

TITLE

SUSTAINED RELEASE DRUG DELIVERY SYSTEM.

TECHNICAL FIELD

The present invention describes sustained/controlled drug delivery system so as to
5 increase the bioavailability of a drug use of mucoadhesive, swelling polymers and their
derivatives as a carrier for achieving sustained/controlled drug delivery, particularly for
the drug acyclovir and the mucoadhesive swelling polymers like Carbopol, PEO, Sodium
alginate, Hypromellose, hydroxypropyl cellulose, sodium carboxy methyl cellulose,
Eudragits, Chitosan and its derivatives.

10 BACKGROUND OF INVENTION

Some of the drug and macromolecules are poorly absorbed across the mucosal membrane
or those that are sparingly/slowly soluble resulting in the fact that during limited time
they remain in the gastrointestinal tract, enough of them is not released or absorbed and
major portion passes out unabsorbed. One of the greatest challenge to the scientists is
15 drug delivery of such molecules which show poor bioavailability as limited amount of the
dose reaches the plasma in a specified period. Low bioavailability leads to variation in
the drug absorption amongst the patients and become very difficult to administer the
effective dosage. Hence, it has been a long awaited requirement of the drug delivery
scientists to enhance the bioavailability of the such orally administered drugs. It was
20 thought that a carrier which can deliver drug in intact form at target site, stays there for
prolonged time and increases the permeability of the mucosal membrane to achieve
unhampered and better absorption of the drug shall be a remedy, provided the carrier is
safe and does not affect the properties of the mucosal epithelium. Acyclovir, one of the
subject matters of present invention is a drug in this category: poorly water soluble, has
25 poor and variable oral bioavailability (10–20%), the elimination half-life of aciclovir is

approximately 3 hours, is renally excreted, partly by glomerular filtration and partly by tubular secretion.

The present invention deals with carriers, including but not limited to polymers like chitosan (and its derivatives not limited to Thiolated chitosan, Trimethyl chitosan, hypermellose, polyethylene oxide, Carbopol, sodium alginate, sodium carboxymethyl cellulose, xanthan gum and similar products, derivatives of these polymers, their various combinations and the like which are mucoadhesive, swellable and which increases the G.I. retention, bioavailability of the drug during the delivery.

PRIOR ART

10 Genta et al.¹ (1997), have described use of microparticles of polylactides and polylactide-co-glycolide to achieve sustained delivery of acyclovir.

Thanau et al.² (2001) have extensively reviewed methods of use of chitosan and trimethyl chitosan as absorption enhancers of hydrophilic macromolecular drugs including peptide drugs. The experiments conducted in pigs and rats showed increased bioavailability of a peptide drug.

There are several examples of use of thiolated chitosan and TMC as drug delivery vehicle of macromolecular polymeric drugs such as insulin, laminin peptide, FITC-dextran 4000 as model lipophilic drug³⁻⁵.

Grafted chitosans have been used as absorption enhancers in drug delivery systems.

20 Rokhade et al.⁶ (2007) have described semi-interpenetrating polymer network (IPN) of microspheres of acrylamide grafted on dextran and chitosan prepared by emulsion cross-linking method using glutaraldehyde as a cross linker and encapsulation of acyclovir into the microspheres and drug release was found to be extended upto 12 hours. They have,

however, used the drug theophylline and its physicochemical properties are totally different than Acyclovir in view of the fact that its bioavailability is 100%..

Hu et al.⁷ (2009) have used stearic acid grafted chitosan oligosaccharide which form micelle wherein the drug is incorporated for improved absorption of the drug

5 Doxorubicin.

US6340475⁸ discloses a controlled-release oral drug dosage form for releasing a drug whose solubility in water is greater than one part by weight of said drug in ten parts by weight of water, said dosage form comprising a solid polymeric matrix with said drug dispersed therein at a weight ratio of drug to polymer of from about 15:85 to about 80:20, 10 said polymeric matrix being one that swells upon imbibition of water thereby attaining a size large enough to promote retention in the stomach during said fed mode, that releases said drug into gastric fluid by the dissolution and diffusion of said drug out of said matrix by said gastric fluid, that upon immersion in gastric fluid retains at least about 40% of said drug one hour after such immersion and releases substantially all of said drug within 15 about eight hours after such immersion, and that remains substantially intact until all of said drug is released. In other claims that pertain to several groups and examples of drugs, the claims limit the period required for release of substantially all of the said drug to within about 10 hours. In this patent the dosage form is designed for treating the local disease of stomach like Ulcer not for systemic absorption.

20 US 20040185105⁹ discloses a controlled release dosage form comprising a polymer matrix and a pharmacologically active agent dispersed in the said polymer matrix comprised of a biocompatible, hydrophilic polymer where the dosage form upon imbibition of water swells unrestrained dimensionally to a size effective to promote gastric retention and maintains its size for an extended period of time before it is 25 diminished by erosion.

US20070160678¹⁰ discloses the invention of microcapsules of polymer insoluble in GIT fluid. The *in vivo* release pattern of acyclovir has also been described. Invention showed that 80% acyclovir was released in first 3 hrs. There is no information on the bioavailability and reduction of dosing frequency.

- 5 None of the above cited references describe *in vivo* studies conducted to know exactly how the drug works in the *in vivo* atmosphere. The present invention along with disclosure on novel and improved ways to use of various mucoadhesive carriers used to deliver drugs having low bioavailability describes *in vitro* as well as *in vivo* studies on controlled release compositions conducted and illustrated more particularly on a drug
- 10 acyclovir entrapped in chitosan, thiolated chitosan and Trimethyl chitosan or acyclovir associated with other polymers to achieve an objective of improving the uniformity of drug release and to increase the period for which the drug is released from the dosage form. The methods and compositions of this invention illustrated on Acyclovir are applicable on any other drug having same or similar properties as well as problems in the
- 15 context of drug delivery, efficacy and treatment.

SUMMARY OF INVENTION

- The invention discloses a controlled release dosage form comprising a therapeutically effective amount of a pharmaceutically active agent that would release in about 12 hours not more than about 90% of the said active agent in a simulated gastric juice in a first
- 20 order rate of release in a USP type 1 dissolution test, and not containing a solubilizer or a swelling enhancer or both, comprising (a) a tablet made from polymer matrix of at least two biocompatible polymers, at least one of which is mucoadhesive, the said pharmaceutically active agent and pharmaceutically permitted excipients; the said tablet capable of rapid swelling without disintegration in the said simulated gastric juice to a
- 25 size that shall result in its gastric retention in the stomach and start controlled release of

the said active agent by starting controlled erosion immediately after coming into contact with the said gastric juice, or (b) microspheres of ungrafted chitosan or a chitosan derivative, or Carbopol incorporating the said active agent, wherein the said pharmaceutically active agent is not a polymeric molecule and after administration in stomach, the said microspheres adhere to the gastric mucosa for a long time releasing the active agent in a controlled way. The two polymers illustrated in the tablet of this invention are Carbopol 974P and Polyethylene oxide; however, any other pair of polymers can be used that shall result, in an appropriate proportion that have the drug release characteristics defined above. The permitted pharmaceutical excipients of this invention comprise a binder, a diluent, a pH modifier, a glidant, a lubricant, a film former, an anti-adherent, a coating agent, a colorant.

In one particular illustration of this invention, the tablet comprises Acyclovir, Carbopol 974P, Polyethylene oxide, Avicel PH 101, Povidone K30, Magnesium stearate, and Colloidal silicon oxide. More particularly, the said tablet contains following ingredients for every 1000 mg of the dosage form: Acyclovir 763.37mg, Carbopol 974P 75mg, Polyethylene oxide 25mg, Avicel PH 101 93.83mg, Povidone K30 30mg, Magnesium stearate 7.5mg, Colloidal silicon oxide 5.0mg.

The chitosan derivative used in making microspheres comprise Trimethyl chitosan or Thiolated chitosan. Mixtures may also be used, if expedient to achieve the desired drug release profile.

The microspheres of this invention may be packed in a sachet or are used as an ingredient with optional addition of permitted pharmaceutical ingredients and excipients to make a solid unit dosage form comprising a tablet or/and a capsule.

