9. Comparative study of different nanocarriers as potential carrier for oral delivery of cromolyn sodium

9.1 Encapsulation efficiency

The EE (%) of prepared different optimized nanocarriers (i.e., CS-PNs, CS-SLNs, CS-PLHNs and CS-PCCSNs) was determined indirectly by following the same estimation protocol and same instrument as mentioned in the ***sub-section 5.1.4.2.*** The results of EE (%) of different nanocarriers are described in the Table 9.1.

Table 9.1 Comparative EE (%) of different optimized nanocarrier systems

CS-SLNs	CS-PNs	CS-PLHNs	CS-PCCSNs
24.84 $\pm$ 0.56 %	34.26 $\pm$ 1.3 %*^{a}	57.8 $\pm$ 1.32 %*^{a,b}	77.34 $\pm$ 1.1 %*^{a,b,c}

*All values reported are mean ± S.D; n=3. *Significant at p<0.05; a vs CS-SLNs, b vs CS-PNs and c vs CS-PLHNs; One-way ANOVA followed by Tukey's multiple comparison test*

The EE of different optimized nanocarrier systems varied considerably. The EE was obtained in the range of 24.84 % to 77.34 % for different nanocarrier systems. The lower EE was obtained for the CS-SLNs and CS-PNs as compared to CS-PLHNs and CS-PCCSNs as indicated in Table 9.1. The probable reason for lower EE could be the hydrophilic nature of the CS, which would have prevented the retention of CS inside the lipophilic carrier matrix by imparting its faster partition into the external aqueous phase during the formulation [29, 30]. Alternatively, higher EE was obtained for the CS-PLHNs and CS-PCCSNs, which might be due to the core-shell architecture. They would have allowed the encapsulation of CS inside the polymeric core by forming the molecular barrier surrounding the same, during the preparation. The formed molecular barrier impedes the faster diffusion of CS into an external aqueous phase and hence, potentially increases drug encapsulation [23, 34-36]. Additionally, the highest

encapsulation for CS-PCCSNs might be due to the cationic chitosan shell, which would have further enhanced the encapsulation of anionic CS [45]. Hence, CS-PCCSNs showed superiority for encapsulating the CS inside the nanocarriers.

9.2 *In-vitro* drug release

The *in-vitro* drug release study of optimized nanocarriers (i.e., CS-PNs, CS-SLNs, CS-PLHNs and CS-PCCSNs) was performed using modified dialysis bag diffusion technique in phosphate buffer pH 7.4. The same protocol was followed for *in-vitro* drug release as mentioned in the ***sub-section 5.1.4.5.*** *In-vitro* drug release profile of different nanocarrier systems is depicted in Figure 9.1. The results of *in-vitro* drug release of different nanocarrier systems in phosphate buffer pH 7.4 are shown in the Table 9.2. At the end of 24 hr, CS-SLNs and CS-PNs exhibited ~95 % and ~89 % drug release, respectively. The faster drug release compared to core-shell nanostructures might be due to the hydrophobic nature of carrier and hydrophilic nature of CS [39, 49]. Oppositely, the core-shell nanostructures, CS-PLHNs and CS-PCCSNs exhibited slower release and showed ~73 % and ~63 % drug release at the end of 24 hr. Moreover, CS-PLHNs and CS-PCCSNs also exhibited drug release up to 48 hr might be ascribed to the molecular barrier (i.e., lipid/polymer), which would have restricted the penetration of release medium and curtailed the faster immobilization of the CS from the polymeric core to the external release media and thereby, extended the release [36]. Further, slowest release for the CS-PCCSNs was also might be due to the ionic interaction between the drug and carrier [45]. Hence, CS-PCCSNs showed slowest release amongst all the developed nanocarrier systems and extended release up to 48 hr.

