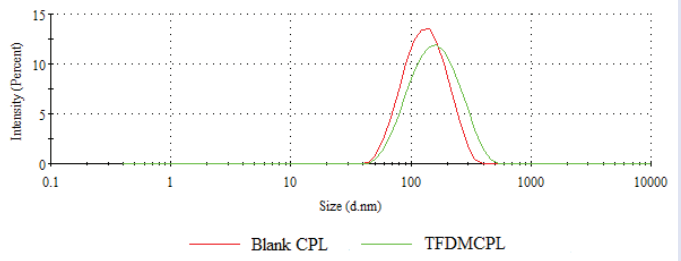

Figure 4: The particle size and PDI of Blank CPL and TFDMCPL

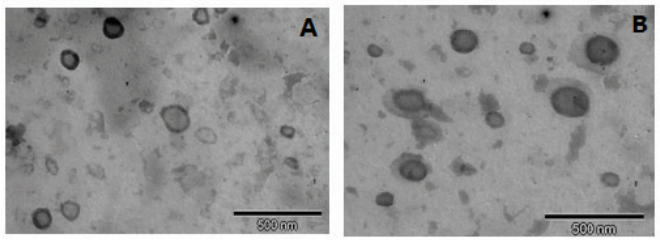

Figure 5: Transmission electron microscope images of morphology of A (Blank CPL) and B (TFDMCPL)

Table 1: The mean size and zeta potential of drug-free and drug-loaded CPL

Batch	Mean size ± SD [a] (nm)	Zeta potential ± SD [a] (mV)
Drug-free CPL [b]	106.4 ± 2.4	-26.7 ± 0.8
Drug-loaded CPL [b]	136.2±3.7	-19.8 ± 1.2

[a] standard deviation (n=3). [b] The mean of 3 batches.

The drug-loaded CPL suspension had an upper zeta potential compared to drug-free formulation. Indeed, the zeta potential was -26.7 mV for the drug-free CPL suspension and became -19.8 mV for the drug-loaded CPL suspension. Zeta potential measurements give information about the surface properties of the carrier and therefore can be useful to determine the type of the association between the active substance and the carrier (whether the drug is encapsulated in the body or simply adsorbed on the surface).

In our study the negative surface charge was further shielded in the presence of the drug, suggesting that at least a part of the association was surface-adsorption and the rest was incorporated within the lipidic matrix.[11] These zeta potential data allowed predicting a very good stability of the preparations (a negative zeta potential higher than 15 mV was sufficient to prevent vesicle coalescence).[12]

The desirable therapeutic effect of CPL as drug carriers can be achieved if they are loaded with a sufficient amount of an active compound. Therefore, suitable entrapment efficiencies of drugs are required. In our study, a relatively higher encapsulation efficacy was found with more than 22% in comparison to traditional liposomes. Thus, the high EE was believed to be due to the high lipophilicity of TFDM and its good solubility in phospholipids.

Surface morphology study by transmission electron microscope(TEM)

As shown in Figure 5, the morphological investigation using transmission electron microscopy revealed nanometric sized and spherical shaped CPL. According to TEM micrographs, CPL ranged in size from 100 to 150 nm correlating well with measurement obtained. Vesicle membranes were composed of phospholipids bilayers.

Stability study of CPL

The mean particle size, PDI of CPL and the EE of TFDM-CPL were examined after 6 months of storage at 4°C. An increase of size of empty CPL from 106.4 ± 2.4 nm to 128.3 ± 5.6 nm and PDI from 0.127 ± 0.012 to 0.167 ± 0.021 and size of TFDM-CPL from 136.2 ± 3.7nm to 186.7 ± 3.7 nm and PDI from 0.158 ±0.015 to 0.205 ± 0.021 were observed. However, the EE of TFDM determined after 6 months of storage at 4°C,were identical to those obtained at t_0 suggesting that CPL retain the TFDM constituent during the storage.

