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REVIEW ARTICLE

NOVEL DRUG DELIVERY SYSTEM

MUCOADHESIVE DRUG DELIVERY: MECHANISM AND METHODS OF EVALUATION

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ABSTRACT

This article gives a brief idea bioadhesive delivery systems based on hydrogels to biological surfaces that are covered by mucus. Techniques that are frequently used to evaluate the mucoadhesive drug delivery systems are discussed. Mucoadhesion can be defined as a state in which two components, of which one is of biological origin are held together for extended periods of time by the help of interfacial forces. Mucoadhesion is a complex phenomenon which involves wetting, adsorption and interpenetration of polymer chains. The concept of mucoadhesion in drug delivery was introduced in the early 1980s. Thereafter, several researchers have focused on the investigations of the interfacial phenomena of mucoadhesion with the mucus. Mucoadhesive drug delivery systems is one of the most important novel drug delivery systems with its various advantages and it has a lot of potential in formulating dosage forms for various chronic diseases.

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KEY WORDS

mucoadhesive, bioadhesive, evaluation techniques, mechanism.

INTRODUCTION

The term bioadhesion refers to any bond formed between two biological surfaces or a bond between a biological and a synthetic surface. In case of bioadhesive drug delivery, the term bioadhesion is used to describe the adhesion between polymers, either synthetic or natural and soft tissues or the gastrointestinal mucosa. In cases where the bond is formed with the mucus the term mucoadhesion may be used synonymously with bioadhesion. Mucoadhesion can be defined as a state in which two components, of which one is of biological origin are held together for extended periods of time by the help of interfacial forces. Generally speaking, bioadhesion is an term which broadly includes adhesive interactions with any biological or biologically derived substance, and mucoadhesion is used when the bond is formed with a mucosal surface.

Mucoadhesive drug delivery systems includes the following,[1]

- Buccal delivery system
- Oral delivery system
- Vaginal delivery system
- Rectal delivery system
- Nasal delivery system
- Ocular delivery system

MECHANISM OF MUCOADHESION

As stated, mucoadhesion is the attachement of the drug along with a suitable carrier to the mucous membrane. Mucoadhesion is a complex phenomenon which involves wetting, adsorption and interpenetration of polymer chains. Mucoadhesion has the following mechanism,[2]

1. intimate contact between a bioadhesive and a membrane(wetting or swelling phenomenon)[3,4]
2. penetration of the bioadhesive into the tissue or into the surface of the mucous membrane(interpenetration)[3,4]

THEORIES OF MUCOADHESION:[2]

Wettability theory:

The ability of bioadhesive or mucus to spread and develop intimate contact with its corresponding substarte is an important factor in bond formation. The wetting theory, was developed predominantly in regard to liquid adhesives, uses interfacial tensions to predict spreading and in turn adhesion.[5-7] The study of surface energy of polymers and tissues to predict mucoadhesive performance has been given considerable attention.[8-11] The contact angle(Q) which should ideally be zero for adequate spreading is related to interfacial tensions(g) as per the Youngs equation,

$$g_{tg} = g_{bt} + g_{bg} \cos Q$$

where the subscripts t,g and b represent tissue, gastrointestinal contents and bioadhesive polymer respectively, for spontaneous wetting to occur,[12]

$$g_{tb} \geq g_{bt} + g_{bg}$$

the spreading coefficient, $S_{b/t}$ can be given by,

$$S_{b/t} = g_{tg} - g_{bt} - g_{bg}$$

For the bioadhesion to take place, the spreading coefficient must be positive, hence it is advantageous to maximize the interfacial tension at the tissue-GI contents interface and minimizing the surface tension at the other two interfaces. The interfacial tension can be measured by methods like the Wilhelmy plate

method.[13,14] It has been shown that the BG-tissue interfacial tension can be calculated as,

$g_{bt} = g_b + g_t - 2F(g_b g_t)^{1/2}$

where the values of F(interaction parameter) can be found in published papers[15,16] thus by the wetting theory it is possible to calculate spreading coefficients for various bioadhesives over biological tissues and predict the intensity of the bioadhesive bond.

