PREPARATION AND CHARACTERIZATION OF (−)-EPIGALLOCATECHIN GALLATE LIPID BASED NANOPARTICLES FOR ENHANCING AVAILABILITY AND PHYSICAL PROPERTIES

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Abstract:

(−)-Epigallocatechin gallate (EGCG) is the most abundant and biologically active polyphenol found in green tea. (−)-Epigallocatechin gallate (EGCG) is highly potent antioxidant therefore, it has great therapeutic effect against cancer disease. Furthermore, it has antibacterial and anti-inflammatory effect. but it has some limitations, such as the poor permeability, the highly affinity to intestinal P-glycoprotein efflux mechanism and short half-life. Therefore, it has very low oral bioavailability.

In current study, Precirol® ATO 5 (P), glyceryl monostearate (GMS) and Compritol® 888 ATO (C) were selected as solid lipids and miglyol (M) and sesame oil (SO) as liquid lipids, to develop EGCG-NLC (nanostructured lipid carrier) and EGCG-SLN (solid lipid nanoparticles). EGCG was successfully encapsulated into lipid nanoparticles to improve its bioavailability. EGCG-NLC and EGCG-SLN were formulated using a hot homogenization-ultrasonication technique, and the physicochemical properties were characterized. The developed EGCG-NLCs and EGCG-SLNs showed small and homogeneous particle size approximately (308 and 379 nm) with entrapment efficiency around (76 and 49%), respectively. Also, the resulted formulation EGCG-NLC3 appeared under transmission electron microscope in almost spherical shape.

Keywords: Epigallocatechin gallate, particle size, entrapment efficiency, transmission electron microscope and in-vitro release

INTODUCTION

(−)-Epigallocatechin gallate (EGCG) is the most abundant and biologically active polyphenol found in green tea. The total amount of catechins in one brewed cup of green tea consists of 50–80% of EGCG, which makes approximately 200–300 mg **(Klinski, 2013)**.

(−)-Epigallocatechin gallate (EGCG) has many biological functions such as anti-tumor, anti-aging, antibacterial and anti-inflammatory promising use as therapeutic agents due to their potent antioxidant activity and diverse biological properties **(Granja**

et al., 2016).

Unfortunately, there are some limitations associated with EGCG, which negatively influences its functional benefits. These are, generally, the short half-life and the high sensitivity to light and heat **(Grumezescu, 2016)**.

Furthermore, EGCG is unstable in alkaline and neutral medium **(Kanwar et al., 2012)** and, therefore, subjects to extensive degradation by gastrointestinal secretion. Furthermore, the poor permeability and the highly affinity to intestinal P-glycoprotein efflux mechanism. All of the introduced drawbacks leading to great low oral bioavailability of EGCG despite its high water solubility **(Shtay et al., 2019)**. Hence, the previously studies reported to overcome these drawbacks and enhancing the oral bioavailability of EGCG through incorporation of EGCG in nanodelivery systems as liposomes **(Zou et al., 2014)**.

In current study, to repair previously mentioned drawbacks and to enhance oral bioavailability, lipid–based drug delivery systems like nanostructured lipid carrier (NLCs) and solid lipid nanoparticle (SLNs) can be employed. Lipid nanoparticles systems (LNs) which have two generations. first, solid lipid nanoparticles (SLN) and second, nanostructured lipid carrier (NLCs) can improve the lymphatic transport of the hydrophilic drugs as EGCG and hence, increase its oral bioavailability **(Mathur et al., 2019)**. LNs systems were recorded as an advanced drug carrier system than polymeric nanoparticle **(Poonia et al., 2016)**.

Advantages of lipid nanoparticles delivery system over the advantages of polymeric nanoparticles because of the lipid component matrix and its properties, which is physiologically tolerated, resulted in avoidance of acute and chronic toxicity. In addition to, as good biocompatibility, protection for the incorporated compound against degradation and controlled release of drugs **(Mendes et al., 2019)**.

Nanostructured lipid carriers (NLCs) composed of both solid and liquid lipids in certain proportion. Therefore, they offer various advantages over solid lipid nanoparticles (SLN) such as higher encapsulation efficiency, smaller size and low polymorphic changes **(Rawal and Patel, 2019)**.

Generally, nanostructured lipid carriers (NLCs) are nano-drug delivery carrier, which own the advantages of polymeric nanoparticles, emulsion, and liposomes. Furthermore, (NLCs) are essentially composed of a biocompatible lipid core with entrapped lipophilic drugs and surfactant at the outer shell.

The aim of this study was to develop of NLCs and SLNs using Epigallocatechin gallate (EGCG) as a model drug. Furthermore, the physical characterization and quality issues of developed formulations were described and determined. Therefore, this study can offer the sequence steps for the development of NLCs and evaluation of their quality characteristics.

Materials and method

Precirol® ATO 5 (P) and Compritol® 888 ATO (C) were provided by Gattefossé (Nanterre, France). Glyceryl monostearate (GMS), Pluronic® F68 (polyoxy ethylene-polyoxypropylene polysorbate 60), miglyol-812, Epigallocatechin gallate (EGCG, 98% purity), Methanol and Acetonitrile HPLC grade were purchased from Sigma Aldrich, Inc. (St. Louis, MI, USA). Sesame oil, Sodium hydroxide, potassium dihydrogen orthophosphate, Potassium Chloride, and Hydrochloric acid (HCl) were supplied by El-Nasr Pharm. Chem. Company, Cairo (Egypt). Membrane filter (0.22 μm) Millipore Iberica S.A.U.; Madrid (Spain).