Table 9.2 Comparative *in-vitro* drug release data of the optimized nanocarriers in phosphate buffer pH 7.4

Time (hr)	Cumulative % drug release			
	CS-SLNs	**CS-PNs**	**CS-PLHNs**	**CS-PCCSNs**
0	0	0	0	0
1	24.13 ± 2.53	19.76 ± 1.31	16.99 ± 0.31	14.50 ± 0.62
2	27.76 ± 0.87	24.02 ± 0.84	20.06 ± 0.32	18.85 ± 0.22
3	30.87 ± 0.84	27.91 ± 0.55	22.19 ± 0.33	20.44 ± 0.50
4	36.89 ± 1.39	32.63 ± 0.69	25.93 ± 0.52	24.84 ± 1.39
5	41.80 ± 0.55	35.69 ± 0.84	29.67 ± 1.25	26.62 ± 1.69
6	47.26 ± 1.12	40.13 ± 1.54	31.52 ± 0.55	28.93 ± 0.97
7	51.06 ± 0.84	45.69 ± 2.46	34.30 ± 0.73	32.17 ± 1.69
8	58.84 ± 0.89	50.41 ± 1.67	36.43 ± 1.28	34.95 ± 1.39
10	66.15 ± 1.57	56.52 ± 1.15	40.04 ± 1.05	37.91 ± 2.00
12	73.10 ± 1.15	64.86 ± 3.24	45.69 ± 2.00	42.73 ± 3.15
18	88.75 ± 2.37	71.80 ± 1.25	61.71 ± 2.12	54.58 ± 5.00
24	95.97 ± 1.38	89.02 ± 1.78*^{a}	73.84 ± 1.69*^{a,b}	63.47 ± 1.38*^{a,b,c}
36	-	-	91.89 ± 0.97	83.10 ± 3.21
48	-	-	92.91 ± 1.21	91.80 ± 1.54

*All values reported are mean ± SD, (n=3); *Significant at p<0.05; a vs CS-SLNs, b vs CS-PNs and c vs CS-PLHNs at the end of 24 hr; One-way ANOVA followed by Tukey's multiple comparison test*

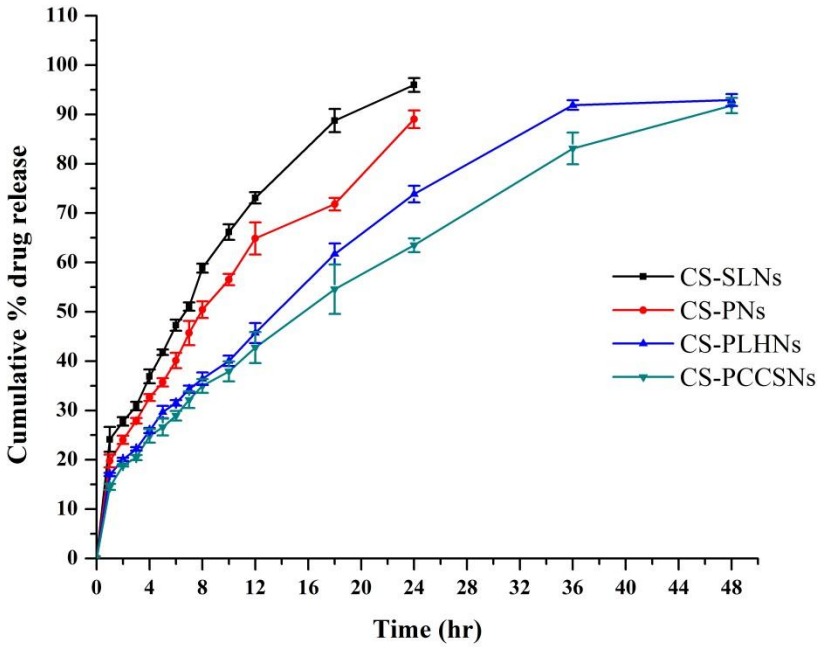

Figure 9.1 Comparative *in-vitro* drug release profiles of different optimized nanocarriers in phosphate buffer pH 7.4 (vertical bar represents ± S.D; n=3)

9.3 Accelerated and real time storage stability study

The stability of optimized nanocarrier systems (i.e., CS-PNs, CS-SLNs, CS-PLHNs and CS-PCCSNs) was assessed over a period of 6 month at room temperature (25 ± 2 ^{o}C), refrigerated condition (4 ± 1 ^{o}C), and accelerated condition (40 ± 2 ^{o}C/75 ± 5 % RH) as per ICH guideline by following the same protocol as mentioned in the ***sub-section 5.1.4.6.*** As indicated by the results of stability study, CS-PNs and CS-PCCSNs showed highest stability at all the different environmental conditions as compared to the CS-SLNs and CS-PLHNs stored for 6 months. CS-SLNs and CS-PLHNs exhibited stability upon storage at refrigerated condition (4 ± 1 ^{o}C), whereas the significant change in the physicochemical properties was observed upon storage at accelerated condition (40 ± 2 ^{o}C/75 ± 5 % RH). This might be due to the substantial aggregation of lipidic material of the nanocarriers, which would have increased the

particle size along with PDI and expelled the drug molecules from the nanocarriers by breaking the native structure [23, 49]. Alternatively, CS-PNs and CS-PCCSNs showed insignificant change in their physicochemical properties after storage of 6 month at all the different environmental conditions, indicating the highest stability of CS-PNs and CS-PCCSNs amongst all the developed nanocarrier systems.

