TABLE 2. Pharmacokinetic Parameters of Different Samples Following Administration[a]

sample		dose (IU/kg)	AUC (mIU·h/L)	PA (%)	*F* (%)
Insulin	s.c.	5	246.95 ± 20.06	-	100
Insulin	oral	75	7.45 ± 6.66	1.38 ± 0.55	0.20 ± 0.18
NCs	oral	75	53.59 ± 19.28	2.60 ± 0.60	1.45 ± 0.25
NPs-1	oral	75	111.19 ± 24.64	6.61 ± 0.62	3.02 ± 0.66

[a] Data are means ± SD (n = 5).

Orally administered NPs-1 generated a slower rise in serum insulin concentration, which reached the maximum concentration at 4 h. The pharmacokinetic parameters at the dose of 75 IU/kg were also shown in Table 2; NPs-1 exhibited a relative bioavailability of 3.02%, which is significantly higher than that of NCs (1.45%).

DISCUSSION AND CONCLUSIONS

The lack of nanocarriers with efficient mucus permeation and transepithelial absorption represents a significant barrier for safe and convenient oral applications of protein drugs in the treatment of chronic disease. One challenge in the oral nanocarrier development is that mucus permeation and epithelial absorption require different or even contradictory surface properties of the nanocarrier. The NP platform prepared herein was rationally designed to solve the dilemma in sequential mode, in which an expandable "mucus-inert" material was applied to disguise the NP as it permeated through the mucus, while the CPPs were subsequently revealed as transepithelial transport enhancers. To achieve optimal efficiency in both of these two processes, the NP was demonstrated to have several unique features.

One unique feature is that the hydrophilic coating is adjustable in charge density, which enables the screening of NP with a range of surface change. Surface property of particle is the most important factor that determines their diffusion in mucus.[35] In contrast with previously reported mucus penetrating NPs, which are generally covalently conjugated with PEG or coated with PEG containing surfactants,[12,30] our distinctive NPs are assembled with a hydrophilic polymer that are amenable to different modifications. The pHPMAs synthesized with different ratios of MA-GG-OH monomer possessed a range of negative charges (Figure 1A, B). Therefore, the varied charge densities of the pHPMAs enabled not only a successful self-assembly of the material with the cationic NCs core as demonstrated by the FRET analysis (Figure 1F), but also the screening of the NP formulation to achieve the optimal surface property for mucus permeation (Figure 1E). Notably, all pHPMAs coated NPs possessed very low mucin affinity (Figure 2A), which suggested effectiveness of our "mucus-inert" strategy by concealing the cationic CPP-rich core under a high hydrophilic and mildly negatively charged polymer. However, for the permeation across mucus, the NPs with relatively neutral surface (NPs-1 and NPs-2) exhibited better ability than those with higher negative charges (NPs-3 and NPs-4) (Figure 2B). This result is consistent with previous reports regarding the PEG-containing MPPs, and suggests that relative electroneutrality of NP surface is essential for efficient mucus permeation by avoiding both muco-adhesion and muco-repulsion.[10] NPs-1 that showed excellent mucus permeation also demonstrated much less obstructed Brownian motion in mucus relative to the uncoated NCs (Figure 2C,D), and this phenomenon may largely increase the odds for the NPs to get access to the epithelial surface.

Another unique feature of the NP that enables its efficient absorption on epithelial tissue is the dissociation of pHPMA molecules from the NP surface in mucus, which simultaneously reveals the CPP-rich core, as shown in the schematic image of Figure 6. Neutral or negatively charged hydrophilic surface of NPs have been widely demonstrated to prevent the interaction of NPs with the cell membrane and thus inhibit their cellular uptake.[16] For the developed NP platform, the pHPMAs that assembled on the particle surface gradually detached from cationic NC core during their permeation through the mucus (Figure 3A,B). Notably, this process happened in a much slower rate in saline solution than in mucus, which is a positive factor for the stability of the NPs and protection of encapsulated drugs in digestive intestinal fluid. Therefore, this technology avoids the drawback of low cell affinity of the traditional MPP. It is worth noting that, although pHPMA molecules dissociated from the NP as it was getting close to the epithelial cells, a large proportion of CPP−insulin nanocomplex core remained intact even after the internalization in the epithelial cells (Figure 3D). As expected, compared with the uncoated NCs, the coating of pHPMA-1 (NPs-1) boosted the uptake of the particles on mucus covered epithelial cells (Figure 2E). However, reduced cell uptake was observed for NPs-3 and NPs-4 in comparison to the NCs (Figure 2E). This might be attributed to their lower surface charge that could reduce their affinity with cells, and might as well be the result of a slower dissociation rate of the pHPMA-3, which possessed stronger interaction with the cationic NC core. Therefore, our study suggested the importance of the screening of the "mucus-inert" material with different charge density for an optimal overall performance.

Orally administered drug needs to be transported across the epithelium to be absorbed into systemic circulation. The insulin encapsulated in the CPP-rich nanocomplex was transported across the epithelial cell monolayer at a rate of 2.3-fold higher than of free drug (Figure 3E), and NPs-1 demonstrated even higher transport rate compared with the NCs. As we noted similar intracellular progression of NPs-1 and NCs