All the above materials were in analytical grade and were used without further purification.

Preparation of SLNs and NLCs

EGCG nanostructured lipid carriers (EGCG-NLC) and EGCG solid lipid nanoparticles (EGCG-SLN) were prepared by hot homogenization - ultrasonication technique but with some few modifications. Briefly, a weighted amount of selected solid lipids for SLN and a weighted amount of selected solid-liquid binary lipids mixture for NLC (5% w/v) were melted at 5 °C above the melting point of solid lipid. A known concentration of EGCG (5 % w/v of lipids) was dissolved in the aqueous phase containing selected surfactant (2.5 % w/v) then heated to the same temperature of lipid phase and added drop by drop to the lipid phase under magnetic stirring at 1500 rpm for 5 min. After that, homogenization of the resultant pre-emulsion was performed at high speed of mixing about 20,000 rpm using an Ultra-Turrax T25 homogenizer (WiseMix™ HG15A, Daihan Scientific, Seoul, Korea) for 10 min **(Anwar et al., 2020; Cortesi et al., 2017)**. The resultant o/w nanoemulsions were subjected to probe sonication (ultrasonic processor, GE130, probe CV18, USA) at 60 % amplitude for 10 min. The obtained NLC dispersion was left beside to reach room temperature. All formulations contents were illustrated in table 1.

Table (1): Suggested formulations of EGCG-NLCs and EGCG-SLNs

F. No.	Solid lipids			Liquid lipids		Surfactants		water	ECCG mg
	P	GMS	C	MYGLYOL	Sesame oil	Tween 60	Pluronic® F68		
NLC1	7			3		3		87	50
NLC2	7				3	3		87	50
NLC3	7			3			3	87	50
NLC4	8.5			1.5		3		87	50
NLC5			7	3		3		87	50
NLC6			7		3	3		87	50
NLC7			7	3			3	87	50
NLC8			8.5	1.5		3		87	50
NLC9		7		3		3		87	50
NLC10		7			3	3		87	50
NLC11		7		3			3	87	50
NLC12		8.5		1.5		3		87	50
SLN1	10					3		87	50
SLN2	10						3	87	50

P=Precirol® ATO 5 GMS=glyceryl monostearate C= Compritol® 888 ATO

Particle size and polydispersity index

The mean diameter and polydispersity index of particle of nanostructured lipid carriers loaded with EGCG was determined using a Zetasizer Nano-ZS (Malvern Instruments, Worceshtire (UK), equipped with a 10 mW He-Ne laser employing the wavelength of 633 nm and a back-scattering angle of 90° at 25 °C. Before Photon correlation spectroscopic (PCS) analysis, EGCG-NLCs formulations should be diluted with a certain amount of double-distilled water (1:200) to get appropriate scattering intensity. The analysis, of Particle size was determined using Mie theory with the refractive index and absorbance of lecithin at 1.490 and 0.100, respectively **(Gu et al., 2019; Li et al., 2016; Shah et al., 2014; Wolf et al., 2018**).

Zeta potential analysis

The zeta potential of NLC formulations was measured via electrophoretic mobility measurements using a Zetasizer Nano-ZS (Malvern Instruments, Worceshtire (UK). The zeta potential was calculated by applying the Helmholtz–Smoluchowski equation (n = 3) **(Marques et al., 2017).**

Entrapment efficiency (EE)

The entrapment efficiency of EGCG into NLC formulations were determined by the indirect method by measuring the concentration of the free EGCG. Initialy, 2 ml of NLCs formulations were centrifuged at 100,000 rpm for 1 h at 4 ˚C to evaluate the unentraped EGCG using cooling ultracentrifuge (Beckman Instruments TLX-120 Optima Ultracentrifuge) **(Nafee et al., 2018; Safwat et al., 2017; Tatke et al., 2019; Tiwari and Pathak, 2011).** The aqueous layer was aspirated and filtered using Millipore® membrane (0.22 μm) and diluted with appropriate amount of double distilled water and measured by UV-Vis spectrophotometer (Shimadzu, the model UV-1800 PC, Kyoto, Japan) at 273 nm to determine the unencapsulated EGCG. Consequently, entrapment efficiency of EGCG into NLCs were determined by the following equations

EE% = {(weight of initial drug - weight of free drug) / (weight of free drug) X 100}

In-vitro drug release study

The *in vitro* release of EGCG from EGCG solution and EGCG-NLCs was performed by a dialysis bag diffusion technique. The receptor compartments consist of phosphate buffer solution (PBS) of pH 6.8 **(Shtay et al., 2019)**. The donor compartment is cellulose membrane dialysis bags (MWCO‑12 000, Sigma, USA) were soaked in dissolution media overnight prior experiment. 1 ml of freshly prepared EGCG-NLCs and EGCG solution (equivalent to 0.5 mg of EGCG) were diluted with 5 ml of dissolution media and which tightly closed from two sides by thermo-resistant thread. The bags were immersed in Dissolution apparatus, (six-spindle dissolution tester, Pharmatest, type PTWII, Germany) automatically adjusted at 37 ± 2 °C and 100 rpm. 2 ml sample was aspirated at a predetermined time interval (1, 2, 4, 6, 8, 10, 12 and 24 h) and the same amount of media was replaced to maintain sink condition. The release of free EGCG from NLC was compared to that from solution. The aspirated samples were measured using UV-Vis spectrophotometer at 273 nm.

