6. Development of Pan-coated PR formulations

After successful development of time-controlled PR platform formulation employing compression coating technology, pan coating technology was also explored to develop PR formulation. Since the prime purpose was to compare the competence of two processes, the effort was made to develop the PR pan-coated tablet (PCT) formulation while keeping the formulation parameters as similar as possible to the developed CCT formulation with no change in core tablet composition.

6.1. Methods

6.1.1. Analytical methods for determination of drugs

As mentioned in chapter 5

6.1.2. Preparation and characterization of core tablets

As mentioned in chapter 5

6.1.3. Preparation of PCTs

The previously formulated core tablets were coated using perforated pan coating machine (Solace Engineers (Mktg) Pvt. Ltd., Vadodara, Gujarat). The coating solution was prepared by sequentially dissolving EC N10, HPMC E5 and dibutyl sebacate (DBS) in 50:50 % w/w dichloromethane (DCM): isopropyl alcohol (IPA) solvent mixture with continuous stirring and slow addition. Here also, similar to CCT formulation, EC and HPMC were taken as functional polymers in the concentration ratio of 83:17% w/w whereas DBS was selected as plasticizer (1-3) in the concentration of 10% w/w of total polymer content (4). Total solid content was kept as 5% w/w. Coating process was carried out by following below mentioned parameters: pre-warming of tablets, 35°C for 10 minutes; inlet air temperature, 35-45°C; bed temperature, 28-33°C; spray nozzle diameter, 1 mm; atomizing air pressure, 1.2-1.5 bar; rotating speed of pan, 5-10 rpm; and spray rate, 3-8 g/min. After completion of the spraying, tablets were subjected to post-drying in the pan itself at 50°C for 30 min. Different coating weights were applied in order to obtain 4-6 h of lag time. Initial optimization was carried out using PRS tablets and subsequently other core tablets were also one-by-one coated using same optimized formulation and process parameters.

6.1.4. Characterization of PCTs

The PCTs were characterized for appearance, weight variation, thickness, hardness, assay, and drug release. The weight variation, hardness, thickness, and assay were determined in the same manner as mentioned for the core tablets. The release study was carried out using 500 mL of 0.1 N HCl for first 2 h followed by pH 6.8 phosphate buffer as dissolution medium with basket apparatus operated at 100 rpm agitation and 37.0±0.5°C temperature. In case of poorly soluble compounds i.e. NIF and LOR, the medium volume was taken as 900 mL instead of 500 mL. Similarly, alcohol-induced dose dumping study was also carried out using 5% v/v ethanol in 0.1 N HCl for first 2 h followed by pH 6.8 phosphate buffer as dissolution medium. The dissolution profile was obtained using six dosage units and data was recorded at every 1 h of repeated interval up to complete release of drug. In each case, the time to rupture the outer coat was observed visually and noted down. Moreover, after rupturing of outer coat (visual observation), additional data points were taken at every 15 min of repeated interval up to the complete release of drug to examine burst release pattern after lag time. At each point, 5 mL sample was withdrawn and equivalent amount of fresh medium was replaced. Each withdrawn sample was filtered through 0.45 µm membrane filter and analyzed by RP-HPLC method. Further, SEM analysis was carried out to determine coating thickness as well as surface characteristics of an optimized PCT before and after drug release study. The optimized PCTs were further evaluated to examine the effect of curing by exposing them at 60°C for 24 h in hot air oven and subsequently analyzed for hardness, assay, and drug release.

6.1.5. Packaging and stability study

The developed PCTs were subjected to short term stability testing according to ICH guidelines (5). Forty PCTs along with a silica bag were packed into high density polyethylene bottle with induction cap sealing. The sealed bottles were subjected to accelerated (40±2°C/75±5% RH) and long term (25±2°C/60±5% RH) stability studies for three months. The samples were periodically withdrawn at 0, 30, 60, and 90 days and examined for hardness, assay, and drug release.

6.1.6. *In vivo* pharmacokinetic study of DIC PCT formulation

In vivo pharmacokinetic study was conducted to compare and evaluate DIC PR PCT formulation with similar dose of conventional IR formulation using New Zealand White (NZW) rabbits (6, 7). All procedures were carried out in accordance with the Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA) guidelines, Department of Animal Welfare, Government of India (8) with approved protocol No. MSU/IAEC/2014-15/1403.

6.1.6.1. Quantification of DIC in rabbit plasma using bio-analytical LC-MS method

To determine the concentration of DIC in rabbit plasma, bio-analytical liquid chromatographic-mass spectrometric (LC-MS) method was developed with slight modification from RP-HPLC chromatographic conditions as mentioned in section 5.3.2.1.

The chromatographic separation was performed using ekspert^{TM} ultra LC with ekspert^{TM} ultra LC 100 pump system (eksigent-AB Sciex, USA) coupled with 3200 QTRAP mass analyser (AB Sciex, USA), located at Dr. Vikram Sarabhai Science Center, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India. The temperature of the column oven (ekspert^{TM} ultra LC 100, eksigent-AB Sciex, USA) was maintained at 40°C. The separation was achieved using Kromasil C18 (250 x 4.6 mm, 5 µm) column at a flow rate of 1 mL/min with 7 min run time. The mobile phase was isocratic composition of 0.1% v/v glacial acetic acid in 25:50:25 % v/v/v mixture of water: methanol: acetonitrile. The mobile phase was vacuum filtered through 0.22 µm nylon membrane filter followed by degassing with an ultrasonicator prior to use. The injection volume was kept as 20 µL.

MS analysis was conducted using 3200 QTRAP mass spectrometer (AB Sciex, USA) equipped with electro spray ionization (ESI) source. The mass spectrometer was operated in the positive ion mode with a potential of 5.5 kV applied on the electro spray ionization needle. The ionization source temperature was kept at 450°C. DIC was identified and quantified using Multiple Reaction Monitoring (MRM) mode. The curtain gas (CUR) pressure was kept at 25.0 psi, the nebulizer source gas 1 was at 50.0 psi and the turbo ion source gas 2 was also at 50.0 psi. The optimized declustering potential and entrance potential were 35.0 V and 4.5 V respectively. The fragmentation

of DIC was achieved by collisionally activated dissociation (CAD) with nitrogen gas. The collision gas pressure was fixed at 5.0 psi for MRM quantification. The optimized collision energy was found to be 19.81 V and collision cell exit potential was 5.0 V. Dwell time of 200 ms was used. The precursor ion (Q1) with 296.00 Da and product ion (Q3) with 214.00 Da were selected for selective determination and quantification of DIC.

