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Lipid-Based Drug Delivery Systems forthe Enhancement of Topical Delivery of Benzocaine

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Abstract:*This study was aimed to develop solid lipid nanoparticles (SLNs) as well as nanostructured lipid carriers (NLCs) and evaluate their potential as carrier systems for topical delivery of benzocaine to improve the drug entrapment efficiency and sustained release. SLNs and NLCs were prepared by high shear homogenization followed by ultrasonication method, using different types of solid lipids such as glyceryl monostearate, stearic acid and Compritol 888 ATO. Isopropyl myristate, as a liquid lipid in formulation of NLCs was used in different ratios to study the influence of liquid lipid content on the particle size, entrapment efficiency and zeta potential of the formulated benzocaine loaded lipid nanoparticles. The results indicated that entrapment efficiency and particle size depend on the concentration and the lipid mixture employed. The selected NLCs (NLC_6) was further incorporated in 5% carboxymethyl cellulose (CMC) hydrogel and then characterized for appearance, pH and in-vitro drug release. The prepared SLNs and NLCs possessed an average particle size of 214 – 440 nm, zeta potential of (-19.6) to (-25.9) mV and 55.65 – 94.62 % entrapment efficiency. The prepared NCL_6 loaded hydrogel showed a smooth texture hydrogel free from any agglomeration of lipid nanoparticles with pH value of 6.10.The release studies of the investigated NLC_6 showed an initial fast release that lasted for 0–1 hr, followed by a sustained release for 8 hrs.These promising findings encourage the potential use of benzocaine loaded lipid nanoparticles for future topical application improving its therapeutic efficacy for topical treatment of pain.*

Keywords:*Benzocaine, Nanostructured lipid carriers, Solid lipid nanoparticles, Topical delivery, Hydrogel*

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I. Introduction

One of major advances in pharmaceutical research is focused on development of new delivery systems of existing drugs (1). To find new modified vesicles possessed properties that allowed them to improve the bioavailability of drugs while at the same time minify their toxic effects. Colloidal particles research has being investigated recently for their potential as drug carriers (2). They are polymeric nanoparticles fabricated from synthetic/natural polymers and ideally suited to optimize drug delivery and reduce toxicity.The particles size of colloidal particles ranging between 10 and 1000 nm are known as nanoparticles.The successful enforcement of nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and their stability in the nanometer size. However, there are problems associated with the colloidal drug delivery systems, such as instability, non-biodegradability (3,4) the lack of safe polymers with regulatory approval and their high cost have bounded the wide spread application of nanoparticles to clinical medicine (5). To beat these limitations of polymeric nanoparticles, lipids have been put forward as an alternative carrier, particularly for lipophilic pharmaceuticals (6). Lipid based drug delivery system is an accepted, proven, commercially viable strategy in formulation of pharmaceuticals. The widening availability of lipid excipients with specific characteristics offers flexibility of their application (7).Lipid based nanoparticles are broadly varied classified into different classes, such as solid lipid nanoparticles (SLNs), which are attracting wide attention of formulators all over the world (6). SLNs are colloidal carriers developed in the last decade as an alternative system to the existing traditional carriers (emulsions, liposomes and polymeric nanoparticles).SLNs showdistinguish properties such as smallparticle size with large surface area, high drug loading and the interaction of phases at the interfaces, and are attractive for their potential to improve performance of pharmaceuticals, neutraceuticals and other materials (8). SLNs gather the advantages and avoid the drawbacks of several colloidal carriers of its class. Nanostructured lipid carriers (NLCs) are colloidal carriers characterized by a solid lipid core consisting of solid and liquid lipids (9),they consist of a lipid matrix with a special nanostructure (10). This nanostructure improves loading of the drug and firmly retains the drug during storage. NLCs system minimize some problems associated with SLNs such as low payload for some drugs; drug expulsion on storage and high water content of SLNs dispersions (9). So if systematically

investigated, SLNs and NLCs may open new skyline in research and remediation (11).Local anesthetics are vastly used to prevent or treat acute pain; to treat inflammatory, cancer related, and chronic pain; and for diagnostic and prognostic purposes. Topical anesthesia is ordinarily used approach applied at the site of needle insertion prior to anesthesia (12, 13). Fear of needles and pain can cause anxiety in patients awaiting procedures in the outpatient setting (14, 15). Application of topical anesthetic before or in place of injection of local anesthetic can help to relieve anxiety, pain and discomfort during dermatologic (16).