Electronic theory:

The electronic theory depends on the assumption that the bioadhesive material and the target biological material have different electronic surface characteristics. Based on this, when two surfaces come in contact with each other, electron transfer occurs in an attempt to balance the Fermi levels, resulting in the formation of a double layer of electrical charge at the interface of the bioadhesive and the biologic surface. The bioadhesive force is believed to be present due to the attractive forces across this double layer.[17,18]

Fracture theory:

This is by-far the most accepted theory on bioadhesion. It explains the forces required to separate the two surfaces after adhesion has taken place. It measures the maximum Tensile stress($s_{m)}$ produced during detachment as follows,[19]

$s_m = F_m/A_o$

where F_m and A_o represent the maximum force of detachment and the total surface area respectively. In a uniform single-component system, fracture strength(s_f), which is equal to the maximum stress of detachment($s_{m),}$ is proportional to the fracture energy($g_{c),}$ Youngs modulus of elasticity(E) and the critical crack length(c) of the fracture site as follows,[20]

$s_f \square (g_c E/c)^{1/2}$

fracture energy can be obtained by the sum of the reversible work of adhesion, W_r(work done to produce new fracture surfaces) and the irreversible work of adhesion ,W_i(work of plastic deformation),

$g_c = W_r + W_i$

Adsorption theory:

This theory states that the bioadhesive bond formed between an adhesive substrate and the tissue is due to the weak van der waals forces and hydrogen bond formation. It is one of the most widely accepted theories of bioadhesion.[21,22]

Diffusion theory:

The concept of the interpenetration and entanglement ob the bioadhesive polymer chains and mucous polymer chains is supported by the diffusion theory. The bond strength increases with the increase in the degree of the penetration.[23] This penetration is dependant on the concentration gradients and the diffusion coefficients. It is believed that interpenetration in the range of 0.2-0.5µm is required to produce an effective bond strength. The penetration depth(l) can be estimated by,[24]

$l = (tD_b)^{1/2}$

where t is the time of contact and D_b is the diffusion coefficient of the bio adhesive material in the mucus.

FACTORS AFFECTING MUCOADHESION:[25]

The mucoadhesion of a drug carrier system to the mucous membrane depends on the below mentioned factors.

- polymer based factors molecular weight of the polymer
  concentration of polymer used
  flexibility of polymer chains
  swelling factor
  stereochemistry of polymer

- physical factors pH at polymer substrate interface
  applied strength
  contact time
- physiological factors mucin turn over rate
  diseased state

ADVANTAGES OF ORAL MUCOADHESIVE DRUG DELIVERY SYSTEMS:[26]

- prolongs the residence time of the dosage form at the site of absorption, hence increases the bioavailability.
- Excellent accessibility, rapid onset of action.
- Rapid absorption because of enormous blood supply and good blood flow rates
- Drug is protected from degradation in the acidic environment in the git
- Improved patient compliance

DISADVANTAGES OF MUCOADHESIVE DRUG DELIVERY SYSTEMS:[26]

- occurrence of local ulcerous effects due to prolonged contact of the drug possessing ulcerogenic property
- one of the major limitations in the development of oral mucosal delivery is the lack of a good model for in vitro screening to identify drugs suitable for such administration.
- Patient acceptability in terms to taste, irritancy and mouth feel is to be checked

POLYMERS USED FOR MUCOADHESIVE DRUG DELIVERY:[2]

These polymers are classified as,

Hydrophillic polymers

Contains carboxylic group and possess excellent mucoadhesive properties.These are,

- PVP(Poly vinyl pyrrolidine)
- MC(Methyl cellulose)
- SCMC(Sodium carboxy metyhyl cellulose)
- HPC(Hydroxyl propyl cellulose)