9.4 *Ex-vivo* intestinal permeation study

The permeation potential of different optimized nanocarrier systems (i.e., CS-PNs, CS-SLNs, CS-PLHNs and CS-PCCSNs) across the rat intestine was assessed by *ex-vivo* intestinal permeation study using non-everted gut sac technique by following the same method as described in ***sub-section 5.1.4.7.2.*** Comparative *ex-vivo* intestinal permeation potential of different nanocarriers is shown in Figure 9.2. The comparative results of *ex-vivo* intestinal permeation study across the rat intestine are shown in the Table 9.3.

Table 9.3 Comparative *ex-vivo* permeation data of the CS solution and different optimized CS encapsulated nanocarriers across rat intestine

Time (min)	Cumulative % drug permeated				
	CS solution	**CS-SLNs**	**CS-PNs**	**CS-PLHNs**	**CS-PCCSNs**
0	0	0	0	0	0
15	1.21 ± 0.039	2.17 ± 0.72	3.33 ± 0.125	4.28 ± 0.08	5.03 ± 0.46
30	2.40 ± 0.021	3.10 ± 0.75	4.47 ± 0.318	9.34 ± 1.02	12.58 ± 1.25
45	3.33 ± 0.053	5.25 ± 0.58	7.34 ± 0.733	12.08 ± 1.08	24.95 ± 1.84
60	4.39 ± 0.052	7.80 ± 1.62	10.69 ± 0.747	20.09 ± 2.23	36.32 ± 2.28
90	4.98 ± 0.083	12.28 ± 2.33	19.13 ± 1.237	29.15 ± 1.83	47.87 ± 1.89
120	5.71 ± 0.539	18.63 ± 1.91	25.84 ± 1.255	36.47 ± 1.94	53.19 ± 1.64
180	6.78 ± 0.504	24.86 ± 2.00	32.71 ± 1.250	44.89 ± 2.81	58.61 ± 2.42
240	7.71 ± 0.588	31.58 ± 2.53	41.28 ± 1.723	54.40 ± 2.72	64.16 ± 3.88

All values reported are mean ± SD, (n=3)

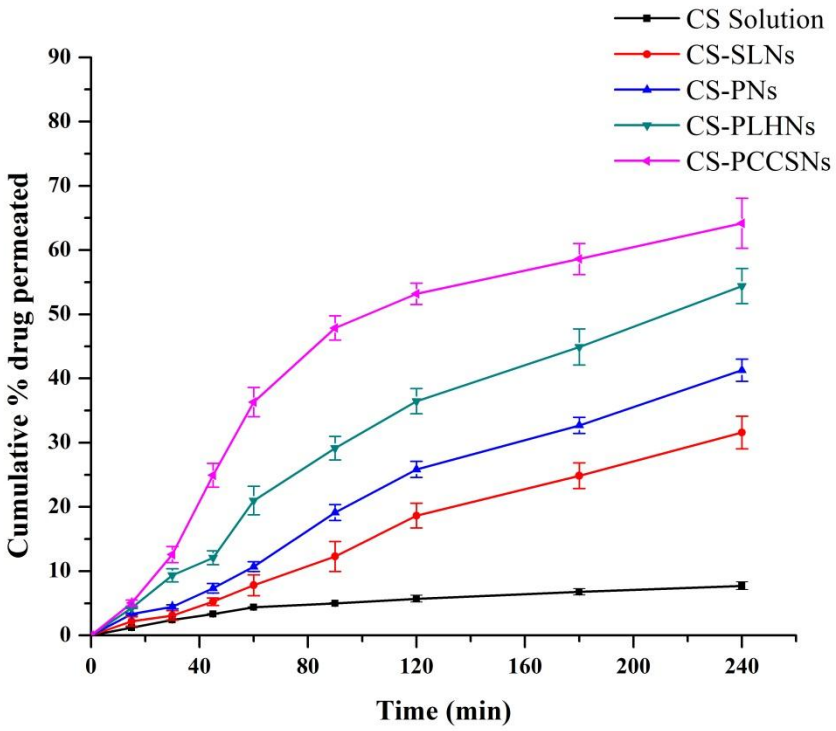

Figure 9.2 Comparative *ex-vivo* permeation studies of CS solution and different optimized CS encapsulated nanocarriers across rat intestinal membrane (vertical bars represent ± SD; n=3)