6.1.6.1.1. Preparation of stock solution and calibration standards

An accurately weighed quantity of DIC (100 mg) was dissolved in 100 mL of acetonitrile to obtain the standard stock solution having concentration of 1000 µg/mL. One mL of this standard stock solution was transferred into 10 mL volumetric flask, 1 mL of rabbit plasma was added into the same and volume was made up to mark with acetonitrile. The flask was vortex mixed for 60 s followed by centrifugation at 4000 rpm for 10 min. The supernatant was suitably diluted with mobile phase to obtain the standard solutions of 0.1, 1, 10, 50, 100, 500 and 1000 ng/mL, and calibration curve was plotted for area vs. concentration. Similarly, validation standards were also prepared at 50, 100 and 500 ng/mL. The retention time of DIC was found to be 5.78±0.01 min. The precision of the method was found to be less than 2% RSD and % recovery for extracted plasma samples was greater than 95%. The LC-MS chromatogram of the lowest selected concentration i.e. 0.1 ng/mL is depicted in Figure 6.1 whereas that of highest selected concentration i.e. 1000 ng/mL is depicted in Figure 6.2. Figure 6.3 displays the calibration plot of DIC (0.1-1000 ng/mL) with its regression equation and correlation coefficient.

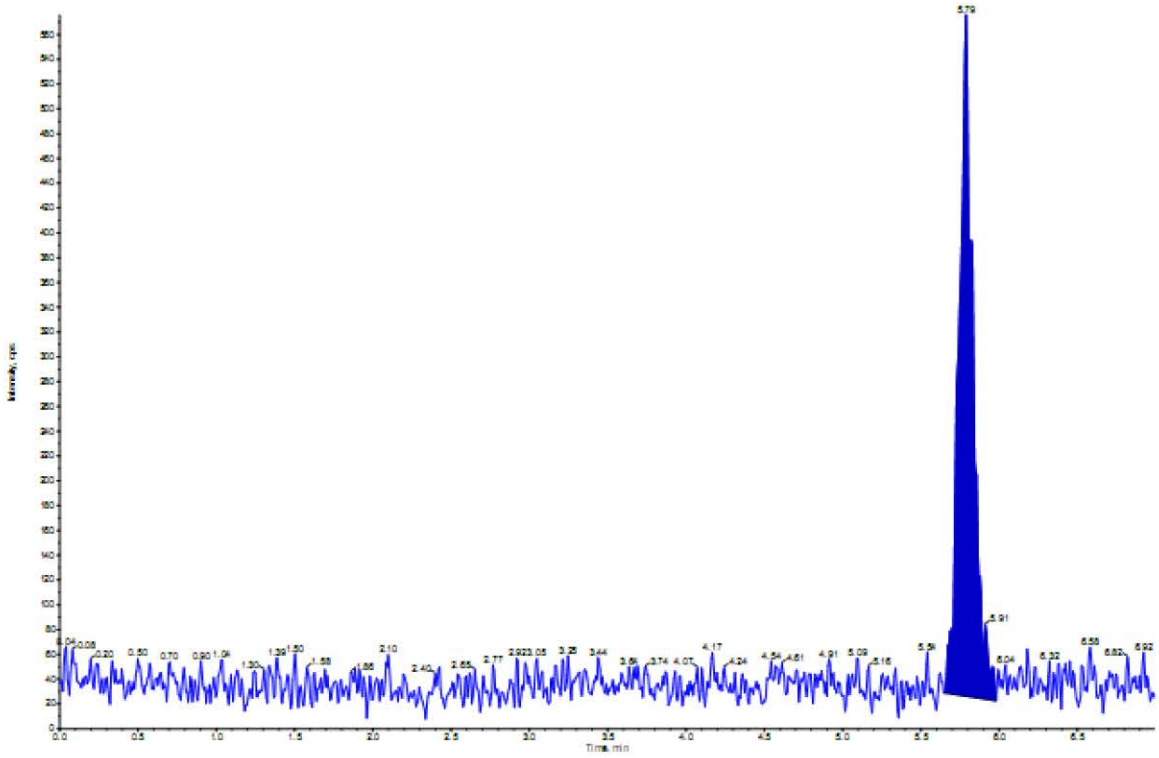

Fig. 6.1 LC-MS chromatogram of 0.1 ng/mL DIC standard solution

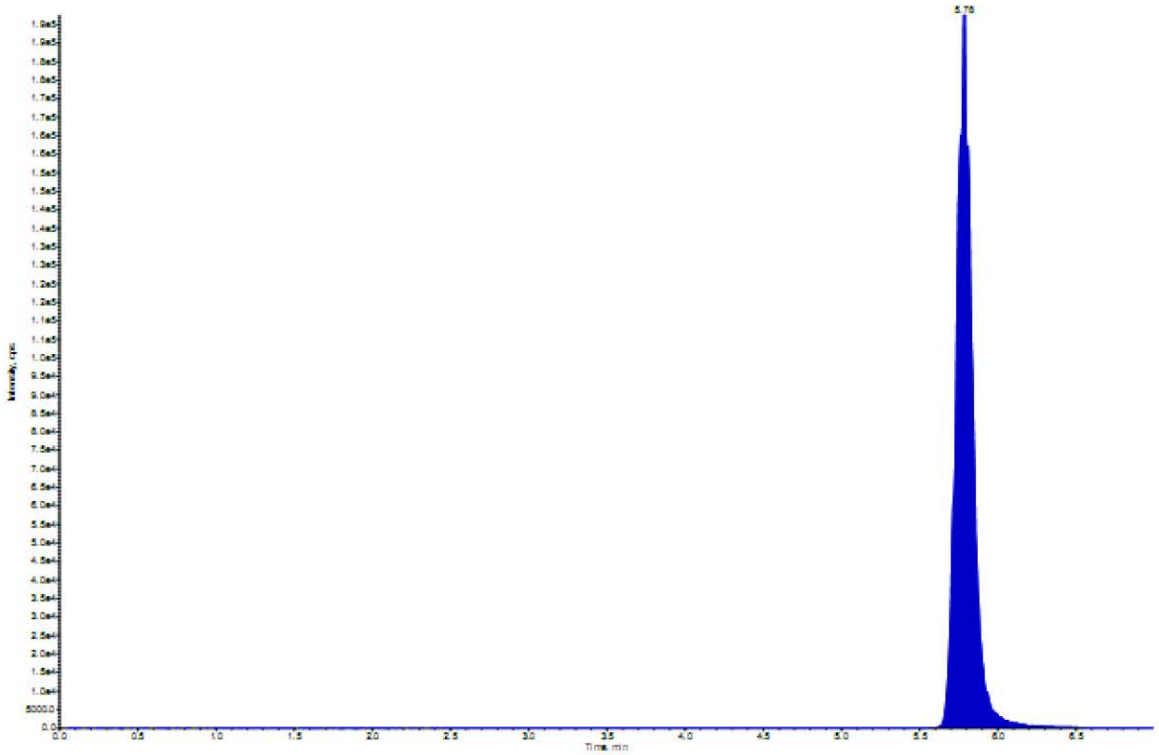

Fig. 6.2 LC-MS chromatogram of 1000 ng/mL DIC standard solution

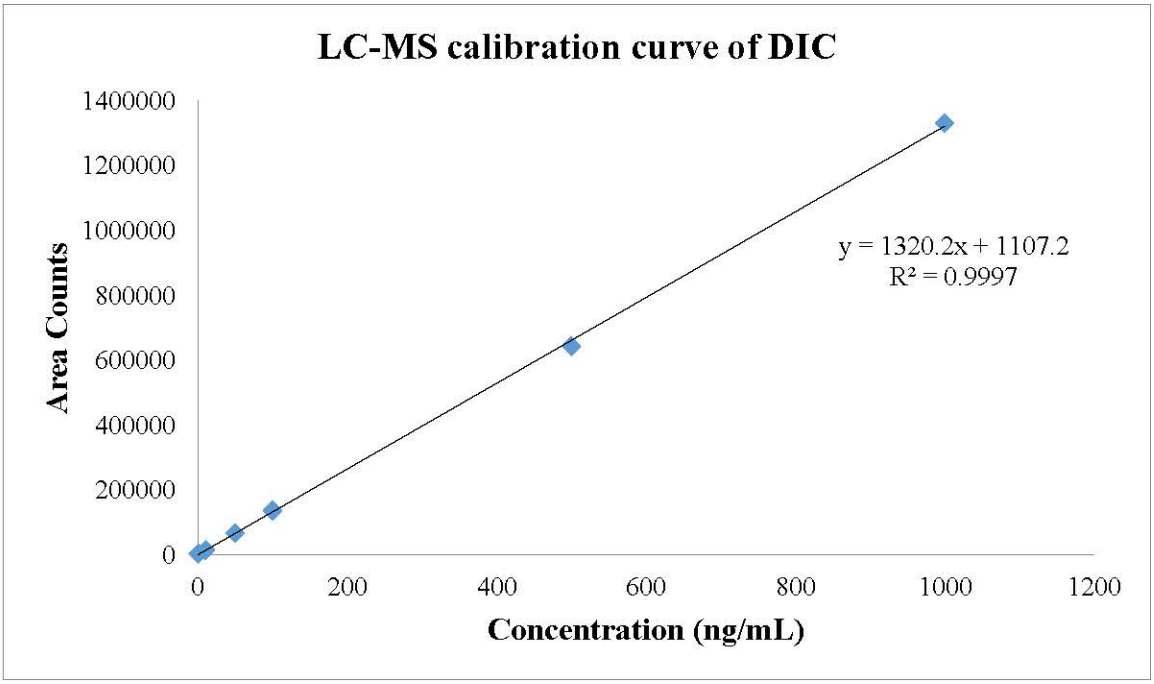

Fig. 6.3 LC-MS calibration curve of DIC (0.1-1000 ng/mL)

6.1.6.2. Animal grouping and dosing procedure