Particle morphology

Morphology of the EGCG loaded NLC was determined by transmission electron microscopy (JOEL Transmission electron microscope (JTEM) model 1010 Tokyo, Japan) to investigate the outer layer of nanoparticles. The sample for TEM observation was prepared by placing few drops of EGCG loaded NLC dispersion that was previously diluted 50-fold with double-distilled water onto a 400-mesh carbon film coated copper grid followed by negative staining with 1% phosphotungstic acid for 10 s. The sample was dried in air before TEM observation **(Anwar et al., 2020).**

RESULTS and DISCUSSION:

Physicochemical characterization of EGCG-NLCs and EGCG-SLNs formulations

Particle size, polydispersity index (PDI) and zeta potential (ζ) measurement

Particle size, PDI and zeta potential are the physical properties of the colloidal dispersion determining stability of the formulation. Particle sizing is a significant method for confirming nanosized particle manufacturing. Also, Particle size played a crucial role in the gastrointestinal uptake and their clearance by the reticuloendothelial system. Hence, the precise determination of the particle size was very important. Usually, particle size less than 300 nm was advisable for the intestinal transport **(Harde et al., 2011; Wang et al., 2013)**.

As represented in table (2) and figure (1) the observations revealed that all the designed formulations were in the colloidal nanometer range (≤ 380 nm). It can be concluded that particle size of Precirol ® ATO5 nanoparticles (NLC1 to NLC4), GMS nanoparticles (NLC5 to NLC8), and Compritol® 888 ATO nanoparticles (NLC9 to NLC12) ranged from (241.37±10.61 to 295.43 ±7.18 nm), (253.34±8.67 to 308.45±8.94 nm) and (261.21±9.17 to 301.89±7.94 nm) respectively. The obtained results were clearly distinguished that formulations which contain pluronic F68 give the small particle size than that contain tween 60 as in F3, F7 and F11 (205.34±10.58, 220.35±7.71 and 214.44±8.01 nm) this findings may be attributed to HLB values of surfactants where HLB value of pluronic F68 (HLB ~ 29) higher than that of tween 60 (HLB ~ 15.6) **(Anwar et al., 2019; Malkani et al., 2014)**.

On the other hand, the largest particle size was exhibited in formula SLN1 and SLN2 (379.34±9.47 and 364.11±9.68) respectively, which contain solid lipid only Precirol® ATO5 as lipid phase to form solid lipid nanoparticles. this observation can be explained by the absence of liquid lipid in lipid phase **(Bahari and Hamishehkar, 2016; Nafee et al., 2018)**.

Table (2): Particle size, and polydispersity indices of EGCG-NLCs and EGCG-SLNs formulations

Formulae Number	Particle size (nm)	RO	PDI	RO
NLC1	241.37±10.61	4	0.15 ± 0.09	2
NLC2	273.67±9.47	7	0.12± 0.06	1
NLC3	205.34±10.58	1	0.18± 0.09	5
NLC4	295.43 ±7.18	10	0.25± 0.04	11
NLC5	253.34±8.67	5	0.20± 0.05	8
NLC6	288.22±7.84	8	0.35± 0.04	14
NLC7	220.35±7.71	3	0.17± 0.04	4
NLC8	308.45±8.94	12	0.28± 0.05	12
NLC9	261.21±9.17	6	0.15± 0.06	2
NLC10	292.24±8.47	9	0.21± 0.04	9
NLC11	214.44±8.01	2	0.24± 0.02	10
NLC12	301.89±7.94	11	0.28± 0.04	12
SLN1	379.34±9.47	14	0.19± 0.04	7
SLN2	364.11±9.68	13	0.18± 0.03	5

