4.2.8. Zeta potential change

Mucoadhesion can also be quantified by observing the change in zeta potential of mucin suspension by adding the polymer solution. For this purpose, a uniform submicron suspension of mucin is prepared by sonicating its coarse suspension. The particle size and zeta potential are measured for known concentration of mucin suspension alone and in the presence of known concentration of polymer in a suitable buffer system. The change in particle size and change in zeta potential reflects the mucoadhesion. The critical ratio of polymer to mucin at which the zeta potential reaches nearly zero from negative represents the charge neutralization of the mucin and the mucoadhesion. Takeuchi et al. determined the degree of mucoadhesion of chitosan and carbopol by this method [27, 31].

4.2.9. Residual mucin

In this method, the microparticles or beads containing mucoadhesive polymer are suspended in aqueous solutions of different conc. of mucin ranging from 50 to 200 μg mL^{-1} and incubated for 1 h at 37°C. This mixture is then centrifuged, and the supernatant is subjected to residual mucin quantification through protein assay such as Lowry protein assay or by protein analysis kit using mucin for standard curve [32]. This is a quantitative technique that can be used for particulate dosage forms in micro ranges, large enough to centrifuge.

4.3. Rinse Methods

4.3.1. Half-pipe method

In this analytical method, the residual amount of marker loaded to the microspheres, particles etc., which still retained on the mucosa after a washout period, is determined. In detail a 5–8 cm long PVC pipe is cut into half longitudinally. On the inner side of the half-pipe, a piece of mucosal layer from either intestine, buccal or vagina is mounted by means of acrylic glue. This half pipe is then fixed at an angle of 45 degrees in an incubator. The conditions of incubator are adjusted to 37°C temperature and 100% humidity. The mobile phase is made to flow on top end of the tissue at a constant flow rate depending upon the type of mucosal layer, by help of a peristaltic pump at 37°C. After predetermined time points, the mucosa with the remaining marker on it is quantified. Bernkop-Schnürch et al. prepared chondroitin sulfate nanoparticles and used fluorescein diacetate (FDA) as marker to assess the mucoadhesive properties on mucosal membrane mounted on half-pipe. Similarly, in another study rhodamine was covalently attached to thiolated cyclodextrin in order to evaluate mucoadhesive properties on conjunctival fornix using half-pipe method [33].

4.3.2. Wash-off test

The wash-off test uses USP tablet disintegrating test apparatus [34] to estimate the degree of mucoadhesion of particulate dosage forms such as microspheres and beads. In this technique, freshly excised intestinal mucosa from the animal source is fixed to a glass slide with glue or thread. Precounted number of microspheres or beads are dispersed on the wet mucosal membrane and this slide is hung on one of the groves of USP tablet disintegrating apparatus. The apparatus is turned on and is paused on predetermined intervals to count the number of remaining adhered microspheres or beads [35, 36]. This method can be used for semiquantitative mucoadhesion studies.

4.3.3. Adhesion number

Another method for quantification of particulate dosage form mucoadhesion is adhesion number. In this method mucous membrane is fixed on a solid support, i.e., glass or polyethylene slide. The microspheres or microparticles are spread over the mucus membrane and incubated for 30 min under the specified conditions so that the polymer gets hydrated without mucus getting dried. The total adhered microspheres are counted under the microscope and later this mucus membrane along with support is placed at a 45 degree angle. A buffer is allowed to flow over it for next 5 min with a flow rate of 1 mL min^{-1}. Later, the microspheres retained by the mucus membrane are counted in the same field area. The adhesion number can be calculated by the following equation [37].

$$N_a = \frac{N}{N_0} \times 100$$

where N_a is the adhesion number, N_0 is the total number of particles in a particular area, and N is the number of particles attached to the mucosa after washing.

4.3.4. Rotating cylinder

In this method, detachment time of polymeric tablet from mucosa attached to rotating cylinder was used to evaluate the mucoadhesive strength of the polymers. A piece of intestinal mucosa is fixed on the rotating cylinder by means of cyanoacrylate glue by keeping the mucosal layer outward. The test polymer or formulation in the form of small tablets/discs or beads in predetermined numbers are attached to this intestinal mucosa. This cylinder is then immersed in a buffer solution rotated at a desired rpm at 37°C [38–40]. The time for the detachment of the beads or small tablets is visually observed by naked eye or camera ranging from 2 h to 72 h, depending upon the type of study or