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# Mucoadhesion of colloidal particulate systems in the gastro-intestinal tract[1]

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**Abstract**

The oral route is the preferred route for drug delivery. However, numerous drugs remain poorly available when administered by this route. In order to circumvent this problem, it has been proposed, successfully for several of them, to associate drugs with colloidal polymeric particle systems. Orally administered nano- and microparticles can follow at least three different pathways: (i) capture by gut-associated lymphoid tissue; (ii) mucoadhesion; and (iii) direct faecal elimination. The relative importance of these different mechanisms is discussed. Emphasis has been laid on mucoadhesion which has been assessed in vitro and in vivo by using polystyrene and poly(lactic acid) nanoparticles as models. On the one hand, in vitro adsorption and desorption studies have shown that particles could be captured to a considerable extent by the mucous gel layer lining the gastro-intestinal tract through a mucoadhesion mechanism. On the other hand, the in vivo behaviour of the particles in the intestinal lumen has been accurately investigated by means of radiolabelled particles. Direct particle translocation through the intestinal mucosa was not predominant. On the contrary, a significant fraction of the particles was captured by the mucous gel layer while the remainder of the particles underwent unmodified transit. It can be concluded that the therapeutic potential of colloidal drug carriers after oral administration is probably not to deliver the drug directly into the blood flow but to increase bioavailability by protecting the drug from denaturation in the gastro-intestinal lumen, or by increasing the drug concentration for a prolonged period of time directly at the surface of the mucous membrane. © 1997 Elsevier Science B.V.

*Keywords:* Oral route; Colloidal suspensions; Nanoparticles; Microparticles; Mucoadhesion; Adsorption; Gastrointestinal mucosa; Radiolabelled particles; Distribution studies

## 1. Introduction

The oral route is the preferred route for drug delivery. However, numerous drugs remain poorly available when administered by this route. Among other reasons, this can be due to: (i) low mucosal permeability for the drug; (ii) permeability restricted to a region of the gastro-intestinal tract; (iii) low or very low solubility of the compound which results in a low dissolution rate in the mucosal fluids and elimination of a fraction of the drug from the alimentary canal prior to absorption; and (iv) lack of stability in the gastro-intestinal environment, resulting in a degradation of the compound prior to its absorption (e.g. peptides).

In order to circumvent some of these problems, it has been proposed to associate drugs with colloidal polymeric particulate systems (or small particles in the range of the micrometer in size). Different oral administration experiments in animals have been reported in the literature, which have helped to improve the pharmacokinetics of several drugs [1–8]. However, the exact mechanism of improvement by polymeric nanoparticulate systems remains unknown.

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Orally administered nano- and microparticles can follow at least three different pathways: (i) capture by gut-associated lymphoid tissue; (ii) mucoadhesion; and (iii) direct faecal elimination. The relative importance of these different mechanisms has been assessed in vitro and in vivo by using polystyrene and poly(lactic acid) nanoparticles as model particles. First, in vitro adsorption and desorption data available from the literature are presented, and particle uptake by the mucous gel layer lining the gastro-intestinal tract is discussed in terms of a mucoadhesion mechanism. Secondly, the in vivo behaviour of the particles in the intestinal lumen investigated using radiolabelled particles is presented.

## 2. Mucoadhesive properties of colloidal polymeric particulate systems in vitro

Mucoadhesive properties of nanoparticles have been studied in vitro by only a very few research groups. Thus, experimental data are very scarce. Data have been obtained through adsorption studies either under flow [10,11] or static conditions [13,17–19], and desorption studies [20] have also been reported from the literature.

