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Characterization of fatty acid based nanostructured lipid carrier (NLC) and their sustained release properties

Han-Choi Yew, Misni Misran
Department of Chemistry, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia

Abstract : Nanostructured lipid carrier (NLC) is a second generation lipid nanoparticle formed by blends of solid and liquid lipids through hot homogenization technique. In this study, arachidic acid (C20) and erucic acid (C22:1) were used as solid lipids while oleic acid as liquid lipid in the preparation of NLC. Five types of NLC were prepared by varying the amount of oleic acid (C18:1) with respect to arachidic acid while maintaining the amount of erucic acid. Physical stability of the prepared NLC was characterized by its size and zeta potential for a period of 28 days. The results showed that the size of NLCs were between 200 to 260 nm with zeta potential of -55 to -40 mV. Differential scanning calorimetry (DSC) data showed that presence of oleic acid reduces the crystallinity of nanoparticle. Apart from that, depending on the compositions, the morphology of NLC examined under transmission electron microscopy (TEM) was round to elongate in shape. Then, active ingredients of ascorbic acid, caffeine and lidocaine with varied hydrophilicity were then loaded into the NLC. Lidocaine has an encapsulation efficiency of nearly 78% while caffeine reaches 43%. Surprisingly, ascorbic acid which is hydrophilic gave a comparative amount of encapsulation efficiency as caffeine at low concentration. This might relate to attraction between opposite charge of NLC and ascorbic acid. When the active ingredients loaded NLC was subjected to in vitro release, the active ingredients' release profile suggested that NLC exhibit sustained release properties whereby the rate of release is concentration dependent.

Keywords: Nanostructured lipid carrier (NLC); fatty acids; encapsulation; kinetic model

***Correspondence to:** Han-Choi Yew; hc1018@hotmail.com, Misni Masran; misni@um.edu.my. Department of Chemistry, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia.

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Introduction

Nanostructured lipid carrier (NLC) has been explored for the past decade for its usage as an alternative carrier for preservation and delivery of active ingredients in dermal and oral application[1]. It is derived from the first generation solid lipid nanoparticle (SLN) by replacement of solid lipid[2] with liquid lipid while maintaining the NLC as a solid lipid at body temperature[3]. The NLC can be easily produced by hot homogenization technique. By which, the solid and liquid lipids were melted at a suitable temperature and dispersed in solution by high speed homogenization. The emulsion droplets were then crystallized in cold environment to form lipid nanoparticle[4].

NLC has been widely used in cosmetic, pharmaceutical and even food industry to enhance the stability of active ingredients and act as a more effective system for delivery. NLC helps to enhance the stability of active ingredients by solubilizing the ingredients during preparation and incorporating them in the lipid core upon recrystallization. The formation of solid particle matrix in NLC minimize the possibilities of active ingredients being diffuse or release to the surrounding environment followed by degradation[5]. Besides, high loading efficiency of NLC gave an advantage as it aids in reduction of active ingredient wastage. NLC which is more amorphous has the possibility to load more active ingredients as compared to rigid SLN. This is because the blend of different types of lipids, especially liquid lipids affects the orientation of the molecules during crystallization of NLC. Thus, the formation of imperfect structure of NLC gave more room to accommodate active ingredients than the perfect structured SLN[6,7]. Moreover, NLC allows deeper skin penetration and helps in increasing skin hydration[8].

From the above, NLC which has a lot of advantages could serves as a potential active ingredients delivery vehicle. Hence, our main aim in this study is to investigate the encapsulation and release profile of active ingredients with different hydrophilicity from NLC. It is important to understand the nature of the active ingredients and their suitability to be incorporated in the NLC as it can give a better insight of the system as an active ingredient delivery carrier. With this, the active ingredients of ascorbic acid, caffeine and lidocaine were selected based on their solubility in water.

Material and Methods

Materials

Arachidic acid, oleic acid, L-ascorbic acid, caffeine and lidocaine were purchased from Sigma-Aldrich, USA. Erucic acid and Span 40 were from Fluka, Switzerland. Deionized water was dispensed from Barnstead NANOpure Diamond ™ (Thermo Scientific, USA) with 18.2 MΩ-cm. Disposable capillary cell was from Malvern, UK. DSC hermetic pan was from TA instruments, USA.