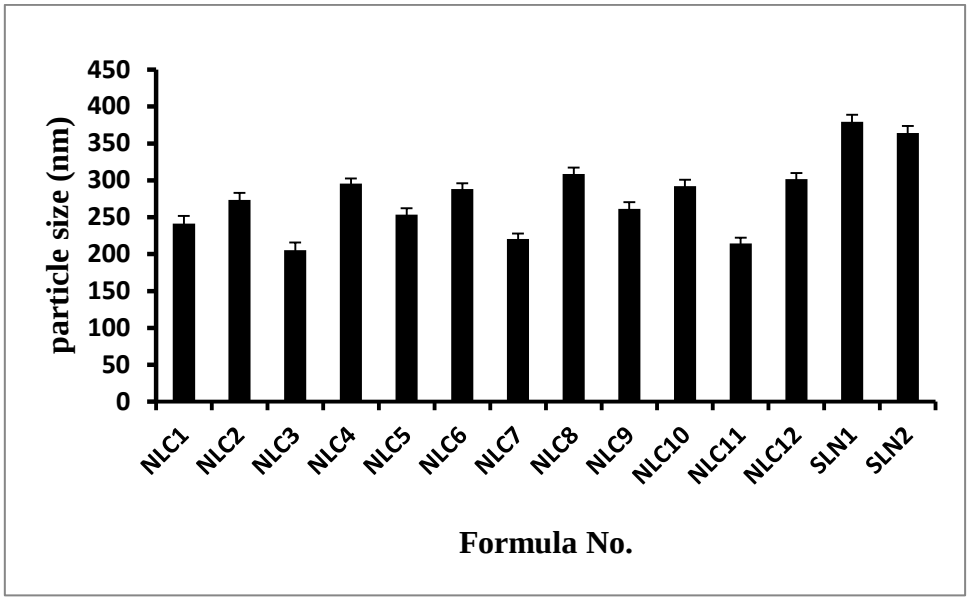

Figure 1: Mean particle size of EGCG-NLCs and EGCG-SLNs formulations

the polydispersity index as an indicator of the size distribution width of the particle. The PI value that reflects dispersion quality typically varies between 0 and 1. Most researchers recognize PI values ≤ 0.3 as optimum values; however, values ≤ 0.5 are also acceptable **(Anwar et al., 2020; Kaur et al., 2008)**.

Table (2) Also, give an overview of the results of polydispersity index measurements. The prepared NLC and SLN dispersions had a PI value ranged from (0.12± 0.06 to 0.35± 0.04) due to preparation method used indicating a homogenous and narrow size distribution of nanoparticles of NLCs.

The zeta potential value is a crucial parameter that influences the storage stability of nanocarriers, since it is related to the surface charge of nanoparticles and indicates the degree of repulsion between closely positioned and similarly charged particles in dispersion **(López-García and Ganem-Rondero, 2015; Montenegro et al., 2016)**.

From the factors which mainly influence zeta potential of lipid-based nanoparticles structure of solid and liquid lipid and the medium composition **(López-García and Ganem-Rondero, 2015; Noh et al., 2017)** also it depends on higher steric stabilization and lower an electrostatic stabilization of nonionic surfactants which perfectly forming a coat around the particles of NLCs. Result in surface coverage of NLC decreases the electrophoretic mobility of nanoparticles and thus lower the zeta potential values**(Burra et al., 2013; Dudhipala and Veerabrahma, 2016; Martins et al., 2012; Shah et al., 2014; Tatke et al., 2019).**This phenomenon explains the higher stability of lipid based nanoparticles despite having a lower zeta potential value.

Zeta potential values of all designed formulations are shown in table (3) and represented in figure (2). The results revealed that the ZP of the various formulations was consistently negative surface charge. ZP values of Precirol® ATO 5 nanoparticles (F1 to F4), Compritol® 888 ATO nanoparticles (F5 and F8) and GMS nanoparticles (F9 to F12) in between (-16.6 ±2.81 to -15.4±2.64 mV), (-19.7±3.17 to -16.7±2.64 mV) and (-30.2±2.97 to -27.6±2.07 mV) respectively.

Surfactants were used as stabilizer for the lipid nanoparticles prepared does not contribute with additional charges to zeta potential because of non-ionic behavior of these molecules. Thus, this molecule does not contribute with additional charges to zeta potential.

Furthermore, the solid lipids were used in developed NLCs composed of mixture of acylglycerols: Precirol® ATO 5 composed of glyceryl tripalmitostearate (25% - 35%), glyceryl dipalmitostearate (40% - 60%) and glyceryl monopalmitostearate (8% - 22%) **(Martins et al., 2011; Raymond C Rowe, 2009)** and Compritol® 888 ATO composed of glyceryl tribehenate (28% - 32%), glyceryl dibehenate (52% - 54%) and glyceryl monobehenate (12% - 18%) **(López-García and Ganem-Rondero, 2015; Raymond C Rowe, 2009),** both of them being glycerol esters of long chain-length fatty acids (C18, C16) and (C22) respectively. so that they provide neither charge nor polarity that contributes to zeta potential. Also, GMS composed of triacylglycerols (5 – 15%), diacylglycerols (30 – 45%) and monoacylglycerols (40 – 55%) (Raymond C Rowe, 2009). In such case due to high content of partial emulsifying glycerides (mono and diglycerides) of GMS and presence of non-esterified hydroxyl group of the glycerol, this molecule exhibits certain polarity that contributes to zeta potential.

On the other hand, the liquid lipids were used in developed NLCs composed of diacylglycerol of medium-chain-length fatty acids. Liquid lipids provide the majority impaction and contributes to zeta potential due to its polarity which result from non-esterified hydroxyl group of the glycerol and the length of the fatty acids. These observations are in line with studies reported by **(López-García and Ganem-Rondero, 2015; Teeranachaideekul et al., 2008)** which stated that, it might be due to the accumulation of oil at the surface of NLC. Being the melting point of the solid lipid higher than that of the oil, when preparing NLC, the solid lipid recrystallizes first, holding a portion of the oil within the solid lipid matrix. Subsequently, the excess of oil remains in the outer shell of nanoparticles, then the oil contributes largely to zeta potential **(Doktorovová et al., 2010; Jores et al., 2005)**.

the obtained results can be concluded that GMS nanoparticles (F9 to F12) possessed high zp values than that of Precirol® ATO 5 nanoparticles (F1 to F4) and Compritol® 888 ATO nanoparticles (F5 and F8) this fact can be explained by the following reasons. certain polarity and emulsifying properties of GMS resulted from none esterified hydroxyl group of glycerol and the length of the fatty acids. Other reason was attributed to the negative charged carboxylic groups of MCT (Miglyol) which composed of diesters of caprylic/capric triglyceride. A similar explanation has been reported by Teeranachaideekul et al, 2007 **(Teeranachaideekul et al., 2007)**, these revealed the higher ZP values of GMS nanoparticles than other nanoparticles.

