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CHARACTERISATION AND *IN VITRO* EVALUATION OF SOME NATURAL MUCOADHESIVE AGENTS

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ABSTRACT

Mucus is an aqueous gel complex with a constitution of about 95 % water, high molecular weight glycoprotein (mucin), lipid, salts, etc. Mucus appears to represent a significant barrier to the absorption of some compounds. Some natural edible substances are in consideration for candidates as mucoadhesive agents to claim more effective controlled drug delivery as an alternative to the currently used synthetic mucoadhesive polymers. Purified mucoadhesive agents were subjected for evaluation of the said property by various in vitro methods like thumb test, shear stress, falling sphere, Wilhelmy's and Robinson's method. Predetermined concentration of selected natural mucoadhesive agents were used and their potential was measured in terms of force required to detach in all the studied methods except falling sphere method, in which the time period was measured. Materials obtained from natural sources such as *Pithecellobium dulce* (PD), *Acacia sinuata* (AS), *Acacia arabica* (AA), *Abelmoschus esculanthus* (AE), synthetic substances like Sodium carboxy methyl cellulose (SCMC), Hydroxy propyl methyl cellulose (HPMC) and Guar gum (GG) were subjected for study. Mucoadhesive agent obtained from various source possess mucoadhesive characteristics. Natural agents such as AA, AS, AE are less strong than PD, HPMC and GG. Pithecellobium dulce demonstrated significantly different mucoadhesive strength characteristics in the demonstrated in vitro models. Further it may not be toxic, since it is edible.

Key words: Mucoadhesion, Detachment force, Pithecellobium dulce, Controlled drug delivery, In vitro models.

INTRODUCTION

Oral bioavailability is highly desirable property for molecules under investigation in the drug discovery process[1]. The gastrointestinal absorption of orally administered drugs depends upon the permeability of the drug in gastrointestinal mucosa and the gastrointestinal residence time of the dosage form[2]. Mucoadhesion/ bioadhesion are technically related terms, which may

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be expressed as the degree of adherence of a substance to a mucosal area/ biological substrate was reported and was predicted for their adhesion[3–6]. Several drug discovery systems are based on mucoadhesive polymers which are able to swell rapidly and therefore, exhibiting a controlled drug release. Since the first presentation of the concept of mucoadhesion, many attempts have been undertaken to improve the adhesive properties of such polymer systems[7,8]. Many of the substances used in bioadhesive drug delivery formulations are polymers and several are polysaccharides[9].

Thiol groups present in polymers play a role in binding with mucin by means of covalent bonds[10]. Mucosal adhesives or mucoadhesives are synthetic or natural polymers, which interact with the mucus layer covering the mucosal epithelial surface and mucin, a glycoprotein molecule[11]. The main constituents of mucus, irrespective of its origin, are glycoproteins, lipids, water, sloughed epithelial cells, electrolytes, and bacteria. Overall mucus contains water (~95%), glycoproteins and lipids (0.5–5%), mineral salts (0.5–1%) and free proteins (1%)[12]. Mucin is a major constituent of mucus and is found in two forms, soluble secretary mucin and membrane bound mucin. Secretary mucins form various gels due to their high molecular weights and their ability to form intermolecular disulfide bridges but membrane bound mucins lack this. Sugar residues are directly linked to the protein backbone of mucin. Monosaccharides commonly found in mucin are *N*–acetyl galactosamine, galactose, fucose and sialic acid[13].