In vivo pharmacokinetic study was performed with two-way, two treatment, parallel design in adult healthy NZW rabbits of either sex aged between 8-12 months and weight of 2-3 kg (7). The animals were supplied with pelleted standard lad diet and normal tap water *ad libitum* and lab condition was maintained at 12/12 h light and dark cycle at 65 ± 10% RH. All the experimental procedures carried out in this study were in accordance with the CPCSEA guidelines, Dept of animal welfare, Govt of India. Total 6 rabbits were randomly divided into two groups of 3 animals each and the study was performed in a parallel way as mentioned below Table 6.1. The animals were fasted 12 h before initiating the study.

Table 6.1 Animal grouping and dosing treatment of DIC pharmacokinetic study

Group	No. of animals	Administered samples
Group I	3	Conventional IR formulation (Reference) (Dose (7): 25 mg, once P.O.)
Group II	3	DIC PR PCT formulation (Test) (Dose: 25 mg, once P.O.)

Following oral administration, in group I, blood samples (0.5 ml) were withdrawn from the marginal ear vein into heparinized collection tubes, at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 12 h of time intervals; whereas in group II, blood samples were withdrawn at 0, 4, 4.25, 4.5, 4.75, 5, 5.5, 6, 6.5, 7, 8, 10, 12 and 16 h of time intervals. The samples were immediately centrifuged at 4000 rpm for 10 min at 4ºC temperature and plasma was extracted out which was stored at -20 °C until analyzed. The protein precipitation was carried out using chilled acetonitrile with vortex mixing followed by centrifugation at 4000 rpm for 10 min. The supernatant was suitably diluted with mobile phase and analyzed for drug content using developed LC-MS method to determine the pharmacokinetic parameters viz. peak concentration (C_{max}), Area under the curve (AUC_{0-t}, $AUC_{0-\infty}$), time to achieve peak concentration (T_{max}), elimination half life ($T_{1/2}$), absorption rate constant (Ka), elimination rate constant (Kel) etc.