The lowest ZP values were observed in formulations of SLN1 and SLN2 (-8.3±1.54 and -9.2±1.64) respectively, due to the absence of liquid lipid in lipid phase and presence only precirol® as solid lipid which has no role at ZP values as mentioned above **(López-García and Ganem-Rondero, 2015; Raymond C Rowe, 2009).**

Table (3): zeta potential of EGCG-NLCs and EGCG-SLNs formulations

Formulae Number	Zeta potential	RO
NLC1	-16.6 ±2.81	10
NLC2	-13.3±2.64	12
NLC3	-18. ±1.27	7
NLC4	-15.4±2.64	11
NLC5	-19.7±3.17	6
NLC6	-17.4±1.35	8
NLC7	-20.4±2.94	5
NLC8	-16.7±2.64	9
NLC9	-30.2±2.97	2
NLC10	-23.8±1.08	4
NLC11	-32.8±1.84	1
NLC12	-27.6±2.07	3
SLN1	-11.3±1.54	14
SLN2	-13.2±1.64	13

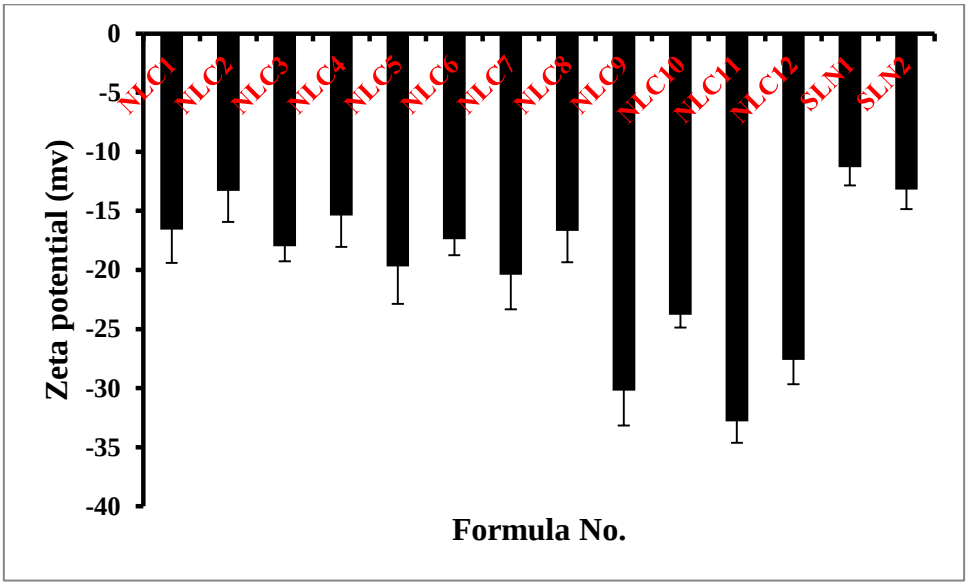

Figure 2: Zeta potential of EGCG-NLCs and EGCG-SLNs formulations

Entrapment Efficiency of EGCG -NLCs and EGCG-SLNs

The quantity of drug encapsulated in the nanoparticles and the drug content in the lipid matrix are a further significant consideration for the optimization of NLC. the quantity of drug encapsulated in the lipid matrix depends on many factors as the type of

lipids used, physicochemical properties of the drug, miscibility and solubility of drug in the molten lipid (Indu Pal Kaur et al., 2014), physical and chemical nature of the lipid matrix and crystalline state of lipid matrix and also surfactant was found to affect encapsulation efficiency (Chantaburanan et al., 2017; Müller et al., 2000; Tatke et al., 2019). Entrapment efficiency of all lipid-based nanoparticles formulations are showed in table (4) and demonstrated in figure (3). The entrapment efficiency was determined and found to be in between 74.88 ±6.51 % to 47.34± 2.06%.

The using of a combination of highly ordered with less ordered lipids, which caused several crystal defects in lipid matrix and provided much imperfections leading to void spaces in which more drug molecules could be accommodated as depicted in NLCs formulations compared with SLN formulations **(Mendes et al., 2019; Tatke et al., 2019; Tran et al., 2014; Wang et al., 2013)**.

It was observed that E.E of EGCG in Precirol® ATO 5 nanoparticles (F1 to F4) were varied from 74.88 ±6.51 % to 62.46 ± 3.65%, in Compritol® 888 ATO nanoparticles (F5 and F8) were varied from 72.23 ± 1.38% to 60.97 ± 2.32 % and in GMS nanoparticles (F9 to F12) also were ranged from 67.67± 2.94% to 55.23± 1.82%.