Methods

Preparation of NLC

Unloaded NLC was prepared by hot homogenization technique. Mixture of fatty acids as shown in Table 1 with Span 40 was melted in ratio 8:2 at 90 ˚C. The molten was added with 2 ml of same temperature deionized water and homogenized using T25 basic homogenizer (IKA, Germany) for 10 minutes with the speed at 13000 rpm. The sample was then poured in ice cooled deionized water to crystallize the lipid emulsion. For active ingredients encapsulation, NLC was fabricated in the same way by first dissolving the active ingredients in their similar phase.

Table 1. Compositions of lipid components for the preparation of NLC

Sample	Fatty Acids (weight percentage)		
	Arachidic acid	Oleic acid	Erucic acid
NLC 1	80	10	10
NLC 2	70	20	10
NLC 3	60	30	10
NLC 4	50	40	10
NLC 5	40	50	10

Characterization of NLC

Particle size characterization and zeta potential

The mean z-average of particle size, particle distribution and zeta potential of NLC were measured using Malvern Zetasizer Nano Series (Nano ZS, Malvern Instruments, UK) at 25 ˚C. The NLC solution was loaded in U-shape clear capillary cell (DTS 1070). An average particle size was obtained from triplicate measurements of the -Required skill(s): Photoshop, Illustrator, After Effect, Premiere Pro, video editing and motion graphic whereby each measurement consists of 12 runs on the sample. Short term physical stability was conducted for NLC with a period of 28 days at room temperature (around 30 ˚C). The measurement was taken on the 1st, 4th, 7th, 10th, 14th, 21st and 28th day. This physical characterization was also performed after loading of active ingredients in NLC.

Differential Scanning Calorimetry (DSC)

The onset temperature, peak temperature and melting enthalpy of NLC were monitored by employing DSC Q20 (TA instruments, USA). 2-3 mg of sample was weighted in 40 µl hematic aluminum pan and the analysis was performed with a scan rate of 5 ˚C per minute from 20 to 100 ˚C. An empty pan was used as the reference. The sample was repeated for 3 cycles.

Transmission Electron Microscopy (TEM)

The morphology of NLC was observed under TEM (Carl Zeiss Libra 120, Germany) using negative-staining method conducted in School of Biological Sciences, University Sains Malaysia (USM, Malaysia). One drop of NLC suspension was placed on the 400 mesh carbon coated copper grid and left for 2 minutes. The excess liquid was removed using filter paper and air-dried for 1 minute. Then, 1 % phosphotungstic acid solution was dropped on the sample and left for 1 minute. The solution was blotted away with filter paper and air-dried before visualized under TEM with acceleration voltage of 120 kV.

Loading and Encapsulation of active ingredients

The amount of active ingredients loaded or encapsulated in NLC is calculated by estimating the amount of free active ingredients in the continuous phase. Loading efficiency (Eq.1) of active ingredients was calculated by dividing the amount of active ingredients encapsulated towards initial amount of basic ingredients present. Encapsulation efficiency (Eq.2) on the other hand was calculated by dividing the amount of active ingredients encapsulated with initial amount of active ingredients used. The loaded NLC sample was passed through 0.22 µm Nylon syringe filter and a clear solution was collected. The free active ingredient was measured by UV-Vis spectrophotometry (Cary 50, Agilent Technologies, USA) and the loading and encapsulation efficiency was calculated as the equation below:

Loading efficiency: (b-c)/a **Eq. 1**

Encapsulation efficiency: (b-c)/b **Eq. 2**

Where a= weight of basic ingredients, b= weight of initial active ingredients, c= weight of free active ingredients

In vitro release of active ingredients

The cumulative percentage release of the active ingredients was studied at 37 ˚C for a period of 24 hours using Franz diffusion cell (Hanson Research, USA). Dialysis membrane with 5000 molecular weight cut off (MWCO) was mounted between the donor and receptor part. The donor part consists of 500 ng active ingredients while receptor part was filled with phosphate buffer pH 7.4. The sample was automatically collected for 14 times at first 30 minutes subsequently first hour, second hour and every consecutive 2 hour. The active ingredient released was estimated by employing UV-Vis spectrophotometry using quartz cuvette with 1 cm path length. The experiment was conducted for six replicate. Active ingredient release model was determined by fitting the result into zero-order model, first-order model, Higuchi model, Hixson Crowell model and Korsmeyer-Peppas model. For zero-order model, cumulative percentage release was plotted against time; first-order model, log of remaining percentage release was plotted against time; and Higuchi model, cumulative percentage release was plotted against square root of time; Hixson-Crowell model, cube root of active remaining was plotted against time; Korsmeyer-Peppas model, log cumulative percentage release was plotted against log time.