Table 9.4 Comparative apparent permeability coefficients (Papp) along with permeability enhancement ratio for CS-solution and different optimized nanocarriers across the rat intestinal tissue

Formulation	Papp × 10^{-5} (cm/s)	Permeability enhancement ratio
CS solution	0.909 ± 0.049	1
CS-SLNs	2.696 ± 0.315*^{a}	2.96 ± 0.63*^{a}
CS-PNs	3.625 ± 0.182*^{a,b}	3.98 ± 0.36*^{a,b}
CS-PLHNs	5.441 ± 0.373*^{a,b,c}	5.98 ± 0.74*^{a,b,c}
CS-PCCSNs	7.781 ± 0.413*^{a,b,c,d}	8.55 ± 0.82*^{a,b,c,d}

*All values reported are mean ± S.D; n=3. *Significant at p<0.05; a vs CS-Solution, b vs CS-SLNs, c vs CS-PNs and d vs CS-PLHNs; One-way ANOVA followed by Tukey's multiple comparison test*

The Papp for CS solution was found to be 0.909 (± 0.049) × 10^{-5} cm/s, due to poor permeation across rat intestine as a result of high hydrophilic nature [43, 45]. The different nanocarriers exhibited significant improvements (p<0.05) in the CS permeation across excised rat intestinal membrane compared to CS solution. Amongst the nanocarrier systems, the intestinal permeation decreased in the following order; CS-PCCSNs>CS-PLHNs>CS-PNs>CS-PNs, as indicated in Table 9.4. The higher permeability for the nanocarriers is likely due to their nanosize structure, which imparts larger specific surface area as well as their specific absorption mechanisms across the GIT (i.e., paracellular transport, transcellular transport and endocytosis through M cells of PP in lymphoid tissues) [106, 107]. CS-PNs exhibited significantly higher intestinal permeation as compared to CS-SLNs, which might be due to their comparatively smaller size. Whereas, CS-PLHNs and CS-PCCSNs, both showed significantly higher intestinal permeation as compared to CS-PNs and CS-SLNs due to their core-shell architecture. In case of CS-PLHNs, the outer phospholipid envelop might have improved the cellular interaction of the nanocarriers with the lipophilic biological membrane, and thereby, enhanced the intestinal permeation [23, 35, 232, 249]. Interestingly, CS-PCCSNs showed highest intestinal permeation potential as compared to other nanocarrier systems. This might be due to their smallest size as well as outer chitosan cover, which would have manipulated the intercellular tight junction between the enterocytes and improved the intestinal permeation by paracellular transport along with transcellular transport. Additionally, the enhanced residence in the intestinal tract through mucoadhesive interaction with biological membrane could be the added advantage to attain higher permeation [45, 232, 257, 258]. Hence, CS-PCCSNs exhibited superiority for enhancing the intestinal permeation of CS amongst all other nanocarriers.

9.5 *In-vivo* pharmacokinetic study

In-vivo pharmacokinetic study was performed by oral administration of CS-solution and different optimized nanocarriers (i.e., CS-PNs, CS-SLNs, CS-PLHNs and CS-PCCSNs) as described in ***sub-section 5.1.4.7.4.*** The comparative plasma drug concentration-time profile data of different nanocarriers are shown in the Table 9.5. The comparative plasma drug concentration-time profiles obtained following the single dose oral administration of the CS solution and different optimized CS encapsulated nanocarriers in rats (20 mg/kg) are depicted in Figure 9.3.