Benzocaine is a water-insoluble ester-type local anesthetic agent that is mainly useful for topical application.Benzocaine topical products are used in many dermatological procedures showing interesting clinical results(17). Benzocaine is distinguished by a fast onset of anesthesia with a short duration compared to the duration of pain (18).

Lipid carriers are advantageous in comparison to other topical carriers in various aspects. These include sustaining the encapsulated drug release, drug targeting, appropriate for incorporation lipophilic and hydrophilic drug molecules, protection of labile drug, bypass the use of organic solvents, large scale production, negligible skin irritability and ease of sterility (19, 20). In addition, they provide an occlusive impact (21) and high percutaneous absorption because of their large surface area and the penetration enhancing ability of their components (22). Accordingly, the primary objective of this study is to develop and characterize a novel topical drug delivery system for enhancing the entrapment efficiency and sustained release of benzocaine.

II. Materials and Methods

Materials

Benzocaine was kindly gifted from Amoun, Pharmaceutical Company, Egypt. Compritol 888 ATO was kindly supplied by Gattefossé, France. Glyceryl monosterate (GMS) was obtained from loba chemic Pvt. Ltd Mumbi, India. Stearic acid, and ethanol were obtained from El Nasr Pharmaceutical Chemicals, Egypt. Isopropyl myristate and tween 80 were purchased from Sigma Chemical Company, St. Louis, USA. Carboxymethyl cellulose (CMC) was obtained from ADWIC, Egypt. All other reagents were of analyticalgrade.

Methods

Preparation of benzocaine-loaded lipid nanoparticles

The high shear homogenization technique followed by ultrasonication was chosen to formulate benzocaine loaded solid lipid nanoparticles (SLNs) andnanostructured lipid carriers (NLCs)(23, 24).The composition of all formulations is shown in Table 1. Briefly, the lipid phase consisted of glyceryl monostearate (GMS), stearic acid or Compritol 888 ATO as solid lipid with/without isopropyl myristate (IPM) as liquid lipid at different ratios was melted 5 °C above the melting point of the lipid used and benzocaine was dispersed therein to obtain a drug–lipid mixture. The aqueous phase of surfactant was prepared by dissolving tween 80 in distilled water and heating up the solution to same temperature of the molten lipid phase.The hot lipid phase was poured onto the hot aqueous phase and homogenization was carried out at 25000 rpm for 5 minutes. The resulted hot O/W emulsion was sonicated for 30 minutes. Benzocaine loaded nanoparticles were obtained by allowing hot nanoemulsion to cold down to room temperature.Blank SLNs and NLCs were prepared using the same procedure.

TABLE NO. 1: Composition of benzocaine-loaded SLNs and NLCs

Formula code	Formula composition (gm)		
	0.05gm benzocaine + 0.5 gm Tween 80		
	Solid lipid	Liquid lipid	Solid lipid: liquid lipid
SLN_{GMS}	Glyceryl monostearate (GMS)	Isopropyl myristate (IPM)	1 : 0
NLC_1			0.9: 0.1
NLC_2			0.7: 0.3
SLN_{ST}	Stearic acid		1 : 0
NLC_3			0.9: 0.1
NLC_4			0.7: 0.3
SLN_{COM}	Compritol 888 ATO		1 : 0
NLC_5			0.9: 0.1
NLC_6			0.7: 0.3

Characterization of benzocaine-loaded lipid nanoparticles

Particle size analysis

Particle size of benzocaine-loaded SLNs and NLCs dispersions was measured by photon correlation spectroscopy (Malvern ZetasizerNano ZS, UK). Before measurement, samples were diluted appropriately with distilled water.

Measurement of zeta potential

Zeta potential of benzocaine loaded SLNs and NLCs dispersions was determined by the measurement of the electrophoretic mobility at 25 °C using a Zetasizer (ZetasizerNano ZS; Malvern). Field strength of 20 V/cm was employed and measurements were done after appropriate dilution.

Drug entrapment efficiency (EE%)

The entrapment efficiency (EE%) of theSLNs and NLCs were obtained indirectly by measuring the concentration of free benzocaine in the supernatant after centrifugation .The unentrapped benzocaine was determined by adding 0.5 ml of benzocaine loaded SLNs or NLCs to 9.5ml ethanol and then this dispersion was centrifuged at 9000 rpm for 30min. The supernatant was collected, filtered through the millipore membrane filter (0.45µm) then diluted with ethanol and analyzed for unencapsulated benzocaine at 293nm using validated UV-spectrophotometric method.