This invention also discloses a method of orally administering a therapeutically effective amount of a pharmaceutically active agent from a controlled release dosage form to a patient, the said dosage form would release in about 12 hours not more than about 90% of

the said active agent in a simulated gastric juice in a first order rate of release in a USP type 1 dissolution test, and not containing a solubilizer or a swelling enhancer or both, comprising (a) a tablet made from polymer matrix of at least two biocompatible polymers, the said pharmaceutically active agent and pharmaceutically permitted excipients; the said tablet capable of rapid swelling without disintegration in the said simulated gastric juice to a size that shall result in its gastric retention in the stomach and start controlled release of the said active agent by starting controlled erosion immediately after coming into contact with the said gastric juice, or (b) microspheres of ungrafted chitosan or a chitosan derivative incorporating as the said active agent, wherein the said pharmaceutically active agent is not a polymeric molecule and after administration in stomach, the said microspheres adhere to the gastric mucosa for a long time releasing the active agent in a controlled way.

The polymers used in the method of this invention illustrated in this invention comprise Carbopol 974P and Polyethylene oxide. However, any other pair of polymers providing the defined drug release profile as above may be used in their place.

The pharmaceutical active illustrated for method of this invention is Acyclovir. However, any other pharmaceutically active agent with same properties as Acyclovir and same drug release problems as Acyclovir may be used in its place.

The permitted pharmaceutical excipients used in the method of this invention comprise a binder, a diluent, a pH modifier, a glidant, a lubricant, a film former, an anti-adherent, a coating agent, a colorant.

The controlled release dosage form of the method of this invention comprises Acyclovir, Carbopol 974P, Polyethylene oxide, Avicel PH 101, Povidone K30, Magnesium stearate, Colloidal silicon oxide, Ethanol.

In a particular embodiment of method of this invention, every 1000 mg of the dosage form the said controlled release dosage form comprises Acyclovir 763.37mg, Carbopol

974P 75mg, Polyethylene oxide 25mg, Avicel PH 101 93.83mg, Povidone K30 30mg ,
Magnesium stearate 7.5mg, Colloidal silicon oxide 5.0mg.

Chitosan derivative used in making microspheres of this invention comprises Trimethyl
chitosan or/and Thiolated chitosan.

- 5 This invention also comprises a method wherein the said microspheres are packed in a
sachet or are used as an ingredient with optional addition of other pharmaceutically
permitted ingredients and excipients to make a solid unit dosage form comprising a tablet
and a capsule.

A process of making oral dosage form of this invention comprises, for tablet form, the
10 steps of making wet granulation of a mixture comprising the said pharmaceutically active
ingredient, the polymers and excipients, adding a gliadent and pressing into a tablet.

A process for making microspheres of this invention comprises preparing a solution of
chitosan or a chitosan derivative in acetic acid, adding aqueous solution of the
pharmaceutically active agent, adding this mixture to continuous phase consisting of light
15 liquid paraffin and heavy liquid paraffin (1:1) containing a surfactant under constant
stirring to form a water-in-oil emulsion, adding gluteraldehyde drop wise over a period of
time, continuing stirring for a period of time, separating the microspheres formed by
centrifugation, washing with petroleum ether to remove liquid paraffin, suspending in a
sodium bisulfite solution and stirring for a period of time to remove residual
20 gluteraldehyde, washing finally with distilled water, drying the microspheres.

BRIEF DESCRIPTION OF FIGURES AND LEGENDS

Figure 1. SEM photomicrograph of Chitosan (A), N-TMC (B), Thiolated chitosan (C),
Carbopol (D) and Methocel K15M (E) microsphere (X 10,000). Scale bar = 50 μ m

Figure 2. % Swelling measurement of microspheres formulations. Data are represents as
25 mean $\pm$ SD (n =3)

Figure 3. % *In vitro* drug release of acyclovir from microspheres formulations. Data are
represented as mean $\pm$ SD (n =3)

Figure 4. Penetration of 6-CF (1 ml of 0.3 % w/v) as fluorescence probe across the duodenum section of intestinal mucosa after 3 hr administration as solution (A), thiolated chitosan (B), TMC (C), chitosan (D), Carbopol (E) and Methocel K15M (F) microsphere formulation (X 450). Scale bar = 250 μ m; MU = Mucosal surface; VI = Villi.

- 5 **Figure 5.** Plasma concentration of acyclovir after administration as drug solution and microsphere formulations. Data are represents as mean $\pm$ SD (n =3)

Figure 6. Showing plasma concentration-time profile of this simulation

Figure 7. Showing the comparison of simulated plasma concentration-time profiles of Acyclovir IR 200 mg & 400 mg

- 10 **Figure 8.** Plasma levels of multiple dose administration of Acyclovir IR 200

Figure 9. Shows the comparison of plasma concentrations of Acyclovir IR 200 mg during the dosing day on day 1 and day 5.

Figure 10. Comparative plasma concentration profiles of acyclovir immediate release and acyclovir controlled release tablets.

- 15 **Figure 11.** Predicated *in vitro* drug release profile of Acyclovir CR formulation

Figure 12. In vitro drug release profile from gastroretentive tablets prepared using Carbopol 974P as polymer (Batch Acy-ER-1A & Acy-ER-1B).

Figure 13. In vitro drug release profile from gastroretentive tablets prepared using Polyethylene oxide (Batch Acy-ER-2A & 2B).

- 20 **Figure 14.** In vitro drug release profile from gastroretentive tablets prepared using combination of Carbopol 974P and Polyethylene oxide (Batch 3A, 3B & 3C).

Figure 15. Comparative In vitro drug release profile from different batches of gastroretentive tablets and target release profile for sustained release acyclovir formulation generated by computer simulation study.

- 25 **Figure 16.** % Swelling profile from different batches of gastroretentive tablets.

Figure 17. Mucoadhesive strength measurement of different batches of gastroretentive tablets.

DETAILS OF INVENTION

- The polymer/s of present invention include water soluble or water insoluble one/s, further
- 30 including chitosan and chitosan derivatives exemplified by thiolated chitosan and trimethyl chitosan, Carbopol, HPMC, alginates, pectins, Eudragits, Hypromellose,

polyethylene oxide their combinations and the like. The pharmaceutically acceptable excipient of present invention may be selected from the group of binder, diluent, pH modifier, glidant, lubricant, film formers, anti-adherent, coating agents colorant and the like.

- 5 The sustained release delivery system may be in the form of single or multiple units. It can be in the form of tablets, capsules, microspheres, pellets, granules, and like. It can be packed in blister, bottles, sachets and like.

The delivery system may be prepared by the processes well known in the art of formulation development, such as wet granulation, dry granulation, direct compression,
10 blending, extrusion & spheronization, coating and the like.

The sustained release delivery system of present invention may be administered as the dosage required by the patient.

The present invention also deals with pharmacokinetic simulations for a drug, more particularly Acyclovir controlled release (CR) to:

- 15
- determine the dose and absorption rate required for Acyclovir CR to achieve comparable C_{max} and C_{min} with that of immediate release (IR).
 - predict the blood levels of Acyclovir CR and compare with that of IR.
 - simulate in-vitro dissolution profiles from absorption rate constants.

- 20 One of the polymer used for sustained release is Chitosan which is a polysaccharide comprising copolymers of glucosamine and N-acetyl glucosamine. It is biodegradable, biocompatible, mucoadhesive polymer and has been used in the formulation of particulate drug delivery system. Chitosan opens the epithelial tight junctions in concentration and pH dependent way. At acidic pH, chitosan is effective in increasing the
25 permeability of certain drugs, but as the pH increases, its effectiveness is decreased.¹¹

So, to overcome the solubility limitations of chitosan at increased pH values, an N-Trimethyl quaternized chitosan (N-TMC) derivative was synthesized and used for comparison studies. N-TMC has shown potential as an absorption enhancer across intestinal epithelial cells even in neutral environments.² The mechanism of drug
5 absorption enhancement by N-TMC is the same as that of chitosan.¹² It opens the tight junctions between the adjacent epithelial cells by means of interaction between the positive charges on the polymer molecules and the anionic components on the surface of epithelial cells.