Results and Discussion

Stability of NLC

The particle size analysis of prepared NLC was shown to be affected by the amount of oleic acid present in the formulation. From Figure 1, gradual increase of oleic acid by 10 wt. % of the lipid mixture in the formulation caused an increase in the particle size of the NLC when the formulation was evaluated on the first day. Moreover, it shows a slight changes in the particle size for all NLC formulation when the stability of the NLC prepared was evaluated. However, beyond Day 14th, the NLC reaches its maximum particle stability whereby the formulation has no or minimum changes in its particle size. In addition, the polydispersity index of the NLC which has a value of 0.22 for all NLC (data not shown) indicates that the formulation has high tendency to be unimodal distribution. The increase in particle size with respect to higher amount of oleic acid in the formulation can be explained from the nature of oleic acid which has an unsaturated double bond at C9-C10, formed a kink in the structure, prevents the formation of compact and rigid NLC with smaller particle size. Furthermore, higher amount of liquid lipid might also caused a more disordered crystalline structure which affects the particle size of the NLC[9].

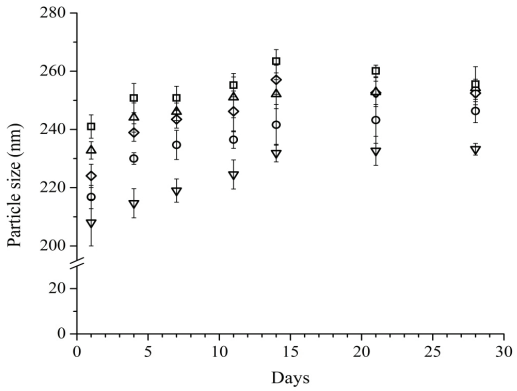


Figure 1. Changes in particle size of aqueous NLC solution prepared using different fatty acid compositions for a storage period of 28 days at 30 ˚C. The symbols ▽ = NLC 1. ○ = NLC 2. ◇ = NLC 3. △ = NLC 4. □ = NLC 5.

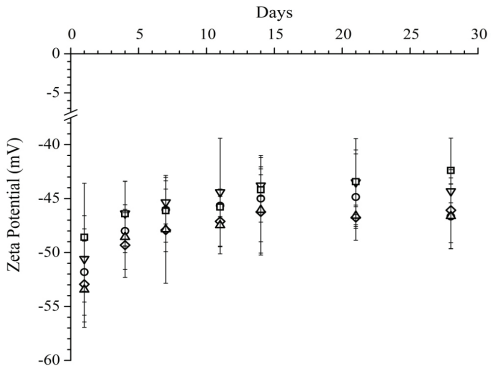


Figure 2. Change in zeta potential of NLC in aqueous suspension with varied fatty acid compositions as a function storage time of 28 days at 30 ˚C. The symbols ▽ = NLC 1, ○ = NLC 2, ◇ = NLC 3, △ = NLC 4, □ = NLC 5.

Besides particle size, on the first day after preparing the NLC, the zeta potential which fluctuate within -52 ± 3 mV for all NLC formulations showed no significant difference in the surface charge (Figure 2). Nevertheless, the changes in surface charge of the NLC has a similar pattern with the particle size whereby it showed an overall a slight increase of zeta potential followed by stable zeta potential around -47 mV after Day 10th. In general, zeta potential with a magnitude of more than positive or less than negative 30 mV is said to be a stable dispersion. The stability of NLC is enhanced by the electrostatic repulsion between the NLC particles of similar surface charge thereby preventing the NLC from aggregating.

Thermal profile of NLC

In addition, DSC analysis was performed on NLC sample in order to determine the heat gain or heat loss for a certain mass of sample with respect to time. The results may be affected by the physical or chemical changes of the sample. Thus, from the thermal parameters of onset temperature, peak temperature and melting enthalpy obtained from DSC curve, the structure of the sample can be predicted. The onset temperature is known as the melting point, peak temperature is the temperature at which maximum reaction rate occurs while melting enthalpy is calculated by the integration of the area above or below the baseline. These three parameters are highly affected by the compositions which made up the NLC. Therefore, those with more liquid lipid has a lower onset temperature, peak temperature and enthalpy changes compared those with higher solid lipid content (Table 2).

