mucoadhesive properties of the polymer. This technique is simple and easy to perform in comparison to other mucoadhesive methods and is suitable for solid dosage forms such as discs, tablets, beads, and pellets.

4.3.5. Immersion

Mucoadhesion study by immersion technique can be performed by attaching 10 cm long and 1 cm wide previously washed mucosa to a plastic strip such that the mucosal side stays exposed. This strip is then immersed in 10 mL suspension of fluorescent labeled nanoparticles in a tube and incubated for half an hour in a shaking water bath. Percentage mucoadhesion is then calculated by comparing the fluorescence of the suspension before and after the immersion of the mucosal tissue.

$$\text{Mucoadhesion}\% = \frac{\text{Initial fluorescence} - \text{Remaining fluorescence}}{\text{Initial fluorescence}} \times 100$$

This technique was used to study vaginal mucoadhesion of the tenofovir-loaded chitosan nanoparticles [41]. It is a quantitative technique that can be used for solutions and suspensions.

4.4. Cellular Methods

4.4.1. Cell adhesion studies

Various studies used mucus producing cell line HT29-MTX to evaluate the mucoadhesion of the polymers. For this purpose, HT29-MTX cells are incubated up to 14 days to attain a uniform monolayer. Later this monolayer is incubated with fluorescent labeled polymer formulation such as microparticle suspension, solution or gel for 1 to 2 h. In the next step the formulation is removed from top of the monolayer. Mucoadhesion can then be evaluated directly by determining the fluorescent intensity of the monolayer or indirectly by determining the residual polymers fluorescence of the incubated polymer. Adamczak et al. used this method to evaluate the mucoadhesion of liposome coated with pectin, alginate, chitosan, and ethyl hydroxyethyl cellulose [42]. This method is also reported to measure the mucoadhesion of probiotic alginate microcapsules by counting the number of *Lactobacillus reuteri* released from the capsules on the monolayer [43].

4.5. Optical Methods

4.5.1. Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) also termed as confocal microscopy is a type of microscopy in which out of focus light from the subject is blocked by a spatial that results in an image of high resolution and contrast. To use this technique for evaluating mucoadhesion, the mucosal surface is incubated with the formulation and mucosal surface is cut into squares and observed by confocal microscope. Images are then taken by the microscope and analyzed by software for the colorimetric intensity. The quantitative results of the polymer can be represented as percent of the blank. For example, Guo et al. determined the mucoadhesion of 10-hydroxycamptothecin nanogel (HCPT/NG) by CLSM [44]. They instilled HCPT/NG solution into anesthetized male BALB/c mice bladder by 24-gauge lubricated catheter. At predetermined time intervals they excised the bladder, opened it, and washed with phosphate-buffered saline. Sections of urothelial surfaces were then prepared and mucoadhesion of the HCPT/NG by measuring the optical fluorescence intensity of HCPT by CLSM. This is also very sensitive technique which can be used for solutions, gels, and nano-drug delivery systems such as SEDDS, nanoparticles, liposomes and other nano-carrier drug delivery systems [27, 45].

4.5.2. Turbidimetry

Turbidity represents the cloudiness or fogginess which is caused by the diffraction of light due to the presence of large number of particles which are invisible to the naked eye. In this technique, the stock solution of mucoadhesive polymer and mucin is prepared and their absorbance is measured at a wavelength of 500 nm or 650 nm on spectrophotometer separately. The theoretical absorbance, A_{theor} of the mixture is calculated by adding the absorbance of individual solutions. Then the actual absorbance of mixture of the two A_{act} is determined at the same wavelength. The difference $\triangle A$ between A_{theor} and A_{act} is calculated, which is directly proportional to the degree of interaction and hence mucoadhesion [46, 47]. When large molecules of polymer interact with mucus to form complex, it has a bigger size than the sum of the contributing molecular size. The large complex size scatters the light more, and the intensity of light passing through the sample is reduced. As a result, spectrophotometer shows higher absorbance and solution becomes turbid hence is known as turbidimetry. This technique can be used for comparative study and is suitable for polymers that form clear solutions [48].

5. MUCOADHESIVE POLYMERS

For the development of dosage forms, excipients are important in designing delivery system in terms of the shape, physical characteristics, and release of entrapped drug. Various factors are involved to define the release