6.2. Results and discussion

6.2.1. Evaluation of PCTs

Different PRS PCTs with 5-16% weight gain were prepared and characterized for *in vitro* release testing. Out of them, PCTs with 12.5% weight gain was found to be satisfactory for obtaining 4-6 h of lag time which was therefore taken as an optimized formula. The coating thickness of an optimized PRS PCT was determined using SEM analysis which was found to be around 170-190 µm as shown in Figure 6.4. Further, the SEM images were also taken before and after drug release study to examine the tablet surface characteristics. As shown in Figure 6.5, the images displayed that the tablet surfaces after dissolution testing was veritably rough in comparison to as such tablet surfaces. This was due to the removal of solid particles upon exposure to the aqueous medium which would thereafter allowed penetration of dissolution medium to the swellable core tablet and ultimately ruptured the rigid outer coat providing burst drug release. Subsequently, other core tablets were also coated using same optimized formulation and process parameters.

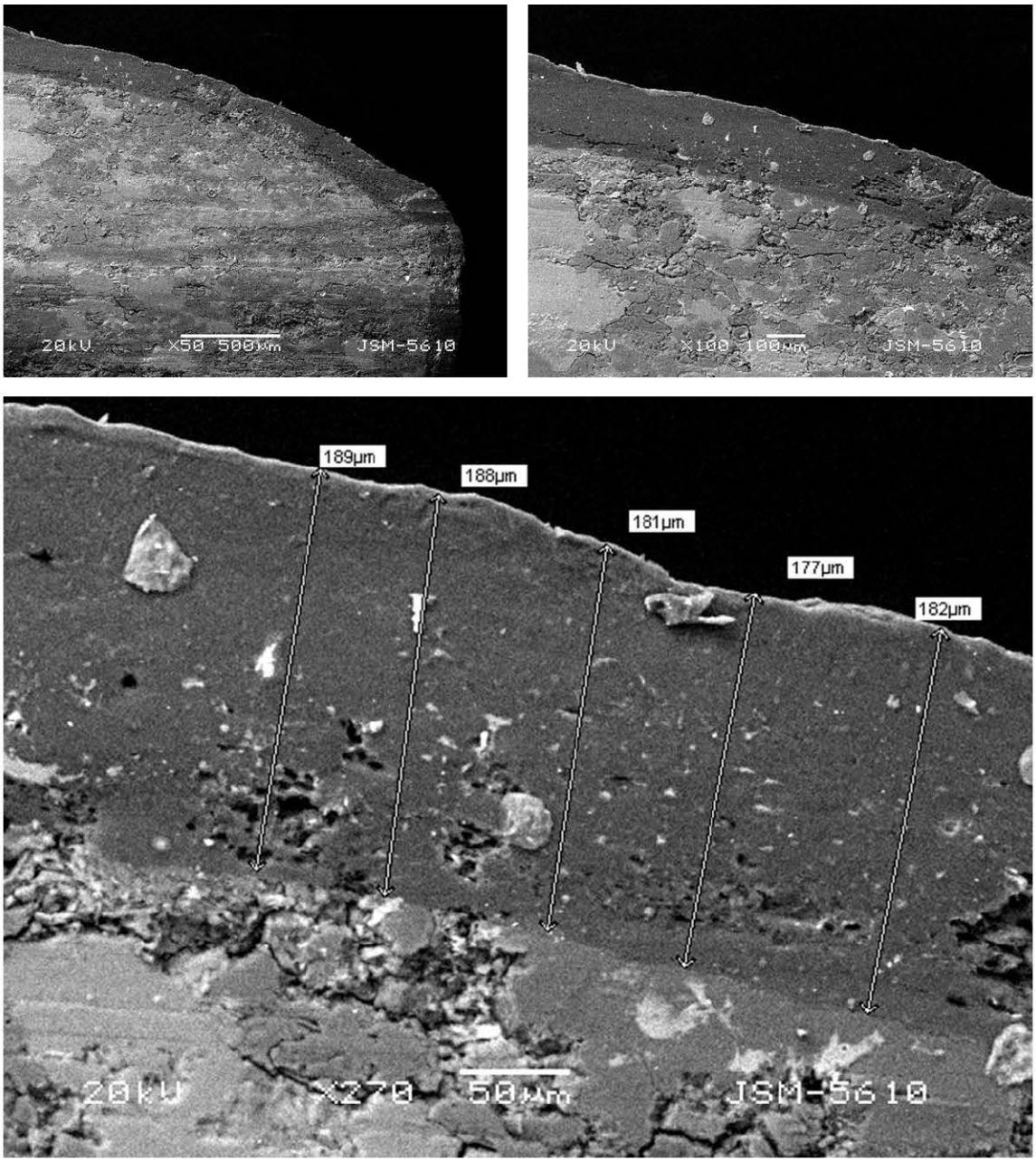

Fig. 6.4 SEM images of coating thickness analysis of optimized PRS PCT

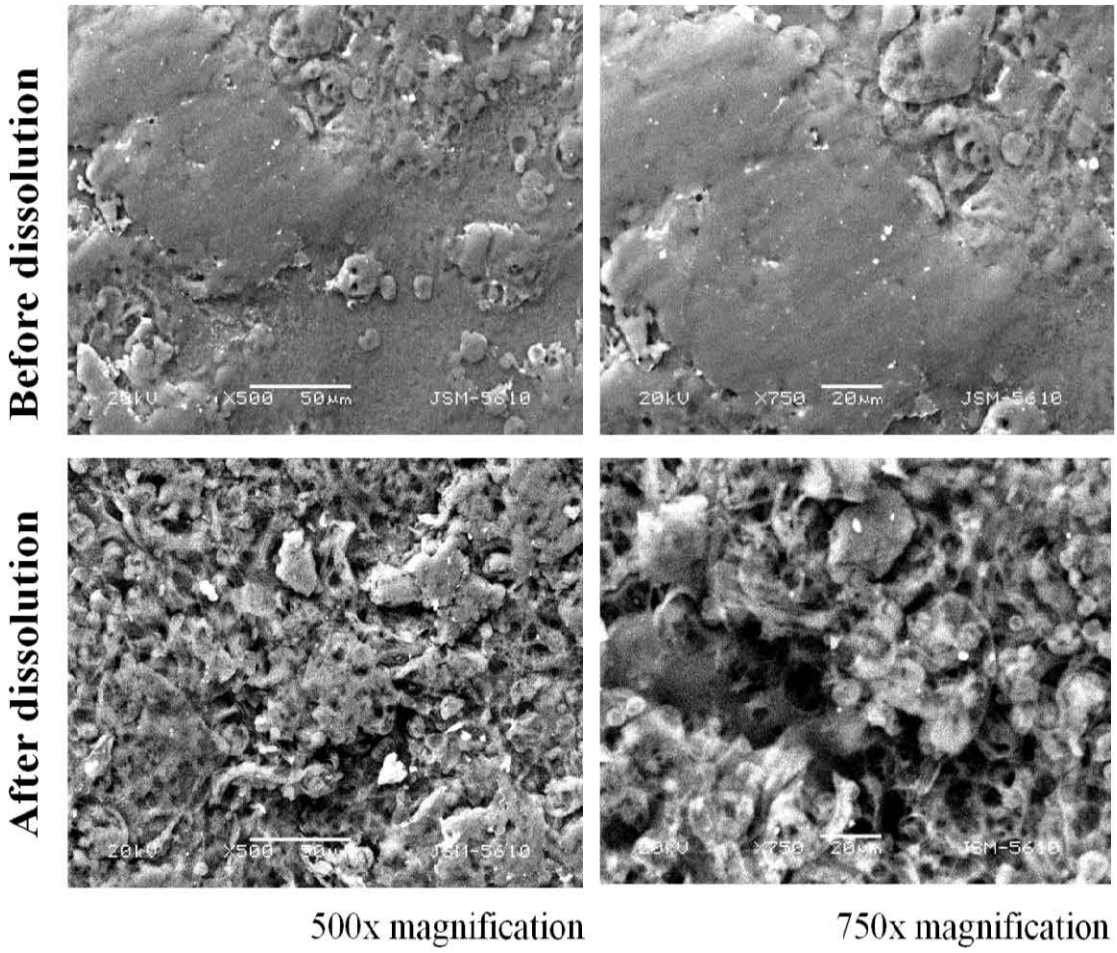

Fig. 6.5 SEM images of optimized PRS PCT before and after drug release study

All PCTs exhibited satisfactory appearance, 8-12 kp hardness and 4.1-4.4 mm thickness. The assay and weight variation results were found to be within ±5% limits for all six PCTs. The lag time results of *in vitro* release testing are displayed in Figure 6.6. Here, the lag time of PRS, MPR, DIC and LOR PCTs were found to be within 4-6 h whereas those of DIL and NIF PCTs were