### *2.1. Adsorption studies under flow conditions*

One of the first approaches to study the mucoadhesive potential of nanoparticles on the intestinal mucosa was based on a model of intestinal absorption of solutes [9]. Teng et al. [10,11] studied the in vitro adsorption of various micron-sized particles (2–3 $\mu$m) from a flowing liquid film of dilute suspensions on to the mucous surface of rat intestinal strips. The steady-state fraction of adsorbed particles increased with the length of the intestinal strip and with decreasing flow rate. The adsorption was mathematically described by a mass transfer coefficient depending on the diffusion of the latex particles in the liquid film and a collision barrier factor [10,11]. For adsorption, negatively-charged particles had to overcome a potential energy barrier of electrical repulsion between the particles and also negatively charged mucus. This energy barrier decreased dramatically when the electrolyte concentration was increased. Generally, no dependence on electrolyte concentration was observed for positively-charged latexes, i.e. latex particles coated with a positively charged polymer. At pH 7.6, a flow rate of 0.103 ml/min and an intestinal strip length of 55 cm, maximal fractions of over 0.5 were adsorbed, when the zeta potential of the particles was positive or close to zero ( − 10 mV), e.g. due to charge shielding by electrolytes. On the contrary, the fraction adsorbed of a positively-charged aminated latex did not exceed 0.36, very probably due to surface-fouling by free soluble mucous glycoproteins. This suggested that, in practice, mucus contamination of particles, whose surfaces have been designed to promote adhesion to the mucus on the intestinal wall, could lead to unfavourable conditions for adhesion. In fact, the fraction adsorbed of mucus-coated latex particles was only 0.26. For very negatively-charged poly(vinyltoluene) latex particles, the fraction adsorbed was never higher than 0.09.

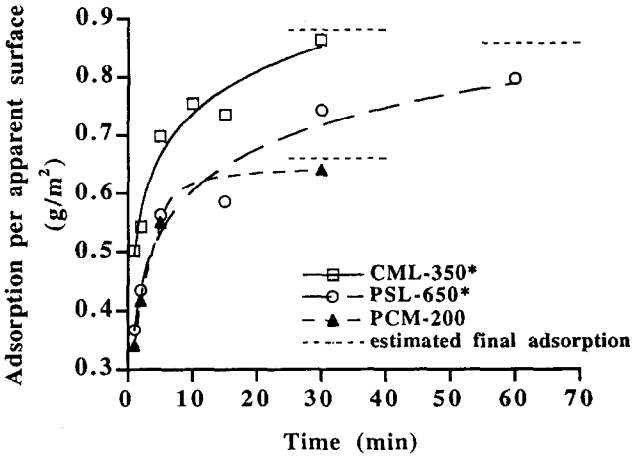


Fig. 1. Kinetics of adsorption of three polystyrene latexes on rat intestinal mucosa. Modified after [13]. Nominal and corresponding measured particle sizes: PCM-200 (230 nm), CML-350* (320 nm), PSL-650* (670 nm).

Generally, the fraction adsorbed was not affected by the latex concentration ranging from $4.5 \cdot 10^6$ to $13.6 \cdot 10^6$ particles/ml or $0.6 \cdot 10^{-4}$ to $1.9 \cdot 10^{-4}$ volume fractions [10]. At latex concentrations of $13.6 \cdot 10^6$ particles/ml, which corresponds to a polymer concentration of 0.2 g/l, the adsorbed latex fraction of 0.5 occupied about 5% of the apparent mucous surface [10]. For a particle size of 3 $\mu$m, 5% coverage of the apparent

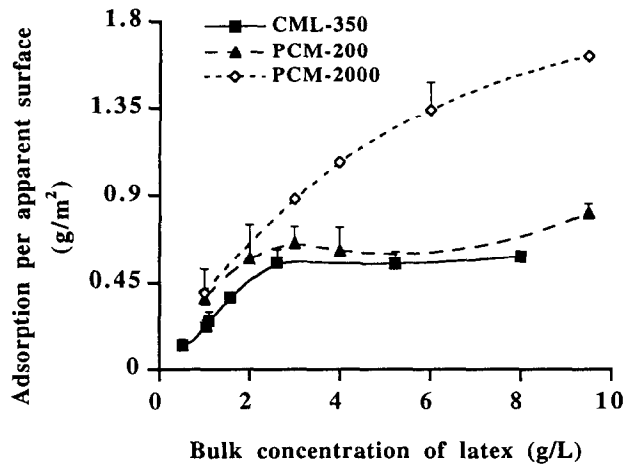


Fig. 2. Adsorption isotherm of three polystyrene latexes on rat intestinal mucosa. Modified after [17]. Nominal and corresponding measured particle sizes: PCM-200 (230 nm), CML-350 (314 nm), PCM-2000 (2045 nm).