Mucoadhesive oral dosage forms interact with mucin present in gastrointestinal tract and thus the gastric retention time of the dosage form is increased. For an ideal oral sustained release preparation, mucoadhesive drug delivery systems are one of the reliable and best methods. The exact mechanism of the mucoadhesion is not well known but may be assumed that the agents with more hydrogen bond forming hydrophilic functional groups such as –OH, –COOH, $-SO_3H$ and $-NH_2$ appear to play a major role in wet adhesion[14–16]. Among hydrophilic polymers, polysaccharides are the choice material due to their non toxicity and acceptance by regulating authorities[17]. Polysaccharides like cellulose ethers, xanthan gum, scleroglucan, locust bean gum and guar gum were evaluated for drug delivery system[18–22]. Several other polysaccharides were also been investigated as carriers for colon specific drug delivery. The polysaccharides that are active investigation for colon specific drug delivery include pectin and its salts, chondroitin sulphate, amylose, dextran and chitosan[23]. Aegle marmolus, karaya gum are also under investigation for their mucoadhesive activities. Mucoadhesive drug delivery system utilize the property of bioadhesion of certain water soluble polymers, which became adhesive on hydration[6] and hence can be used for targeting a drug to a particular region of the body for extended period of time[24]. The release behavior of the drugs varies with the nature of the matrix and its complex interaction of swelling, diffusion and erosion process[25]. The detachment forces between polymeric films or tablets and animal mucosa are frequently determined to evaluate the mucoadhesion strength of polymers. The most important interactions contributing to mucoadhesion are Van der Waals interactions and hydrogen bonds between

mucoadhesive polymers and mucus, thus influencing the mucoadhesion strength[26]. A common method for assessing the in vitro mucoadhesion of a particular test substance is the measurement of peak detachment force, the force required to separate a potential bioadhesive from mucus or mucosa. There are three types of measurements tensile testing, in which the stress is applied evenly and perpendicular to the adhesive joint, and peel which limits the stress to a fine line at the edge of the joint[27]

The objective of the present study was to investigate the mechanical and mucoadhesive strength of various natural polysaccharide like substances obtained from various plant sources of *Pithecellobium dulce* (PD), *Acacia sinuata* (AS), *Acacia Arabica* (AA), *Abelmoschus esculanthus* (AE) and related substances like Guar gum (GG), hydroxy propyl methyl cellulose (HPMC), sodium carboxy methyl cellulose (SCMC) obtained from market.

MATERIALS AND METHODS

Materials

Hydroxy propyl methyl cellulose (HPMC), sodium salt of carboxy methyl cellulose (SCMC) and Guar gum were obtained from S. D. Fine chemicals company (India). Available plant sources of *Pithecellobium dulce Benth* (PD), *Acacia sinuata* (AS), *Acacia Arabica* (AA) and *Abelmoschus esculanthus* (AE) were collected from the local area. PD seeds, bark exudates of AS, AA, fresh tender fruits of AE were sources for the natural mucoadhesive agents represented in this article. Other chemicals used were of analytical grade.

Preparation of mucoadhesive agents

The mucoadhesive agent was isolated from the natural source by the method prescribed earlier[28] in three batches on a laboratory scale.

Isolation of mucoadhesive agent from PD

Fresh seeds of PD were washed with running tap water and the epicarp was removed. 50 g of kernel powder was made into slurry with water and poured in to 900 mL of distilled water. The solution was boiled for 1 hour with continuous stirring under a boiling water bath. The solution was kept overnight undisturbed. The supernatant liquid was centrifuged to obtain a clear supernatant liquid. Thus obtained liquid was poured into thrice the volume of acetone and stirred to get the precipitate. The product was filtered and washed thoroughly with acetone and dried under reduced pressure. The particles between 75 and 150 microns were collected and used for the studies.

Isolation of mucoadhesive agent from AS and AA

The gum exudates obtained from AS and AA are ground to powder to make a slurry. The slurry was subjected to the above mentioned process to obtain the mucoadhesive agent.

Isolation of mucoadhesive agent from CC and AE

Fresh tender fruits of CC and AE were collected and made slurry with water. The slurry was subjected to the above mentioned process to obtain the mucoadhesive agent.

In vitro mucoadhesive strength measurement

The mucoadhesive strength of the agents isolated from the natural sources and obtained from the market was tested by the methods prescribed. Measurements were performed in four replicate.