The percentage of entrapment efficiency (EE%) was calculated using the following equation (25, 26):

$$EE\% = \frac{(W \text{ initial drug} - W \text{ free drug}) \times 100}{W \text{ initial drug}}$$

In-vitro drug release study

In-vitro release of benzocaine from the bestlipid nanoparticles dispersionswas evaluated by the dialysis bag diffusion method using cellulose membrane (molecular weight cutoff 12 000–14 000) (27). About 2ml of the chosen lipid nanoparticles dispersion was added to the dialysis bag and sealed at both ends.Then, sealed bags were placed in 50 ml of phosphate buffer pH 5.5 representing the receptor compartment maintained at 32± 0.5°C and shaken in a water bath shaker at 100 rpm (Memmert GmbH, schwabach, Germany). At specific time intervals, 2ml sample was taken and replenished immediately with the exact volume of fresh phosphate buffer pH 5.5 to keep the sink conditions. The samples were adequately diluted and analyzed for benzocaine content spectrophotometrically at 293 nm.

Preparation of lipid nanoparticles based hydrogel

Carboxymethyl cellulose (CMC), a gelling agent, was chosen to formulate benzocaine-loaded lipidnanoparticles hydrogel. The selected lipid nanoparticles were incorporated into CMC 5% (w/w) gel. CMC was dissolved in distilled water under continuous stirring at 1000 rpm and the formed gel was left at 8°C for 24 hours (28). Nanoparticles were mixed with CMC gel with continuous stirring to form benzocaine-loaded based gel.

Evaluation of lipid nanoparticles based hydrogel

Physicochemical properties

The hydrogels were characterized for physicochemical properties such as color, odor and texture.

Measurement of pH

About 1 gm of benzocaine-loaded lipid nanoparticleshydrogel was dispersed in 20 ml distilled water, then the pH was measured using digital pH at 25 0C (Jenway, Bibby scientific Limited, Stafford-shire, UK).

***In-vitro*drug release study**

In-vitro release of benzocaine was evaluated by the dialysis bag diffusion technique. About 2 ml of the selected lipid nanoparticles or 0.5gm of benzocaine-loaded lipid nanoparticles hydrogel was placed in a cellulose acetate dialysis bag (molecular weight cutoff 12000– 14000), sealed at both ends and the release study was carried as previously mentioned in the section ‘‘*In-vitro* drug release study”

III. Results And Discussion

Preparation of benzocaine loaded lipid nanoparticles

Abundantmethods are available for the preparation of lipid nanoparticles, in the present study, homogenization technique followed by ultrasonication was chosen due to numerous advantages such as simple and easy to perform(29), the short production time and the possibility of production on large scale,the avoidance of organic solvents (30) and yielding small particle size. Ultrasonication also gave a share in to produce smaller particle size along with homogenization (31)

Liquid lipid and solid lipids for the preparation of lipid nanoparticles were selected depending on their ability of carry the drug. A good affinity of the solid and liquid lipid may warrant for high entrapment efficiency, which is an essential qualification of a carrier system (32).

Benzocaine loaded solid lipid nanoparticles (SLNs) dispersions were composed of glyceryl monostearate (GMS), stearic acid or compritol 888 ATO as core matrices stabilized with tween 80 as a surfactant. Tween 80 was used as a surfactant. Tweens are nonionic surfactants providing formulation benefits in a number of pharmaceutical applications having long standing food and pharmacopeia approval [34, 35]. Tweens are characterized with high HLB value which is commonly considered to be ideal for the production of stable o/w emulsion (33).

Benzocaine nanostructured lipid carries were formulated using isopropyl myristate (IPM) as a liquid matrix. The w/w composition of the benzocaine NLCs and their corresponding SLNs formulations is given in Table (1).

Particle size analysis

Particle size analysis is commonly performed to characterize lipid nanoparticles dispersions because of the critical influence size has on the potential applications as well as subsequent stability of the systems. Furthermore, studying particle size also provides insight into the effect of formulation and processing parameters.

Table (2) showed particle size of the prepared benzocaine loaded lipid nanoparticles. The particle size of prepared SLNs and NLCs was ranged from 214±3.1 to 440±3.7. It was found that the sizes of SLNs were being larger than those of NLCs when prepared with the same procedure, surfactant type and total lipid amount. Also, the increase in liquid lipid content showed decrease in particle size of lipid nanoparticles. This observation was attributed to the better emulsification of solid lipid matrix when oil is incorporated in the solid matrix.

