Melting point of mebeverine hcl biology essay

Science, Biology

S. NOMaterialSource1234567891011Mebeverine HClGuar gumGluteraldehydeHydrochloric acidSpan 80Tween 80Eudragit S100Liquid paraffinIsopropyl alcoholPotassiumdihydrogeorthophosphate(LR)Sodium HydroxideShasun Pharmaceuticals, pondy. Indian Sea Foods , CochinLoba Chemicals, MumbaiQualigens, MumbaiLoba Chemicals, MumbaiQualigens, MumbaiEvonik Degussa India Pvt. Ltd. MumbaiQualigens, MumbaiMerck specialties, GermanyMumbai Merck specialties, GermanyQualigens, Mumbai

Table no 4: list of materials used in the formulation

4(a) Equipments used:

S. NOEquipment and model numberSource123456789Electronic Balance Ax 200StirrerDigital PH Meter L 120Optical Microscope HL – 4 (a)Glass WareHomogeniserScanning Electron MicroscopeFT - IRUV spectrophotometerShimadzu , JapanRemi Equipments Pvt. LtdElico, MumbaiWeswoxBorosilRemi Equipments Pvt. LtdHitachi – S520Alpha FT IRLab India

Table no 5: Equipment used in the formulation

4(b) Methodology:

4. 1 Pre formulation studies

4. 1. 1 Solubility study

The solubility of Mebeverine HCl in 10mg/ml of solvent was carried out and its solubility was reported.

4. 1. 2 Melting point determination

Melting point of Mebeverine HCl was determined by open capillary method.

4. 1. 3 Identification of Mebeverine

Study was carried out using FT-IR (BRUKER) where the spectra of pure drug compared with the spectra of the Mebeverine given in the IP. The spectrum of Mebeverine shows the functional groups at their frequencies shown in the figure no 5.

4. 1. 4 Drug – Excipient compatibility study:

Study was carried out using FT-IR (BRUKER) where the spectra of pure drug, Guar gum + pure drug were taken. The specific peaks of drug and the polymers were studied for the interactions. IR Spectra shown in Figure no 7, 8.

4. 2 Determination of λmax

A solution of Mebeverine HCl containing concentration 10µg/ml was prepared in water and UV spectrum was taken using Lab India UV Spectrophotometer and scanned between 200-400nm. The maxima obtained in the graph were considered as λmax for the drug Mebeverine HCl. The spectrums shown in figure no 4.

4. 3 STANDARD CALIBRATION CURVE FOR MEBEVERINE HCl:

4. 3. 1 Preparation of simulated gastric fluid (pH 1. 2 buffer)

8. 5ml of concentrated Hydrochloric acid was dissolved in 1000ml of distilled water.

Principle

Mebeverine showed maximum absorbance at 263nm in simulated gastric fluid and obeyed Beer’s law at the concentration range between 5-30 mcg/ml.

Instrument

Lab India UV spectrophotometer.

Procedure

Stock solution

Weighed quantity of Mebeverine (100mg) was dissolved in pH 1. 2 buffer and the volume was made up to 100ml with the same medium. 1000mcg/ml (stock 1)10ml of SS 1 was then made up to 100ml with the same medium. 100mcg/ml (stock 2)Aliquots of 0. 5, 1, 1. 5, 2, 2. 5 and 3ml of SS 2 were pipette into 10ml volumetric flasks and the volume was made up to 10ml with pH 1. 2 buffer. The absorbance was measured at 263nm against blank (pH 1. 2 buffer). The graphs shown in figure no 9.

4. 3. 2 Preparation of phosphate buffer (pH 6. 8)

6. 8g of potassium dihydrogen phosphate was dissolved in 200ml of distilled water to it add 112ml of 0. 2M NaOH and make up the volume to 1000ml with distilled water.

Principle

Mebeverine showed maximum absorbance at 263nm in simulated intestinal fluid and obeyed Beer’s law at the concentration range between 5-30 mcg/ml.

Instrument

Lab India UV spectrophotometer.

Procedure

Stock solution

Weighed quantity of Mebeverine (100mg) was dissolved in pH 6. 8 buffer and the volume was made up to 100ml with the same medium. 1000mcg/ml (stock 1)10ml of SS 1 was then made up to 100ml with the same medium. 100mcg/ml (stock 2)Aliquots of 0. 5, 1, 1. 5, 2, 2. 5 and 3ml of SS 2 were pipette into 10ml volumetric flasks and the volume was made up to 10ml with pH 6. 8 buffer. The absorbance was measured at 263nm against blank (pH 6. 8 buffer). The graphs shown in figure no 10.