Table 2. Thermal parameters of fatty acids based NLC.

	Onset Temperature, (˚C)	Peak Temperature, (˚C)	Melting Enthalpy, ΔH (J/g)
NLC 1	67.6	71.8	156.7
NLC 2	65.4	70.5	144.9
NLC 3	59.0	67.7	109.4
NLC 4	53.2	65.9	67.82
NLC 5	49.9	62.5	59.51

The changes in the thermal parameters on the NLC prepared is affected by the amount of oleic acid in the compositions. Oleic acid being the only liquid lipid in the formulation tends to melt at room temperature as compared to arachidic acid and erucic acid (Figure 3). Therefore, mixture with less oleic acid is expected to form a more rigid solid structure with higher crystallinity. Hence, the peaks of melting temperature broaden while the peak height gradually decreased when the amount of oleic acid was gradually increased. This indicates the loss in the crystallinity of NLC.

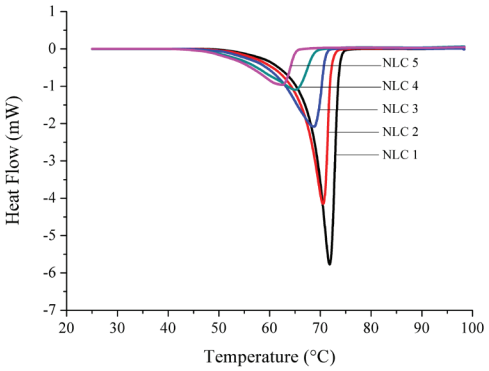
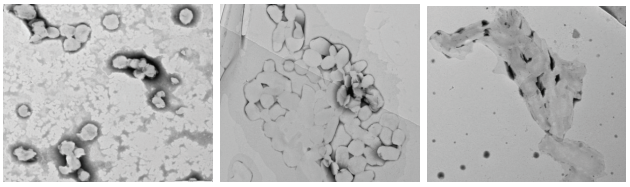


Figure 3. Differential scanning calorimetry thermogram of NLC. The scanning was performed from 25˚C to 100˚C at a scan rate of 5˚C per minute.

Morphology of NLC

As a supplementary to the above results, the TEM micrographs clearly showed the morphology of the NLC prepared (Figure 4). NLC 1 which has lesser oleic acid had particles which were more rounded and uniform in the shape. However, when more oleic acid was added in the formulation, the shape of NLC turned into more elongated in shape and the overall size is larger than those with lesser oleic acid. This might be attributed by the kink structure of oleic acid, prevent the formation of rigid rounded shape of NLC. The size result of TEM is in agreement with the particle size obtained from DLS measurement whereby the order of particle size is NLC 1 < NLC 3 < NLC 5. The micrographs also gave us an idea that the NLC with higher liquid lipid content possess unexpectedly higher encapsulation efficiency. Nevertheless, it was not suitable to have liquid lipid more than 30 percent of the lipid components as the sample might forms oil droplet apart from NLC.

Figure 4. TEM micrographs of NLC where (a) NLC 1 (b) NLC 3 (c) NLC 5.



(a) (b) (c)

Loading and Encapsulation efficiency of active ingredients in NLC and their stability

Active ingredients loaded in NLC

The loading efficiency of active ingredients in NLC is highly affected by the nature of the active ingredients whereby those which is less hydrophilic are more susceptible for higher loading efficiency. When the amount of active ingredient with respect to NLC was increased, the loading efficiency was also increased until it reaches the optimum loading amount (Figure 5). Lidocaine, which is the most hydrophobic active ingredient among the three, has the highest loading efficiency in NLC as compared to caffeine and ascorbic acid. The loading efficiency of lidocaine in NLC is at least four times higher than that of both ascorbic acid and caffeine. This is owing to the lipophilic properties of lidocaine, whereby it can easily incorporate into the lipid matrix of NLC than the other two active ingredients.