Thumb test

Thumb test[29] is an initial screening method and is useful in identifying a material, whether it possess some adhesive character or not. Though the method does not provide any statistical data, but useful in initial screening test parameters. The test is being carried out by means of the force required or the difficulty to pull out the thumb from other finger, when kept in contact by the mucoadhesive agent in specific concentration and volume, by means of force and contact time.

Shear stress method

Several methods have been reported and most of the cases of in vitro models are based on the measurement of shear or tensile strength[14,30]. Two glass plates of 2.5 x 7.5 cm were fixed with the help of an adhesive (Araldite). A nylon thread was sandwiched in between the glasses. Another glass plate of same dimension has been taken and one end was fixed with another nylon thread, which was then passed on a pulley and at the end, provision was provided to add weight. The sandwiched plate was fixed on a flat table and another glass plate fixed (Fig. 1) with nylon thread was kept in contact on the sandwiched plate by placing appropriate concentration of mucoadhesive agent in specified volume and allowed at specified intervals. Finally, force required to detach the plates were measured as a means of adhesive strength.

Falling sphere method

Method reported earlier[31] is simple and easier to follow. Accordingly, a 10 percent mucus solution prepared from fresh goat intestine was poured in a channel and mustard grains coated with various concentration of mucoadhesive agents were introduced on the top of the solution in the channel and the time taken to travel from initial point to end point was measured as a characterization of adhesive strength.

Robinson's method

Mucoadhesive strength characterization reported by Park and Robinson[32] was used with slight modification. Fresh section of animal tissue from fundus portion of goat intestine on a glass vial, was fixed facing mucosal side out and kept in simulated gastric fluid (pH 1.2) without

pepsin. Another portion of mucus side exposed tissue was kept over a rubber stopper and secured with an aluminium cap. The mucoadhesive agents were uniformly spread on the exposed mucus layer (later case), and kept in contact with the former tissue and was then connected with a pan in which the weight can be raised. At specific intervals, applied weight and the force required to detach was measured as mucoadhesive strength.

Wilhelmy's method

Method reported by Smart[33] was used after slight modification for the characterization of mucoadhesive agent. A small glass plate (2 x 5 cm) was coated with the mucoadhesive agent. Fresh goat intestine was utilized to collect the mucus gel. After washing the intestine slowly and the contents of the intestine was collected scrapping softly. Diluted with equal volume of water and centrifuged to isolate the gel like mucus at middle portion. Nylon thread was attached at one end of the glass plate and passed over a pulley. Provision was given to raise the weight at the end of the nylon thread. At specified intervals, weight was added to detach the coated glass plate from gel and the force required to detach was measured as mucoadhesive strength.

Data analysis

The shear or tensile measurements between mucoadhesive agents and tissue mucosa were determined by recording the force required to detach both. The mean and standard error mean of the findings were calculated. The results obtained from the in vitro mucoadhesive strength evaluations were analyzed statistically for significant difference.

RESULTS AND DISCUSSION

The results obtained are shown and the measurement of mucoadhesive strength was appraised based on various parameters, which are unique in nature. Though the exact method to measure the mucoadhesive strength in a single experiment is not possible, the conducted study will definitely provide an appraisal of mucoadhesive agents for their efficiency in delivering a controlled release formulation targeted to GIT by means of mucoadhesion.

Mucoadhesive properties

The shear stress or tensile testing by various methods reported is useful parameters in determining mucoadhesive characterization of a mucoadhesive agent. In the present study, mucoadhesive agents with different mucoadhesive characteristics were incorporated in various in vitro models to investigate their influence on adhesion. The mechanism of mucoadhesion was unclear although it may be assumed that the subjected product was acting as a mucoadhesive was put forward. This study, reported here in, was an effort to establish a standard reference system to investigate the potential mucoadhesion of novel mucoadhesive agents in in vitro experimental models.

Thumb test

Though the thumb test does not involve any instrument, it has provided a rough idea about mucoadhesiveness of the products taken for study.