Hydrogels

These swell when in contact with water and adhere to tne mucus membrane . these are further classified according to their charge
Anionic polymers- carbopol, polyacrylates
Cationic polymers- chitosan
Neural/ non ionic polymers- eudragit analogues

METHODS OF EVALUATION:

Mucoadhesive polymers and drug delivery systems can be evaluated by testing their adhesion strength by both in vitro and in vivo tests.[26]

In vitro tests / exvivo[2,27-31]

- methods determining tensile strength
- methods determining shear stress
- adhesion weight method
- fluorescent probe method
- flow channel method
- mechanical spectroscopic method
- falling liquid film method
- colloidal gold staining method
- viscometer method
- thumb method
- adhesion number
- electrical conductance
- swelling properties
- in vitro drug release studies
- mucoretentability studies

In vivo methods[26,32,33]

- use of radioisotopes
- use of gamma scintigraphy
- use of pharmacoscintigraphy
- use of electron paramagnetic resonance(EPR) oximetry
- X ray studies
- Isolated loop technique

These techniques are less common due to high cost, time consuming and ethical factors. But these are important to asses the true mucoadhesive potential specially an the case of oral mucoadhesive drug delivery.

The GI transit time can be measured by using one of the many radio opaque markers like barium sulphate which is coated to the bioadhesive dosage form so as to assess the GI transit by means of X-ray inspection. By means of gamma scintigraphy both the distribution and retention can be studied.

In 1985 Chng et.al. studied the transit of various 51 cr radio labeled polyacrylic acid beads through the rat GI tract,. The beads were fed to the rats and at various tome intervals after which the rats were sacrificed. The rats intestine was then systemically dissected into 20 equal parts and the amount of radiation in each part measured thus allowing, the transit overtime to be realized.[34] The development of a non invasive technique to determine the transit time of mucoadhesive polymers was done by Davis. The transit time could be imaged via labeling of the polymer with a gamma emitting nucleotide which was determined with the help of gamma scintigraphy.[35]

A recent technique by Albrecht et al. was to use magnetic resonance imaging to localize the point of release of thiolated polymers from dosage forms via the use of gadolinium. In vivo mucoadhesion was determined by ascertaining thre residence time of the fluorescently tagged thiomer on intestinal mucosa of rats after 3 hours.

***Shear stress method**:*

The measurement of the shear stress gives an direct correlation to the adhesion strength. In a simple shear stress measurement based method two smooth, polished plexi glass boxes were selected one block was fixed with adhesive araiditeon a glass plate, which was fixed on leveled table. The level was adjusted with the spirit level. To the upper block, a thread was tied and the thread was passed down through a pulley, the length of the thread from the pulley to the pan was 12cms. At the end of the thread a pan of weight 17gms was attached into which the weights can be added.[36,37] A recent method involves the measurement of mucoadhesion by use of a stainless steel rotating cylinder which was coated with freshly excised porcine intestinal mucosa to which polymer discs were attached. The cylinder was placed in a dissolution apparatus and rotated at 125rpm. It was analysed every 30 mins for the attachement of the polymers discs.[38]

***Detachment force measurements**:*

The Wilhelmy plate method is one of the traditional methods for the measurement of the force of adhesion of various bioadhesive dosage forms. The method involves the measurement of the dynamic contact angles and utilizes a microtensiometer and a microbalance.[39] The CAHN dynamic contact angle analyzer is used for this purpose. Wilhelmy plate method measures the bioadhesive force between the mucosal tissue and the polymer/dosage form attached to a metal wire and suspended into the microtensiometer.[169] The mucosal tissue(usually rat jejunum) is used which is placed in the tissue chamber, this chamber is raised so as to make contact between the tissue and the test material. After a certain period(7 mins for microspheres) the stage is lowered and the force of adhesion is meadured. This apparatus measures the following parameters:

Fracture strength: force per unit area required to break the adhesive bond.