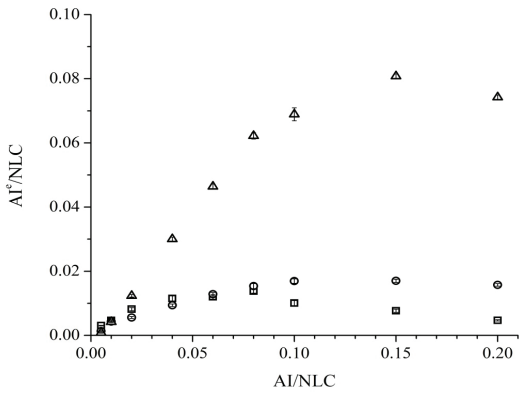


Figure 5. Active ingredients loaded NLC at different weight ratio of active ingredients to NLC. Where symbol □ = ascorbic acid, ○ = caffeine, △= lidocaine while AI = active ingredients, AI^e = loaded active ingredients.

Encapsulation efficiency

On the other hand, encapsulation efficiency of active ingredients showed that the encapsulation efficiency will increase gradually until it reaches its optimum percentage, followed by a decrease in encapsulation efficiency (Figure 6) even though the loading efficiency is still increasing. Lidocaine, which is more hydrophobic, has the highest encapsulation efficiency of around 78% as compared to caffeine of around 43%. Surprisingly, ascorbic acid which is more hydrophilic, theoretically should have lower encapsulation efficiency than caffeine and lidocaine, anyhow gave a high encapsulation efficiency when the ratio of active ingredients to NLC is less than 0.05. This might be attributed to the absorption of positively charged ascorbic acid on the surface of the NLC.

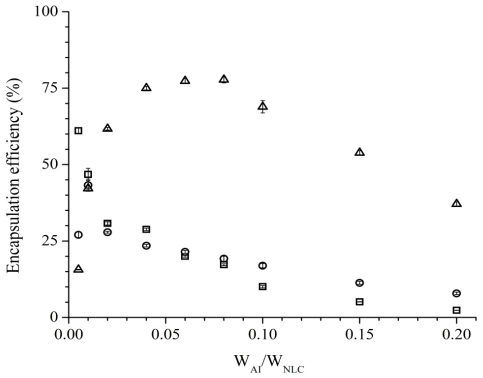


Figure 6. Encapsulation efficiency of active ingredients in varied weight ratio of active ingredients to fatty acid. Where symbol □ = ascorbic acid, ○ = caffeine, △= lidocaine while W= weight, AI= active ingredients.

Physical stability of encapsulated NLC

The changes in particle size and zeta potential of active ingredients loaded NLC is affected by the nature of active ingredients selected. Ascorbic acid, caffeine and lidocaine gave a positive, neutral and negative charge in deionized water respectively. The encapsulation of caffeine and lidocaine in NLC, do not show any significant changes in their particle sizes. But, ascorbic acid encapsulated NLC showed a drastic change in particle size by two folds as compared to unencapsulated NLC (Figure 7). This is plausibly because the changes in the pH of the aqueous phase after addition of ascorbic acid induce aggregation to the NLC. Nevertheless, all samples showed only a slight increase in particle size over time. This indicates that despite there are changes in the particle size, the active ingredients encapsulated NLC still being remained as a stable formulation.

Furthermore, the zeta potential of encapsulated NLC deviates in respond to the active ingredients loaded in the NLC (Figure 8). The surface charge of ascorbic acid, caffeine and lidocaine encapsulated NLC has an increase, no change and decrease in zeta potential respectively. This might due to the surface charge on the active ingredients itself when solubilized in water. Ascorbic acid which is positively charged neutralized the negative charge of NLC while lidocaine gave more negative effect towards the NLC. Other than that, there is a slight increase in the zeta potential of the active ingredients loaded NLC when evaluated for four weeks. Nevertheless, it was followed by stable zeta potential after day 7^{th}.

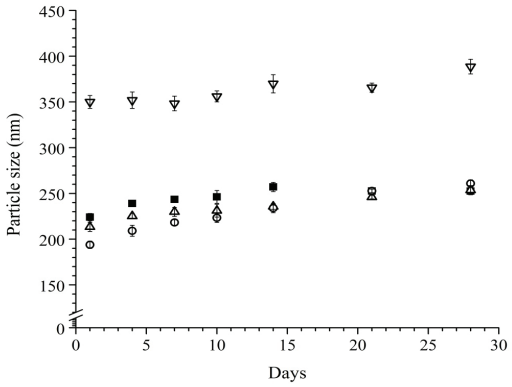


Figure 7. Effect of active ingredients towards the stability of NLC with respect to storage time at 30 ˚C. Where symbols = unloaded NLC, ▽ = ascorbic acid, △ = caffeine, ○ = lidocaine.

