polymer – lipid hybrid nanoparticles for gastrointestinal tract cancer targeting and improved *in vitro* cytotoxicity against GIT cell lines

Abstract: In the present study, orcinol glucoside-loaded nanostructured lipid carrier (NLC) coated with polyethylene glycol - 25/55 - stearate (PEG-25/55-SA) were formulated and evaluated for oral delivery of orcinol glucoside (OG) to improved *in vitro* cytotoxicity against GIT cell lines such as Hepatocellular carcinoma cell line HepG2, hepatocyte-derived carcinoma cell line Huh-7, humancolorectal carcinoma cell line HCT-116 and human gastric adenocarcinoma AGS cells. Orcinol glucoside loaded PEG-NLCs (OG-PEG-NLCs) consisting of tristearin, oleic acid and PEG - 25/55 - stearate were prepared by hot homogenizations followed by altrasonocation technique. Orcinol glucoside loaded NLC (OG-NLC) were also prepared as control. The characteristics of OG-PEG-NLCs and OG-NLC such as particle size, zeta potential, TEM, SEM, AFM, entrapment efficiency and drug loading were investigated in details. The mean particle size was about 160 to 230 nm and the zeta potential value was about -8 to -20 mV. TEM, SEM and AFM images revealed spherical and smooth surface morphology of NLCs. The differential scanning calorimetry analysis established that orcinol glucoside was not in crystalline state in NLC. Drug release pattern with burst release initially and sustained release afterwards was obtained. The OG-PEG-NLCs exhibited superior anticancer activity compared with OG-NLC and OG solution against GIT cancer cell lines. This is first report demonstrating a practical approach for oral delivery of OG for GIT cancer targeting, warranting further *in vivo* cancer study for superior management of GIT cancer.

1. Introduction

Gastrointestinal (GI) cancers involve malignancy of the GI tract, and associated organs, which include the esophagus, stomach, bowel and rectum, liver, and pancreas[1]. In this respect, 'oral chemotherapy' has provided a major leap forward, when it comes to "Chemotherapy at Home". This holds great promise to radically upgrade the clinical practices of chemotherapy and immensely improve the overall quality of life of the patients, as oral route is a widely practiced and readily compatible means of drug administration[2]. As such, it is now possible to administer a decent number of small anticancer drugs orally[3-11]. The past decade has seen some major advances in the effectiveness of chemotherapeutic treatment for patients suffering from GI cancers. Due to the possible complications and adverse after-effects of surgical interventions in treating GI carcinoma, oral chemotherapy has gained much popularity with the prospect of providing bedside treatment for cancers. Notwithstanding the potential benefits of the oral route, oral formulations encounter several typical issues, when it comes to treating carcinoma of the GI tract, *e.g.* poor stability in the gastric environment and low solubility and/or bioavailability. A major drawback is their inability to penetrate the mucosal barrier, disrupting drug absorption in the GI tract[12]. There have been few advances recently to address these in the light of the effectiveness of chemotherapy for treating patients with GI cancers. Amongst the various alternatives to treatment considered so far, the oral chemotherapy – as novel and attractive it may seem – continues to be a major challenge when it comes to formulations[13-15].

Recently, a lot has been probed into nanoparticle-based drug delivery systems for delivering cancer therapeutic molecules by oral route[2,16-20]. Amongst these, nanostructured lipid carriers (NLCs) have proven their potentiality as an efficient drug delivery system for GI targeting *via* passive mechanisms[21-24]. The potential benefits of having such a system are good tolerability, low drug leakiness, controlled drug release, high oral bioavailability, large-scale production, and less acute and chronic toxicity[24-28]. Hence nowadays, oral delivery of NLCs is considered to be the preferred mode of drug administration, which due to their distribution on a larger surface area, provide better physical stability coupled with enhanced protection of incorporated drugs from

degradation, improvement in consistency of plasma level[25,29], sustained drug release[26], a modest decrease in bioavailability compared to single-unit systems and site-specific targeting[30]. However, one of the greatest challenges in the development of an efficient nano-carrier for oral administration is to overcome the absorption barrier of intestinal mucosa, consisting of intestinal epithelial cells as well as a mucus layer[12].

In our previous work, we have reported that development of ursolic acid-loaded nanostructured lipid carriers (UA - NLC) could be propounded as an effective strategy to modify the poor aqueous solubility and to prolong the half-life of UA[31]. Consequently, nanostructured lipid carriers (NLCs) (regarded as the second-generation of lipid nanoparticles), developed from solid lipid nanoparticles (SLNs) systems, attract considerations for an alternative colloidal drug delivery system. However, reports of NLC being rapidly absorbed from the systematic circulation by the reticuloendothelial system (RES), also known as mononuclear phagocyte system and shuttled out of circulation to the liver, spleen, or bone marrow, and non-specific binding of NLC to non-targeted or non-diseased regions are not a typical[32,33]. There is also an enhanced risk of *in vivo* NLC toxicity due to RES accumulation; such accumulations could further result in NLC entrapment in the liver, lungs, *etc.* mainly due to capillary occlusion[34,35]. To counteract this issue, surfaces of drug carriers have been adjusted with low molecular weight (MW) polyethylene glycol (PEG).These have been reported to facilitate improved performance by (a) sterically stabilizing particles, avoiding plasma protein binding, thereby reducing the elimination by the RES and resulting in extending the half-life of drugs in circulation, (b) reducing immunogenicity, and (c) enhancing the effect of permeability and retention in tumor tissues[36-38]. In addition, it has also been shown that low MW PEG coupled with their large surface area could possibly improve the NLC surface hydrophilicity by decreasing hydrophobic or electrostatic interactions, thus minimizing mucoadhesion. Thus, adjusting the macroscopic properties of nanoparticles by low MWPEG facilitated rapid mucosal penetration and transport, while unmodified nanoparticles were thoroughly trapped by highly viscoelastic and adhesive GI mucosa[39]. Additionally, the FDA approved PEG's non-toxic, non-immunogenic and non-antigenic nature accompanied by its high water solubility renders it to have de minimis interference with the drug release[40].

To the best of our knowledge to date, there is no report of employing PEG-25/55-stearate coated NLC carriers for molecules such as orcinol glucoside oral drug delivery. Therefore, investigation of the parameters guiding particle uptake could potentially lead to the design of novel and factual site-specific delivery nanocarriers in the future.

Herbal medicines with anti-carcinogenic properties and high safety margins present novel pharmacotherapeutic leads in the treatment of cancer[31,41-45]. In particular, orcinol glucoside (β-D-Glucopyranoside 3-hydroxy-5-methylphenyl; OG) is an active constituent isolated from the rhizomes of *Curculigo orchioides* Gaertn[46]. This is a conventional medicine with diverse beneficial biological and pharmacological activities, including treatment of GI degenerative disease, immunomodulatory activity[47], anti-oxidant[48], antidepressant[49], adaptogenic activities neuroprotective activity[50,51], treating reproductive system deficiencies and liver and renal conditions. Lately, a number of studies have focused on the anticancer properties of OG[52,53]. These studies provide relevant insights about OG's capabilities in regulating CYP3A in L02 cells through cAMP-PKA signal pathway. This study reports, for the first time, a preliminary characterization of physicochemical properties and biological activity of orcinol glucoside, together with an evaluation of their cytotoxic activities against several GI tract cancer cell lines.

In the present study, PEG-coated orcinol glucoside-loaded nanostructured lipid carriers (OG-PEG-NLC) were prepared with the purpose of targeting GItract cancer with improved anticancer activity for oral delivery of OG. The physicochemical characterization such as the particle size, polydispersity index (PDI), zeta potential (Z.P.), TEM (transmission electron microscopy), SEM (scanning electron microscopy), AFM (atomic force microscopy), drug loading capability, crystalline form and *in vitro* release were also investigated and monitored in detail. Finally, the relative cytotoxicity of OG-PEG-NLC to OG-NLC and OG suspension were evaluated in GItract cancer cell lines.