Deformation to failure: it is the distance required to move the stage before complete separation occurs.

Work of adhesion

Another method used to measure the in vitro mucoadhesive capacity of different polymers is a modified method developed by Martti to assess the tendency of mucoadhesive materials to adhere to the oesophagus. The intestine was removed from the sheep and kept in tyrode solution at 4c. segments of 6-7cm long were cut from the intestine , the lower end tied to the glass tube of diameter 15mm. The 6mm paracetamol plane tablets, paracetamol tablets layered on one side with mucoadhesive polymer, and the paracetamol in matrix tablets(2:1) ratio were prepared. VH/AB fine hole drilled in the tablets to be tested with fine needle in the centre. A thread was passed through it and tied around the tablet. The other end of the thread is tied to the glass rod suspended from the stand. To the other end of the glass rod, a pan was tied in which a beaker was placed. After inserting vh/ab tablet into gi segment and lightly pressing the gi segment with tablet by a forceps, the assembly is kept undisturbed for 30 mins to 1 hour.

Then water is added to burette slowly drop by drop into the beaker. The amount of water required to pull out the tablet from the intestinal segment represents the force required to pull the tablet against adhesion.[36-38]

F=0.00981 W/2

W= amount of water.

Two new methods used for the measurement of the force of attachement are the modified Wilhelmy plate method[40] and the modified dual tensiometer method[41]. The modified Wilhelmy plate method consists of a glass plate which is coated with the polymer layer, suspended from a microbalance into a beaker containing mucus. The work done to detach the polymer from the mucus is found. This system has the demerit of not involving any living tissue. The modified dual tensiometer method was developed by Leung and Robinson. The texture analyzers have also been used like the TA-XT2 texture analyzer.[42]

Swelling studies*:*

buccal adhesive dosage forms were weighed individually(w1) and placed separately in Petri dishes containing 4ml of phosphate buffer ph 6.6. at regular intervals(.5,1,2,3,4,5,6 hours) the dosage forms were removed from the Petri dishes and excess surface water was removed using filter paper. The dosage form were reweighed and swelling index(SI) was calculated as follows,[43]

SI= (W2-W1)/W1

In vitro drug release*:*

these are performed in phosphate buffer ph 6.6 , 150ml at 37c in a modified dissolution apparatus which consists of a 250ml beaker and a glass rod attached with a grounded glass disk(2cm dia) as a donor tube. The back surface of the NBAS was attached to the glass disk with an adhesive cynoacrylate adhesive. The donor tube was dipped into the medium and stirred at constant rpm 5mlaliquots were withdrawn at preset times (.08, .16, 1,2,3,4,5,6 hours), filtered through a 0.2 micron filter and absorbance measured at 290 nm.[43]

Rheological measurement of mucoadhesion*:*

Madsen and colleagues determined the interactions between four mucoadhesive polymers (noveon, pemulenTR2, carageenan, SCMC) and a homogenized mucus gel. Using a dynamic rheological method it was seen that the incorporation of a mucoadhesive polymer into mucus produced rheological behavior that was indicative of a weakly cross linked gel.[44]

Novel electromagnetic force transducer technique:

It is remote sensing instrument that utilizes a calibrated electromagnet to detach a magnetic loaded polymer from a tissue. It measures the adhesive force by monitoring the magnetic

force required to exactly oppose bioadhesive force.[45]

Tests for mucoadhesive microspheres:
Adhesion number- It is the ratio of the number of particles attached to the substrate to the total number of applied particles. It is expressed as a percentage.[39]

Falling liquid film method- It as a quantitative, in-situ technique. In this method the percentage of particles which get retained on a mucosal tissue, spread on a plastic slide in an inclined position, when a suspension of the microspheres is allowed to flow down the tissue. The quantification can be done by the aid of coulter-current method.[46]