Another polymer selected in the present study is thiolated chitosan. Thiolated chitosan
10 represents a new promise in the field of mucoadhesive polymers. The higher mucoadhesive properties of thiomers are reported to intensify the contact with gastric mucosa, providing an increased epithelial permeability for many drugs.¹³⁻¹⁵ In addition, these polymers are reported to increase the intestinal permeability of drugs that shall be beneficial for increasing the intestinal permeability of acyclovir along with
15 mucoadhesiveness.

In one of the embodiment of this invention drug Acyclovir which is used for the treatment of herpes simplex virus infections, is most widely used drug for infections such as cutaneous herpes, genital herpes, chicken pox, varicella zoster infections and herpes keratitis.¹⁶ Acyclovir is currently marketed as capsules, tablets and suspension for oral
20 administration, intravenous injection and topical ointment. Oral acyclovir is mostly used in dose strength as 200mg tablets, 5 times a day. In addition, long term administration of acyclovir (six month or longer) is required in immunocompetant patient with relapsing herpes simplex infection.¹⁷ The presently available conventional therapy is associated with a number of drawbacks such as highly variable absorption and low bioavailability
25 (10-20%) and requirement of frequent administration (5 times a day) resulting in poor patient compliance. Furthermore, with increase in dose, there was seen decrease in

bioavailability. Moreover, the mean plasma half life of the drug being 2.5 hrs, necessitates repeated administration of high dose of the drug (200mg five times a day). As a result, most of the drug is excreted in the faeces (50-60%) in unabsorbed form.¹⁸ Acyclovir is soluble in acidic pH and is predominantly absorbed from upper regions of gastro intestinal tract (GIT).¹⁹

One of the embodiment of the present investigation discloses mucoadhesive microspheres for gastroretentive delivery of a drug. Polysaccharide Chitosan, thiolated chitosan, Trimethyl Chitosan, Carbopol 71G and Methocel K15M and their various combinations were used as mucoadhesive polymers. Microsphere formulations were prepared using emulsion-chemical crosslinking technique and evaluated *in vitro*, *ex-vivo* and *in-vivo*. These microspheres may be administered in dosage forms, including a sachet, as a tablet, through a capsule and the like.

Another embodiment, discloses gastroretentive tablets of acyclovir were prepared from one or more the polymers and excipients for sustained delivery. ,

In further embodiment of this invention the drug pharmacokinetic simulation was done for Acyclovir CR to:

- determine the dose and absorption rate required for Acyclovir CR to achieve comparable C_{max} and C_{min} with that of IR.
- predict the blood levels of Acyclovir CR and compare with that of IR.
- simulate in-vitro dissolution profiles from absorption rate constants.

In the following are described experiments conducted that serve as non limiting illustrations of how the invention is performed. Any modifications or variations in the parameters including but not limited to polymers used, their combination used, drugs used, chemicals and their concentrations used, various procedures for assaying the drug, simulation of the drug are merely illustrative and any equivalents of them that are obvious to a person skilled in the art and that are capable of achieving the same objective

if used in their place shall be considered as included in the content / scope of this specification

MATERIALS AND METHODS

Materials

5 Chitosan (degree of deacetylation 82% and molecular weight 650, 000) was obtained as gift sample from Central Fisheries Research Institute, Cochin. Methocel K15M and Carbopol 71G were obtained as gift sample from Colorcon Ltd., Mumbai and Degussa Ltd., Mumbai, respectively. 2-Iminothiolane-HCl, 6-Carboxyfluorescein (6-CF) and Traut's reagent were procured from Sigma-Aldrich Ltd., USA. Ethanol, acetonitrile, 10 methanol and xylene were procured from E. Merck Ltd., Mumbai, India. Thiolated Chitosan and N Trimethyl chitosan (N-TMC) was prepared in the lab according to the method reported by Bernkop-Schnurch et al.¹⁴.

Preparation of Microspheres formulations

Microspheres formulations using chitosan, N-TMC and thiolated chitosan as polymers 15 were prepared using the emulsification cross linking method (Wang et al.²⁰). Emulsification cross linking method was optimized for different process and formulation variables.

A solution of chitosan, (1.0 to 2.0 % w/v) was prepared in acetic acid (2% v/v) and aqueous solution of drug (0.1 to 0.5% w/w) was added to their respective solution. This 20 was further added to continuous phase (consisting of light liquid paraffin and heavy liquid paraffin (1:1) containing Span 80 (0.5% w/v) as surfactant) under constant stirring (1200-2000 rpm) using a three blade propeller stirrer to form w/o emulsion. This was followed by addition of glutaraldehyde (0.25 to 1.0 ml, 25%v/v) drop wise at 15, 30, 45 and 60 min, respectively. The stirring was continued for 3.5 hours. The microspheres so 25 obtained were separated by centrifugation and washed with petroleum ether to remove liquid paraffin. The microspheres were suspended in 5%w/v sodium bisulfite solution and

stirred for 15 minutes to remove residual glutaraldehyde. Final washing was done with distilled water, microspheres were dried and stored in a vacuum desiccator. Thiolated chitosan and N- Trimethyl chitosan, microsphere was prepared using same method using optimized process and formulations variables.

- 5 Microspheres of Carbopol 71G and Methocel K15M were prepared by spray drying techniques as reported by Harikarnpakdee et al.²¹ Methocel K15M or Carbopol 71G were dissolved in deionised water. Acyclovir was separately dissolved in distilled water. Colloidal silicon dioxide (Aerosil), maltodextrin and propylene glycol were then mixed with the polymer solution. The solution of each batch was spray dried employing inlet
10 temperature of 120⁰C for Carbopol 71G and 130⁰C for Methocel K15M, pump setting of 5ml/min; spray flow rate of 400 nano liter/min.

Fluorescently loaded microspheres were prepared in the same way. For this purpose drug solution was replaced with 0.3% w/v solution of 6-CF and microspheres were prepared using the procedure described above.

15 **Characterization of Microspheres**

Morphological Examination

- The morphology of microspheres was examined by scanning electron microscopy (SEM, JSM-5310LV scanning microscope Tokyo, Japan). The microspheres were mounted on
20 metal stubs using double-sided tape and coated with a 150 ⁰A layer of gold under vacuum using gold coater. Stubs were visualized under scanning electron microscope.

Particle Size Measurement

- The particle size of the microspheres was measured using stage micrometer scale. Dry microspheres (5 mg) were suspended in distilled water and ultrasonicated for 5 seconds.
25 A drop of suspension was placed on a clean glass slide and microspheres were counted under stage ocular micrometer. Minimum of 200 microspheres were counted per batch.

Swelling Measurement

The swelling of microspheres was conducted in phosphate buffer pH 6.8. The size of dried microsphere and after incubating in phosphate buffer (pH 6.8) for 0.3, 1.0, 3.0 and 5.0 hr was measured by using microscopic method. The percentage of swelling at different time interval was determined by taking difference between diameter of microspheres at time t (D_t) and initial time ($t = 0$ [D_0]) as calculated from the following equation

$$\text{Swelling \%} = \frac{D_t - D_0}{D_0} * 100 \quad (1)$$

Production Yield

- 10 The production yield (% w/w) was calculated from the ratio of average weight of dried microspheres (W_1) recovered from each of three batches to the sum of initial dry weight of starting materials (W_2).