2. Materials and methods

2.1. Materials

Orcinol glucoside (OG) was isolated and provided by Dr. Parasuraman Jaisankar Laboratory, Chemistry division, Indian Institute of Chemical Biology (IICB) (West Bengal, India), with a purity of 99%. Tristearin (TS), oleic acid (OA), polyethylene glycol- 25- stearate (PEG-25-SA) and polyethylene glycol-55- stearate (PEG-55-SA) were purchased from TCI Chemicals, India. Dialysis bag (12 kDa MWCO), DMEM and RPMI 1640 medium with L-glutamine, fetal calf serum (FCS), trypsin (Gibbco, USA), HEPES, sodium pyruvate, penicillin - streptomycin (Biowest, Germany), gentamycin (Nicholas, India), MTT [3-(4,5-dimethylthiozol-2-il)-2,5-2,5- dipheniltetrazoliumbromide] were purchased from SRL. Tween 80 was purchased from Sisco Research Laboratory, India. AR grade disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$), sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$) and sodium chloride (NaCl) were the products of Merck Specialties Pvt. Ltd, India. Dimethyl sulfoxide (DMSO), sodium bi-carbonate, and other chemicals and reagents were of analytical grade and purchased from local firms. All the chemicals used were stated to be ≥99% pure and used as received. Double distilled and HPLC grade water were used throughout the study.

Hepatocellular carcinoma cell line HepG2, hepatocyte-derived carcinoma cell lineHuh-7, colorectal carcinoma cell line HCT-116 and gastric adenocarcinoma AGS cells were obtained from National Facility for Animal Tissue and Cell Culture, Pune, India. The HepG2, Huh-7 and HCT-116 cells were cultured in DMEM and AGS cell was RPMI 1640 medium routinely maintained and supplemented with 10% heat inactivated fetal calf serum, 1% penicillin - streptomycin and were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. During the sub culturing of cells, the adherent property can be diminished by adding 1x Trypsin solution in the cell. In all the experiments, untreated Hepatocellular carcinoma cell line HepG2, hepatocyte-derived carcinoma cell lineHuh-7, colorectal carcinoma cell line HCT-116 and gastric adenocarcinoma AGS cells were termed as control group.

2.2. Methods

OG-PEG-NLC and OG-NLC were prepared by the hot homogenization - ultrasonication method as previously described[31]. Briefly, 5 wt. % orcinol glucoside (weight percentage of drug to the total amount of lipids), 100 mg of lipids (TS: PEG-25/55-SA: OA = 4:4:2, w/w/w) were weighted and then completely co-dissolved into chloroform-methanol mixture (3:1, v/v); the solvent was removed using a rotary evaporator. The thin film thus obtained was melted at 75 °C and dispersed in the preheated aqueous surfactant solution (Tween 80, 200mg). The coarse emulsion was exposed to high speed dispersion for 1 h; the obtained pre-emulsion was sonicated for a period of 1h with a probe sonicator (Takashi U250, Takashi Electric, Japan) at 150 W/kHz maintaining the same temperature to produce nanoemulsions which were allowed to cool down to room temperature in order to acquire OG-PEG-NLC dispersions. The OG-NLC dispersions were prepared by the same process; except that PEG-SA was replaced by an equivalent amount of TS. The obtained NLCs were stored at 4 °C for further study.

Mean particle size, population distribution, polydispersity index and zeta potential of the NLCs were measured in a dynamic light scattering spectrometer using Malvern Zetasizer Nano Series ZS90 (Malvern Instruments, Malvern, UK) at 25 °C. Calorimetric measurements were performed using DSC 1 STAR^e system (Mettler Toledo, Switzerland). The DSC studies were performed in the temperature range of -30 °C to 100 °C with a scan rate of 2.5 °C/min. The phase transition temperature and other relevant thermal parameters were evaluated from the obtained DSC thermograms of respective samples using STAR^e Software Version 11.00. Shape, morphology and surface topology of the NLCs were investigated by TEM (Hitachi, Japan), SEM (FEI Quanta-200 MK2, Oregon, USA) and tapping mode AFM (Nanoscope III, Bruker, Switzerland) studies[31]. *In vitro* drug release of entrapped drug from PEG-NLC and NLC formulations were studied by employing dialysis tube diffusion techniques[31]. 5 ml of OG-NLC and OG-PEG-NLC dispersion equivalent to 5% OG-NLC (w/v) was individually kept in a dialysis membrane (MWCO-12 kDa Himedia, India) which was tied at both ends and placed in separate beaker containing 20 ml phosphate buffered saline (PBS), pH 7.4, 6.8 and 1.2 at 37 °C with constant stirring at 100 rpm. Drug releasing medium (2 mL) was withdrawn at predetermined intervals and replaced

with equal volume of fresh buffer to maintain sink conditions. The withdrawn samples were analyzed for OG content by measuring the absorbance at 273 nm in a UV- spectrophotometer. The experiment was performed thrice.

Entrapment efficiency (EE) and drug loading (DL) were determined by the standard methods as reported elsewhere[54]. OG content was estimated by UV-spectrophotometer method, measuring the absorbance at 273 nm. Entrapment efficiency (EE) and drug loading (DL) capacity of NLCs were calculated using the following equations:

$$EE\% = \frac{W_{TC} - W_{FC}}{W_{TC}} \times 100 \quad (1)$$

$$DL\% = \frac{W_{TC} - W_{FC}}{W_{TC} - W_{FC} + W_{TL}} \times 100 \quad (2)$$

where, W_{TC}, W_{FC} and W_{TL} represent total amounts of OG, free OG and total amount of lipid respectively.

2.3. *In vitro* cytotoxicity study

All the cancer cells ($1x10^5$) were seeded in 96-well plates and incubated inside a CO_2 incubator for 24 h before treatment. The cells were treated with freshly prepared OG-PEG-25-SA-NLC, OG-PEG-55-SA-NLC and OG-NLC comprising different concentrations (0.625, 1.25, 2.5, and 5 µm/ml) for 24, 48 or 72 h at 37 °C in a humidified atmosphere containing 5% CO_2. Untreated cells served as control. The cell growth inhibition studies were performed by MTT assay and the absorbance of this colored solution can be quantified by measuring at a certain wavelength (HepG2, Huh-7 and HCT at 540 nm and AGS cells at 492nm) by microplate manager (Reader type: Model 680 XR Bio-Rad laboratories lnc.). IC_{50} values for all the cell lines were determined for 24 and 48hrs.

3. Results and discussion

3.1. Disperions / solution behavior of the NLCs

3.1.1. Dynamic light scattering (DLS) studies

The average hydrodynamic diameter (d_h), polydispersity index (PDI) and zeta potential (Z.P.) are the physical characteristics of the colloidal dispersion for

the evaluation of the drug carriers. The influence of the PEG on d_h, PDI and Z.P. values of different NLCs was assessed keeping the weight % of TS: OA and TS: PEG-25-SA/PEG-55-SA: OA fixed at 80:20 and 40:40:20 respectively and using Tween 80 (200 mg) as the dispersion medium as well as the stabilizer. The resulting non-PEGylated NLC and PEGylated NLCs were TS+OA, TS+PEG-25-SA+OA, and TS+PEG-55-SA+OA.

Table 1. Photon correlation spectroscopy dimensional analysis, entrapment efficiency and drug loading capacity of blank as well as orcinol glucoside loaded non - PEGylatd and PEGylated NLCs.