not completely within the desired specification range. Hence, unlike CCT formulation, PCT formulation was not found to be a platform technology. It was anticipated that the higher lag time of DIL PCTs might be due to the higher binding property of API whereas that of NIF PCTs would be due to the COP (employed to prepare ASD) which itself acts as binder. Similar effects were also observed with the CCT formulation i.e. slightly higher lag time with DIL and NIF CCTs in comparison to other CCTs, however the effects were relatively lesser and their lag times were strictly confined to 4-6 h of specification range. Moreover, it should be noted that the variability in lag time of PCT formulations was found to be relatively higher than that of the CCT formulations (Table 6.2); which is also a downside of PCT

formulation. However, after completion of lag time, the burst release profile of each individual PCT was found similar to respective CCTs i.e. >85% within 15 min for PRS, MPR, DIC, DIL and LOR PCTs and >85% within 30 min for NIF PCTs. Furthermore, PCT formulations were found to be more sensitive to hydro-alcoholic medium. The results of 5% v/v alcohol in 0.1 N HCl for first 2 h followed by pH 6.8 phosphate buffer are depicted in Figure 6.7. Here, mere 5% alcohol concentration had reduced the lag time of all PCTs below lower specification limit (i.e. 4 h). Hence, the studies with higher alcohol concentration were not carried out. Further, the results of curing study exhibited negligible effect of curing on either of appearance, hardness, assay of drug release as shown in Table 6.3. Thus, it can be construed that the stated post-drying conditions were sufficient for adequate curing process.