Measurement of zeta potential

Almost all particles in contact with a liquid acquire an electric charge on their surface. The electric potential at the shear plane is called the zeta potential. The zeta potential (ζ) of the particles could be used as a measure of overall charges acquired by particles in a particular medium and is considered as one of the benchmarks of stability of a colloidal system.

Results of zeta potential measurements of benzocaine loaded SLNs and NLCs are given in Table (2). The surface charge of the different samples was consistently negative. The zeta potential values ranged from -19.6 to -25.9 mV indicating a relatively good stability and dispersion quality.

Drug entrapment efficiency (EE%)

Table (2) illustrates the entrapment efficiency of the prepared lipid nanoparticles. The entrapment efficiency of prepared formulations ranged from 55.65 ± 0.12 to 94.62 + 0.35. Generally, it was observed that replacing solid lipid by increasing percent of liquid lipid in all lipid nanoparticles led to gradual increase in EE%. This was in accordance with previously reported results that NLCs showed higher EE% compared to SLNs (25, 34).This could be attributed to that the incorporation of liquid lipid to solid lipid can lead to massive crystal order disturbance. The resulting matrix of lipid particles indicates great imperfections in the crystal lattice which provide sufficient space for large amount of drug to lodge successfully, thus leading to improved drug entrapment efficiency(25,35,36).

Generally, high drug entrapment efficiency for the drugs that are sparingly soluble in water is one of the major advantages of lipid nanoparticles (37). Since benzocaineis sparingly soluble in water, the solubility of the drug in the lipid or oil used can provide some clues about the entrapment efficiency of lipid nanoparticles.

Thus, the higher drug entrapment observed upon the addition of increasing amounts of isopropyl myristate in NLCs formulations can be ascribed to the better solubility of benzocaine in liquid lipid compared to the drug solubility observed in the lipid melt alone used for SLNs preparation.

The data clearly showed that the formulations containing Compritol 888 ATO as solid lipid had the highest entrapment efficiency. Compritol 888 ATO is believed to be one of the most applied and cited excipient in preparing SLNs and NLCs (38). Moreover, it shows high drug entrapment efficiency percentage (EE%) due

to the presence of large amount of mono-, di-, and tri-glycerides in the structure of Compritol 888 ATO, that helps in drug solubilization. Also, the less-defined mixture of acylglycerol provides further space for entrapping drug molecules (39).

The data clearly shows that glyceryl mono-stearate lipid nanoparticles exhibited the lowest entrapment efficiency of benzocaine compared to Compritol 888 ATO and stearic acid lipid nanoparticles. This can be attributed to the difference in composition and chain length of the three lipids used. The higher drug EE% noticed with Compritol 888 ATO and stearic acid was attributed to the high hydrophobicity due to the long chain fatty acids attached to the triglycerides resulting in increased accommodation of lipophilic drugs.

TABLE NO. 2: Characterization of the investigated lipid nanoparticles by particle size, zeta potential and entrapment efficiency (EE%)

Formula code	Particle size (nm)	Zeta potential (mV)	Entrapment Efficiency (EE%)
SLN_{GMS}	440±3.7	-20.9	55.65±0.12
NLC_1	337±2.9	-24.3	57.14±2.07
NLC_2	315±4.4	-22.0	58.50±1.57
SLN_{ST}	400±3.6	-19.6	56.25±0.84
NLC_3	381±3.9	-25.9	61.10±1.76
NLC_4	312±3.4	-22.5	84.62±0.99
SLN_{COM}	330±2.8	-23.9	72.37±1.14
NLC_5	287±4.1	-21.1	81.13±2.11
NLC_6	214±3.1	-20.0	94.62 ±0.35

Preparation of lipid nanoparticles based hydrogel

For the evolution of more proper formulations for topical administration, loading of NLCs and SLNs dispersions into hydrogels could be an interesting approach to develop better lipid nanoparticles systems.Amongst all prepared NLCs and SLNs systems, the nanostructured lipid carriersNLC6 containing Compritol 888 ATO as solid lipid and isopropyl myristate as liquid lipid, in ratio (0.7:0.3) solid lipid to liquid lipid, were chosen for incorporation in hydrogel for more convenient topical application. NLC6 showed the best performance, as it is characterized by thehighest entrapment efficiency (94.62±0.35%), smallest particle size (214±3.1 nm) and zeta potential value of -20.0 mV indicating a relatively good stability and dispersion quality.

In the current study, carboxymethyl cellulose (CMC) was selected as a gelling agent for the production of the hydrogel of NLC6. Carboxymethyl cellulose is a polyelectrolyte derived from natural materials. It has been extensively studied as a hydrogel polymer (40). CMC at the concentration of 5 % (w/w) was able to gel the selected NLCs dispersion (NLC6).