Entrapment efficiency

- Acyclovir loaded microspheres (10mg) of chitosan, N-TMC or thiolated chitosan were digested in HCl (0.01M). Carbopol 71G and Methocel K15M microspheres were dispersed in 0.1M NaOH and 0.05M phosphate buffer (pH 6.8), respectively for overnight with intermittent shaking. The mixture was filtered and filtrate was assayed spectrofluorometrically (Elico Spectrofluorometer, SL-174, Delhi, India) at excitation wavelength of 256nm and emission wavelength of 374nm according to the method reported by Darwish et al.²² The entrapment efficiency was calculated from the ratio of actual amount of the drug present in the formulation to the initial amount of the drug added

Mucoadhesion Measurement Study

- The mucoadhesion property of microsphere formulations was determined according to the method described by Vyas et al.²³ A 5 cm long piece of freshly cut pig intestine was obtained from a local abattoir within 1 hr of killing of animal was further cleaned by

washing with isotonic saline solution. An accurate weight of microspheres was placed on the mucosal surface to which was attached a polyethylene plate that was fixed at an angle of 40° relative to the horizontal plane. Phosphate buffer (pH 6.8) warmed at 37±1 °C was flown at a rate of 5ml/min over the tissue. The time required for detaching all the
5 microspheres from mucosal surface of the pig intestine was recorded by visual inspection.²¹

In Vitro Drug Release Study

In vitro release of acyclovir from microspheres was determined by carrying out dissolution test using USP paddle method at a stirring rate of 50 ± 5 rpm at temperature
10 37±0.5 °C. 900ml of HCl buffer (pH 1.2) was used as dissolution medium for first hr and phosphate buffered saline (PBS, pH 6.8) was used for next 11 hrs. The dried microspheres were filled in hard gelatin capsules and were placed in dissolution vessels. 5ml sample was withdrawn at various time intervals and volume of the media was replenished with an equal amount of dissolution media. The samples were then analyzed
15 spectrofluorometrically.

G.I.T Distribution

Rats (Sprague dawley strain), 6 to 8 months old, weighing 200-220 gm were kept on fasting for 16-20 hr before commencement of the experiment. Water was provided ad libitum. The protocols for these investigations were approved by the Institutional Animal
20 ethics committee in accordance with the disciplinary principles and guidelines of CPCSEA. Six groups were employed in the present study with each group containing 15 rats. First group received oral administration of aqueous solution of 6-CF (1 ml of 0.3% w/v). The second, third, fourth, fifth and sixth groups received microsphere of 6-CF prepared from chitosan (M-CH), thiolated chitosan (M-TCH), N-Trimethyl chitosan (N-
25 TMC), Carbopol 71G or Methocel K15M, respectively. Oral administration of microspheres was accomplished by suspending 20 mg sample of microspheres

corresponding to 3.0 mg of 6-CF in 1.0 ml normal saline and force-feeding via a rubber tube under non-anesthetic conditions. The rats were sacrificed after 2, 4, 6, 8 or 10 hr of administration, stomach (section 1) along with entire length of small intestine that was further subdivided into 6 sections (sections 2-7; length of each section 14 cm) were isolated immediately. The stomach and intestinal sections were cut opened to expose the inner mucosal surface. All microspheres located in each part were collected by scratching the mucosa with a spatula. To the collected sample, 10 ml of 0.1 N HCl was added in case of chitosan, thiolated chitosan and N-TMC. The 0.1 N NaOH and phosphate buffer (0.05 M) were added in case of Carbopol 71G and Methocel K15M microsphere, respectively. The mixture was mashed using homogenizer to extract 6-CF and kept for 24 hr. After centrifugation at 3000 rpm for 20 min, the supernatant was analyzed fluoremetrically at $\lambda_{\text{excitation}}$ 489nm and $\lambda_{\text{emission}}$ 515nm for 6-Carboxyfluorescein. The extraction efficiency of 6-CF using this method was found to be approximately 95%. In addition a 2 cm portion of section 2, 3 and 4 was taken out and further processed for fluorescence microscopy.

Fluorescence Microscopy

Fluorescence microscopy was performed to determine the extent of distribution and penetration of microsphere formulations. The excised tissue sections of GIT were blotted with tissue paper. The wiped tissue was fixed in fixative solution (3:1 absolute alcohol: chloroform) for 3 hr. The pieces were first transferred to absolute alcohol for 0.5 hr and then in absolute alcohol and xylene for 1 hr. Wax scrapings were added in this solution till saturation and were kept for 24 hr. Paraffin blocks were made by embedding the tissue in hard paraffin, matured at $62 \pm 1.0^\circ\text{C}$. The sections (5 μm thickness) were cut using microtome (Erma optical works, Japan) and examined under fluorescence microscope (Leica, DMRBE, Bensheim, Germany).

Hemolytic Toxicity Assay

The procedure from literature was followed to perform the hemolytic toxicity studies.¹⁹ Blood from healthy donors was collected and anticoagulated with 3% sodium citrate. Erythrocytes were separated from blood plasma by centrifugation (3000 x g, 5 min) and
5 suspended in phosphate buffer saline (PBS) of pH 7.4. The RBC suspension (1%) was mixed with distilled water, which was considered as producing 100% hemolysis, and normal saline producing no hemolysis hence acting as blank. 0.5 ml 2% w/v dispersion of microspheres formulations in PBS (pH 7.4) was added to 4.5 ml of normal saline and interacted with 1 ml RBC suspension. Similarly, 0.5 ml of 0.3% w/v solution of acyclovir
10 in PBS were mixed with 4.5 ml of normal saline and interacted with RBC suspension and kept in incubator for 1 hr at 37±1.0 °C. After 1 hr, mixture was centrifuged and supernatants were taken and diluted with an equal volume of normal saline and absorbance was taken at 540 nm against supernatant of normal saline diluted similarly as blank. The percentage hemolysis was thus determined for each sample by taking
15 absorbance of water as 100% hemolytic sample.

Pharmacokinetic Study

Rats (Sprague dawley), 6 to 8 months old, weighing 200-220 gm were divided into 6 groups, each consisting of 5 animals. Rats were kept on fasting 12 hours before drug administration and until 24 hours post dosing. Water ad libitum was given throughout the
20 study. The dose selected of acyclovir was 5 mg/kg.²⁴ First group received oral administration of 0.3 % w/v drug solution in PBS (pH 7.4). Second, third, fourth, fifth and sixth group received oral administration of chitosan, thiolated chitosan, trimethyl chitosan, Carbopol or Methocel microspheres, respectively. A 20 mg sample of microsphere corresponding to 3.0 mg of acyclovir were suspended in 1.0 ml saline and
25 administered orally using a rubber tube under non-anesthetic condition. At 2.5, 5, 10, 15 and 24 hrs time intervals, blood was collected from jugular vein in ependorff tubes and

centrifuged at 2000 rpm for 10 min (REMI Equipment, Mumbai, India). Supernatant was collected and acetonitrile was added to precipitate the proteins. The precipitated proteins were settled by centrifugation at 2000 rpm for 15 min. The supernatant was collected and filtered through a 0.45 μ m filter into volumetric flask and drug concentration was determined by spectrofluoremetric assay.

PHARMACOKINETIC SIMULATIONS PERFORMED FOR ACYCLOVIR CONTROLLED RELEASE

Assumptions considered for simulation performance study are as follows:

1. The product will be indicated for initial and intermittent therapy of genital herpes. These conditions require a dosage of 200 mg every four hours, 5 times daily for 10 days.
2. The product will be a bioadhesive dosage form, which would be designed to be retained in upper part of GIT for a prolonged period of time.
3. The CR formulation will be administered two times a day.
4. Bioavailability of CR formulation is 25% higher than that of IR formulation due to prolonged residence in absorbable areas of GIT.
5. The absorption process is controlled by the release from the dosage form. The intrinsic absorption rate constant of the drug is far higher than the drug release from the dosage form ($K_a \gg \text{drug release rate}$) and hence absorption of any amount of drug release from the dosage form is instantaneous.

Simulations Performed

The simulation process involved following steps.

1. As a first step the plasma concentration profiles of Acyclovir / Acyclovir IR tablets were calculated using the pharmacokinetic parameters reported in the literature^{16, 18}.
2. These parameters were then used to calculate required properties of CR dosage form.
3. Then simulations for CR dosage form were performed using parameters obtained in step 2.

The methodology and results are summarized in table 6-9 and figure 6-11.

Platform Used

Computer PIV (1.7 dual processor), 1 GB Ram, 200 GB Hard disk with software Microsoft Excel 2003.