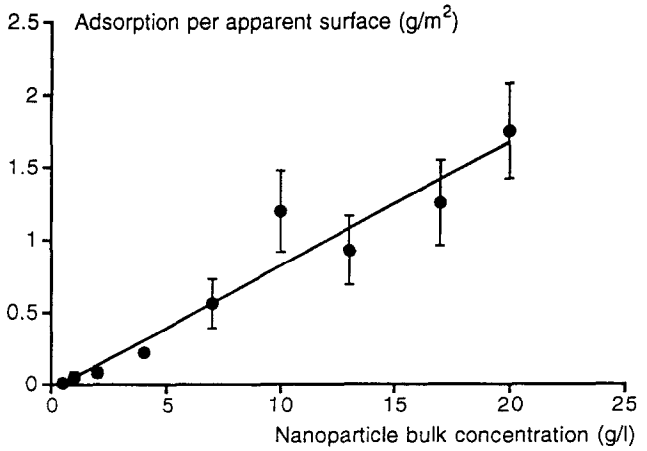


Fig. 3. Adsorption isotherm of poly(isobutyl cyanoacrylate) nanospheres on rat intestinal mucosa. Modified after [21].

surface, i.e. 5% of the adsorption in a monolayer, corresponds to 0.1 g/m$^2$.

From wash-out experiments at the end of adsorption studies it was concluded that the adsorbed particles were bound irreversibly to the mucous surface [10].

### 2.2. Equilibrium adsorption under static conditions

Another approach to study interactions between disperse systems and macroscopic substrates, such as intestinal mucosa, consists of performing kinetics and adsorption isotherms [12]. This approach has been used in our group. The reasons for this choice are: (i) adsorption mimics the in vivo process; (ii) adsorption studies give quantitative data; and (iii) experimental simplicity and reproducibility.

A new model to study the adsorption of latexes to rat intestinal mucosa ex vivo under static conditions was therefore developed [13]. Fig. 1 presents some kinetics of adsorption obtained with different-sized poly(styrene) latexes in physiological saline. 90% of equilibrium was reached after 10 min for a particle size of 230 nm, 20 min for a particle size of 320 nm, and 30 min for a particle size of 670 nm. The differences in the observed rate constants were bigger than expected for simple diffusion of the particles in the suspension medium. In another study of latex adsorption [14], it was proposed that the adsorption kinetics were controlled by the phase of attachment that followed the transport phase. Similar kinetics were found in colloid aggregation, which were initially limited by diffusion and ended up by a reaction-controlled process [15]. Considering particle size, the mucous gel constitutes a porous rather than a smooth adsorbent, and the adsorption kinetics may be controlled in a second phase of transport by diffusion into the mucous network [16].

The adsorption of poly(styrene) latexes [17–19] and poly(isobutyl cyanoacrylate) nanoparticle suspensions

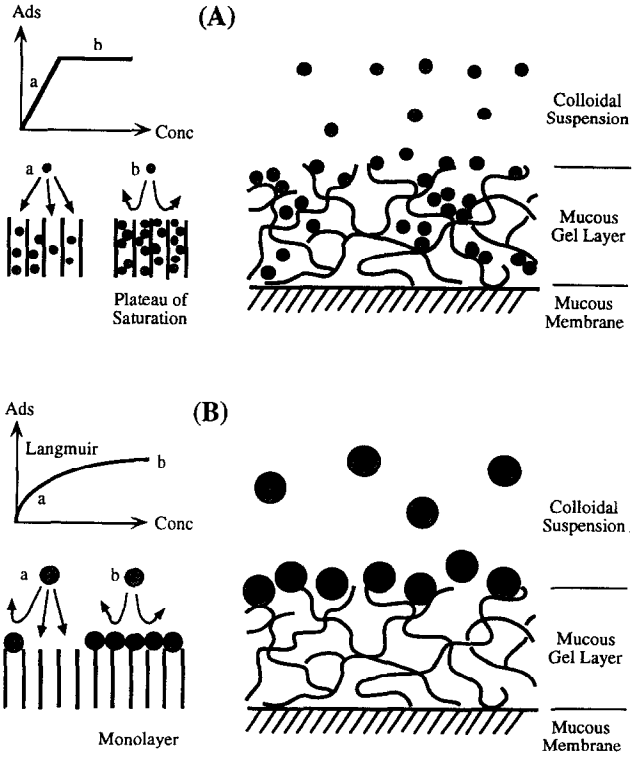


Fig. 4. Adsorption isotherm shapes and corresponding adsorption models. A: case of particles < 1 μm. B: case of particles > 1 μm.