Evaluations of lipid nanoparticles based hydrogel

The formulated gel was white in color, odorless with smooth texture and free from any agglomeration of lipid nanoparticles. The pH value was found to be 6.10 ± 0.15, which is considered to be the recommended value for topical formulation and compatible with the pH of the skin (41).

***In-vitro* drug release study**

*In-vitro*drug release study of the investigated hydrogel is a pivotal step in the development stages of new formulations and a routine quality-control test to assure the final product uniformity.

Figure (1)shows the release of benzocaine for 8 hours from the NLC6 in comparison to free benzocaine. The percentage of benzocaine released after 8 hours was 79.53±1.11% forNLC6, showing a sustainedrelease profile. While fast release was observed with free benzocaine, as 92.10±3.5% after four hours only.

The release profiles of the investigated NLC6 showed an initial fast release that lastedfor 0–1 hr, followed by a sustained releasefor 8 hrs. It may be ascribed to the diffusion of unentrapped drug at first followed by diffusion from the NLCs surface and thereafter from the core. Also, This might be contributed to the effect of the present surfactant, which was responsible for partitioning of benzocaine molecules between the melted lipid phase and the aqueous Tween surfactant phase during the production of benzocaine NLCs(42).

From a therapeutic standpoint, both initial fast release and sustained release are recommended for topical products. Initial fast release can be useful to enhance the drug penetration whereas sustained release prolongs the residence time after topical application, as well as minimizing the systemic absorption (43).

The release of benzocaine from the hydrogel revealed that NLC6 hydrogel showed a controlled release of the drug as thepercentage of drug released were 66.37±2.31% after 8 hours.

From the previous results it was revealed that the release of benzocaine from the prepared hydrogel is slower than that of NLC6. The obtained result may relate to the release retarding effect of polymericmatrix of the gelling agents (44). The release pattern of both investigated NLC6and its hydrogel formulations, showed a drug sustained release for 8 hours.

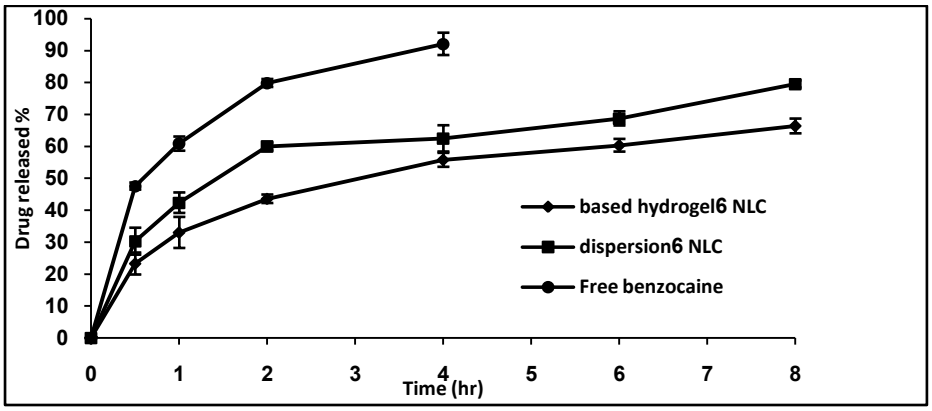

FIGURE NO. 1:*In-vitro*release profile of benzocaine from NLC_6 dispersions and NLC_6 based hydrogels using the dialysis bag diffusion technique

IV. Conclusion

In conclusion, this study proved that both solid lipid nanoparticles and nanostructured lipid carriers are considered as potential carrier systems for topical delivery for benzocaine.The results showed that the entrapment efficiency and particle size depend on the concentration and the lipid mixture employed. Where increasing the liquid lipid content resulted in higher entrapment efficiency, smaller particle size and a relatively good stability and dispersion quality.

Furthermore, the selected benzocaine-loadedNLC dispersion showed the highest entrapment efficiency with smallest particle size amongst the investigated lipid nanoparticles. Also, the selected benzocaine-loaded NLC hydrogel was meaningfully for benzocaine topical delivery showing optimum characters concerning adequate pH, and reasonably fast, yet, extended release.

Such findings proved that lipid nanoparticles produced in this study can potentially be exploited as promising carrier of benzocaine, thus achieving maximum drug efficacyfor prolonged and effective pharmacological action.

Conflict of interest

The author confirm that this article content has no conflict of interest

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