Formulations	SIZE (nm)	PDI	ZP	EE%	DL%
TS+OA	160±3	0.30±0.003	-20.4±0.3	–	–
TS+-PEG-25-SA+OA	182±5	0.15±0.001	-8.5±0.5	–	–
TS+PEG-55-SA+OA	186±4	0.14±0.006	-8.1±0.7	–	–
TS+OA+OG	230±2	0.37±0.012	-13.5±0.1	93.70±1.7	4.52±0.04
TS+-PEG-25-SA+OA+OG	203±4	0.19±0.009	-9.5±0.2	99.62±1.5	4.74±0.07
TS+PEG-55-SA+OA+OG	207±6	0.18±0.013	-9.2±0.6	99.30±0.9	4.73±0.09

N= 3: Mean±SD

OA: Oleic acid: common in all the systems; TS: tristearin; PEG-25-SA: polyethylene glycol - 25 stearate; PEG-55-SA; polyethylene glycol - 55 - stearate; PDI: Polydispersity index; EE: encapsulation efficiency; DL: drug loading; Blank NLC: NLC with no drug; OG+NLC: NLC with drug (5mg).

The size distributions curve (data not shown) as well as from the PDI values shown in Figure 2 were revealed that the particles were fairly monodispersed. Sizes of the non-PEGylated NLC and PEGylated NLCs ranged from 160 to 230 nm with a unimodal distribution. The d_h-time profiles for NLC formulations have been presented in Figure 1; d_h values were mainly dependent on the PEG. NLC formulations were studied upto 120 days. As shown in Table1, particle sizes of TS+OA, TS+PEG-25-SA+OA, and TS+PEG-55-SA+OA were found to be 160 ± 3 nm, 182 ± 5 nm, and 186± 4 nm respectively.

From the Table 1, it was found that the size of PEGylated NLCs were higher than those of the non-PEGylated NLCs. The average diameters also increased which suggests that the PEG chains are located on the surface of the NLC[55]. Incorporation of orcinol glucoside (OG) into PEGylated and non-PEGylated NLC resulted, significant effects on the particle size and the PDI

values. Incorporation of OG into PEGylated NLCs the size was found to be 203 nm and 207 nm for OG-PEG-25-SA NLC and OG-PEG-55-SA NLC respectively. However, in case of OG loaded in non-PEGylated NLC the size was found to be 230nm. Hence the drug loaded PEGylated and non-PEGylated NLC size was higher than the corresponding bare PEGylated and non-PEGylated NLCs.

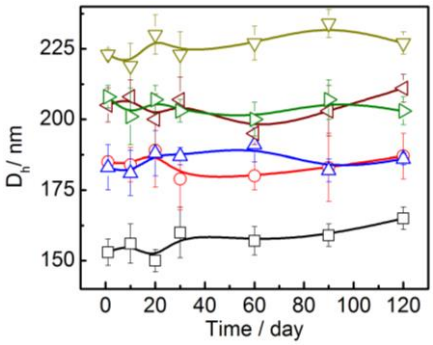

Figure 1. Variation in the hydrodynamic diameter (d_h)–time profile of NLCs (black): TS+OA; (red): TS+PEG - 25 - SA+OA; (blue): TS+PEG - 55 - SA+OA; (dark yellow): TS+OA+OG; (wine): TS+PEG - 25 - SA+OA+OG and (olive): TS+PEG - 55 - SA+OA+OG. 100mg NLC was dispersed in 200mg Tween 80 in each case. OG concentration was (5mg). Temp. 25 °C.

The size enhancement was more prominent in case of non-PEGylated NLC. This could be attributed to the fact that due to the amphiphilic nature of the drug, the accumulated drug form shell enriched type NLC with conventional NLC as base.

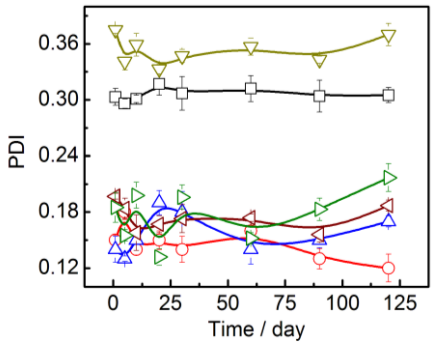

Figure 2. Variation in the polydispersity index (PDI)–time profile of NLCs (black): TS+OA; (red): TS+PEG - 25 - SA+OA; (blue): TS+PEG - 55 - SA+OA; (dark yellow): TS+OA+OG; (wine): TS+PEG - 25 - SA+OA+OG and (olive): TS+PEG - 55 - SA+OA+OG. 100mg NLC was dispersed in 200mg Tween 80 in each case. OG concentration was (5mg). Temp. 25 °C.

However, in case of PEGylated NLCs, the hydrophilic head group of PEGylated lipids assists the drug molecules to reside in the lipophilic part of the PEGylated NLCs. Thus, partitioning of the drug in lipophilic part of the formulation gets increase. As the drug was getting inserted into the morphological defects of the NLC, the size enhancement was not higher in comparison to the conventional NLCs.

The analysis of zeta potential was a useful way in order to predict the physical storage stability of colloidal carriers. The zeta potential value was affected by the presence of the PEG chains; the zeta potential of TS+PEG-25-SA NLC and TS+PEG-55-SA NLC (-8.5±0.5 and - 8.1±0.7 mV) respectively, are lower than those of the non-PEGylated NLC (-20.4±0.3 mV) as presented in Figure 3. The results indicated that a strong negative charge of NLC surface is partially neutralized by PEG coating. Rationally, tristearin nanoparticles presented a marked negative charge due to the presence of anionic lipids in their composition. This negative charge was reduced in the case of PEG-coated nanoparticles due to the extension in the plane of shear produced by the presence of this polymer on the surface[56,57].

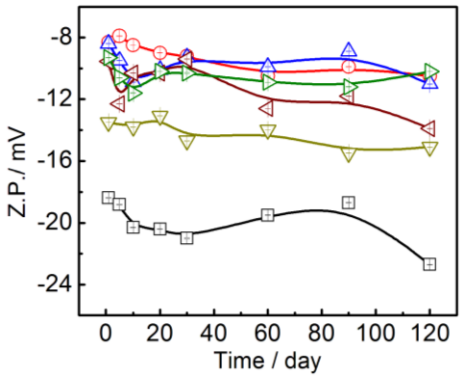

Figure 3. Variation in the zeta potential (Z.P.)–time profile of NLCs (black): TS+OA; (red): TS+PEG - 25 - SA+OA; (blue): TS+PEG - 55 - SA+OA; (dark yellow): TS+OA+OG; (wine): TS+PEG - 25 - SA+OA+OG and (olive): TS+PEG - 55 - SA+OA+OG. 100mg NLC was dispersed in 200mg Tween 80 in each case. OG concentration was (5mg). Temp. 25 °C.

It also can be explained that the steric stabilization effects resulting from Tween-80 avoid aggregation of the nanoparticles in the PEGylated NLCs system.

Meanwhile, the stability of the lipid suspension could be further improved by hydration of hydrophilic Tween 80 coating[58].

In case of OG loaded PEGylated and non-PEGylated NLCs the zeta potential value was found to be -13.5±0.1, - 9.5±0.2 and -9.2±0.6 mV for TS+OA+OG, TS+OA+PEG-25-SA+OG and TS+OA+PEG-55-SA+OG NLCs. In case of OG loaded PEGylated NLCs no significant zeta potential variation was observed. However, in case of non-PEGylated NLC marked decreased zeta potential values was noted. This could be explained by the fact that in case of conventional NLC, the surface accumulated drug mask the surface charge, hence reduced the magnitudes of zeta potential. On the contrary, due to the accumulation of the drug in the morphological defects, no significant variation in zeta potential values was noticed for drug loaded PEGylated NLCs.

3.2. Morphology studies

3.2.1. TEM, SEM and AFM studies

TEM and SEM images were performed in order to investigate the morphology, shape and size of the bare as well as OG loaded PEGylated and non-PEGylated NLCs. Figure 4 (TEM) and (SEM) shows the image of non-PEGylated NLC and PEGylated NLCs.