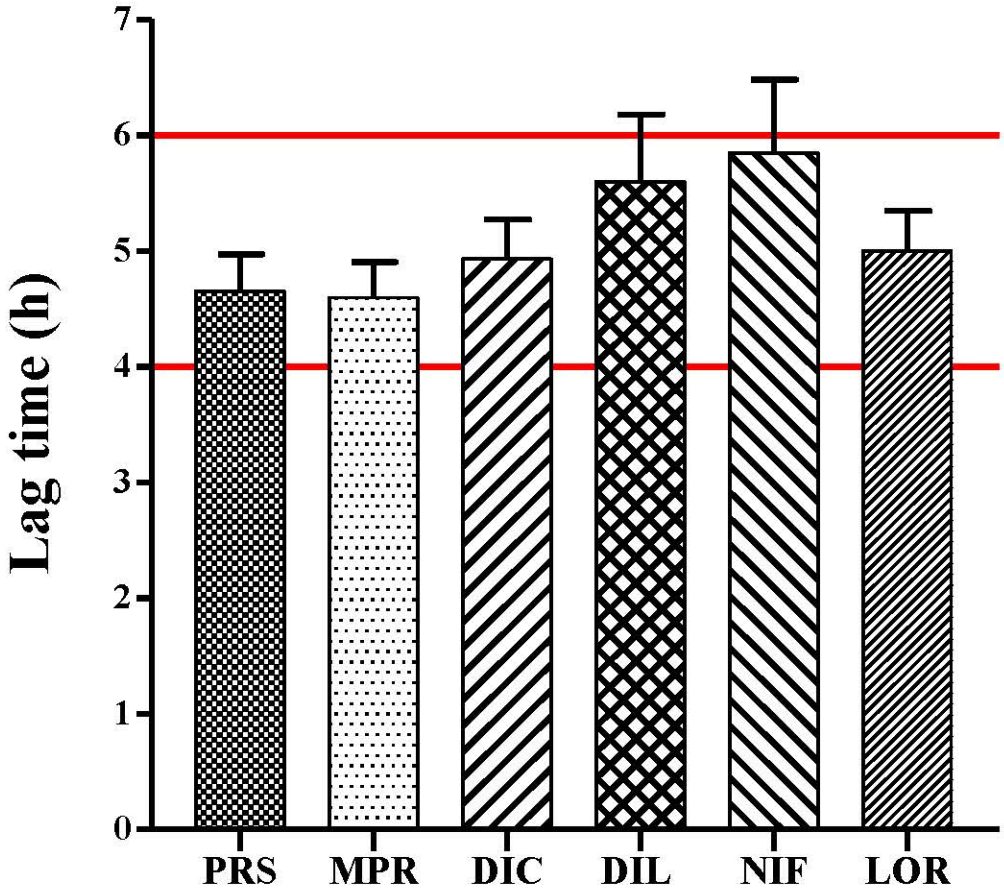

Fig. 6.6 The lag time results of different PCTs obtained using 500 mL of 0.1 N HCl for first 2 h followed by pH 6.8 phosphate buffer; basket apparatus; 100 rpm; 37.0±0.5°C temperature. [In case of NIF and LOR, medium volume 900 mL]

Table 6.2 % RSD (n=6) of lag times for developed PCT and CCT formulations obtained using 0.1 N HCl for first 2 h followed by pH 6.8 phosphate buffer; basket apparatus; 100 rpm; 37.0±0.5°C temperature.

Drug	% RSD of lag time (n=6)	
	PCT formulation	CCT formulation
PRS	6.8	3.3
MPR	6.7	3.7
DIC	6.9	3.9
DIL	10.4	4.8
NIF	10.9	5.4
LOR	6.8	3.9

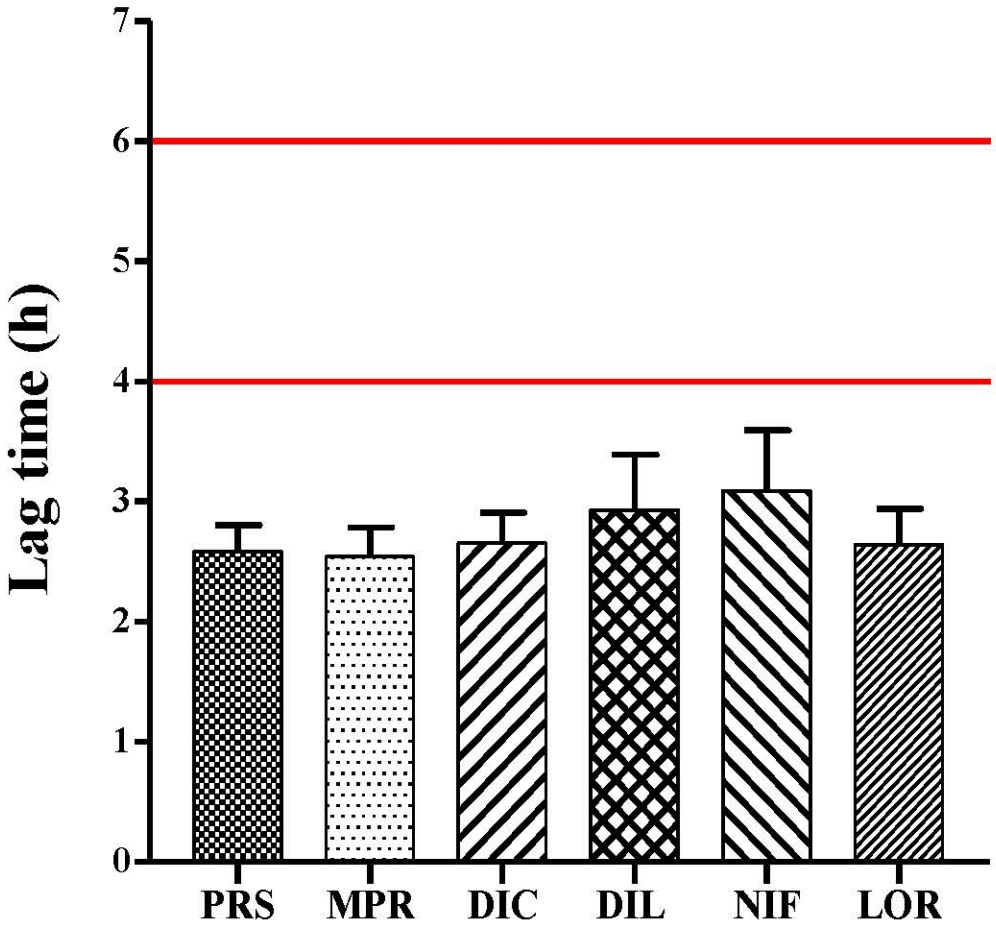

Fig. 6.7 The lag time results of different PCTs obtained using 500 mL of 5% v/v alcohol in 0.1 N HCl for first 2 h followed by pH 6.8 phosphate buffer; basket apparatus; 100 rpm; 37.0±0.5°C temperature. [In case of NIF and LOR, medium volume 900 mL]

Table 6.3 Results of curing study for all six PCTs

PCT	Effect of curing	Hardness	Assay	Lag time*	Burst release
PRS	Before curing	8-12 kp	99.7 %	4.65±0.32 h	>85% in 15 min
	After curing	No change	99.4 %	4.74±0.34 h	>85% in 15 min
MPR	Before curing	8-12 kp	99.7 %	4.60±0.31 h	>85% in 15 min
	After curing	No change	99.8 %	4.65±0.36 h	>85% in 15 min
DIC	Before curing	8-12 kp	100.4 %	4.93±0.34 h	>85% in 15 min
	After curing	No change	100.1 %	4.99±0.36 h	>85% in 15 min
DIL	Before curing	8-12 kp	99.6 %	5.60±0.58 h	>85% in 15 min
	After curing	No change	99.3 %	5.58±0.61 h	>85% in 15 min
NIF	Before curing	8-12 kp	99.4 %	5.85±0.64 h	>85% in 30 min
	After curing	No change	99.1 %	5.85±0.63 h	>85% in 30 min
LOR	Before curing	8-12 kp	99.5 %	5.00±0.34 h	>85% in 15 min
	After curing	No change	99.2 %	4.96±0.29 h	>85% in 15 min

*mean±SD; n=6

6.2.2. Packaging and stability

The results of stability study exhibited negligible change for either of appearance, hardness, assay, or drug release under stated storage conditions. Since all results were found within their respective specification limits (Table 6.4), the PCTs were found to be stable under the selected packaging material and stated storage conditions.