Table 9.5 Comparative plasma drug concentration time profile data of CS solution and different optimized CS encapsulated nanocarriers following single dose oral administration in rats

Time (hr)	Plasma concentration of CS (ng/ml)				
	CS solution	CS-SLNs	CS-PNs	CS-PLHNs	CS-PCCSNs
0	0	0	0	0	0
0.25	11.42 ± 1.36	6.92 ± 0.54	7.33 ± 1.12	7.70 ± 1.04	9.72 ± 1.74
0.5	50.59 ± 4.30	37.26 ± 3.97	39.79 ± 2.23	24.11 ± 2.08	40.06 ± 2.62
1	112.23 ± 5.90	69.66 ± 5.84	77.34 ± 2.04	59.55 ± 2.35	82.21 ± 4.56
2	70.08 ± 2.86	146.84 ± 4.24	165.32 ± 11.78	109.38 ± 6.83	183.16 ± 7.89
4	28.75 ± 2.10	83.55 ± 3.72	99.54 ± 8.55	268.20 ± 9.59	349.42 ± 11.21
8	ND	35.46 ± 4.20	55.97 ± 3.75	153.65 ± 7.78	259.83 ± 7.82
12	ND	7.84 ± 1.20	24.02 ± 2.16	101.29 ± 5.34	151.49 ± 3.46
24	ND	ND	7.52 ± 0.98	37.58 ± 3.45	71.87 ± 4.54
48	ND	ND	ND	7.42 ± 0.68	9.08 ± 3.68

All values reported are mean ± SEM, (n=6); ND: Not detected

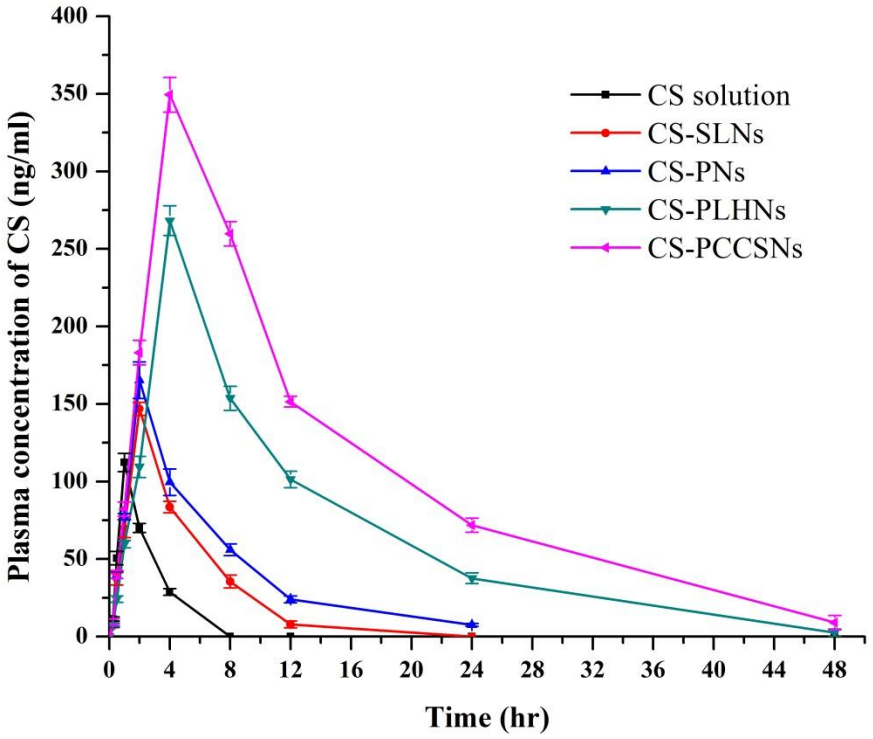

Figure 9.3 Comparative plasma drug concentration time profiles of CS solution and different optimized CS encapsulated nanocarriers following single dose oral administration in rats; Dose: 20 mg/kg (vertical bars represent ± SEM; n=6)

The different nanocarriers exhibited significant improvements (p<0.05) in the pharmacokinetic parameters of CS as compared to CS solution upon single dose oral administration of different nanocarriers in the rats. Various pharmacokinetic parameters for CS solution and different nanocarrier systems, obtained by non-compartmental analysis are summarized in Table 9.6. Amongst the nanocarrier systems, the improvement in all the pharmacokinetic parameters (C_{max}, T_{max}, $T_{1/2}$, MRT, $AUC_{0-\infty}$, Fr) increased in the following order; CS-PNs<CS-SLNs<CS-PLHNs<CS-PCCSNs, as indicated in Table 9.6. The poor pharmacokinetic parameters for the CS solution was due to its high hydrophilic nature, which resulted in the poor permeation across the intestine and thereby, oral bioavailability [43, 45].