From the results it was clearly distinguished that Precirol® ATO 5 nanoparticles and Compritol® 888 ATO nanoparticles showed higher entrapment efficiency than that of GMS nanoparticles such fact was attributed to the chemical composition of each one where the imperfect matrix structure of Precirol® ATO 5 and Compritol® 888 ATO molecules, which are formed due to the mono-, di- and triglyceride contents that expected to exhibit lower crystallinity and highly porous structural characteristics which allows higher solubility and easier accommodation of more drug **molecules (Anwar et al., 2020; Khames et al., 2019; Vivek et al., 2007a).**

Also, Precirol® ATO 5 is a di-glyceride with two different chain length fatty acids palmitic and stearic acid (C16 and C18); therefore, it is expected to have less ordered lipid network compared to GMS, and thus lead to the more drug molecules could be entrapped **(Khames et al., 2019; Vivek et al., 2007b).**

Regarding the type of surfactant, it was clearly observed that lipid-based nanoparticles formulation prepared using Pluronic® F68 higher E.E. than that prepared using another surfactant as demonstrated in F3, F7, F11 and SLN2. This fact might be attributed to high value of hydrophilic - lipophilic balance of pluronic® F68 (HLB ~ 29) compared to another surfactant.

Table (4): Entrapment efficiency of EGCG-NLCs and EGCG-SLNs formulations

Formulae Number	Entrapment Efficiency±SD (%w/w)	RO
NLC1	74.88 ±6.51	2
NLC2	70.39 ± 2.14	5
NLC3	76.12 ± 1.62	1
NLC4	62.46 ± 3.65	9
NLC5	72.23 ± 1.38	4
NLC6	69.87 ± 4.62	6
NLC7	74.76 ± 2.74	3
NLC8	60.97 ± 2.32	10
NLC9	67.67± 2.94	8
NLC10	60.55± 1.05	11
NLC11	68.56± 2.45	7
NLC12	55.23± 1.82	12
SLN1	47.34± 2.06	14
SLN2	49.45± 3.06	13

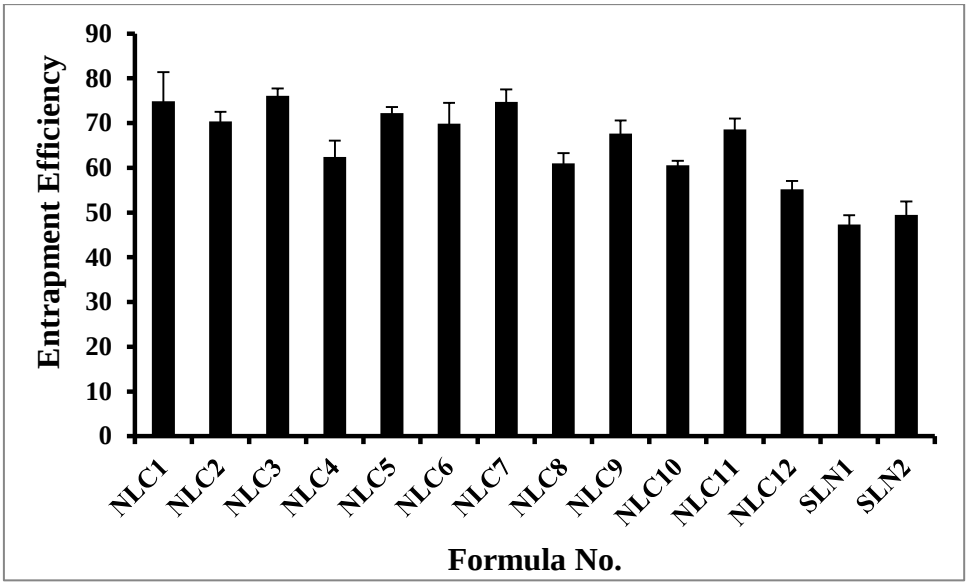

Figure 3: Entrapment efficiency of EGCG-NLCs and EGCG-SLNs formulations

In-vitro release study

In-vitro release study was achieved for all formulations in addition to pure EGCG solution. The release condition monitored in PBS (pH 7.4) to achieve "sink" conditions during a dissolution test for all formulation. It was observed that EGCG solution showed almost complete drug release (100%) within 4 h.

On the other side, the EGCG-NLCs and EGCG-SLNs formulations exhibited prolonged release over the incubation time ranged from (67.1±8.1 to 51.2±6.5% and

72.2±9.1 to 66.9±6.8%) respectively, at the end of the incubation in dissolution media as depicted in figures (4-7). This was expected and indicates the release of encapsulated EGCG from the NLCs and SLNs, Due to the large surface area and its nanosized **(Shtay et al., 2019; Zhang and Zhang, 2018).** In addition, there was no significant difference in the release profiles of all developed formulations.