In vitro drug release of TFDM- CPL

To investigate *in vitro* drug release of TFDM-solution and TFDM-CPL, the amount of drug released from CPL was drafted as a function of time. The cumulative amount of TFDM in the receptor over 24 h was charted after administration of TFDM-CPL ,as shown in Figure 6, and the equation matching the results to the release behavior are seen in Table 2. It was obvious that the drug release from CPL lifted significantly. When comparing the results, which were consistent with the general summary, that composite phospholipidliposomal entrapment of drugs promotes their release, and the release behavior of TFDM-CPL, was similar to the one order equation. According to a recent report, CPL, was a novel carrier of nano-formulation delivery system. This could make the drug molecule transported between CPL and cell membranes and might indicate more initial burst release in intestinal tract. Therefore, CPL had a potential application in enhancing the oral bioavailability of TFDM *in vivo*.

Pharmacokinetics studies

The aim of experiment is to evaluate the viability of the CPL as a means of increasing the oral bioavailability of TFDM, the pharmacokinetics of TFDM-CPL was compared with that of TFDM-solution in rats by an HPLC method. The content of TFDM (luteolin glucuronide, rosmarinic acid and tilianin) was more than 82.6%, the content of luteolin glucuronide, rosmarinic acid and tilianin in rat plasma was determined respectively. The method was validated for factors such is linearity, precision, accuracy and stability.

Figure 7 shows the plasma concentration versus time profiles of TFDM-solution and TFDM-CPL. The plasma concentration of TFDM-solution increased with time over the 15 min and decrease slowly up to 12 h and the plasma concentration of TFDM-CPL increased with time over the 30 min and decrease slowly up to 12 h.

Table 3 lists the relevant pharmacokinetic parameters of TFDM-solution

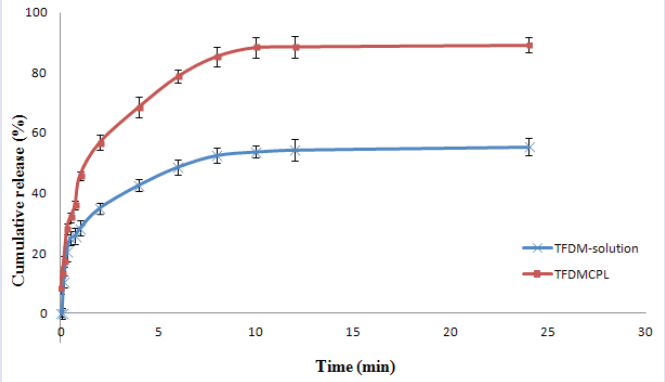

Figure 6: The release of TFDM and TFDMCPL (n=3)

Table 2: Results of release curve fitting

Release	TFDM-solution	r^2	TFDMCPL	r^2
Zero order	Q = 0.3166t+0.0574	0.9249	Q = 0.3568t+0.1139	0.9553
First order	Ln(1-Q) = 0.9214t+0.1123	0.9521	Ln(1-Q) = 0.4996t+0.1112	0.9725
Higuchi	$Q = 0.5861t_{1/2}$+0.1225	0.9342	$Q = 0.3804t_{1/2}$+0.9617	0.9617
Weibull	InIn[1/(1-Q)] = 1.1706Int-0.433	0.9482	InIn[1/(1-Q)] = 0.4405Int-0.637	0.9352

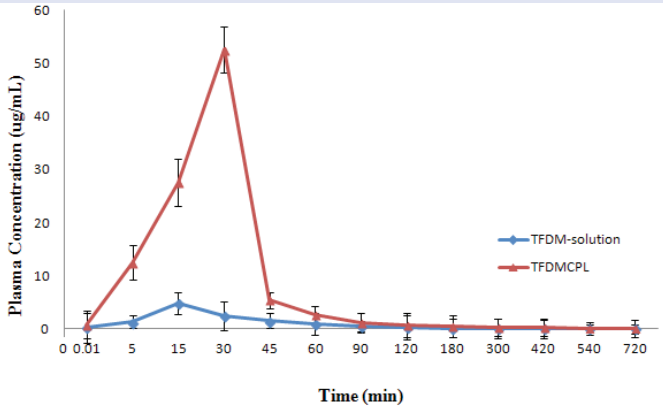

Figure 7: Mean ± SD plasma concentration-time profile of TFDM after oral administration of free TFDM solution and TFDMCPL at 300 mg/kg dose to rats