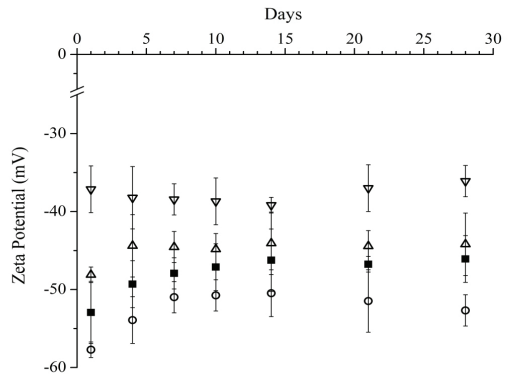


Figure 8. Changes in zeta potential and stability of active ingredients loaded NLC as a function of storage time at 30 ˚C. Where symbols ■ = unloaded NLC, ▽ = ascorbic Acid, △ = caffeine, ○ = lidocaine.

Release profile of active ingredients

The release profile of active ingredients from NLC showed a similar pattern as that of unencapsulated active ingredients, which are in the ascending order of release are lidocaine < caffeine < ascorbic acid (Figure 9). Nevertheless, the rate of caffeine and lidocaine released from encapsulated NLC were much lower than that of uncapsulated active ingredients while ascorbic acid was almost similar to unencapsulated active ingredients (data not shown). This suggested that ascorbic acid which majorly adsorbs on the surface of the NLC could detach and permeate into the receptor chamber when there is a drop in the chemical potential than the other to active ingredients which are embedded in the matrix core of NLC.

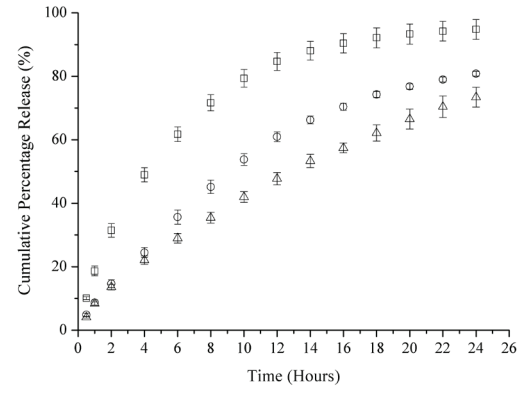


Figure 9. Release profile of active ingredients encapsulated NLC. Where symbol □ = Ascorbic Acid, ○ = Caffeine, △=Lidocaine.

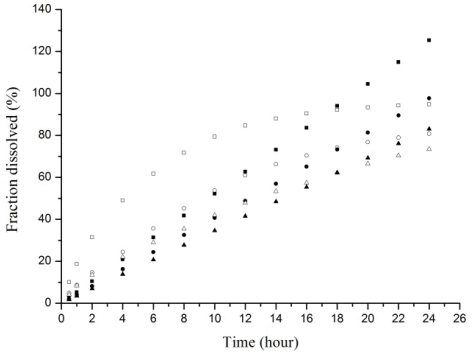
From the cumulative percentage release profile, various kinetic models were employed to describe the release kinetic[10,11]. The curve fitting showed that the release kinetic fits well in all models except zero-order model (Figure 10). Among the four kinetic models which have R^2 values of at least 0.939 (Table 3), first-order model has the best linear regression towards the original plots. Additionally, in the model, it was found that the rate constant of active ingredients' release from NLC is at least 30% slower than the unencapsulated active ingredients. Hence, we hypothesized that at the initial phase of release, the active ingredients released were mostly from the continuous phase of the prepared NLC solution. This has caused a burst effect on the rate of active ingredients' release, where most of the unencapsulated active ingredients could permeate into the receptor chamber. When the chemical potential gradient decreased, the encapsulated active ingredients from NLC will gradually partition into the continuous phase. At this stage, the release rate would be much lower as more energy was required to overcome the phase barrier for it to be release to the continuous phase.

Table 3. Curve Fitting of Active Ingredients Released from NLC by using diferent kinetic models.