5 FABRICATION OF GASTRORETENTIVE ACYCLOVIR TABLETS

Matrix tablets were prepared by wet granulation method. Acyclovir, polymer and Avicel were weighed and sifted together through the sieve #40 ASTM and blended in a polybag for 5 min. Blended material was granulated (what are steps / method of granulation?) using ethanolic solution of PVP-K30. The wet mass was dried in a tray dryer for 30 min at 40 °C and dried materials passed through a sieve # 20 ASTM. Granules were blended with magnesium stearate and compressed using 19mm X 9 mm, modified capsules shaped, concave punch. The formulation ingredients of various batches are summarized in Table 9. The hardness of the tablets was kept in the vicinity of 19.6-22.6 kp and thickness was 5.94 and 6.03mm.

Characterization of Tablets

15 The properties of the compressed acyclovir gastroretentive tablets, such as hardness, friability, thickness, weight variation, and content uniformity were determined using reported procedure. Briefly, hardness was determined by using Monsanto hardness tester. Friability was determined using Roche friability testing apparatus. Weight variation and uniformity of drug content were performed according to the IP procedures.

20 In Vitro Drug Release Studies of Acyclovir Gastroretentive tablets

The in vitro dissolution studies were performed using USP-2 type dissolution apparatus at 50 rpm) and temperature was maintained at 37°C ± 0.5°C. Release testing was carried out in 900 ml of different dissolution media: simulated gastric fluid (pH 1.2) and phosphate buffer (pH 6.8). An aliquot (10 mL) was withdrawn at specific time intervals and drug content was determined by UV-visible spectrophotometer) at 255 nm. It was made clear that none of the ingredients used in the tablet formulations interfered with the assay.

Water uptake kinetics

Water uptake studies were performed by equilibrium weight gain method using USP type I dissolution test apparatus. The tablets were accurately weighed and placed in a dissolution basket. The basket was immersed in a dissolution vessel containing 900 ml 0.1 N HCl (pH1.2) maintained at 37 ± 0.5 °C; speed of rotation was 50 rpm. At regular intervals, the basket-matrix system was removed from the dissolution vessel, blotted with tissue paper to remove excess water, and reweighed. The percentage water uptake (i.e., the degree of swelling due to absorbed medium) was calculated using following equation.

$$\% \text{ Water Uptake} = \frac{W_t}{W_0} \times 100 \quad (2)$$

Where W_0 and W_t are weights of dry and swelled tablet at time t , respectively.

Matrix erosion studies

Matrix erosion studies were performed by a method reported by Ebube et al.²⁵ USP type I dissolution test apparatus was used for this purpose. The dry tablets were weighed, placed in dissolution basket containing 900 ml of 0.1 N HCl (pH1.2) maintained at 37 ± 0.5 °C and the basket was rotated at 50 rpm. At regular intervals, the whole basket-matrix assembly was removed from the dissolution vessels and dried to a constant weight in a hot-air oven at 50 °C. The matrix erosion (E) at time, t , was estimated from Eq. 3.

$$\text{Matrix erosion } \% = \frac{W_{dt}}{W_0} \times 100 \quad (3)$$

W_{dt} and W_0 are weights of dried tablet and initial weight of dry tablet at time t respectively.

Mucoadhesive measurement study of acyclovir gastroretentive tablets

Mucoadhesive strength of acyclovir gastroretentive tablets were determined by detachment force measurement method using pig intestine. Immediately after slaughter, different parts of intestine were removed and transported to Tyrode solution kept at 4 °C. The composition of tyrode Tyrode solution (g/L) is NaCl, 6; KCl, 0.2; CaCl₂ 2H₂O,

0.134; NaHCO₃, 1.0; sodium hydrogen phosphate, 0.05; glucose-H₂O, 1.0. During the experiment, the solution was aerated with pure oxygen and kept at 37 °C . The intestinal tissue was fixed on glass plate of receptor compartment. The tablet was placed over intestinal tissue and on the other end of the glass rod a pan was attached in which a beaker was placed. After keeping the preparation for 30 min, the water was added with a burette dropwise to the beaker. The force needed to detach the tablet was measured using a modified prescription balance. The force was used as a parameter for adherence. The force F in newtons was calculated by the equation

$$F = 0.00981 \frac{W}{2} \quad (4)$$

10 Where W is the amount of water in the beaker in grams.

Statistical Analysis

Data are expressed as the mean $\pm$ standard deviation (SD) of the mean. Statistical analysis was carried out employing the student's t test using the Graph-pad PRISM software (Version 2.01, San Diego, CA). A value of p <0.05 was considered statistically significant.

RESULTS

Preparation and In Vitro Characterization

Table 1 shows the composition of different microsphere formulations prepared using chitosan, thiolated chitosan and trimethyl chitosan as polymers and results were compared with microsphere prepared with widely used mucoadhesive polymers Carbopol 71G and Methocel K15M.

Twenty different types of microsphere formulations were prepared using four different formulations and process variables. Initially, chitosan microspheres were prepared using different drug concentrations (batch CH-A1 to CH-A5 containing 0.1 to 0.5 % w/v acyclovir), different polymer concentrations (batch CH-B1 to CH-B5 containing 1.0 to 5.0 % chitosan), different volume of cross linker (batch CH-C1 to CH-C5 containing 0.25

to 1.0 ml glutaraldehyde) and different speed of stirring (batch CH-D1 to CH-D5 prepared at 1200 to 2000 rpm). Optimization of each variable was carried out on the basis of their characterization in terms of surface morphology, particle size, entrapment efficiency and *in-vitro* drug release and data is summarized in Table 2.

- 5 Batch CH-D5 (acyclovir con. 0.3% w/w, chitosan, 2% w/v, volume of cross linker, 1.0 ml and speed of stirring at 2000 rpm) was selected as optimum batch because it exhibited high entrapment efficiency (88.0 ± 4.1 %), possessed perfectly spherical shape and sustained the drug release (71.1 ± 2.8 % drug release at 11 hr). Two batches of microsphere formulation M-TMC and M-TCH using all the above optimized values was
10 prepared using N-Trimethyl chitosan chloride and thiolated chitosan instead of chitosan as polymer.

Figure 1 (A-E) depict the photomicrographs of microspheres prepared using chitosan, N-TMC, thiolated chitosan, Carbopol 71G and Methocel K15M polymers. All microsphere formulations were spherical in shape and possessed smooth surface as visualized under
15 SEM. The size of different microspheres formulation was found to range from 11.2 ± 0.4 to 21.3 ± 1.0 μm (Table 2). The particle size was dependent mainly on the concentration of the polymer and stirring speed. Table 2 shows the effect of stirring rate on the particle size of microspheres. The results showed that increasing the stirring speed from 1200 to 2000 rpm decreased the particle size from 21.3 ± 0.9 μm to 12.5 ± 0.3 μm . This can be
20 attributed to the greater energy generated at higher stirring speed, which was able to efficiently disperse the bigger droplets to smaller ones having less particle size.

Increase in the concentration of polymer from 1 to 3% w/v produced a significant increase ($p < 0.05$) in the particle size. However, thereafter further increase in polymer concentration (from 3 to 5% w/v) didn't significantly ($p < 0.05$) influence the particle size.

- 25 When the chitosan concentration was increased from 1 to 3 %, the viscosity of chitosan solution increased and bigger droplets of the internal phase during emulsification step

were produced. This increase is high enough to result in difficult dispersion and subdivision of droplets thus, resulting in large size of microspheres. It was observed that the volume of glutaraldehyde (cross-linker solution) did not have a significant influence ($P < 0.05$) on the particle size and entrapment efficiency of the microspheres. On the other hand, stirring speed did significantly effect ($P < 0.05$) the particle size of microspheres. An increase in the stirring speed from 1200 to 2000 rpm significantly decreased ($p < 0.05$) the particle size. The geometric mean diameter decreased from 21.3 ± 0.9 to 12.5 ± 0.3 μm with an increase in the stirring speed. Microspheres prepared at higher stirring speed were perfectly spherical compared to lower stirring speed, which resulted in the formation of clumps. Microspheres became more discrete with increase in the stirring speed from 1200 to 2000 rpm (Table 2).