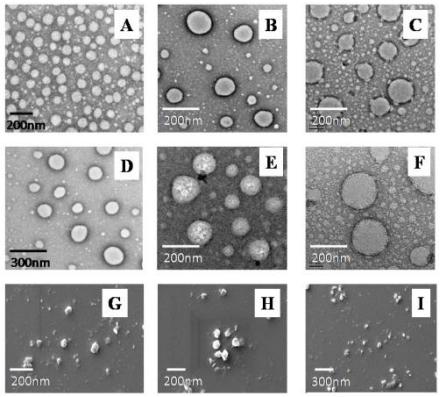

Figure 4. TEM and SEM images of (A and G) TS+OA, (B and H) TS+PEG-25-SA+OA and (C, I) TS+PEG-55-SA+OA NLC respectively; (D, E and F) are the corresponded orcinol glucoside loaded formulations.

From the TEM and SEM micrographs, it was evident that particles were spherical in shape and homogeneously distributed with size ranging between 160 and 230 nm. It was found that the size of the NLCs as determined from the TEM

and SEM image analysis correlated well with the particle size data that was obtained from DLS[31,59]. As shown in the representative images of the bare as well as OG loaded non-PEGylated NLCs and PEGylated NLCs shown in the Figure 4, all NLCs showed spherical morphology and smooth surface. This also indicates that all the systems are monodispersed, and incorporation of OG did not seem to cause morphological changes as was observed by DLS.

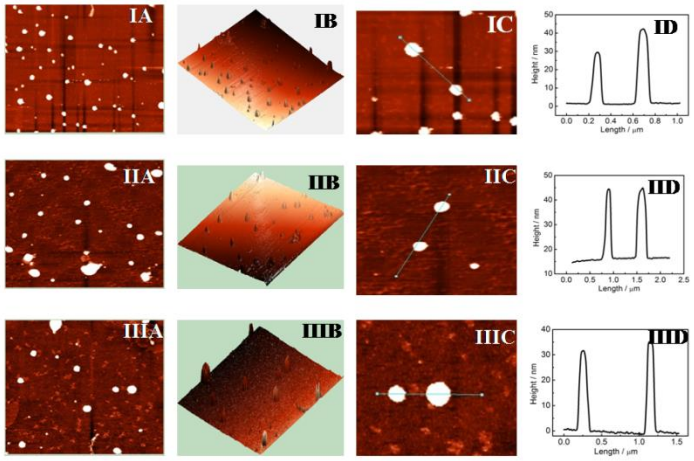

Figure 5. AFM images of (I) TS+OA (II) TS+PEG-25-SA+OA and (III) TS+PEG-55-SA+OA NLC formulations. (A) Two-dimensional images,(B) Three-dimensional images and (C and D) section analysis.

Further surface morphology of bare as well as OG loaded non-PEGylated and PEGylated NLCs comprising TS+OA, TS+PEG-25-SA+OA, TS+PEG-55-SA+OA and TS+OA+OG, TS+PEG-25-SA+OA+OG, TS+PEG-55-SA+OA+OG respectively were evaluated by AFM analysis in detail. AFM tapping mode images are shown in Figure 5 and 6, showing both planner and 3D images in terms of height. The shape of non-PEGylated and PEGylated NLCs was found spherical and smooth surface which is similar to TEM and SEM analysis. NLCs were separate from each other indicating the absence of aggregated species. The particle size range of 160 - 230 nm (Figure 5 and 6) obtained with AFM was in comparison to TEM, SEM and DLS results. The average height of closely packed non-PEGylated and PEGylated NLCs were found 30 to 40 nm.

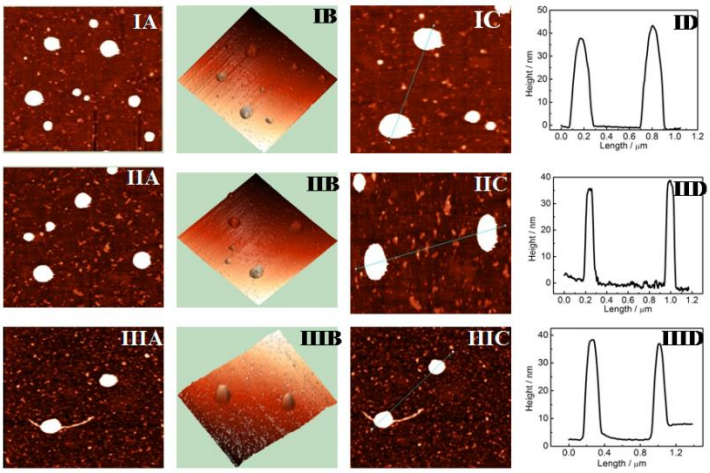

Figure 6. AFM images of (I) TS+OA+OG (II) TS+PEG-25-SA+OA+OG and (III) TS+PEG-55-SA+OA+OG NLC formulations. (A) Two-dimensional images, (B) Three-dimensional images and (C and D) section analysis.

3.3. Differential scanning calorimetri(DSC) investigations

DSC studies were carried out in order to investigate the influence of orcinol glucoside on the melting, polymorphic transition and crystallization behavior of the NLCs as well as to perceive the possibility of modification in the crystallization process with and without PEG-25/55- stearate. The phase transition temperature (T_m) of the individual components *viz*., tristearin , PEG-25-SA, PEG-55-SA, oleic acid, and the drug orcinol glucoside were recorded separately and were found to be 60.88, 43.61, 51.66,14.63 and 122.17 °C respectively as shown in Figure 7.

In case of the physical mixtures (where the components were dissolved in appropriate organic solvents and then dried under vacuum), the sharp peak of OG, which appeared at122.17 °C in its pure state disappeared as demonstrated in Figure 8. This indicates complete solubilization or amorphous state of orcinol glucoside in the lipid matrix[60,61]. Initially, physical mixture of lipids *viz*., lipids (TS+OA), lipids + OG, lipids + polymer, and lipids + polymer + OG were evaluated and the results are presented in Figure 9 (panel C and D). The endothermic T_m values of the physical mixture of TS+OA, TS+OA+OG, TS+PEG-25-SA+OA, TS+PEG-25-SA+OA+OG, TS+PEG-55-SA+OA and TS+PEG-55-SA+OA+OG appeared to be 61.43, 59.04, 57.13, 55.69, 57.83 and 56.06 °C however, the exothermic T_m values were found to be 44.38, 41.29,

39.83, 37.12, 38.64 and 35.91 °C respectively as presented in Figure 9 (panel C and D respectively) .

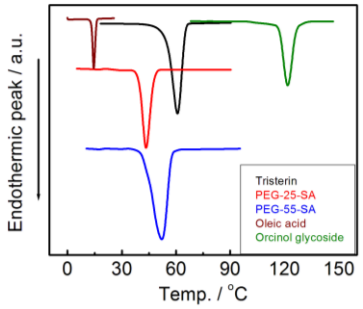

Figure 7. DSC heating thermograms of orcinol glucoside and the pure lipid components; systems mentioned inside the figure. Scan rate: 2.5 °C min^{-1}.

The T_m value of the TS+OA+OG, as well as TS+OA+PEG-25/55-SA+OG however, decreased significantly with OG incorporation into lipid as well as polymer - lipid, which indicates the change in the crystal order structure of the lipid matrix; this may be attributed to OG entrapment in the case of NLCs. In addition TS+OA+OG mixture, as well as TS+OA+PEG-25/55-SA+OG although the peak was broader compared to the corresponding physical mixture of TS+OA and TS+OA+PEG-25/55-SA mixture as shown in the Figure 9 (panel C and D respectively), which may be attributed to the entrapment of OG in the NLCs.