Shear stress method

Shear stress testing provides transparency in finding products capable to adhere. The adherence is characteristic for molecules and the application area. It is been observed that there is a statically significant difference in the concentration of the product applied. The concentration, product force required to detach with respect to time interval is focused in and Fig. 1. It has shown that the force required is increased to a higher level of 500 when the time allowed is 30 minutes. This is probably the time required for effective mucoadhesion. From the table, it is possible to observe that the PD exhibited a high degree of tensile strength and is concentration and time dependent. Though other products like HPMC, and AS are gaining more tensile strength after allowing the contact time to a specific period, PD produced the maximum force required to detach in the same specified time duration of 30 minutes.

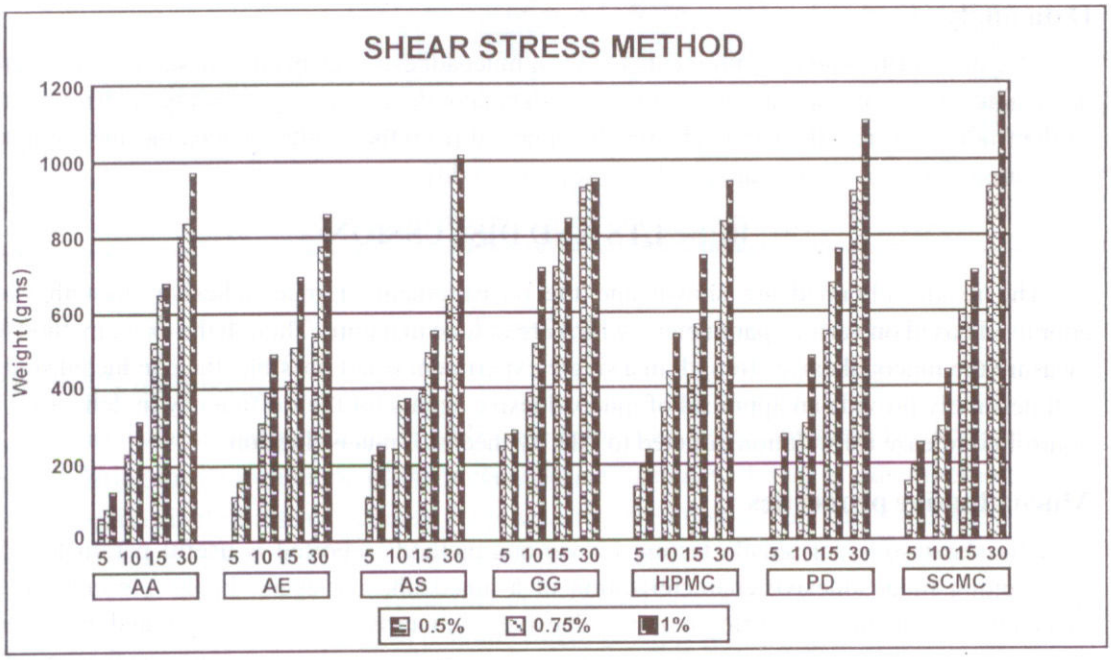

Figure 1

Falling sphere method

Mucus is the major portion present in the lumen of gastrointestinal tract. Mucus consists of mainly water about 95%. Remaining are glycoprotein, mineral salts, carbohydrates etc. As per

the method prescribed earlier[31], the static and dynamic performance of the mucoadhesive agent is measured by means of time to travel between two specified points.

Natural products of AS and PD exhibited a long duration of time of about 11–12 seconds. The behavior of passing the distance might have been influenced by the characteristics of the individual product probably due to attraction between the constituents of mucus liquid and the mucoadhesive products. A wide variation is observed statistically between PD and HPMC in this demonstration. PD showed a better concentration dependant behavior than other agents. The values obtained are shown in Fig. 2.