Entrapment efficiency of different microsphere formulations was found 55 ± 1.8 to $91.6 \pm 3.1\%$ (Table 2). Entrapment efficiency was found to be mainly dependent on the concentration of polymer used. Other formulation and process variable didn't show any significant ($p < 0.05$) effect. The result of entrapment efficiency measurement was well correlated with the previous report of Thanoo et al.²⁶.

The production yield of optimized microsphere formulations was found to be 74.5 ± 3.5 , 72.4 ± 2.8 , 76.3 ± 3.8 , 69.4 ± 4.1 and 54.1 ± 3.0 , respectively, for chitosan, N-TMC, thiolated chitosan, Carbopol 71G and Methocel K15M polymers. The entrapment efficiency (%w/w) of acyclovir in optimum batch of microspheres prepared from chitosan, N-TMC, thiolated chitosan, Carbopol 71G or Methocel K15M was found to be 88.0 ± 2.6 , 91.3 ± 4.5 , 86.8 ± 3.1 , 91.4 ± 4.2 and 77.3 ± 4.2 , respectively (Table 3).

Mucoadhesive Measurement

Table 3 summarized the results of mucoadhesive measurement of different microspheres formulation in pig intestine. The adhesion time of microspheres followed the rank order of thiolated chitosan (8.0 ± 0.8 hr) > N-TMC (4.9 ± 0.6) chitosan (3.1 ± 0.4 hr) > Carbopol

71G (1.1 ± 0.2) > Methocel K15M (0.2 ± 0.1 hr). Without getting bound to any theory we have following understanding on various observations in this inventions. Comparatively poor mucoadhesion of Methocel microspheres could be attributed to its non-ionic property. On the contrary, strong electrostatic attraction seems to have contributed to good mucoadhesion between mucin and Carbopol 71G or chitosan. Numerous hydrophilic functional groups such as carboxyl groups in chitosan molecules have an ability to form hydrogen bonds with the mucus molecules. This interaction is reported to be responsible for mucoadhesive property of this polymer.²¹ N-TMC microsphere has significantly higher mucoadhesion in comparison to chitosan microsphere due to ionic nature of N-TMC bearing the positive charge that will form a strong bond with -SH group of mucin resulting in strong mucoadhesion. Carbopol microspheres possessed negative charge, which in presence of investigating medium PBS buffer (pH 6.8) could have been repelled by the negatively charged mucus leading to poor mucoadhesion. The excellent mucoadhesion was observed in thiolated chitosan microspheres may be due to presence of thiol groups, which are known to enhance the mucoadhesive property of chitosan because of formation of strong covalent bonds (disulfide bonds) with mucin. The formation of disulfide bonds between the thiomers and the mucus gel layer takes place either through thiol/disulfide exchange reaction or via a simple oxidation process of thiol groups.²⁷ However, other polymers like chitosan or Carbopol form non-covalent bonds like hydrogen bonds, van der Waal's forces or ionic interactions thereby resulting in weak mucoadhesion.

Swelling Study

Figure 2 shows the percentage swelling of different microsphere formulations at different time intervals. The results revealed that all microsphere formulations swelled rapidly when immersed in phosphate buffer (pH 6.8). It is reported that adhesive properties and cohesiveness of mucoadhesive polymers are generally affected by their swelling

behavior.²⁸ Mucoadhesive microspheres are supposed to take up water from underlying mucosal tissue by absorbing, swelling, and capillary effects, leading to considerable stronger adhesion.²⁹ % Swelling of different microsphere formulation was found to follow the rank order 248.3 ± 18 , 260.1 ± 20 , 198.2 ± 15 , , 279.1 ± 26 and $164 \pm 15\%$, respectively, after 2 hr for microsphere prepared from chitosan, N-TMC, thiolated chitosan, Carbopol 71G and Methocel K15M. After 5 hr of incubation % swelling was observed to be 295.5 ± 28 , 309.2 ± 24 , 273.2 ± 24 , 340.7 ± 30 or $173.1 \pm 15\%$, respectively. Chitosan and Methocel K15M microspheres showed significantly less ($p < 0.05$) swelling as comparison to thiolated chitosan, N-TMC or Carbopol 71G microsphere. It was observed that N-TMC and thiolated chitosan microspheres swell slowly and produced higher mucoadhesive strength. This is perhaps because slow swelling avoids the formation of over hydrated structure that loses its mucoadhesive properties before reaching the target.³⁰ On the other hand, highest swelling observed in microspheres of Carbopol 71G could be due to its high ionization at pH 6.8, which is capable of absorbing high amount of water.³¹

In Vitro Drug Release

Figure 3 shows the release of acyclovir from various mucoadhesive microspheres. Drug powder enclosed in hard gelatin capsules was completely released ($95.3 \pm 4.1\%$) within 1 hr. The time taken to release 75% of acyclovir (t_{75}) from chitosan, N-TMC, thiolated chitosan, Methocel K15M or Carbopol 71G microspheres was 5.0 ± 0.4 , 8.0 ± 0.6 , 9.5 ± 0.7 , 4.0 ± 0.3 and 5.5 ± 0.6 hr, respectively. The significantly higher time required ($p < 0.05$) by the thiolated chitosan microspheres to release acyclovir may be due to its better stability in acidic medium, which contributed significantly less amount of drug release during initial 1 hr of dissolution ($29.3 \pm 1.1\%$ and $20.5 \pm 0.5\%$ drug released from chitosan or thiolated chitosan microsphere during 1 hr). This initial higher release of acyclovir from chitosan microspheres may be attributed to the higher solubility of chitosan in acidic medium. Chitosan is soluble in acidic medium but crosslinking with glutaraldehyde through its amino group stabilized the microspheres matrix and provide the sustained

release.²⁶ Significantly lesser drug release from thiolated chitosan microspheres is due to the presence of disulphide bonds in microsphere matrix further stabilized the structure along with glutaraldehyde as cross linking agent. Higher amount of drug released from Methocel K15M microspheres could be assigned to its linear structure and low viscosity at pH. It was surprising that whereas the microspheres of acrylamide grafted dextran and CS released 20 to 40 % acyclovir in first hour itself, microspheres from trimethyl chitosan released only about 7% chitosan in the first hour leading to most uniform drug release profile over a period of 12 hours ultimately leading to 80% release in 12 hours, while microspheres from other investigated compositions released drug ranging from about 20% (for microspheres of carbopol) to about 35% (for microspheres of Methocel) in first hour.

The release rate from microsphere depend on many factors like concentration of polymer used, method of preparation, amount of cross linker used and amount of drug used, dissolution conditions etc.,

For the characterization of the release kinetics the *in vitro* drug release data was fitted to zero order, first order and Higuchi equation

$$M_t / M_f = Kt^n$$

Where, M_t is the amount of drug release at time t ; M_f is the amount of drug release after infinite time; K is the release rate constant; n is the diffusional exponent indicative of the operating release mechanism.

Quantitative GIT Distribution

Table 4 shows the time course of distribution of mucoadhesive microspheres loaded with 6-CF in the GI tract, including the stomach (Section 1) and small intestine (Section 2-7) after oral administration has been determined. 6-CF was selected as fluorescence marker because of its hydrophilic nature, higher extraction efficiency (>95%) and lower detection limit (1.0 ng/ml). Following oral administration, more than 30% 6-CF solution was recovered from stomach, but less than 10% was located after 4 hr. Maximum amount of 6-CF was transferred to the lower part of intestine after 8 hrs of its administration in

rats. The reason for poor retention of 6-CF at absorption site is its soluble nature and its very little affinity to GIT tissue. On the other hand oral administration of 6-CF loaded thiolated chitosan microspheres revealed a different GI distribution pattern. After 2 hr, 22.3±3.1% formulation was recovered from stomach (Section 1) and after 4 hr, nearly 41.6±2.9% was recovered from Section 2, 3 and 4 (duodenum and jejunum portion of intestine). Further, after 10 hr of administration, 26±2.1% formulation was recovered from Section 2, 3 and 4. The significantly higher quantity of acyclovir recovered ($p<0.05$) from sections 2, 3 and 4 of GIT suggest gastroretentive characteristic of thiolated chitosan microsphere formulation. In comparison chitosan, Carbopol 71G and Methocel K15 microspheres showed 33.5±4.2, 17±2.8 and 9.6±1.4% recovery from section 2, 3 and 4 of GIT after 4 hr of oral administration and 12.9±1.2, 2.5±0.3, 0% recovery after 10 hr of administration. The 2-fold higher GI retention of thiolated microspheres in comparison to chitosan, Carbopol 71G and Methocel K15M microsphere formulation may be attributed to the better mucoadhesive properties of thiolated chitosan in pH 5-6 that is the pH of duodenum and jejunum region of intestine.³² The main problem with the conventional therapy of acyclovir is its poor retention in duodenum and jejunum region resulting in very poor absorption of drug and nearly more than half of the drug is recovered in the faeces in unchanged form.