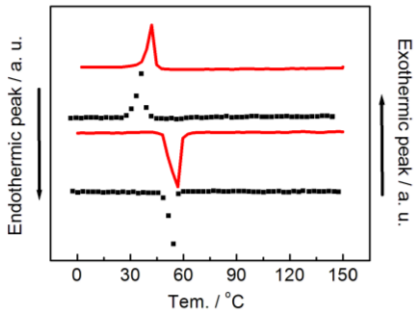

Figure 8. Representative DSC thermograms of (red) TS+OA+OG and (black) TS+PEG-25-SA+OA+OG, 80: 20:5 and 40:40:20:5 mg/mg/mg/mg) comprising physical mixture. Scan rate: 2.5 °C min^{-1}.

In comparison to the bulk OG + lipid and OG + PEG-25/55-SA + lipid, both the melting point and enthalpy of all NLC formulation decreased. The endothermic peak maximum of bulk TS+OA+OG, TS+OA+PEG – 25 – SA+OG

and TS+OA+PEG-55-SA+ OG lipid decreased from 59.04, 55.69, 56.06 to 52.43, 52.46 and 51.62 °C, however, the exothermic peak maximum the values were 41.29, 37.12 and 35.91 °C to 26.11, 26.51 and 25.16 °C when the corresponding systems were formulated as NLCs. Such decrease in the melting point can be explained by the small particle size in the nanometer range, the higher specific surface area, and stabilized by aqueous surfactant[58,62,63]. The melting point depression owing to the reduction of particle size to the nanosize range can be attributed to the Kelvin effect which is described by the Thomson equation as well as due to the presence of drug[31,63,64].

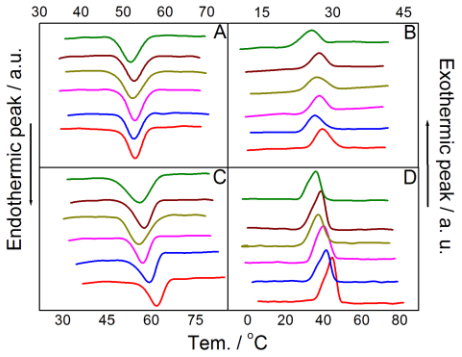

Figure 9. DSC endothermic (panel A) and exothermic curves (panel B) of bare as well as orcinol glucoside-loaded non-PEGylated and PEGylated NLCs: (red) TS+OA, (blue) TS+OA+OG, (pink) TS+PEG-25-SA+OA, (dark yellow) TS+PEG-25-SA+OA+OG, (brown) TS+PEG-55-SA+OA and (green) TS+PEG-55-SA+OA+OG.Panel C and panel D are the physical mixture of corresponded NLC. An orcinol glucoside concentration was 5 mg. The scan rate was 2.5 °C/min.

Both the endothermic and exothermic peak were considered in order to determine the other associated thermal parameters, *viz.* change in enthalpy(ΔH), change in heat capacity (ΔC_p) and width of half height at the peak ($\Delta T_{1/2}$). The DSC endothermic and exothermic thermograms of free as well as orcinol glucoside loaded non-PEGylated and PEGylated NLCs *viz.* TS+OA, TS+OA+OG, TS+PEG – 25 – SA+OA, TS+PEG – 25 – SA+OA+OG, TS+PEG – 55 – SA+OA, and TS+PEG – 55 – SA+OA+OG NLCs are presented in Table 2.

Considering the drug free systems, significant changes in the T_m values were noted between non-PEGylated and PEGylated NLC. In case of drug free system

for endothermic and exothermic thermograms, the T_m values were 53.13, 52.73, 52.67 °C and 27.76, 27.09, 27.19 °C for TS + OA, TS + PEG – 25 – SA + OA and TS + PEG – 55 – SA + OA respectively.

Table 2. Temperature for maximum heat flow (T_m), change in enthalpy (ΔH), the width at half peak height ($\Delta T_{1/2}$), heat capacity (ΔC_p) and crystallinity index of orcinol glucoside loaded PEGylated and non - PEGylated NLCs.

Heating thermogram					
Formulations	***T_m / °C***	***ΔH/kcal.mol^{-1}***	***$\Delta T_{1/2}$ / °C***	***ΔC_p/kcal.mol^{-1}C^{-1}***	***% CI***
NLC-B	53.13	13.41	3.23	4.15	17.19
NLC-OG	52.43	9.54	3.43	2.78	12.23
NLC-PEG-25-SA	52.73	12.82	4.03	3.18	16.44
NLC-PEG-25-SA-OG	52.46	11.1	5.04	2.20	14.23
NLC-PEG-55-SA	52.67	12.92	4.24	3.05	16.56
NLC-PEG-55-SA-OG	51.62	11.23	5.25	2.14	14.40
Cooling thermogram					
Formulations	***T_m / °C***	***ΔH/kcal.mol^{-1}***	***$\Delta T_{1/2}$ / °C***	***ΔC_p/kcal.mol^{-1}C^{-1}***	***%CI***
NLC-B	27.76	20.84	3.79	5.50	62.70
NLC-OG	26.11	11.11	3.97	2.80	33.42
NLC-PEG-25-SA	27.09	15.15	4.15	3.66	45.58
NLC-PEG-25-SA-OG	26.51	6.23	4.87	1.28	18.74
NLC-PEG-55-SA	27.19	10.19	4.69	2.17	30.66
NLC-PEG-55-SA-OG	25.16	4.67	4.91	0.96	14.05

OA: Oleic acid: common in all the systems; TS: tristearin; PEG-25-SA: polyethylene glycol - 25 stearate; PEG-55-SA; polyethylene glycol - 55 - stearate; OG: orcinol glucoside.

The significant decrease in T_m value with incorporation of polymers could be attributed to the fact that the PEG chain incorporated / interacted into the lipid matrix. Wide peaks indicate the presence of multicrystalline entities in the NLC formulation. The effect of polymers on the thermal behavior of NLCs were further scrutinized as shown in the Table 2. From the results, it implies that the

$\Delta T_{1/2}$ values were higher for PEGylated systems over non-PEGylated system. Results further support our proposition as already mentioned previously[65,66]. Interaction of the lipidic core by hydrocarbon chain of PEG -25/55-stearate, resulted in increasing the crystal imperfection, led to increase the $\Delta T_{1/2}$ values. Changes in enthalpy values for the PEGylated NLCs were lower than the corresponding non-PEGylated NLC. Increase in multicrystallinity with adding PEG - 25/55 - SA would effectively result in decreased ΔH value. Similar trend was observed for change in heat capacity (ΔC_p) profile, further supports our proposition[63]. Crystallinity index (CI) is another DSC derived thermal parameter which can highlight the effect of PEG- 25/55 - SA and drug on the molecular packing of the NLC components. The pegylated NLC crystallinity decreased with adding PEG- 25/55- SA. This was due to the interaction / incorporation of PEG – 25 / 55 – SA into the lipidic components.

Furthermore, in order to investigate the impact of OG on the PEGylated and non-PEGylated NLC, the associated thermal parameters were also evaluated. In case of drug loaded PEGylated and non-PEGylated NLC systems the phase transition temperature (T_m values), change in enthalpy (ΔH) and change in heat capacity (ΔC_p) and crystallinity index (CI) were appreciably decrease with incorporation of drug could be explained due to the incorporation of drug in to the NLCs. Nevertheless, the $\Delta T_{1/2}$ values were higher with addition of drug in to PEGylated and non-PEGylated systems as demonstrated in Table 2. Results further support our proposition.