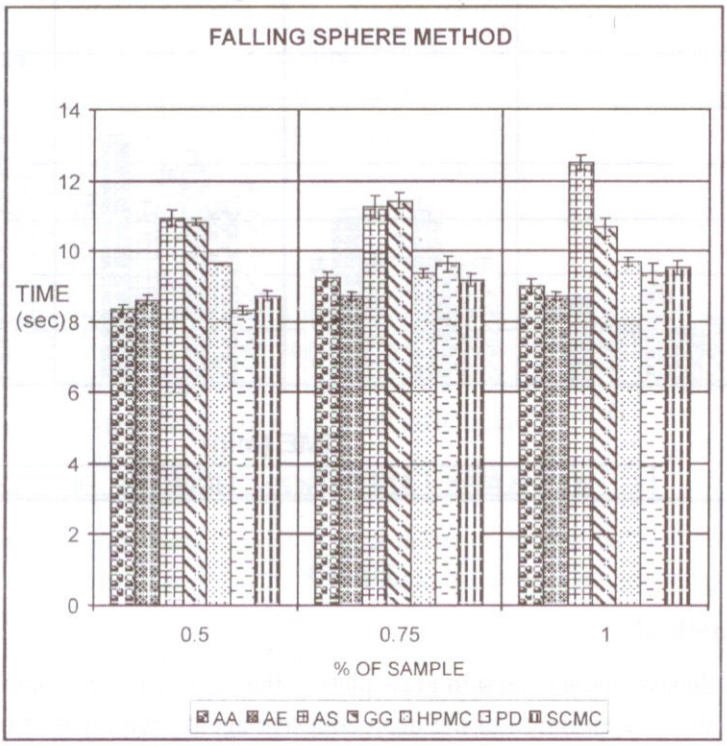

Figure 2

Robinson method

Tensile strength measurements are more or less based on the force required to break the adhesion between either mucus or a polymer surface and mucoadhesive agent. This may reflect the effects of charge density, hydrophillicity, ionic strength and pressure applied during the process between two membranes on mucoadhesion. The values obtained are focused in Fig. 3. PD required more force to detach, when compared to other products. As the concentration of mucoadhesive agent is increased, the degree of mucoadhesiveness also increased. This may be

due to attraction between the hydrophilic groups present in mucoadhesive agent and the binding sites of animal intestinal membrane. Though the degree of adhesion of taken mucoadhesive agents was different in other tests, PD exhibited a high detachment force in other tests too.