4. 3. 3 Preparation of phosphate buffer (pH 7. 4)

6. 8g of potassium dihydrogen phosphate was dissolved in 200ml of distilled water to it add 195. 5ml of 0. 2M NaOH and make up the volume to 1000ml with distilled water.

Principle

Mebeverine showed maximum absorbance at 263nm in simulated colonic fluid and obeyed Beer’s law at the concentration range between 5-30 mcg/ml.

Instrument

Lab India UV spectrophotometer.

Procedure

Stock solution

Weighed quantity of Mebeverine (100mg) was dissolved in pH 7. 4 buffer and the volume was made up to 100ml with the same medium. 1000mcg/ml (stock 1)10ml of SS 1 was then made up to 100ml with the same medium. 100mcg/ml (stock 2)Aliquots of 0. 5, 1, 1. 5, 2, 2. 5 and 3ml of SS 2 were pipette into 10ml volumetric flasks and the volume was made up to 10ml with pH 7. 4 buffer. The absorbance was measured at 263nm against blank (pH 7. 4 buffer). The graphs shown in figure no 11.

4. 4 General methods for the preparation of Microspheres:

Following processes are generally used for preparation of MicrospheresSingle emulsion techniqueDouble emulsion techniqueNormal PolymerizationInterfacial PolymerizationPhase separation Coacervation TechniqueSpray Drying and Spray CongealingSolvent extraction method

4. 4. 1 Single emulsion technique: 38

The current preferred manufacturing process for preparation of Microspheres is Single emulsion technique. In this method, the biodegradable polymer (GG) was slowly dissolved in water with constant stirring and allow to swell it for two hours, later the dissolved drug was added to the aqueous guar gum and mix thoroughly to form a creamy viscous solution(dispersed phase). Take required amount of light liquid paraffin in a clean beaker and add desired concentrations of span and tween 80 (continuous phase). The resultant continuous phase was kept for stirring; to it add above prepared aqueous guar gum creamy solution (dispersed phase) slowly with constant

stirring at 4000rpm for 4 hours. Add 2-3 ml of 0. 1M HCl to the emulsion and add gluteraldehyde with slow sturring and allowed it to an overnight. The microspheres formed were collected by sedimentation followed by decantation of oil and then washed with several fractions of IPA and dried. Thus formed were resultant free flowing microspheres.

4. 4. 2 Eudragit S 100 coated Microspheres: 40

Weighed amount of microspheres were dispersed in minimum amount of organic solvent (alcohol and acetone) in which Eudragit S100 was previously dissolved to give 10: 1 coat: core ratio. This organic phase was poured in light liquid paraffin containing 1-2% span 80. This system was maintained under agitation speed of 1000rpm at room temperature for 3-4 hours to allow the evaporation of solvent. Finally, the coated microspheres were filtered, washed with n-Hexane and dried for 24 hours. All prepared Guar gum microspheres were coated with Eudragit S100. S. noGuar gum conc. (%)Aq. phase: Oil. phase ratioTween 80(%)Span80

(%)

rpmDuration(hours)Gluteraldehyde(ml)Result111: 211100021fails211: 311100021fails311: 312200021fails41. 51: 32. 52200031Cake formation51. 51: 322. 5250031. 5Cake formation61. 51: 322250031. 5Solid matter formed721: 322300031. 5Spheres formed were Aggregates821: 322300032Sphere formation observed921: 322300042Free flowing spheres

Table: 6 List of trials conducted to get optimized formulae

4. 4. 3 Formulation design

Formulation composition for mebeverine HCl loaded guar gum microspheres by varying parameters: Formulation codeGuar gum concentration (%)Gluteraldehyde(ml)Eudragit S 100(%)Rat caecal content (2%)F121. 5

-

F22. 51. 5

-

F32. 52

-

F42. 522F52. 5222

Table 7: Different formulations with varying parameters

4. 5 Evaluation of Mebeverine HCl loaded guar gum microspheres

4. 5. 1 Drug polymer interaction (FT-IR) study

FT-IR spectroscopy was performed on Fourier transform infrared spectrophotometer. The microspheres were scanned in the wave number range of 4000-600cm-1. FTIR study was carried on drug loaded microspheres, Eudragit S100 coated microspheres and the spectra were shown in Figure no7.