3.3. Determination of drug entrapment efficiency and loading capacity

Entrapment efficiency (EE) and drug loading (DL) are two important characteristics to assess the efficiency of carriers. The higher EE and DL represent more powerful ability of carriers. In the present work, the EE of OG-NLC, OG-PEG-25-SA-NLC and OG-PEG-55-SA-NLC were 93.70. ± 1, 99.62 ± 1.5, 99.30 ± 0.9% and the DL were 4.52 ± 0.04, 4.74 ± 0.07, 4.73 ± 0.09% respectively, also summarized in Table 1 along with other data. These data clearly showed that OG-PEG-25-SA-NLC / OG-PEG-55-SA-NLC had excellent drug-loading capacity over OG-NLC. However, compared with OG-NLC, the EE and DL of OG-PEG-25-SA-NLC / OG-PEG-55-SA-NLC were evidently higher. Since the components and the preparation method of OG-PEG-25-SA-

NLC / OG-PEG-55-SA-NLC and OG-NLC were the same, except PEG-25-SA/ PEG–55-SA instead of TS partially, incorporation of PEG-25-SA/ PEG-55-SA in lipid matrix resulted in higher EE% and DL% as compared with OG-NLC might be attributed to the use a combination of highly ordered with less ordered lipids, which caused numerous crystal defects in solid matrix and provided much imperfections to accommodate more drug molecules during the formation of nanoparticles. Therefore, the amount of drug incorporated in OG-PEG-25-SA-NLC / OG-PEG-55-SA-NLC higher considerably[15].

3.5. *In vitro* release studies

The *in vitro* release behavior of OG from PEGylated, non-PEGylated NLC dispersion and native OG were studied by conventional dialysis diffusion method using cellulose as a membrane[31]. The cumulative percentage release of native OG, OG-NLCs, OG-PEG-25-SA-NLC and OG-PEG-55-SA-NLC dispersion over 48 h are shown as in Figure 10. OG showed 94.21, 82.18, 71.48, 60.39% for pH 7.4; 95.99, 85.18, 68.41, 63.17% for pH 6.8; and 92.86, 81.65, 69.54, 62.71% for pH 1.2 release from the free OG, OG-NLCs, OG-PEG-25-SA-NLC and OG-PEG-55-SA-NLC dispersion respectively. The total amount of the drug released from the PEGylated NLCs was significantly lower than the non-PEGylated NLC as well as OG solution. Analogous studies were carried out using the OG separately in the absence of PEGylated and non-PEGylated NLC. It has been established that the diffusion of drug across the dialysis membrane was not the limiting step of the overall diffusion process[67]. From the result, it might be suggested that the OG in the PEGylated-NLCs released steadily over the given period, while the native OG showed more rapid release of OG than OG PEGylated and non-PEGylated NLC. These indicate that release of OG incorporated in PEGylated NLCs were retarded predominantly compared to the same in non-PEGylated NLC as well as OG solution. Thus, the PEGylated NLC dispersion could be a useful carrier with better control of OG release.

Biphasic release behavior was observed for all the PEGylated and non-PEGylated OG-NLCs as established by the initial burst release within 2 h (61.20, 37.94, 27.05, 24.87% for pH 7.4; 62.33, 39.49, 29.59, 27.21% for pH 6.8; and 54.49, 35.49, 26.31, 24.14% for pH 1.2) from the free OG, OG-NLCs, OG-PEG-

25-SA-NLC and OG-PEG-55-SA-NLC dispersion respectively followed by a sustained release for 48h. Results revealed that the initial burst release of OG was higher with non-PEGylated NLCs as shown in the Figure 10 as compared to the PEGylated NLCs. This could be due to covering effect of coating layer. Coating the surface leads to a decrease in the burst release effect compared to non-PEGylated NLCs because the adsorbed orcinol glucoside on lipid surface slowly releases from PEG 25/55-SA coated NLCs. Following the initial burst, the systems provided a sustained and controlled release of the orcinol glucoside. This control release might be attributed to the PEG coating on the TS+PEG-25/55-SA+OA+OG NLCs surface acting as the hydrophilic shield surrounding NLC, due the incorporation of PEG-25/55-SA or affinity of the drug for the lipids as well as the absence of degradation of the lipid matrix under the *in vitro* release conditions[55,68-71]. The initial burst release decreases because of higher thickness of modified NLCs based on Fickian diffusion[55,69-71]. Moreover, in order to investigate whether pH is an important factor on drug release, orcinol glucoside loaded PEGylated, non-PEGylated NLC dispersions and free OG were studied in different simulated gastrointestinal solutions: pH 1.2 gastric solutions, pH 6.8 intestinal solutions and blood pH 7.4 as presented in Figure 10. Results indicated that the drug release in different pH for PEGylated and non-PEGylated NLC systems showed similar trends.

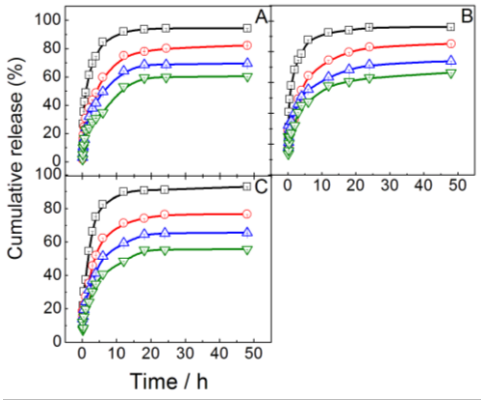

Figure 10. *In vitro* cumulative release of orcinol gluoside from NLCs. Composition of NLCs: (black squares) free OG, (red circles) TS + OA + OG, (blue up triangles) TS + PEG – 25 – SA + OA + OG and (green down triangles) TS + PEG – 55 – SA + OA + OG in PBS panel A (pH 7.4), panel B (pH 6.8) and panel C (pH 1.2) at 37 ± 0.1 °C. Error bars represent the standard deviation (SD) of three different release experiments.

The obtained drug release data were fitted with 4 common release kinetics models: (i) Korsemeyer-Peppas (ii) Higuchi model (iii) Hixson-crowell and (iv) First order by using DDSolver 1.0, an Add-In program for modeling and comparison of drug release mechanism profiles[72]. The highest regression values (r^2) have been used to decide the best fit model among all the four models. The release rate constant and regression co-efficient were calculated from the slope of the appropriate plots. The results are summarized in Table 3. Release kinetics for all the NLCs followed Korsemeyer–Peppas model. The regression coefficient (r^2) values of Korsemeyer–Peppas model for all the NLCs were 0.99. The release exponent (n) determined from Korsemeyer–Peppas model for all the NLCs were found to be less than 0.5 suggesting that the release mechanism was controlled by Fickian diffusion in all the systems. The release rate constants of TS+OA+OG, TS+PEG-25-SA+OA+OG and TS+PEG-55-SA+OA+OG comprising OG-NLCs were 24.26 h^{-1}, 17.69h^{-1} and 15.95h^{-1} respectively.

Table 3. Drug release kinetics profiles of orcinol glucoside loaded PEGylated NLCs and non - PEGylated NLCs.

Formulation	Korsemeyer-Peppas			Higuchi		Hixson-Crowell		First order	
	r^2	k	n	r^2	k	r^2	k	r^2	k
PEG-55-SA+ OG-NLC	0.99	15.95	0.295	0.95	7.87	0.95	0.005	0.97	0.018
PEG-25-SA+ OG-NLC	0.99	17.69	0.283	0.96	8.72	0.96	0.006	0.97	0.025
NLC+OG	0.99	24.26	0.319	0.98	10.93	0.94	0.014	0.95	0.082

*r^2=regression coefficient, k= release rate constant, n=diffusional exponent.

3.6. *In vitro* cytotoxicity of OG-NLCs

To evaluate the cytotoxicity of orcinol glucoside when administered as free forms or loaded in conventional NLC and PEG-25/55-SA coated NLCs, *in vitro* cytotoxicity assays were carried out on GIT cell lines such as hepatocellular carcinoma cell line HepG2, hepatocyte-derived carcinoma cell line Huh-7, human colorectal carcinoma cell line HCT-116and human gastric adenocarcinoma AGS cells by performing the MTT assay. The cytotoxicity of the free drug and drug encapsulated in conventional NLC and PEG-25/55-SA coated NLCs, *in vitro* cytotoxicity assays were carried out on gastric cell lines as

shown in Figure 11 – 13 respectively. The biocompatibility of the bare NLCs was investigated in our previous studies[31].