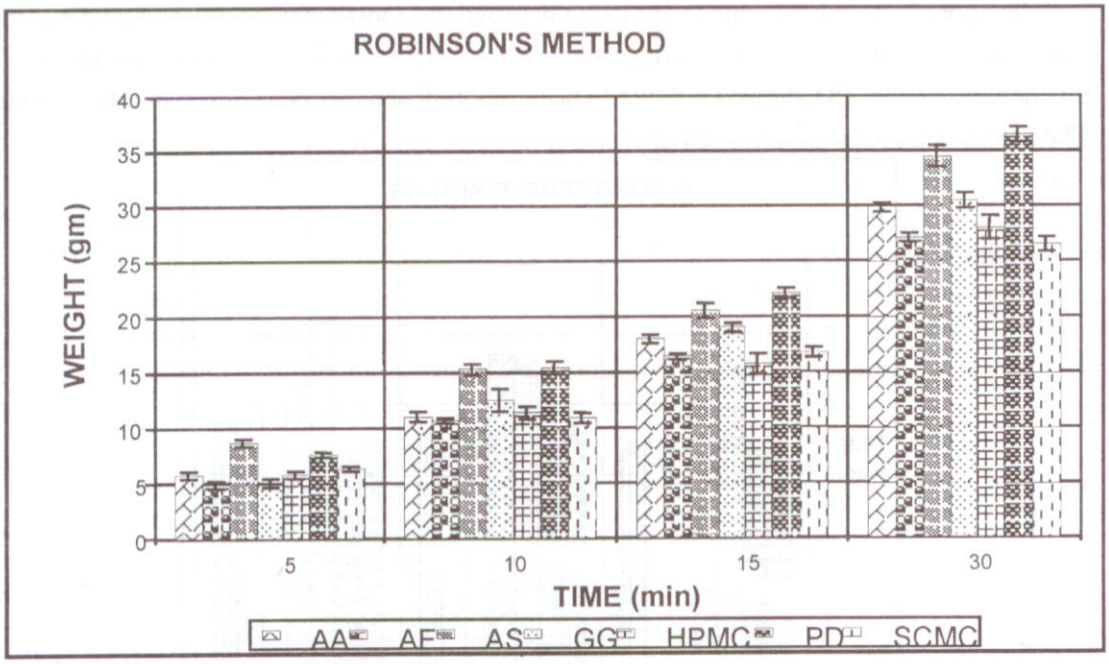

Figure 3

Wilhelmy's method

The mucoadhesive agent coated in glass plate is the area, where the binding force can act. On considering the concentration of the mucoadhesive agents, with that of other method, it is less. Hence, relatively a less detachment force was observed through out this experiment. The values are shown in Fig. 4. HPMC has shown the maximum degree of mucoadhesion. The variation may be attributed to the availability of attraction producing factors. The factors may be physical attraction forces, chemical bonding forces etc. It is interesting to observe that other natural mucoadhesive agents subjected were also constituted of related chemical moieties. The resulting variation might be site specific due to the uniqueness of natural mucoadhesive molecules.

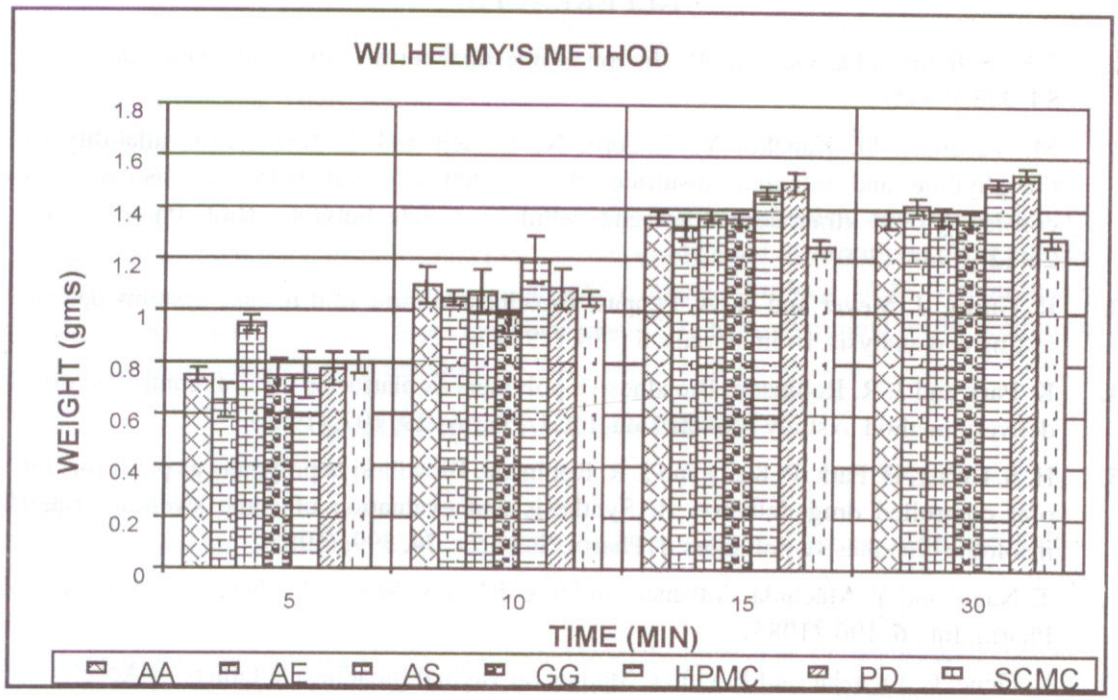

Figure 4

CONCLUSION

The mucoadhesive agent isolated form pithecellobicum dulce seeds can be considered as a candidate for controlled release of drugs through the development of mucoadhesive drug delivery system. The peak force of detachment was observed in a concentration and time dependant manner. Mucoadhesive solid dosage forms for gastrointestinal administration has an advantage of protecting dosage forms from stomach and or small intestine degradation due to pH. It also enhances longer transit time of dosage forms, thereby the drug absorption will be increased to a greater extent.