Other in vitro tests*:*
Park and Robinson determined the effect of various polymer and mucin interactions via the use of fluorescent probes. This technique involved the labeling of the lipid bilayer of cultured human conjuctiva cells with the fluorescent probe pyrene. The adhesion caused a change in the degree of fluorescent which was proportional to the polymer binding.[2]
Batchelor and co workers designed a technique in which fluoroscentely labeled alginate solutions of known rheological profile were delivered onto porcine oesophageal tissue. A washing solution to mimic flow was selected and it was seen that the 20% of dose remained in contact with the tissue for upto 30 mins.[47]

Another imaging technique that did not involve fluorocence was developed by Kockisch and colleagues. Here investigators developed a semi quantitative image analysis based on the technque for the in vitro and in vivi detections of polymers with an affinity for the mucosal surfaces of the oral cavity.[48]
Takaeuchi and co workers measured the mucoadhesion of different polymers via the BIACORE instrumentation. This system was based on the optical phenomenon of surface plasmon resonance(SPR).[48]

CONCLUSIONS

The phenomenon of mucoadhesion is a novel controlled drug delivery approache. The various advantages of the oral mucoadhesive drug delivery systems like prolongation of the residence time of the drug which in turn increases the absorption of the drug are important factors in the oral bioavailability of many drugs.. A number of both in-vitro and in-vivo techniques have been developed for the evaluation of the mucoadhesive drug delivery systems. Mucoadhesive dosage forms extend from the simple oral mucosal delivery to the nasal, vaginal, ocular and rectal drug delivery systems. The most widely studied and accepted polymers for mucoadhesion have been the hydrophilic, high molecular weight, anionic molecules like carbomers. Recently the focus has been on the novel second generation polymers like the thiolated polymers, lectins and lecithins.

REFERENCES

1. S. Ganga, mucosal drug delivery – a review, Vol. 5 issue 6, 2007. http// www.pharmainfo.net. Accesed on 08/07/2010.
2. Andrews G.P., Laverty T.P., Jones D.S., Mucoadhesive polymeric platforms for controlled drug delivery, Eur. J. Pharm. Biopharm., 71: 505–518, (2009).
3. Chowdary K.P.R., Srinivas L., Mucoadhesive drug delivery systems: A