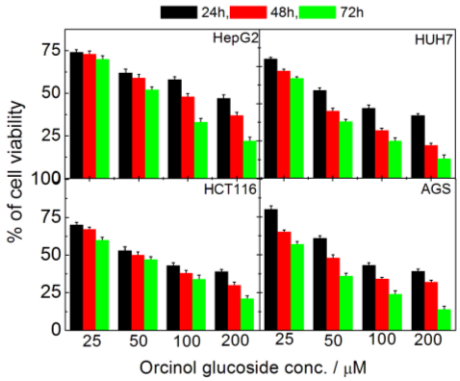

Figure 11. *In vitro* cytotoxicity activity of free orcinol glucoside on the viability of Hepatocellular carcinoma cell line (HepG2), hepatocyte-derived carcinoma cell line (Huh-7), colorectal carcinoma cell line (HCT-116) andgastric adenocarcinoma (AGS) cells. Cell was grown and treated for 24h. Experiments were performed in triplicate, with the results showing the mean and standard deviation of the triplicate of each group.

Herein, the biocompatibility of PEG-25/55-SA coated NLCs was further investigated by formulating NLCs using the same conditions in absence of the drug in order to ensure that the cytotoxicity was caused by the OG itself and not by components of the formulated NLC; cells were incubated with blank PEGylated NLCs.

Table 4. IC_{50} value of pure orcinol glucoside (OG), OG loaded non-PEGylated NLC (OG-NLC), PEG-25-SA coated OG loaded NLC (OG-P_1-NLC) and PEG-55-SA coated OG loaded NLC (OG-P_2-NLC) for Hepatocellular carcinoma cell line (HepG2), hepatocyte-derived carcinoma cell line (Huh-7), colorectal carcinoma cell line (HCT-116) andgastric adenocarcinoma (AGS) cells.

Formulation	HepG2 IC_{50} (µg)	Huh-7 IC_{50} (µg)	HCT-116 IC_{50} (µg)	AGS IC_{50} (µg)
Free OG	70.71	84.61	26.79	77.13
OG-NLC	4.79	3.96	3.78	2.03 (48h)
OG-p_1-NLC	2.26	2.19	1.91	1.32 (48h)
OG-p_2-NLC	2.5	2.24	1.64	2.10

No significant cytotoxicity of the blank PEGylated NLCs were observed after 72 hr of incubation on the gastric cell lines studied, even at the highest concentration. The results are displaced in Figure 12 – 13, which suggested that PEGylated NLCs were safe drug carrier[70].

The IC_{50} values of free OG and OG encapsulated in conventional NLCs and PEG-25/55-SA coated NLCs after 24 h, and 48 h incubation were measured and compared in order to assess the cytotoxicity activity of OG delivered by NLCs for gastric cell lines. The IC_{50} values of the free OG and OG loaded in conventional NLC (OG-NLC) and PEG-25/55- SA coated NLC (OG+PEG-25-SA + NLCs, OG+PEG-55-SA + NLCs) were shown in Table 4 at 24 h and 48 h for Hepatocellular carcinoma cell line HepG2, hepatocyte-derived carcinoma cell line Huh-7, human colorectal carcinoma cell line HCT-116and human gastric adenocarcinoma AGS cells. The IC_{50} values of the OG- NLCs, OG- PEG-25-SA-NLC and OG- PEG-55-SA-NLC at 24 h and 48 h of incubation were significantly lower than that of the free OG for all the cell lines.

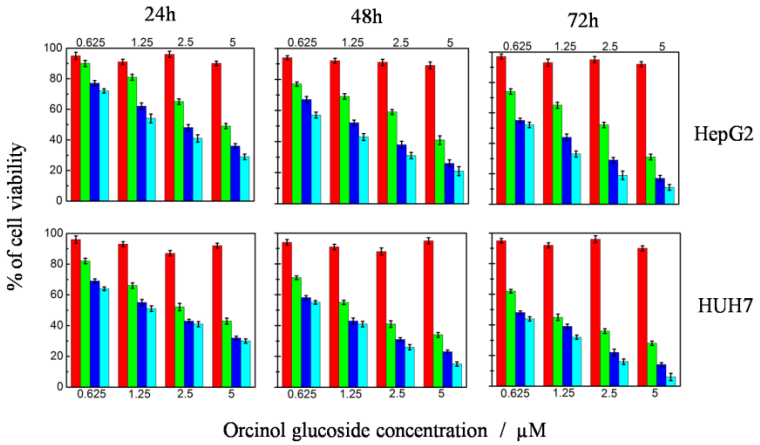

Figure 12. *In vitro* cytotoxicity activity of representative PEG coated bare NLC (—) and orcinol glucoside loaded with different NLCs: TS+OA (—); TS+PEG-25-SA+OA (—) and TS+PEG-55-SA+OA (—) on the viability of HepG2 and HUH7 cell. Cell was grown and treated for 24h, 48h and 72h. Experiments were performed in triplicate, with the results showing the mean and standard deviation of the triplicate of each group.

Moreover, the IC_{50} values of the OG- PEG-25-SA-NLCs and OG- PEG-55-SA-NLsC at 24 h and 48 h of incubation were appreciably lower than the conventional OG-NLCs for all the cell lines, which suggested that the cytotoxicity of the OG-PEG- 25-SA-NLCs and OG- PEG-55-SA-NLs were significantly higher than the conventional OG-NLC as well as free OG. The

minimum IC_{50} values and higher cytotoxicity for the OG- PEG-25-SA-NLCs and OG - PEG-55-SA-NLs for all the cell lines could be attributed to the fact that the increased intracellular drug concentration through avoiding RES recognition, mainly by modifying the NLC surface by PEG to minimize the interaction with opsonins or increased intracellular drug concentration via the transport of OG loaded PEGylated NLCs as well as sustained release and smaller size as reported by others[73-76].

Furthermore, as observed from the Figure 12 and 13 the cell cytotoxicity increased when either the concentration of OG-PEG-25/55-SA NLCs were increased or the incubation time was extended. These results indicated that the cytotoxicity of OG against gastric cell lines occurred in a concentration and time-dependent manner. This dominance may mainly be caused by better internalization of the OG-PEG-25/55-SA NLCs and the sustained release of OG inside the cancer cells[76]. Besides to the above mentioned achievements it is worthwhile to stress that OG non-PEGylated as well as PEGylated NLCs showed a remarkable cytotoxicity in gastrointestinal tract cancer cells. OG NLCs thus hold the promise in treating GItract cancer issue, and this aspect should be extensively exploited in cancer treatment.

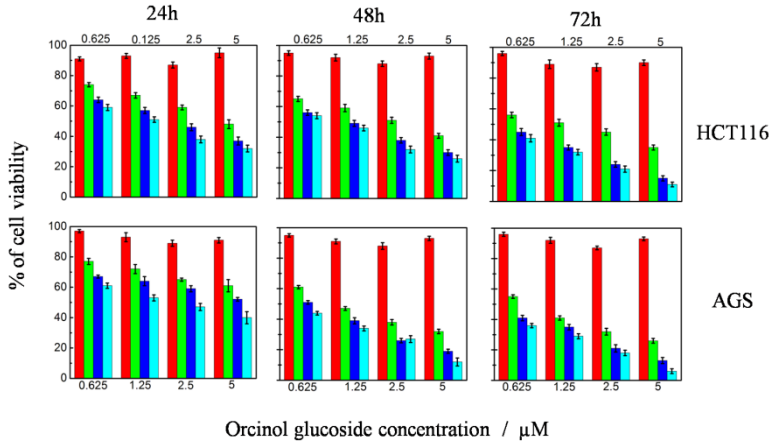

Figure 13. *In vitro* cytotoxicity activity of representative PEG coated bare NLC (—) and orcinol glucoside loaded with different NLCs: TS+OA (—); TS+PEG-25-SA+OA (—) and TS+PEG-55-SA+OA (—) on the viability of HCT116 and AGS cell. Cell was grown and treated for 24h, 48h and 72h. Experiments were performed in triplicate, with the results showing the mean and standard deviation of the triplicate of each group.