Qualitative GIT distribution study

Gastroretentive characteristic and permeation enhancement effects of microsphere formulations was determined by carrying out by determining the extent of penetration across the duodenum and jejunum section (Section 2, 3 and 4) of fluorescence marker (6-CF) loaded microspheres formulation. Figure 4 (A-F) shows the photomicrograph of rat intestine after treatment with 6-CF solution (5A), thiolated chitosan (5B), N-TMC (5C), chitosan (5D), Carbopol 71G (5E) or Methocel K15M (5F) microspheres. The fluorescence photomicrographs revealed better qualitative uptake and localization of

fluorescence marker loaded mucoadhesive microsphere in duodenum and jejunum as compared to its solution. Oral administration of 6-CF loaded thiolated chitosan and N-TMC microsphere showed higher fluorescence intensity accompanied with deep penetration of marker in intestinal tissue (Figure 5B, 5C). This indicates higher mucoadhesiveness and penetration enhancement effect of thiolated chitosan and N-TMC microsphere formulation. Thiolated chitosan and N-TMC is reported to open the tight junction of intestinal epithelium by interacting with intestinal protein this is responsible for its penetration enhancing effect.³² Acyclovir is a Class III drug. Its low permeability is the rate-limiting factor influencing its oral absorption. Hence, these results indicate that the penetration enhancement effect of thiolated chitosan microspheres could be beneficial in facilitating the oral absorption of acyclovir.

Hemolytic Toxicity Assay

Hemolytic assay is a simple method widely used to study polymer-membrane interaction. It gives a quantitative measure of hemoglobin release. Table 5 compares the results of % hemolysis of different microspheres formulations of acyclovir. Thiolated chitosan, N-TMC, chitosan, Carbopol 71 G and Methocel K15 M microspheres showed 13.1 ± 1.2 , 27.2 ± 1.8 , 20.1 ± 2.0 , 26.2 ± 3.4 and 22.0 ± 2.8 % hemolysis, respectively after 1 hr of incubation. Thiolated chitosan microsphere displayed a lower membrane damaging effect causing a significantly lower hemoglobin release than chitosan microsphere. In the case of the thiolated chitosan microspheres the lower membrane-damaging effect in comparison to the chitosan microsphere might be explained by the formation of intra- as well as inter- molecular disulfide bonds, thus leading to a higher rigidity of the microsphere matrix. Rigid molecules have more difficulties to attach to the cellular membrane than flexible molecules and showed toxicity.³³ These findings are in good agreement with previous studies of Guggi et al.³⁴ asserting that chitosan-TBA and

glucosamine-TBA conjugates have a significant less toxic effect on red blood cells in comparison to unmodified chitosan and glucosamine

Pharmacokinetic Study

Figure 5 shows the plasma concentration profile of acyclovir after oral administration in the form of solution and microspheres. Thiolated chitosan microspheres showed superiority over the other formulations in plasma concentration at 24 hours. Nearly 4.0 times higher AUC_{0-24} value of acyclovir for these microspheres (1090.7 ± 51 ng.hour/ml) as compared to drug solution (281.7 ± 28 ng.hour/ml) was observed. For Trimethyl chitosan microspheres, however, this value that was still higher i.e. 1335.5, rise in plasma concentration was earlier and higher, achieved highest plasma concentration at 10 and 12 hours which lowered than Thiolated chitosan at 24 hours. Thus, for a 12 hours dosing, Trimethyl Chitosan may be superior than Thiolated Chitosan, although on account of significantly low haemolytic activity, Thiolated chitosan may be considered preferable over Trimethyl chitosans and best amongst all the microsphere compositions tested here.

Gastroretentive characteristic of microsphere formulations was further supported by significantly higher ($p < 0.05$) relative bioavailability 387% for M-TCH) as compared to drug solution. In addition, thiolated chitosan microspheres showed the ability to maintain the acyclovir plasma concentration through 24 hr as compared to the drug solution that could maintain this level of drug only for 5 hr. These results confirmed the sustained release potential of mucoadhesive microspheres of acyclovir prepared from thiolated chitosan. Hence, the overall better pharmacokinetic performance of thiolated chitosan microsphere in comparison to drug solution is due to (1) increased residence time within upper GI tract as evident by GI distribution study (2) an intensified contact between the intestinal mucosa and microspheres as evident by mucoadhesion study (3) increased drug concentration at site of absorption as evident by in vitro drug release study and (4) facilitated drug permeation through mucosa as evident by fluorescence microscopy study.

Plasma Concentration Profiles of Acyclovir IR 400 mg – Single dose

Three parameters, time for maximum plasma concentration (t_{max}), elimination half life ($t_{1/2}$), and area under plasma concentration profile (AUC) were collected from literature^{16, 18, 35, 36}. Other pharmacokinetic parameters were calculated from these values and provided in the table 6.

A simulation was performed to calculate the plasma concentrations during the course of single dose by using the parameters described in the table 7. The C_{max} was 0.78 mcg/mL. The figure 6-11 shows the plasma concentration-time profile of this simulation.

Plasma concentration profiles of Acyclovir IR 200 mg-Single dose

- 10 Plasma concentrations of Acyclovir IR 200 mg were calculated from the simulated blood levels of Acyclovir IR 400 mg by extrapolation. The pharmacokinetics of Acyclovir is less than dose proportional (non-linear pharmacokinetics). Hence, the plasma concentrations of 400 mg dose were not linearly extrapolated, but a ratio of 0.69 was used (200 mg : 400 mg)¹. The figure 7 shows the comparison of simulated plasma concentration-time profiles of Acyclovir IR 200 mg & 400 mg.

Plasma concentration profiles of Acyclovir IR 200 mg-Multiple dose

Plasma levels of multiple dose administration of Acyclovir IR 200 mg (200 mg every 4 hours, 5 times daily)³⁶ were simulated by super position method as shown in the figure 8

- 20 The figure 9 shows the comparison of plasma concentrations of Acyclovir IR 200 mg during the dosing day on day 1 and day 5. The difference in the maximum and minimum plasma concentrations (C_{max} and C_{min}) achieved during day 1 and day 5 was insignificant. C_{max} was about 0.80 mcg/mL and C_{min} was about 0.10 mcg/mL.

Plasma concentration profiles of CR formulations

- 25 Simulation was performed by two methods. First is by considering that the release of the drug and absorption follow a zero order kinetics (ER-Zero order), and the second by considering first order (ER-First order).

Comparative plasma concentration profiles are shown in figure 10.

- 30 The table no. 7 provides the simulated pharmacokinetic parameters of CR formulations in comparison with IR formulation. It can be observed from the data on the table above that the C_{max} / C_{12h} / C_{24h} plasma levels of CR formulations are close to that achieved after the administration of IR formulations.

An important biopharmaceutics factor that has to be noted here is the slow GI transit time during the second dose of the day where most of the course of the absorption is to take place in a lied down position and reduced gastrointestinal movements (when the patient is asleep). Usually this must result in a better bioavailability than the first dose (morning dose), but with a slow rate of absorption resulting in a lower C_{max} as compared to the morning dose.