review of current status. *Indian Drugs*., 37(9): 400-406, (2000).
4. Gandhi R.B., Robinson J.R., Bioadhesion in drug delivery. *Ind. J. Pharm. Sci*., 50(3):145-152, (1988).
5. N.A. Peppas, P.A. Buri. Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissue. J Controlled Release, 2 : 257-275, (1985).
6. A.G. Mikos, N.A. Peppas, Measurement of the surface tension of mucin solutions. Int J Pharm, 53: 1-5, (1989).
7. A. Baszkin, J.E. Proust, P. Monsengo, M.M. Boissonnade, Wettability of polymers by mucion aqueous solutions. Biorheology, 27: 503-514, (1990).
8. C.M. Lehr, H.E. Bodde, J.A. Bowstra, H.E. Junginger, A surface energy analysis of mucoadhesion. II: Prediction of mucoadhesive performance by spreading coefficients. Eur J Pharm Sci, 1: 19-30, (1993).
9. C.M. Lehr, J.A. Bowstra, H.E. Bodde, H.E. Junginger. A surface energy analysis of mucoadhesion: Contact angle measurements on polycarbophil and pig intestinal mucosa in physiology relevant fluids. Pharm Res, 9: 70-75, (1992).
10. R.T. Spychal, J.M. Marrero, S.H. Saverymuttu, T.C. Northfield. Measurement of the surface hydrophobicity of human gastrointestinal mucosa. Gastroenterology, 97: 104-111, (1989).
11. D.H. Kaelble, J. Moacanin. A surface energy analysis of bioadhesion. Polymer, 18: 475-482, (1977).
12. K.V. Ranga Rao, P. Buri. Bioadhesion and factors affecting the bioadhesion of microparticles. In: High Performance Biomaterials, M. Szycher, Lancaster PA, (eds.), Technomic Publishing Company, New York, 1988, pp. 259-268.
13. C.T. Reinhart, N.A. Peppas. Solute diffusion in swollen membranes. Part II. Influence of crosslinking on diffusive properties. J Membr Sci , 18: 227-239, (1984).
14. B.O. Bateup. Surface chemistry and adhesion. Int J Adhes Adhesive, July: 233-239, (1989).
15. S. Wu. Surface and interfacial tensions of polymer melts. II. Poly(methyl methacrylate), poly(n-butyl methacrylate), and polystyrene. J Phys Chem, 74: 632-638, (1970).
16. R.J. Good. Spreading pressure and contact angle. J Colloid Interface Sci, 52: 308-313, (1975).
17. J.W.Lee, J.H. Park, J.R. Robinmson, bioadhesive based dosage forms: the next generation, J Pharm. Sci, 89: 850-866, (2000).
18. B.V. Derjaguin, Y.P. Toporov, V.M. Muller, I.N. Aleinkova. On the relationship between the molecular component of the adhesion of elastic particles to a solid surface. J Colloid Interface Sci, 58: 528-533, (1966).
19. Mathiowitz E, Chickering III D.E., Definitions, Mechanisms, and Theories of Bioadhesion, In: Bioadhesive Drug Delivery Systems, Fundamentals, Novel Approaches, and Development, Mathiowitz E, Chickering III D.E., Lehr C.M., (eds.), Marcel Dekker Inc. New York, 2010, pp. 4-8.
20. H.W. Kammer. Adhesion between polymers. Acta Polym, 34: 112, (1983).
21. R.J. Good. Surface energy of solids and liquids. Thermodynamics, molecular forces, and structure. J Colloid Interface Sci, 59: 398-419, (1977).
22. D.Tabor. surface forces and surface interactions. J Colloid Interface Sci, 58: 2-13, (1977).
23. S.S. Voyutskii. Autoadhesion and adhesion of high Polymers. In: Polymer Reviews. 1st edn., vol. 4, H.F. Mark, E.H.