4. Summary and conclusions

In this study, orcinol glucoside loaded conventional as well as PEGylated NLCs were successfully formulated for GItract cancer targeting. The findings reveal the influence of PEG-25/55- stearate on particle size, polydispersity index, zeta potential, drug encapsulation efficiency, *in vitro* release behavior and *in vitro* cytotoxicity of the formulations. Spherical morphology with smooth surface was experienced for all the formulations. Significant difference in crystal structure between conventional and PEGylated NLCs were noted whereby the crystallinity of OG was lost due to its incorporation in the NLCs. Release of the drug was sustained for all the NLCs; PEGylated NLCs exhibited slower drug release than non-PEGylated NLCs. The most valuable findings from this report is the significant difference between the cytotoxicity of free OG and OG loaded in PEGylated and non- PEGylated NLCs, which demonstrates the superiority of OG-NLCs over free OG in penetrating cell membrane. OG in PEGylated NLCs showed comparable cytotoxicity in GIT cell lines such as Hepatocellular carcinoma cell line HepG2, hepatocyte-derived carcinoma cell line Huh-7, human colorectal carcinoma cell line HCT-116and human gastric adenocarcinoma AGS cells, and enhanced anticancer activity. Conclusively, orcinol glucoside could be a potent anticancer drug candidate for gastrointestinal tract cancer and both PEGylated and non- PEGylated NLCs formulated in the present study may be used as potential oral delivery systems for OG with improved anticancer activity.

References

References are given in BIBLIOGRAPHY under Chapter IV (pp. 183–187).

SUMMARY AND CONCLUSION

Detail studies involving the composition, functionality and structure of the adsorbed BLES films in the absence and presence of three additives, *viz.*, cholesterol, low density lipoprotein (LDL) and serum protein were performed. The study includes the pathophysiological amount of these materials found in lungs in disease. The additives altered the bilayer and film packing, surface activity and structures of surfactant, suggesting possible molecular rearrangement and disordering of surfactant in disease. Since excess cholesterol or its esters may actually arrive inside the lung through LDL transport, it is cholesterol that is the most potent inactivator of LS, than leaked soluble serum proteins, since lipids are far more hydrophobic and more difficult to remove from films during dynamic cycling. Whole serum, serum proteins and its lipid components may specifically interact with the surfactant films and bilayers by either separate mechanisms, or synergistically. In future studies, specific lipid components of serum such as HDL, one of the major studies requires the specific separation of serum lipid and serum protein fractions and observing their structure function correlations in inactivating surfactant. The specific molecular rearrangements observed in our study of domains in films need to be further explored in detecting the exact composition of the variety of domain structures using possibly fluorescently labeled cholesterol, LDL and serum proteins. However these studies need to be conducted in a manner using similar sets of biophysical methods which yield structure function correlates of bilayers and films, as well as uses pathophysiological amounts of materials, so that an over simplification of the models in previous studies can be rectified.

While considering the lipids mixtures as novel drug delivery system, nanostructured lipid carriers (NLCs) were formulated by using Span 65, soy lecithin and stearic acid dispersed in aqueous Tween 40 or Tween 60 solution. Tween 60 provides better stabilization than Tween 40 because of its longer hydrocarbon chain. Hydrocarbon chains of Tween 60 could penetrate to greater extent than Tween 40 into the NLC matrices. TEM study confirmed the spherical morphology of the NLCs with smooth surface. Higher amount of LIDO could be

encapsulated into the NLC than PRO.HCl. LIDO resides in the core of the NLC for its relatively higher hydrophobic nature. PRO.HCl, being ionic, preferentially adsorbs over the NLC surface. Apart from the DLS studies, DSC and spectroscopic investigations on the drug loaded NLC further supported such proposition. Because of its larger lipophilicity LIDO could be entrapped to greater extent compared to PRO.HCl. *In vitro* drug release study revealed that the lipidic matrices could act as promising vehicles for two most widely used local anaesthetics with controlled and prolonged release. Biphasic release behaviour was experienced by all the combinations. In order to further explore the viability, the formulations may be subjected to *in vitro* studies under biological condition. Besides, the *in vivo* studies as well as some clinical trials are warranted which are considered as the future perspectives.

The impact of saturation and unsaturation in the fatty acyl hydrocarbon chain on the physicochemical properties of nanostructured lipid carriers (NLCs) was investigated to develop delivery systems loaded with the anticancer drug, ursolic acid (UA). The findings reveal the influence of saturated and unsaturated lipids and fatty acids on the particle size, polydispersity index, ζ potential, encapsulation efficiency, *in vitro* release behavior and *in vitro* cytotoxicity of the formulations. The studies of surface pressure (π) − area (A) isotherms of pure components, mixed lipids, and mixed lipids with ursolic acid suggest that ursolic acid alters the interfacial organization of lipids. The spherical morphology of NLCs with a smooth surface was observed for all the formulations. Significant differences in crystal structure between NLCs comprising saturated and unsaturated lipids were noted, whereby the crystallinity of UA was lost because of its incorporation into the NLCs. Release of the drug was sustained for all the NLCs; unsaturated lipids exhibited drug release faster than that of saturated components. The most useful finding from this report is the significant difference between the cytotoxicity of free UA and UA-loaded NLCs, which demonstrates the superiority of UA-loaded NLCs over free UA in penetrating the cell membrane. UA in saturated and unsaturated lipids and fatty acid comprising NLCs showed comparable cytotoxicity in human leukemic cell line K562 and melanoma cell line B16 and enhanced anticancer activity. Conclusively, both saturated and unsaturated lipid-containing NLCs formulated in this study may be used as potential delivery systems for UA with improved anticancer activity.

Orcinol glucoside-loaded nanostructured lipid carrier (NLC) coated with polyethylene glycol - 25/55 - stearate (PEG-25/55-SA) were formulated and evaluated for oral delivery of orcinol glucoside (OG) to improved *in vitro* cytotoxicity against GIT cell lines such as Hepatocellular carcinoma (HepG2), hepatocyte-derived carcinoma (Huh-7), humancolorectal carcinoma (HCT-116) and human gastric adenocarcinoma AGS cells. The findings reveal the influence of PEG-25/55- stearate on particle size, polydispersity index, zeta potential, encapsulation efficiency, *in vitro* release behavior and *in vitro* cytotoxicity of the formulations. Spherical morphology with smooth surface was experienced for all the formulations. Significant difference in crystal structure between conventional and PEGylated NLCs were noted whereby the crystallinity of OG was lost due to its incorporation in the NLCs. Release of the drug was sustained for all the NLCs; PEGylated NLCs exhibited slower drug release than non-PEGylated NLCs. The most valuable findings from this report is the significant difference between the cytotoxicity of free OG and OG loaded in PEGylated and non-PEGylated NLCs, which demonstrates the superiority of OG-NLCs over free OG in penetrating cell membrane. OG in PEGylated NLCs showed comparable cytotoxicity in GIT cell lines such as Hepatocellular carcinoma (HepG2), hepatocyte-derived carcinoma (Huh-7), human colorectal carcinoma (HCT-116) and human gastric adenocarcinoma AGS cells, and enhanced anticancer activity. Conclusively, orcinol glucoside could be a potent anticancer drug candidate for gastrointestinal tract cancer and both PEGylated and non-PEGylated NLCs formulated in the present study may be used as potential oral delivery systems for OG with improved anticancer activity.

As extension of the present work, specific lipid components of serum such as HDL, one of the major studies requires the specific separation of serum lipid and serum protein fractions and observing their structure function correlations in inactivating surfactant are considered to be significant. Besides the potential of nanostructured lipid carrier (NLC) as drug delivery system the *in vitro* and *in vivo* study in different cancer cell line and cancer animal models respectively are warranted in order to ensure the superiority of the formulated drug delivery systems.