Table 6.4 Results of stability study for all six PCTs

PCT	Storage condition	Hardness	Assay	Lag time*	Burst release
PRS	Initial	8-12 kp	99.7 %	4.65±0.32 h	>85% in 15 min
	25±2°C/60±5% RH (3M)	No change	99.4 %	4.69±0.35 h	>85% in 15 min
	40±2°C/75±5% RH (3M)	No change	99.2 %	4.82±0.33 h	>85% in 15 min
MPR	Initial	8-12 kp	99.7 %	4.60±0.31 h	>85% in 15 min
	25±2°C/60±5% RH (3M)	No change	99.3 %	4.50±0.36 h	>85% in 15 min
	40±2°C/75±5% RH (3M)	No change	99.5 %	4.67±0.35 h	>85% in 15 min
DIC	Initial	8-12 kp	100.4 %	4.93±0.34 h	>85% in 15 min
	25±2°C/60±5% RH (3M)	No change	99.9 %	5.01±0.35 h	>85% in 15 min
	40±2°C/75±5% RH (3M)	No change	99.6 %	4.90±0.35 h	>85% in 15 min
DIL	Initial	8-12 kp	99.6 %	5.60±0.58 h	>85% in 15 min
	25±2°C/60±5% RH (3M)	No change	99.4 %	5.63±0.57 h	>85% in 15 min
	40±2°C/75±5% RH (3M)	No change	99.3 %	5.81±0.66 h	>85% in 15 min
NIF	Initial	8-12 kp	99.4 %	5.85±0.64 h	>85% in 30 min
	25±2°C/60±5% RH (3M)	No change	99.2 %	5.76±0.57 h	>85% in 30 min
	40±2°C/75±5% RH (3M)	No change	99.0 %	5.83±0.64 h	>85% in 30 min
LOR	Initial	8-12 kp	99.5 %	5.00±0.34 h	>85% in 15 min
	25±2°C/60±5% RH (3M)	No change	99.4 %	4.89±0.40 h	>85% in 15 min
	40±2°C/75±5% RH (3M)	No change	99.1 %	5.01±0.42 h	>85% in 15 min

*mean±SD; n=6

6.2.3. *In vivo* pharmacokinetic study of DIC PCT formulation

After ensuring desired results with *in vitro* release study, DIC PCT was selected as a model formulation and evaluated for *in vivo* pharmacokinetic study using NZW rabbits. The pharmacokinetics of developed PR PCT formulation (i.e. test) was compared to equivalent dose (25 mg) of the conventional IR formulation viz. uncoated DIC core tablet (i.e. reference). The plasma concentration vs. time profile of both formulations following oral administration is depicted in Figure 6.8. The data are represented in form of mean±SD (n=3).

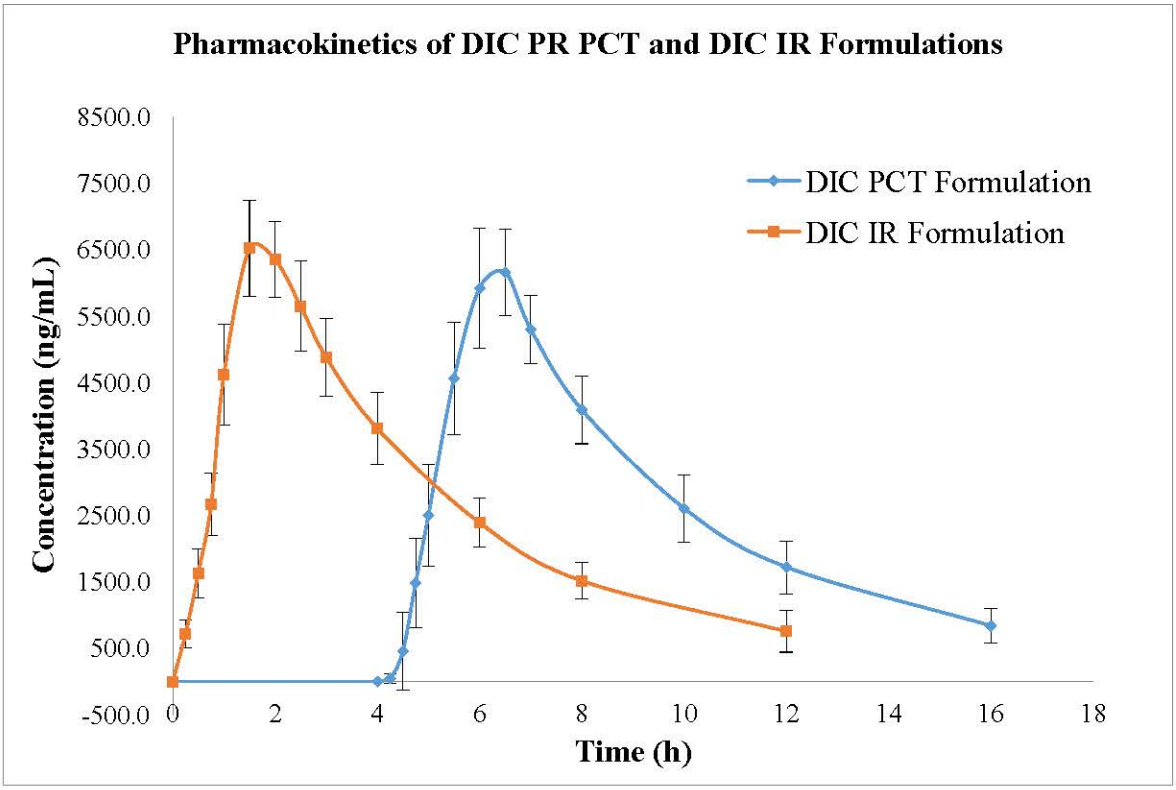

Fig. 6.8 Plasma concentration vs. time profile after oral administration of DIC PCT formulation and DIC IR formulation in NZW rabbits (mean±SD; n=3)