[21] on rat intestinal mucosa were studied under near-equilibrium conditions. The adsorption isotherms of poly(styrene) latexes (Fig. 2) showed a strong dependence on particle size [17,18]. The adsorption isotherm of poly(isobutyl cyanoacrylate) nanoparticles (Fig. 3) showed a linear increase in particle adsorption, at least up to a bulk concentration of 20 g/l in the supernatant [21]. When comparing the slopes of the linear portions of the isotherms, it appears that the slope corresponding to poly(isobutyl cyanoacrylate) nanoparticle suspension is lower than the slope for polystyrene latex (230 nm in diameter), suggesting a lower affinity of poly(isobutyl cyanoacrylate) nanoparticles for the rat intestinal mucosa compared with polystyrene particles.

Table 1
Body distribution of $^{14}$C labelled PLA microspheres in rat 1 h after intragastric administration (2 ml of a 10 mg/ml particle suspension)

| Localization | % Of recovered radioactivity |
|---|---|
| Intestinal level | 97.0 |
| Lumen content | 88.2 |
| Mucosa | 8.8 |
| Faeces | 0 |
| Body level | 3.00 |
| Blood | 1.04 |
| Liver | 1.68 |
| Lung | 0.05 |
| Spleen | 0.03 |
| Urine | 0.20 |

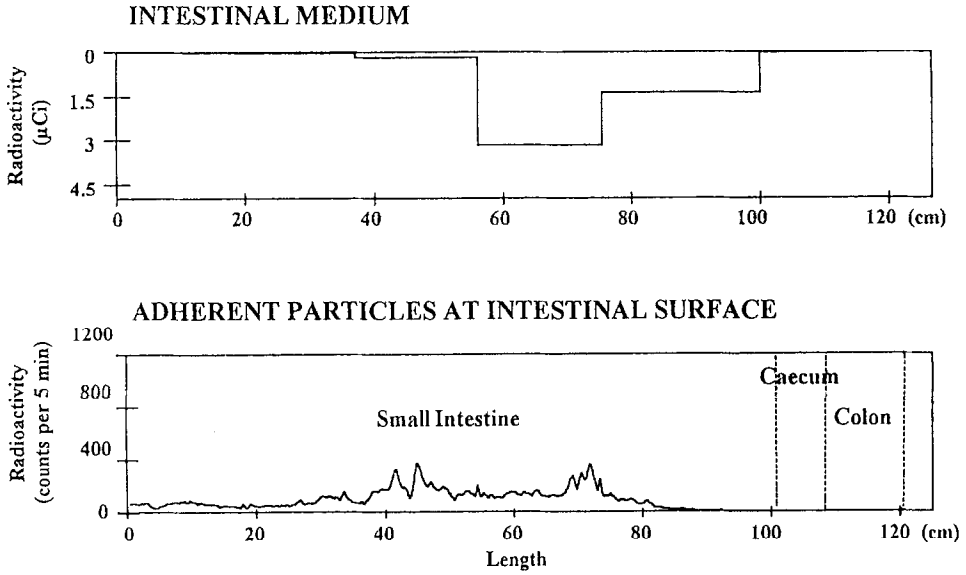


Fig. 5. Intestinal mucoadhesion profiles of poly(lactide) microspheres (mean diameter 1.5 $\mu$m) and corresponding lumen content profile, 0.5 h after intragastric gavage in rat.

These different isotherms have been analyzed according to different isotherm classifications [17]. Isotherms of small latexes, i.e. 200 nm poly(isobutyl cyanoacrylate) nanoparticles, and poly(styrene) latexes with particle sizes of 230 and 670 nm, had the characteristic isotherm shape of adsorbates, which penetrate into the porous adsorbent (Fig. 4a). In this situation, the linear increase in the isotherm corresponds to the creation of new adsorption sites when the bulk particle concentration is increased. These sites are available for further adsorption up to the isotherm plateau which corresponds to a saturation of the available sites. The hypothesis of a diffusion of particles into the mucous layer is supported by a confocal microscopy study by Scherrer et al. [22] showing that fluorescently labelled poly(isobutyl cyanoacrylate) particles (211 nm in diameter) penetrated at least 60 $\mu$m deep into the mucus layer of rat intestine mucosal fragments. Alternatively, the scanning electron microscopic photograph made by Damgé et al. [1] showed 200 nm nanocapsules in close contact with the mucous network a short time after oral administration to rats. In the case of larger particles, such as 2 $\mu$m polystyrene particles, adsorption occurs as a Langmuirian type. The adsorbate adsorbs in a monolayer on the adsorbent surface, which behaves like a smooth surface (Fig. 4b).