Mucoadhesion is a channel that has a great potential for pharmaceutical controlled release dosage forms design and patient compliance. Polymers are playing a vital role in the process of mucoadhesion. The development of mucoadhesive dosage forms with natural polymer depends on the availability of polymers with expected adhesiveness in mucosal area, stability and non toxicity. As the natural polymers studied here, particularly the mucoadhesive agent obtained from *Pithecellobium dulce* is edible, non–toxic, non–irritant, stable in aqueous medium and found to have adhesiveness in in vitro models, will definitely opens up a new era in mucoadhesion.

REFERENCES

1. J. E. William and L. Georgio, Prediction of intestinal permeability, Adv. Drug Del. Rev., **54**, 273 (2002).
2. M. Yasunori, O. Kanako, Y. Shigeru, N. Tsuneji and T. Kozo, Bioavailability of theophylline and thiamine disulfide incorporated into mucoadhesive microspheres consisting of dextran derivatives and cellulose acetate butyrate. Biol. Pharm. Bull., **26(12)**, 1744, (2003).
3. R. Gurny, J. Meyer and N. A. Peppas, Bioadhesive intra–oral release systems design, testing and analysis. Biomaterials, **5**, 336, (1984).
4. K. Park and J. R. Robinson, Bioadhesive polymers as platforms for oral controlled drug delivery method to study Bioadhesion, Int. J. Pharm., **19**, 107, (1984).
5. H. S. Ch'ng, H. Park, P. Kelly and J. R. Robinson, Bioadhesive polymers as platforms for oral controlled drug delivery. II. Synthesis and evaluation of some swelling, water insoluble bioadhesive polymers, J. Pharm. Sciences, **74**, 399, (1985).
6. T. Nagai and Y. Machida, Advances in Drug delivery. Mucosal adhesive dosage forms, Pharm. Int., **6**, 196, (1985).
7. L. Genc, C. Oguzlar and E. Gûler, Studies on vaginal bioadhesive tablets of Acyclovir. Pharmazie, **55**, 297, (2000).
8. A. Bernkop – Schnûrch, V. Schwarz and S. Steininger, Polymers with thiol groups : a new generation of mucoadhesive polymers. Pharm.Res., **16**, 876, (1999).
9. M. U. Adikwn, J. E. Ojile and N. D. Okoli, Effect of dissolution fluid and presence of hog intestinal barrier on the dissolutions of theophylline from bioadhesive granules, Boll. Chim. Farm., **139**, 242, (2000).
10. T. K. Mandal, Swelling controlled release system for the vaginal delivery of miconazole. Eur. Jour. Pharm. Biopharm., **50**, 337, (2000).
11. A. Alka, R. K. Khar and A. Javed, Mucoadhesive drug delivery systems, Drug dev. Ind. Pharm., **23(5)**, 489, (1997).
12. M. J. Rathbone and J. Hadgraft, Absorption of drugs from the human oral cavity, Int. J. Pharm., **74**, 9, (1991).
13. D. M. Carlson, Structures and immunochemical properties of oligosachharides isolated from pig submaxillary mucus, J. Biol. Chem., **243(3)**, 616, (1968).
14. L. C. James and N. C. Gilman, Compositions producing adhesion through hydration. In: R. S. Manly (Eds) Adhesion in biological systems, Academic Press, New York, (1970), p.178.

15. B. G. Rajesh and J. R. Robinson, Bioadhesion in drug delivery, Ind. Jour. Pharm. Sci., **50(3)**, 145 (1988).

16. L. Claws–Michael, A. B. Joke, H. S. Etienne and E. J. Hans, In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers, Int. Jour. Pharm., **78**, 43, (1992).