Table 9.6 Comparative pharmacokinetic parameters of CS solution and different nanocarrier system following single dose oral administration in rats (Dose: 20 mg/kg)

Parameters	CS solution	CS-SLNs	CS-PNs	CS-PLHNs	CS-PCCSNs
C_{max} (ng.ml^{-1})	112.23 ± 5.90	146.84 ± 4.24*a	165.32 ± 11.78*a,b	268.20 ± 9.59*a,b,c	349.42 ± 11.21*a,b,c,d
T_{max} (hr)	1 (± 0)	2 (± 0)*a	2 (± 0)a	4 (± 0)*a,b,c	4 (± 0) *a,b,c
AUC_{0-24h} (ng.hr.ml^{-1})	232.16 ± 12.31	662.36 ± 19.97*a	1077.59 ± 57.42*a,b	2748.82 ± 276.61*a,b,c	4556.94 ± 320.91*a,b,c,d
$AUC_{0-\infty}$ (ng.hr.ml^{-1})	295.53 ± 17.79	694.73 ± 29.38*a	1159.41 ± 55.90*a,b	3033.81 ± 193.77*a,b,c	4978.65 ± 211.40*a,b,c,d
$T_{1/2}$ (hr)	1.52 ± 0.02	2.41 ± 0.23*a	5.97 ± 0.19*a,b	8.22 ± 0.51*a,b,c	9.74 ± 0.56*a,b,c,d
MRT (hr)	2.79 ± 0.03	4.77 ± 0.31*a	9.05 ± 0.20*a,b	13.30 ± 0.59*a,b,c	15.34 ± 0.50*a,b,c,d
Fr	1	2.86 ± 0.08*a	4.69 ± 0.51*a,b	11.89 ± 1.25*a,b,c	19.75 ± 1.74*a,b,c,d

*All values reported are mean ± SEM; n=6. *Significant at p<0.05; a vs CS solution, b vs CS-SLNs, c vs CS-PNs and d vs CS-PLHNs; One-way ANOVA followed by Tukey's multiple comparison test.*

The improvement in all the pharmacokinetic parameters with different nanocarrier system was due to their enhanced permeation thereby, absorption across GIT by virtue of their smaller size and different absorption mechanisms (i.e., paracellular transport, transcellular transport and endocytosis through M cells of PP in lymphoid tissues) [106, 107]. CS-PNs exhibited significantly improved pharmacokinetic parameters as compared to CS-SLNs due to their higher intestinal permeation potential as well as comparative smaller size and extended release potential, which would have resulted in higher oral bioavailability to that of CS-SLNs [39]. Whereas, CS-PLHNs and CS-PCCSNs, both showed significantly higher pharmacokinetic parameters as compared to CS-PNs as well as CS-SLNs due to their core-shell architecture. In case of CS-PLHNs, The significant improvement (p<0.05) in oral bioavailability of CS, achieved with CS-PLHNs might be due to their nano-sized structure and increased surface area, which would have enhanced systemic absorption of CS-PLHNs through specialized absorption mechanisms across GIT. [23, 106, 107, 236]. Additionally, phospholipid envelop over the polymeric core might have improved the bioadhesion of CS-PLHNs with the intestinal membrane and assisted in the CS-PLHNs movement as well as would have extended the *in-vivo* drug release [248, 249]. Interestingly, CS-PCCSNs showed highest oral bioavailability as compared to other nanocarrier systems, due to their smallest size as well as outer chitosan cover, which would have manipulated the intercellular tight junction between the enterocytes and improved the intestinal permeation by paracellular transport along with other absorption mechanisms [232, 258, 259]. Furthermore, the extended *in-vivo* drug release along with enhanced residence in the intestinal tract through mucoadhesive nature of the outer chitosan envelop could also be the plausible reason for attaining the higher plasma concentration [45, 232, 257, 258]. Hence, CS-PCCSNs

exhibited superiority for enhancing the oral bioavailability of CS amongst all other nanocarriers.

9. 6 Summary

After comparing the potentials of all the developed four nanocarrier systems in terms of encapsulation efficiency, *in-vitro* drug release, storage stability, *ex-vivo* intestinal permeation and *in-vivo* pharmacokinetic study, results suggested that PCCSNs have a superior potential for oral delivery of CS, compared to other developed nanocarrier systems.