## 3. Mucoadhesive properties of colloidal polymeric particulate systems in vivo

Obviously, many variables in the alimentary canal can affect significantly the behaviour of nanoparticles after their oral administration. Therefore, it is of interest to examine the fate of the particles and the pattern of their distribution in the gastro-intestinal tract under in vivo conditions. Some direct experimental in vivo data are available in the literature. However, transit was mostly investigated with no special emphasis on mucoadhesion.

After peroral administration of radiolabelled poly(-hexyl cyanoacrylate) nanoparticles to mice, whole-body autoradiography showed that 30 min after administration the particles were exclusively localized in the stomach [25]. After 4 h, a large quantity of radioactivity was found in the intestine in the form of clusters without macroradiographic evidence of accumulation at specific intestinal sites. On the contrary, a persistent film of nanoparticles adhering to the stomach wall was observed. In this study, very little of the radioactivity was found to be absorbed. In a similar study, microautoradiographs confirmed the presence of radioactivity throughout the whole gut [25]. The amount of radioactivity dropped between 30 and 40% of the 90 min value within 4–8 h and to 5%, 24 h after dosing. Histological investigation showed radioactivity adjacent to the brush border, incorporated into the underlying cell layers, and in goblet cells up to 6 days after administration.

In order to gain more detailed particulate distribution patterns in the intestinal tract, mucoadhesion profiles were obtained by our group after intragastric administration of microspheres in the micron-range. $^{14}C$ radiolabelled poly(lactide) microspheres were prepared by a classic solvent evaporation technique (1.5 $\mu$m in diameter), using methylene chloride as the dispersed phase and a 5% poly(vinyl alcohol) aqueous solution as the dispersing medium. Stable radiolabelling