17. M. C. Benferoni, S. Rossi, M. Tamayo, J. L. Pedraz, A. Dominguz_Gil and C. Caramella, On the employment of ë– Carrageenanin a matrix system, I. Sensitivity to dissolution medium and comparison with Na caboxy methyl cellulose and xanthan gum. J. Control. Release, **26**, 119, (1993).

18. J. L. Ford, M. H. Ribinstein, F. McCaul, J. E. Hogan and P. J. Edgar, Importance of drug type, tablet shape and added diluents on drug release kinetics from hydroxypropyl methyl cellulose matrix tablets. Int. J. Pharm., **40**, 223, (1987).

19. M. M. Talukdar and J. Plaizier–Vercammen, Evaluation of xanthan gum as a hydrophilic matrix for controlled release dosage form preparations. Drug Dev. Ind. Pharm., **19(9)**, 1037, (1993).

20. S. Risk, D. Duru, D. Gaudy and M. Jacob, Natural polymer hydrophilic matrix: influencing drug release factors, Drug Dev. Ind. Pharm., **20(16)**, 2563, (1994).

21. J. Sujja–areevath, D. L. Munday, P. J. Cox and K. A. Khan, Release characteristics of diclofenac sodium from encapsulated natural gum mini–matrix formulations, Int. J. Pharm,, **139**, 53, (1996).

22. P. Khullar, R. K. Khar and S. P. Agarwal, Evaluation of guar gum in the preparation of sustained–release matrix tablets. Drug Dev. Ind. Pharm., **24(11)**, 1095, (1998).

23. Y. S. R. Krishnaiah, V. Satyanarayana, B. Dineshkumar and R. S. Karthikeyan, In vitro drug release studies on guar gumbased colon targeted oral drug delivery ststems of 5–Fluorouracil, Eur. Jour. Pharm. Sci.., **6**, 185, (2002).

24. K. R. Kumath and K. Park, Mucoasal adhesive preparations, Encyclopedia of Pharmaceutical Technology, In: J.Swarbrick, J.C. Boylan, Eds. Marcel Dekker, New York, **10**, 133, (1994) p. 133.

25. P. Colombo, R. Bettini, G. Massimo, P. L. Catellani, P. Santi and N. A. Peppas, Drug diffusion front movement is important in drug release control from swellable matrix tablets. J. Pharm Sci., **84(8)**, 991, (1995).

26. B. Marija, V. Tomaz, K. Mojca, D. Ales, G. Iztok and M. Ales, The correlation between zeta potential and mucoadhesion strength on pig vesical mucosa, Biol. Pharm. Bull., **26(5)**, 743, (2003).

27. J. J. Sarah and C. P. Alen, In vitro assessment of the mucoadhesion of cholestyramine to porcine and human gastric mucosa, Eur. J. Pharm. Biopharm., **52**, 121, (2001).

28. S. K. Baveja, K. V. Ranga Rao and A. Jagdish, Examination of natural gums and mucilages as sustaining materials in tablet dosage forms, Indian J. Pharm. Sci., **50(2)**, 89, (1988).

29. N. A. Peppas, and P. A. Buri, Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues, J. Control. Release, **2**, 257, (1985).

30. Y. Madhusudan Rao, G. Vani and R. Balarameshacharry, Design and evaluation of mucoadhesive drug delivery systems. Indian drugs, **35(9)**, 559, (1998).

31. K. V. R. Rao and P. Buri, A novel in situ method to test polymers and coated micro particles for Bioadhesion. Int. Jour. Pharm, **52**, 265, (1989).

32. H. Park and J. R. Robinson, Physico–chemical properties of water insoluble polymers important to mucin epithelial adhesion. J. Control. Release, **2**, 47, (1985).

33. J. D. Smart, I. W. Kellaway and H. E. C. Worthington, An in vitro investigation of mucosal adhesive materials for use in controlled drug delivery, J. Pharm. Pharmacol., **36**, 295, (1984).

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