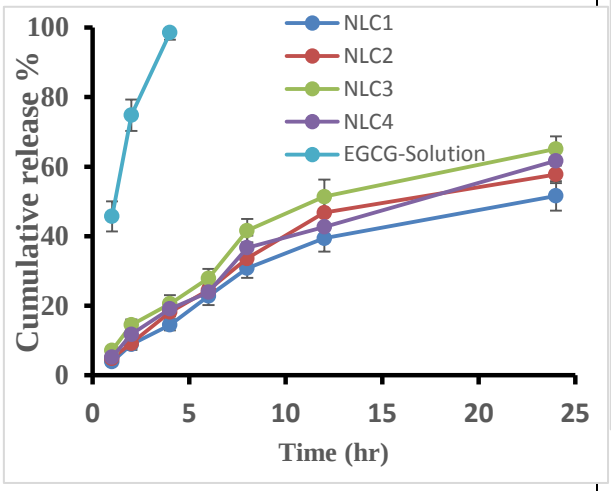

Figure 4: Cumulative release % of EGCG-NLC1 to EGCG-NLC4 formulations

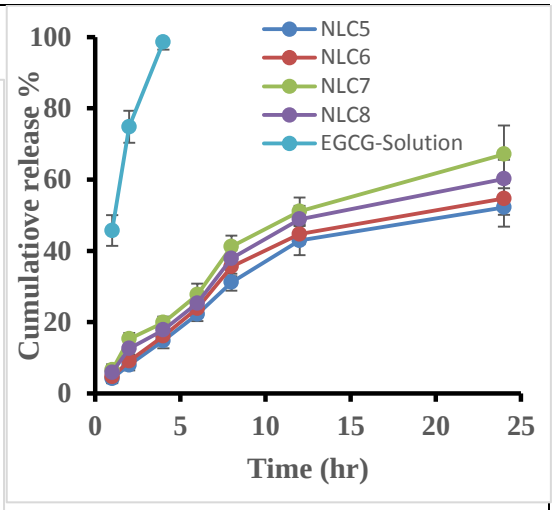

Figure 5: Cumulative release % of EGCG-NLC5 to EGCG-NLC8 formulations

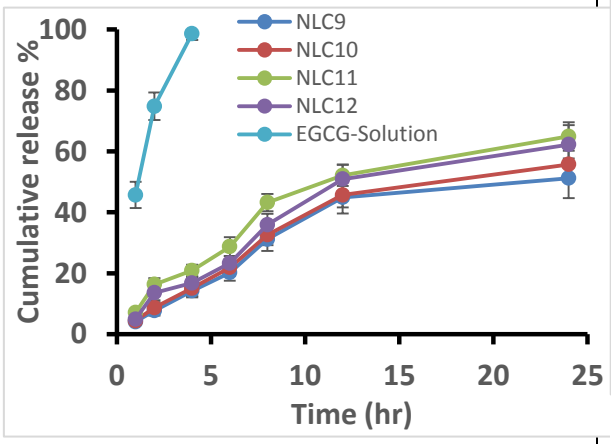

Figure 6: Cumulative release % of EGCG-NLC9 to EGCG-NLC12 formulations

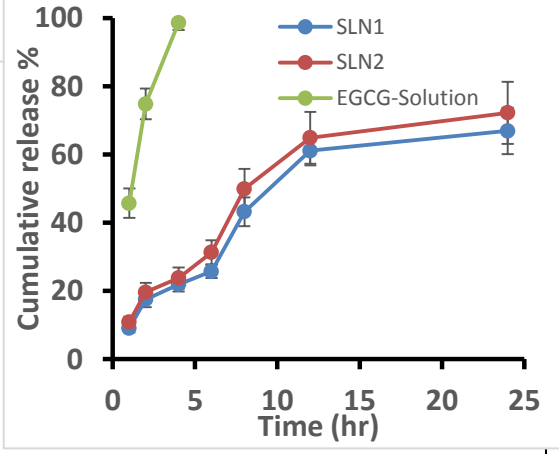

Figure 7: Cumulative release % of EGCG-SLNs formulations

Table (5): Cumulative release % of EGCG formulations

Formula	1hr	2hr	4hr	6hr	8hr	12hr	24hr	RO
NLC1	3.9	8.9	14.5	22.8	30..8	39.4	51.6	2
NLC2	4.9	9.2	18.2	24.5	33.6	46.8	57.8	6
NLC3	7.2	14.5	20.6	27.9	41.6	51.4	65.1	11
NLC4	5.2	11.8	19.1	23.9	36.7	42.7	61.7	8
NLC5	4.2	8	14.8	22.2	31.2	42.9	52.2	3
NLC6	4.7	9.1	16.1	23.9	35.6	44.7	54.7	4
NLC7	6.6	15.3	19.9	27.7	41.2	51.1	67.1	13
NLC8	5.9	12.6	17.8	25.3	37.9	48.9	60.2	7
NLC9	4.1	7.7	14.2	20.2	31.2	44.9	51.2	1
NLC10	4.4	8.7	15.1	21.9	32.6	45.7	55.7	5
NLC11	7.1	16	20.9	28.7	43.2	52.1	64.9	10
NLC12	4.9	13.6	16.8	23.3	35.9	50.9	62.2	9
SLN1	9.1	17.5	21.9	25.7	43.2	61.1	66.9	12
SLN2	10.9	19.6	23.8	31.3	49.9	64.9	72.2	14