Immergut, (eds.), John Wiley & Sons, New York, 1963, pp. 272.
24. A.G. Mikos, N.A. Peppas. Systems for controlled release of drugs. V. Bioadhesive systems. S T P Pharmacol, 2: 705-716, (1986).
25. Chen J.L., Cyr. G.N., Composition producing adhesion through hydration., In: Adhesion in biological system, Mainly R.S., (ed.), New York, Academic Press, 1963, pp.163-181.
26. G.S. Asane, mucoadhesive gastro intestinal drug delivery system : an overview,vol. 5 issue 6,(2007). http// www.pharmainfo.net. Accessed on 06/07/2010.
27. Sam AP, Van Dan Heuij JT, Tukker J. Mucoadhesion of both film-forming and non- film forming polymeric materials as evaluated with the Wilhelmy plate method. *Int J Pharm*, 53: 97-105, (1989).
28. Gupta A, Garg S, Khar RK. Measurement of bioadhesive strenghs of mucoadhesive buccal tablets: Design of in-vitro assembly. *Indian Drugs*, 30(4): 152-155, (1992).
29. Smart JD. An in vitro assessment of some mucosa-adhesive dosage forms. *Int J Pharm*, 73: 69-74, (1991).
30. Park K. A new approach to study mucoadhesion: colloidal gold staining. *Int J Pharm*, 53: 209-217, (1989).
31. Smart JD, Kellaway IW. Pharmaceutical factors influencing the rate of gastrointestinal transit in an animal model. *Int J Pharm*, 53: 79-86, (1989).
32. DavisSS, Hardy JG, Taylor MJ, Stockwell A, Whalley DR, Wilson CG. The in vivo evaluation of an osmotic device (osmet) Using gamma scintography. *J Pharm Pharmacol*, 36: 740-742, (1984).
33. Krishnaiah YSR, Kondala Rao B, Rama Prasad YV, Satyanarayana S. Gamma scintigraphy : an imaging technique for non-invasive in vivo evaluation of oral dosage forms. *Indian Drugs*, 35(7): 387-398, (1988).
34. H.S. Chhhng, H.Park, P.Kelly, J.R.Robinson, bioadhsesive polymers as platforms for voral controlled drug delivery. Ii: synthesis and evalyation of some swelling, water insoluble bioadhesive polymers, J.Pharm. Sci, 74: 399-405, (1985).
35. S.S.Davis, the design, evaluation of controlled release systems for the gastrointestinal tract, J.Control.Release, 2: 27-38, (1985).
36. Seham S. Abd E Hady, Nahed D. Mortada, Gehanne A. S. Awad, Noha M. Zaki, Ragia A. Taha. Development of in situ gelling and mucoadhesive mebeverine hydrochloride solution for rectal administration. Saudi Pharmaceutical Journal, 11(4): 159-171, (2003).
37. Kok Khiang Peh, Choy Fun Wong. Polymeric films as vehicle for buccal delivery: Swelling, mechanical and bioadhesive properties. Journal of Pharmacy and Pharmaceutical Sciences, 2(2): 53-61, (1999).
38. V. Grabovac, D. Guggi, A. Bernkop-Schnürch, Comparison of the mucoadhesive properties of various polymers, Adv. Drug Deliv Rev, 57: 1713–1723, (2005).
39. J.K.Vasir, K. Tambwekar, S.Garg. Bioadhesive microspheres as a controlled drug delivery system. International Journal of Pharmaceutics, 255: 13–32, (2003).
40. J.D. Smart, I.W. Kellaway, H.E. Worthington, An in-vitro investigation of mucosa-adhesive materials for use in controlled drug delivery, J. Pharm. Pharmacol, 36: 295–299, (1984).
41. S.H. Leung, J.R. Robinson, The contribution of anionic polymer structural

features to mucoadhesion, J. Control. Release, 5: 223–231, (1988).
42. M.J. Tobyn, J.R. Johnson, P.W. Dettmar, Factors affecting in vitro gastric mucoadhesion. I: Test conditions and instrumental parameters, Eur. J. Pharm. Biopharm, 41: 235–241, (1995).
43. H.K. Batchelor, D. Banning, P.W. Dettmar, F.C. Hampson, I.G. Jolliffe, D.Q.M.Craig, an in vitro mucosal model for prediction of the bioadhesion of alginate solutions to the oesophagus, Int. J. Pharm, 238: 123-132, (2002)
44. S.Kockisch, G.Rees, S.Young, J.Tsibouklis, J.Smart, a direct staining method to evaluate the mucoadhesion of polymers from aqueous dispersion, J. Control. Release, 77: 1-6, (2001).
45. Hertzog, B.A., Mathiowitz, E., 1999.Novel magnetic technique to measure bioadhesion. In: Mathiowitz, E., Chickering, D.E.,Lehr, C.M. (Eds.), Bioadhesive Drug Delivery Systems—Fundamentals, Novel Approaches and Development, Marcel Dekker, New York, 1998 pp. 147–171.
46. H.Takeuchi, J.Thongborisute, Y.Matsui, H,Sugihara, H.Yamamoto, Y.Kawashima, novel mucoadhesion tests for polymers and polymer coated particles to design optimal mucoadhesion drug delivery systems, Adv. Drug Deliv. Rev, 57: 1583-1594, (2005).
47. H.Junginger, J.Hoogstraate, J.C. Verhoef, recent advances in buccal drug delivery and absorption- in vitro and in vivo studies, J.Control.Release, 62: 149-159, (1999).
48. S.Cafaggi, R.Leardi, B.Parodi, G.Caviglioli, E.Russo, G.Bignardi, preparation and evaluation of a chitosan salt-poloxamer 407 based matrix for buccal drug delivery, J.Control. Release, 102: 159-169, (2005).