Table 3: Pharmacokinetic parameters of oral administration of free and formulated drug in rats

Parameter	TFDM-solution	TFDMCPL
C_{max} (ug/mL)	3.73 ± 0.87	51.76 ± 3.22**
T_{max} (min)	16.07 ± 1.24	29.47 ± 3.08*
$t_{1/2}$ (min)	10.95 ± 0.88	13.34 ± 1.34*
$MRT_{(0-\infty)}$ (min)	16.33 ± 0.56	28.19 ± 1.68*
$AUC0-_{12\,h}$ (min ug/mL)	162.79 ± 5.81	1571.92 ± 32.62**
CL (mL/min·kg)	614.27 ± 8.66	63.62 ± 2.23**

Values are expressed as mean ± SD (n= 6) * p< 0.05, compared with TFDM-solution ** p< 0.01, compared with TFDM-solution

and TFDM-CPL.As Table 3 shows, the C_{max}, AUC_{0-12h} and CL of TFDM-solution and TFDM-CPL showed a highly significant difference.

The T_{max} and $t_{1/2}$ extended to 29.47 ± 3.08 min and 13.34 ± 1.34 min ($p < 0.05$), respectively. The C_{max} in TFDM-solution was 3.73 ± 0.87ug/mL, which was different from that in the TFDM-CPL of 51.76 ± 3.22ug/mL ($p< 0.01$). The relative oral bioavailability of the encapsulated TFDM was 965.61 % as compared with the TFDM-solution, which was significantly enhanced ($p<0.01$).Based on research results it has been indicated that the *in-vitro* drug release and rats pharmacokinetics results have a similar variation trends to the oral absorption of TFDM-CPL.

In Fig 7, a pharmacokinetically well defined plasma concentration profile was observed. The liposomal encapsulation provided a great improvement for *in vivo* absorption of TFDM, supporting other studies that used CPL to enhance the bioavailability of poorly water soluble drugs. One of the main reason for improved oral bioavailability might be that CPL could protect drugs from enzymatic metabolism after oral administration.[13] In addition, the majority of drugs stays inside of vesicles of CPL and thus drugs itself may not have a chance to contact esophagus and get higher oral bioavailability.[14] Also, CPL were formed by phospholipid bilayers, resulting in improving the membrane permeability and cellular absorption. Furthermore, biocompatible polymers have been used for surface coating of CPL to extend the residence time in intestine.[15-16] And the addition of cholesterol to the CPL would also enhance stability of CPL *in vivo*.[17] Therefore, the prolonged retention of liposomes in intestine might contribute to increase the drug absorption. In summary, according to our study on the oral administration, the TFDM-CPL led to a significant improvement in bioavailability compared to the TFDM-solution, with almost10-fold increase in relative bioavailability.

CONCLUSION

For the first timeTFDM, which is a poorly water soluble, and low oral bioavailability compounds were successfully encapsulated in the CPL formulations. They encapsulate TFDM constituents with high EE values. According to the physicochemical properties and drug *in-vitro* release of TFDM-CPL, which with high EE, small size, well suited PDI and the final CPL, was able to potentially promote releasing of TFDM.

According to the Pharmacokinetics Studies, TFDM-CPL could be effective to enhance bioavailability of TFDM after oral administration *in vivo*. They were also stable after 6 months of storage at 4°C.Therefore, the novelliposomalnano formulation could be a promising carrier for poorly water soluble drugs and enhanced oral bioavailability of TFDM.

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Conflicts of interest

There are no conflicts of interest

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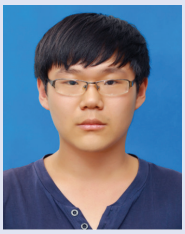
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