Table (6): Total rank order of GCG-NLCs and EGCG-SLNs formulations

Formulae Number	PS	PDI	ZP	EE%	Total	RO	In-vitro release	Total	RO
NLC1	4	2	10	2	18	3	2	5	2
NLC2	7	**1**	12	5	25	7	6	13	5
NLC3	**1**	5	7	1	14	1	11	12	4
NLC4	10	11	11	9	41	11	8	19	7
NLC5	5	8	6	4	23	6	3	9	3
NLC6	8	14	8	6	36	9	4	13	5
NLC7	3	4	5	3	15	2	13	15	6
NLC8	12	12	9	10	43	12	7	19	7
NLC9	6	2	2	8	18	3	1	4	1
NLC10	9	9	4	11	32	8	5	13	5
NLC11	2	10	**1**	7	20	5	10	15	6
NLC12	11	12	3	12	38	10	9	19	7
SLN1	14	7	14	14	49	14	12	26	8
SLN2	13	5	13	13	44	13	14	27	9

Morphology of EGCG-NLC9

The morphology and presence of colloidal nanoparticles of EGCG-NLC3 was determined by transmission electron microscope and was showed in figure (8). TEM images illustrated that EGCG-NLC9 were of uniform distribution, separate and almost spherical shapes. This result might be based on particles developed using chemically polydispersed lipids are almost spherical as in present study (Dingler et al., 1999). The TEM micrograph showed that EGCG-NLC9 were nanometer-sized particles around 200 nm which in proximity with the observations yielded by a Malvern® Zetasizer Nano ZS90 (Malvern® Instruments Limited, Worcestershire, UK) and there were no crystals of free drug observed.

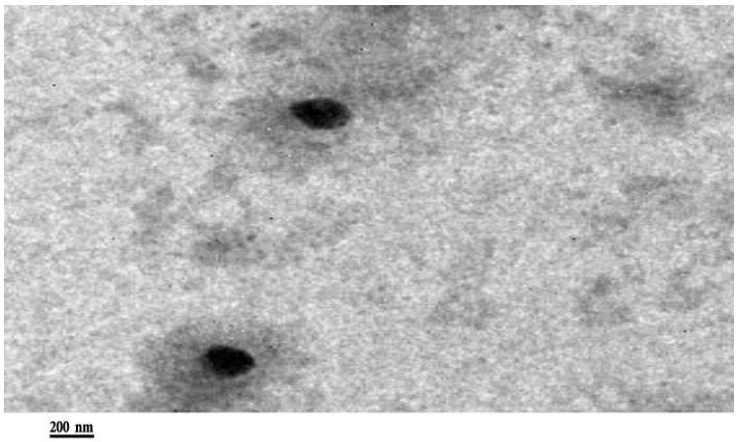

Figure 8: Transmission electron microscope image of EGCG-NLC9

Conclusion

Lipid based nanoparticles (NLCs and SLNs) are adaptable nanoparticles with multipurpose applications. However, quality and successful incorporation of EGCG into NLC and SLN to develop more efficient formulations were perquisite for the oral bioavailability improving. In current study, EGCG–NLCs and EGCG-SLNs were successfully formulated by hot homogenization – ultrasonication technique. Furthermore, the developed formulations were subjected to physicochemical characterization. The developed EGCG-NLCs and EGCG-SLNs showed small and homogeneous particle size approximately (205 and 379 nm) with entrapment efficiency around (47.34 and 76.12%) respectively. Also, the resulted formulation EGCG-NLC9 appeared under transmission electron microscope in almost spherical shape.

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صياغة وتوصيف ابيجالوكاتيكين جالات المحمل علي جزيئات دهنية متناهية الصغر لتحسين اتاحتها الحيوية وخصائصها الفيزيقية

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الملخص :

ابيجالو كاتكين جاليت هى ماده فينوليه ذات نشاط حيوى وعلاجى ومن أكثر المواد ذات الفعالية من مكونات الشاى الاخضر. وهى لها تاثير فعال فى علاج كثير من الامراض على سبيل المثال الاورام ومضاده للبكتريا كما ان لها ايضا تاثير مضاد للالتهابات . ولكن لها بعض القيود التى تقلل من اتاحتها الحيويه مثل ضعف النفاذية ، والتقارب الشديد لآلية تدفق البروتين السكري المعوي ونصف العمر القصير. لذلك ، في هذه الدراسة الحالية.

تم فى هذه الدراسه اختيار ال جلسريل مونو ستياريت وال كومبريتول اى تى او٨٨٨ وال بريسيرول اى تى او٥ كدهون صلبه . وال ميجليول وزيت السمسم كدهون سائله لصياغة وتحميل عقار ابيجالو كاتكين جاليت على جزيئات دهنيه متناهية الصغر والتى تم تحضيرها معمليا بتقنية التجانس الساخن والموجات فوق الصوتيه.

تم عمل التوصيف الفيزيائى والكيميائى للصيغ المحضره معمليا حيث وجد انها فى حجم النانوميتر(٢٠٥و ٣٧٩ نانومتر) مع كفائة تحميل تتراوح بين (٤٧-٧٦ ٪) كما تم تصويرها تحت المجهر الالكترونى النافذ وتبين منها كروية الشكل للجزيئات الدهنيه متناهية الصغر المحضره معمليا

الكلمات المفتاحية : ابيجالوكاتيكين جالات، حجم الجزيئات، سعة التحميل، المجهر الالكتروني النافذ، الاطلاق الحيوي المعملي