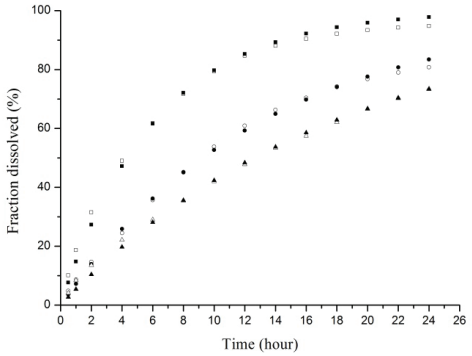
	Ascorbic acid	**Caffeine**	**Lidocaine**
	5.224	4.070	3.461
Zero-order, K_0			
	R^2= 0.454	R^2= 0.870	R^2= 0.927
	0.160	0.075	0.055
First-order, K_1			
	R^2= 0.993	R^2= 0.998	R^2= 0.996
	22.048	16.716	14.125
Higuchi, K_H			
	R^2= 0.939	R^2= 0.963	R^2= 0.964
	0.043	0.021	0.016
Hixson-Crowell, K_{HC}			
	R^2= 0.966	R^2= 0.985	R^2= 0.988
	26.963	11.981	8.767
Korsmeyer-Peppas, K_{KP}			
	R^2= 0.950	R^2= 0.982	R^2= 0.999

Where K_0 is the zero order release constant expressed in units of μg h^{-1}, K_1 is first order rate constant expressed in units of h^{-1}, K_H is the Higuchi dissolution constant expressed in units of μg $h^{-1/2}$, K_{HC} is the constant incorporating the surface-volume relation expressed in units of μg h^{-1}, K_{KP} is the release rate constant expressed in units of h^{-n}.

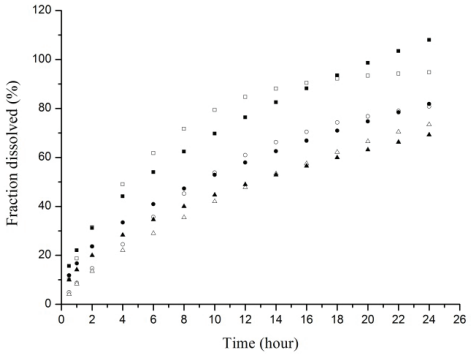
Zero-order



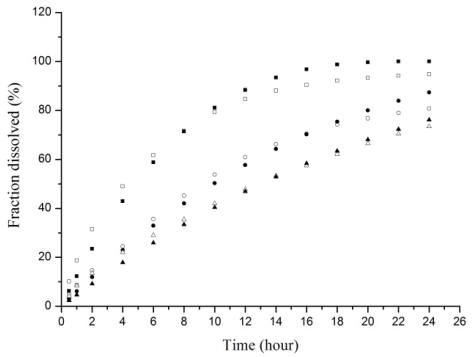
First-order



Higuchi



Hixson-Crowell



Korsmeyer-Peppas

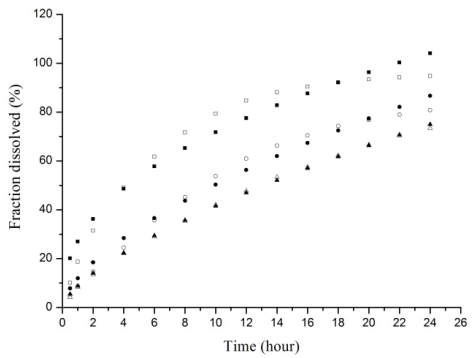


Figure 10. Fitting of cumulative percentage release of active ingredients from NLC in different kinetic models. Where symbol □ = Ascorbic Acid, ○ = Caffeine, Δ=Lidocaine. The opened symbols represents the original plot while the solid symbols represents the predicted plot.

Conclusion

NLC is a versatile system which has the ability to entrap different types of compounds depending on the nature of the active ingredients. Different types of active ingredients will affect the physical properties, encapsulation efficiency of NLC thereby exert an influence on the release profile. NLC 3 is considered as the most suitable NLC for application as the mixture showed high stability, good thermal profile and acceptable shape and size from TEM micrograph. The encapsulation efficiency of active ingredients is as predicted for caffeine and lidocaine. The encapsulation of ascorbic acid gave us an idea that the active ingredients adsorp on the surface of NLC which causes the aggregation. This is further confirmed when the permeation studies showed that the release rate of ascorbic acid encapsulated and free active ingredient is almost the same. For caffeine and lidocaine, the release rate of encapsulated is slower than the free active ingredients. The release profile elucidate that the release of active ingredients were concentration dependent this suggest the sustained release property of NLC. Thus, NLC is a potential carrier for active ingredients which are amphiphilic and lipophilic in nature.

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Conflict of Interest

The authors declared that there is no conflict of interest.

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