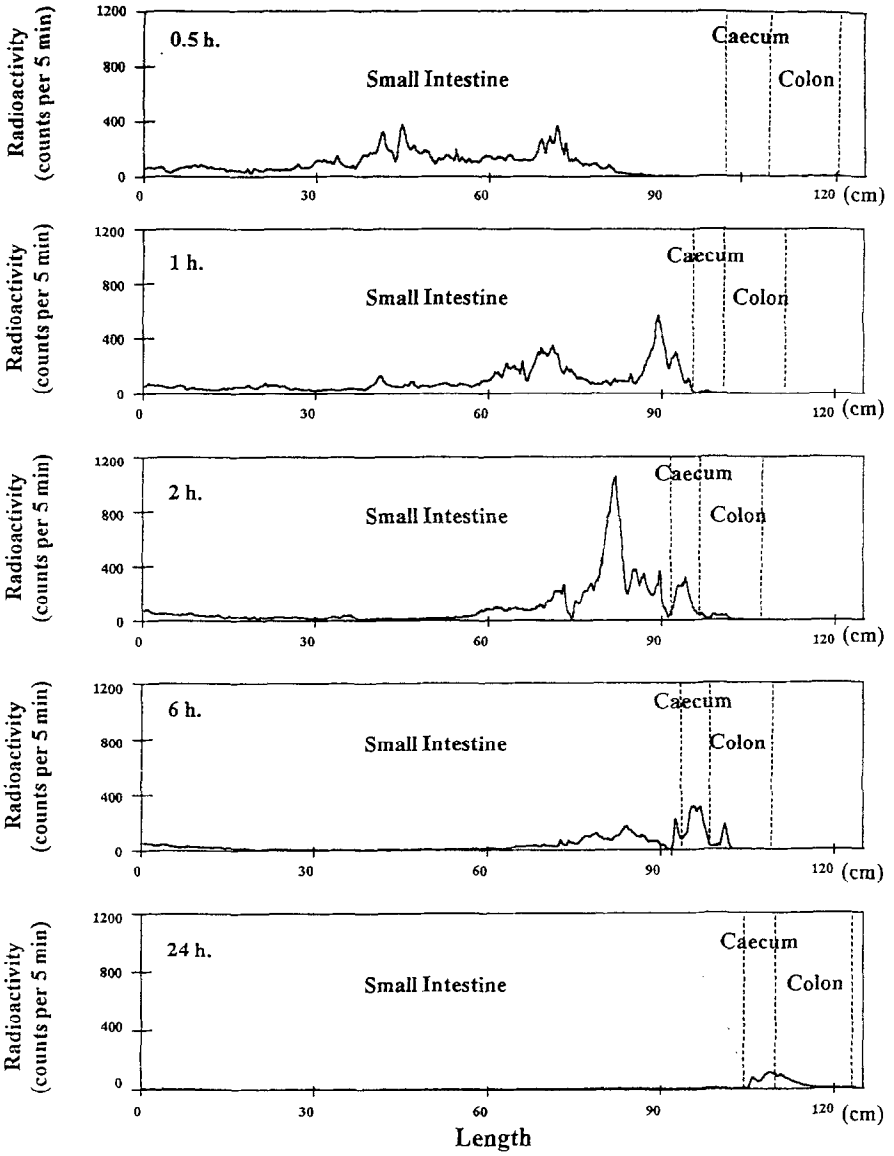


Fig. 6. Intestinal mucoadhesion profiles as a function of time after intragastric gavage in rat.

was obtained by incorporating $^{14}C$ oleic acid during the preparation process. After intragastric administration of 2 ml of a 10 mg/ml particulate suspension, the rats were sacrificed after different transit times. The entire length of the gut was sampled from duodenum to colon and divided into five segments. Each segment was opened lengthwise and rinsed with saline to remove all the non-adhering particles present in the luminal content. Firstly, the radioactivity present in each rinsing fraction was counted subsequently for assessing the amount of non-adhering particles present in the corresponding segment of the intestinal lumen. Secondly, the radioactivity of the rinsed segments, due to the presence of mucoadherent particles, was then measured by means of a linear gas counter, resulting in mucoadhesion profiles along the mucosal segment. Finally, body distribution analysis was determined.

Analysis of the body distribution of PLA particles 1 h after administration showed that 97% of radioactivity was localized in the gastro-intestinal tract (Table 1). Only 3% was recovered in other organs, supporting the idea that particle translocation through the mucosa is a limited process. Analysis of the gastro-intestinal content showed that the amount of radioactivity due to particles adhering on the whole mucosal surface corresponded to about 9% of the recovered radioactivity, whereas 88% of the particles remained in the fluids of the lumen.

A detailed analysis of the distribution pattern of particles adhering to the mucosa is shown in Fig. 5 which presents a typical intestinal mucoadhesion profile and the corresponding lumen content profile obtained 0.5 h after intragastric gavage in the rat. When examining the luminal content profile, it can be concluded that

the fraction of non-adhering particles transits rapidly through the intestine. In the present case, the transit is responsible for the appearance of a high particle concentration in the ileal region. However, the transit of the particles up to the jejunum has resulted in the adhesion of particles on the whole mucosal surface as can be seen on the mucosal profile where radioactivity can be found along the whole length of the intestine, except the caecum and the colon. It may also be observed that the maximal adherence of particles occurs at the jejunal level, in front of the particle-concentrated luminal content, which may result from the adsorption process previously described.

From a quantitative standpoint, the adhering fraction of poly(lactic acid) microspheres remains limited when compared with the non-attached particles fraction in the lumen. However, it must be remembered that the physicochemical properties of the polymer and the surface properties of the particles are likely to affect greatly the mucoadhesion process, as shown in the in vitro section. In this respect, poly(lactic acid) microspheres prepared in the presence of poly(vinyl alcohol) present rather hydrophilic surfaces and may exhibit only poor adsorption properties compared with model polystyrene latexes or poly(isobutyl cyanoacrylate) nanosphere suspensions.

Obviously, the distribution pattern of adhering particles depends on time, as shown in Fig. 6 for a series of intestinal mucoadhesion profiles. On the one hand, the maximum of particles adhering on the mucosal surface progressively reaches the colon in 6 h. On the other hand, radioactivity decreases in the proximal portion of the intestine during this period of time. This is likely due to either progressive detachment of particles from mucus or mucus turnover. By plotting the radioactivity remaining on a mucosal segment as a function of time, the time necessary for the detachment of half of the adhering particles has been estimated to be 1.4 h. This value is in the range of the estimated time for complete renewal of the intestinal mucous gel layer in the rat in situ loop which was reported to range between 47 and 270 min [26]. The turnover of the mucus would therefore constitute the limiting factor for the time period of particle adhesion to the mucosa.