IN-VITRO DRUG RELEASE

Since the absorption of the drug after administration of CR formulation will be independent of the intrinsic absorption rate, as the intrinsic absorption rate is far higher than drug release rate from the dosage form. So, the absorption rates used for the above simulations are considered to be reflective of the drug release. Hence the in-vitro drug release from these formulations was calculated using the general equations governing zero / first order kinetics. Table 8 and figure 11 present the simulated in-vitro release rate.

PREPARATION AND CHARACTERIZATIONS OF ACYCLOVIR GASTRORETENTIVE TABLETS

Table 9 shows the composition of different gastroretentive acyclovir tablets. Carbopol and PEO were selected as mucoadhesive swellable polymers for controlling the release of acyclovir. Carbopol and PEO have many advantages as candidate for extended release tablets like good gel forming ability and mucoadhesive properties. The 763 mg was selected the dose of acyclovir for controlled release tablets as calculated by simulation study (Table 8 and Figure 11). Tablets were prepared by wet granulation method and characterized for different quality control parameters.

Figure 12-14 shows the in vitro drug release of different batches of gastroretentive tablets. Results shows that batch Acy-ER-1A, 1B and Acy-ER-3A, 3B and 3C prolong the drug release to 12 hr period of time. Figure 15 shows the comparative graph of in vitro drug release profile of different batches with target profile generated by computer simulation study. Batch Acy-ER-3B and Acy-ER-1B were found best match with the target release profile.

Swelling is a very important characteristic of polymer that control the drug release and increase the G.I. retention of gastroretentive tablets. Figure 15 shows the % swelling of different batches of acyclovir tablets. Result shows that batch Acy-ER-1B and Acy-ER-3B shows the significantly higher and prolong swelling to 12 hr period of time. Acy-ER-1B and 3B shows the 302.5 and 255.7 % swelling after 45 min and 34.9 and 12.2% swelling after 12 hr period of time. Results are well correlated with the in vitro drug release study where similarly drug release was prolonged for 12 hr period of time. The results also shows the matrix stability to 12 hr period of time.

Figure 16 shows the results of mucoadhesive strength measurements of different batches of gastroretentive tablets. Batch Acy-ER-1B and 3B have shown the higher mucoadhesive strengths in comparison to other batches. This invention is based on the principal of combination of swelling and mucoadhesive mechanism for preparation of gastroretentive tablets of acyclovir. Acyclovir is absorbed predominately from upper G.I tract to duodenum and jejunum region. The results of swelling and mucoadhesive measurement study showed that these both mechanisms are working in combination to develop gastroretentive tablets of this invention.

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CLAIMS

1. A controlled release dosage form comprising a therapeutically effective amount of a pharmaceutically active agent that would release in about 12 hours not more than about 90% of the said active agent in a simulated gastric juice in a first order rate of release in a USP type 1 dissolution test, and not containing a solubilizer or a swelling enhancer or both, comprising:
 - a. a tablet made from polymer matrix of at least two biocompatible polymers, at least one of which is mucoadhesive, the said pharmaceutically active agent and pharmaceutically permitted excipients; the said tablet capable of rapid swelling without disintegration in the said simulated gastric juice to a size that shall result in its gastric retention in the stomach and start controlled release of the said active agent by starting controlled erosion immediately after coming into contact with the said gastric juice, or
 - b. microspheres of ungrafted chitosan or a chitosan derivative incorporating the said active agent, wherein the said pharmaceutically active agent is not a polymeric molecule and after administration in stomach, the said microspheres adhere to the gastric mucosa for a long time releasing the active agent in a controlled way.
2. A controlled release dosage form of claim 1 (a), wherein the said polymers are mucoadhesive, swellable polymers comprising Carbopol 71G and Polyethylene oxide, hypermellose, polyethylene oxide, Carbopol, sodium alginate, sodium carboxymethyl cellulose, Eudragits, xanthan gum.
3. A controlled release dosage of claim 1 (a), wherein the said active agent is Acyclovir or Acyclovir derivatives.

4. A controlled released dosage form of claim 1 wherein said permitted pharmaceutical excipients comprise a binder, a diluent, a pH modifier, a glidant, a lubricant, a film former, an anti-adherent, a coating agent, a colorant.
- 5 5. A controlled release dosage form comprising Acyclovir, Carbopol 974P, Polyethylene oxide, Avicel PH 101, Povidone K30, Magnesium stearate, and Colloidal silicon oxide.
6. A controlled release dosage form comprising, for every 1000 mg of the dosage form, Acyclovir 763.37mg, Carbopol 974P 75mg, Polyethylene oxide
10 25mg, Avicel PH 101 93.83mg, Povidone K30 30mg, Magnesium stearate 7.5mg, Colloidal silicon oxide 5.0mg.
7. A controlled release dosage form of claim 1(b) wherein the said chitosan derivative is Trimethyl chitosan or Thiolated chitosan.
8. A controlled release dosage form of claim 1(b) wherein the said microspheres
15 are packed in a sachet or are used as an ingredient to make a solid unit dosage form comprising a tablet and a capsule.
9. A method of orally administering a therapeutically effective amount of a pharmaceutically active agent from a controlled release dosage form to a patient, the said dosage form would release in about 12 hours not more than
20 about 90% of the said active agent in a simulated gastric juice in a first order rate of release in a USP type 1 dissolution test, and not containing a solubilizer or a swelling enhancer or both, comprising:
 - i. a tablet made from polymer matrix of at least two biocompatible polymers, the said pharmaceutically active agent and pharmaceutically
25 permitted excipients; the said tablet capable of rapid swelling without disintegration in the said simulated gastric juice to a size that shall

result in its gastric retention in the stomach and start controlled release of the said active agent by starting controlled erosion immediately after coming into contact with the said gastric juice, or

- ii. microspheres of ungrafted chitosan or a chitosan derivative incorporating as the said active agent, wherein the said pharmaceutically active agent is not a polymeric molecule and after administration in stomach, the said microspheres adhere to the gastric mucosa for a long time releasing the active agent in a controlled way.

10. A method of claim 9 (a), wherein the said polymers comprise Carbopol 71G and Polyethylene oxide, Avicel PH 101, Povidone K30, Magnesium stearate, and Colloidal silicon oxide.

11. A method of claim 9 (a), wherein the said active agent is Acyclovir.

12. A method of claim 9, wherein said permitted pharmaceutical excipients comprise a binder, a diluent, a pH modifier, a glidant, a lubricant, a film former, an anti-adherent, a coating agent, a colorant.

13. A method of claim 9, wherein the said controlled release dosage form comprises Acyclovir, Carbopol 974P, Polyethylene oxide, Avicel PH 101, Povidone K30, Magnesium stearate, Colloidal silicon oxide.

14. A method of claim 9, wherein for every 1000 mg of the dosage form the said controlled release dosage form comprises Acyclovir 763.37mg, Carbopol 974P 75mg, Polyethylene oxide 25mg, Avicel PH 101 93.83mg, Povidone K30 30mg, Magnesium stearate 7.5mg, Colloidal silicon oxide 5.0mg.

15. A method of claim 9 (b) wherein the said chitosan derivative is Trimethyl chitosan or Thiolated chitosan.

16. A method of claim 9 wherein the said microspheres are packed in a sachet or are used as an ingredient with optional addition of other pharmaceutically

permitted ingredients and excipients to make a solid unit dosage form comprising a tablet and a capsule.

17. A process of making oral dosage form of claim 1 comprising the steps of:

- 5 i. making wet granulation of a mixture comprising the said pharmaceutically active ingredient, the polymers and excipients, adding a gliadent and pressing into a tablet, or
- 10 ii. preparing a solution of chitosan or a chitosan derivative in acetic acid, adding aqueous solution of the pharmaceutically active agent, adding this mixture to continuous phase consisting of light liquid paraffin and heavy liquid paraffin (1:1) containing a surfactant under constant stirring to form a water-in-oil emulsion, adding gluteraldehyde drop wise over a period of time, continuing stirring for a period of time, separating the microspheres formed by centrifugation, washing with petroleum ether to remove liquid paraffin, suspending in a sodium
15 bisulfite solution and stirring for a period of time to remove residual gluteraldehyde, washing finally with distilled water, drying the microspheres.