The pharmacokinetic analysis was carried out using PK Solver add-in software (9) with Microsoft Excel 2007 (Microsoft Corporation, USA) considering non-compartmental analysis with extravascular administration. Table 6.5 depicts various pharmacokinetic parameters of both formulations obtained using linear trapezoid method; which demonstrates that except T_{lag} and so as T_{max}, all other parameters of DIC PR formulation were almost similar to those of DIC IR formulation. As per our expectations, the PCT formulation exhibited 4.25±0.25 h of lag time and hence its T_{max} was delayed by 4-5 h as shown in Table 6.5. Overall data revealed that the developed

PR PCT restricted the drug release for predetermined time and subsequently provided burst drug release similar to conventional IR formulation. Typically, close proximity of Ka as well as Kel between PR and IR formulations ruled out the possibility of differential absorption phenomenon that might have occurred due to the change in absorption sites. This was attributed to the very high Log P value of DIC (i.e. 4.4) which is even higher than metoprolol, a reference standard used for evaluating permeability of other drugs (10).

Table 6.5 Summary of pharmacokinetic parameters of DIC IR formulation and DIC PR formulation

PK parameter	DIC IR formulation*	DIC PR formulation*
C_{max}	6574.5±644.8 ng/mL	6481.6±487.1 ng/mL
AUC_{0-t}	32462.7±4928.1 ng/mL.h	32751.0±4339.6 ng/mL.h
$AUC_{0-\infty}$	36115.0±6783.9 ng/mL.h	37254.8±6113.6 ng/mL.h
Ka	0.48±0.02 h^{-1}	0.44±0.03 h^{-1}
Half life ($T_{1/2}$)	3.25±0.39 h	3.64±0.45 h
Kel	0.22±0.03 h^{-1}	0.19±0.02 h^{-1}
T_{lag}	-	4.25±0.25 h
T_{max}	1.67±0.29 h	6.17±0.29 h

*mean±SD (n=3)

Further, the bioequivalence (BE) of DIC PR formulation (test) was evaluated with respect to DIC IR formulation (reference) by calculating geometric mean ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ at 90% confidence interval (CI) (11). As shown in Table 6.6, all three results were found to be within 0.80-1.25 and hence the test formulation can be considered as bioequivalent to the conventional IR formulation with only difference of lag time (T_{lag}) and thereby T_{max}.

Table 6.6 BE parameters of DIC PR formulation with respect to DIC IR formulation at 90% CI

PK parameter	Geometric Mean Ratio	90% CI Lower	90% CI Higher
C_{max}	0.99	0.92	1.06
AUC_{0-t}	1.01	0.88	1.14
$AUC_{0-\infty}$	1.03	0.87	1.19

6.3. Conclusion

As stated in the beginning, development of PR PCT was carried out by keeping the outer coat ingredients as similar as possible to the developed CCT formulation and employing same core tablet composition. Thus, in order to achieve the time-controlled lag time, outer coat was fabricated using EC N10 and HPMC E5 as functional polymers; the concentration of which were kept almost similar to the developed CCT formulation. Since the process changed from compression coating to pan coating, the lubricant and glidant were replaced with the plasticizer (DBS) in order to form proper and uniform coating. The optimization was performed with PRS tablets by applying different coating weights and subsequently analyzed for drug release testing. Consequently, other core tablets were also coated using optimized formulation and process parameters, and characterized for *in vitro* release testing. The results revealed that the lag times of PRS, MPR, DIC and LOR PCTs were found to be within the desired range of 4-6 h whereas those of DIL and NIF PCTs were not. Thus, unlike CCT formulation, PCT formulation cannot be conferred as a platform technology. Further, the variability in lag time of PCT formulation was found to be higher than that of CCT formulation (Table 6.2). Moreover, the PCT formulations were found to be relatively more sensitive to hydro-alcoholic medium as compared to the CCT formulations. However, after ensuring desired results with *in vitro*, DIC PCT was selected as a model formulation and evaluated for *in vivo* pharmacokinetic study using NZW rabbits in order to compare the pharmacokinetics of PR formulation with that of the IR formulation. The results revealed that the developed PCT exhibited about 4-5 h of *in vivo* lag time which thereby delayed its T_{max} accordingly. Apart from that, all other pharmacokinetic parameters (viz. C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, Ka and Kel) of PR formulation were found to be in accordance with those of the IR formulation (Table 6.5). Moreover, the BE of PR PCT (test) was also assessed with the IR formulation (reference) in terms of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. As shown in Table 6.6, the geometric mean ratios (test/reference) of all three parameters were found to be within 0.80-1.25 limit at 90% CI. Thus, the developed PR PCT was found to be bioequivalent to the conventional IR formulation with only difference of lag time and thereby T_{max}.

In nutshell, like CCT formulation, the developed PCT formulation also exhibited distinct lag time followed by burst release profile, however, the lag times of two (i.e. DIL and NIF PCTs) out of six selected PCTs were not strictly confined to 4-6 h of desired specification range. Hence, unlike CCT formulation, PCT formulation was not emerged as a platform technology which means that development of PR formulation using pan coating technology may stipulate separate optimization of formulation/process parameters according to individual drug requirement. Besides, relatively higher variability in lag time with PCT formulation also makes it inferior in comparison to the CCT formulation. Nevertheless, the ease of feasibility and availability of rather simple, more common and cost-effective pan coaters are the strong points to opt for the same.

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