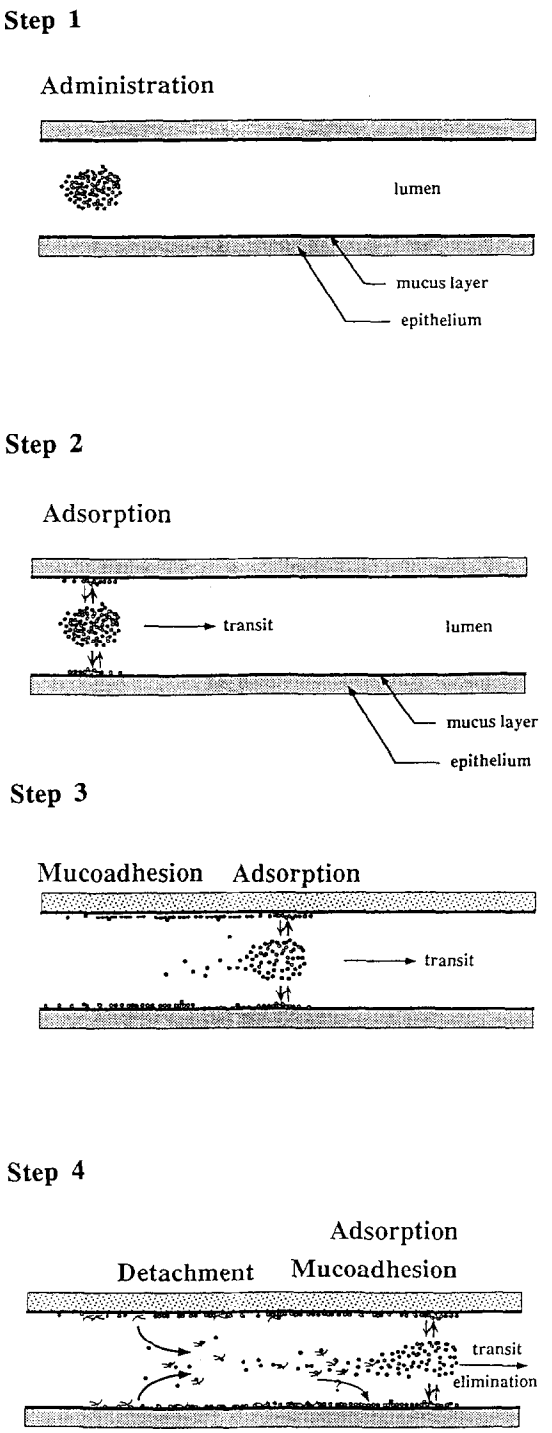


Fig. 7. Mucoadhesive behaviour of colloidal particulate systems following oral administration. Step 1, administration of the colloidal system. Step 2, adsorption of particles. Step 3, mucoadhesion and luminal transit of the reservoir colloidal suspension. Step 4, particle detachment, luminal transit of the colloidal suspension, faecal elimination.

## 4. Mucoadhesion of nanoparticles after oral administration

When considering the different phenomena occurring after oral administration of a suspension of colloidal particles via the oral route, the following general dynamic description, illustrated in Fig. 7, can be given. First, a suspension of particles is administered and immediately enters into contact with a portion of the oral mucosa (step 1). From this moment, the concentrated suspension acts as a reservoir of particles and, very rapidly, an adsorption process takes place, leading to the adsorption of a fraction of the available particles (step 2). Adsorption occurs with the mucous layer and

is an irreversible process. However, the luminal particle suspension transits through the intestine, sweeping progressively the whole mucosa. The simultaneous adsorption process results in a progressive covering of the intestinal mucosa by adhering particles (step 3). Finally, detachment of the particles from the mucosa begins to occur in the proximal region and is progressively extended to the distal region (step 4). Non-adhering particles from the lumen pool and detached particles from the mucoadherent pool are finally eliminated in the faeces. Quantitatively, particle translocation through the intestinal mucosa remains a secondary phenomenon.

## 5. Conclusion

The oral administration of colloidal suspensions of polymeric particles (nanoparticles or microspheres in the micron-range and made from non-swellable polymers) leads to the mucoadhesion of a significant fraction of the particles. Clearly, the particles are captured by the mucous gel layer while the remainder of the particles undergoes unmodified transit.

It can be concluded that the therapeutic potential of colloidal drug carriers after oral administration is probably not to deliver the drug directly in the blood flow, but to increase bioavailability by protecting the drug from denaturation in the gastro-intestinal lumen or by increasing the drug concentration for a prolonged period of time directly at the surface of the mucous membrane.

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