4. 5. 2 Surface morphology (SEM)

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of

fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation. SEM studies were carried out by using model JEOL, JSM-6610LL; Scanning Electron Microscope, Japan. Dry Mebeverine HCl microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of Mebeverine HCl microspheres were taken by random scanning of the stub. The SEM and Projection Microscopic images of trial and optimized formula were shown in Figure no 12.

4. 5. 3 Frequency distribution analysis

Determination of average particle size of Mebeverine HCl loaded guar gum microspheres was carried out by optical microscopy in which stage micrometer was employed. A minute quantity of microspheres was spread on a clean glass slide and average size of 20 microspheres was determined in each batch. 41 In order to able to define a size distribution or compare the characteristics of particles with many different diameters, the size distribution can be broken down into different size ranges, which can be presented in the form of a histogram. Histogram presents an interpretation of the particles size distribution and enables the percentage of particles having a given equivalent diameter to be determined and the histograms shown in figure no 19, 20. The results were shown in Table no 13.

4. 5. 4 Percentage yield42

Percentage practical yield is calculated to know about efficiency of any method, thus it helps in selection of appropriate method of production.

Practical yield was calculated as the weight of drug loaded guar gum microspheres recovered from each batch in relation to the sum of starting material. The percentage yields of different formulations were shown in table no 13. The percentage yield of prepared guar gum microspheres was determined by using the formula:

4. 5. 5 Determination of percentage drug entrapment efficiency (PDE)

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula: The percentage drug entrapment efficiency of different formulations was shown in table no 13. 4. 5. 5. 1 Theoretical drug content was determined by calculation assuming that the entire mebeverine present in the polymer solution used gets entrapped in guar gum microspheres, and no loss occurs at any stage of preparation of guar gum microspheres. 4. 5. 5. 2 Practical drug content40 was analyzed by using the following procedure, The amount of Mebeverine associated was analyzed in terms of surface adsorbed drug and entrapped drug.

4. 5. 5. 3 Estimation of drug adsorbed on Microspheres:

10mg drug equivalent microspheres were dispersed in 10ml of PBS (pH 7. 4) and shaken vigorously for 10minutes and supernatant was kept aside. Similarly the sediment was again treated in the same manner and second supernatant was mixed with first supernatant and analyzed for Mebeverine HCl content spectrophotometrically at 263nm. The amount of Mebeverine in the mixed washings gave the amount of drug adsorbed on the surface of the microspheres.

4. 5. 5. 4 Estimation of entrapped drug in microspheres:

The microspheres obtained after two washings were digested in 30ml of PBS (pH 7. 4) for 12hours. The digested homogenate was assayed for Mebeverine, Spectrophotometrically against pH 7. 4 as blank and calculated for the percentage of drug present in the sample.

4. 6 Swellability/ degree of swelling 40

As guar gum is soluble in water, may swell and dissolve in aqueous GIT fluids in the upper part of GIT and may release the drug there before reaching the colon. Therefore the effect of Eudragit coating on swellability of microspheres was studied. The swelling ability of the microspheres on physiological media was determined by suspending them in the PBS buffer (7. 4). Accurately weighed amount of microspheres was immersed in a little excess of PBS (pH 7. 4) and allowed to swell up to constant weight. The swelling of Mebeverine Microspheres is influenced by the extent of cross linking. The values were tabulated in table no: 13. The formula used for the calculation of swelling of various microspheres is as follows:

$\propto =$

4. 7 Angle of repose: The angle of repose of Guar gum microspheres was determined by the funnel method. The accurately weighed quantity of microspheres was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap of the microspheres. The microspheres were allowed to flow through the funnel freely onto the surface. The diameter of the microspheres cone was measured and angle of repose was calculated using the following equation. θ

= tan -1The Flow property values of different formulations were shown in table no 12. Where h and r are the height and radius of the microspheres cone, respectively.

Angle of Repose

Flow property

< 25Excellent25 – 30Good30 - 40Passable> 40Very bad

Table: 8 Flow properties for different values of angle of repose

4. 8 Determination of bulk density and tapped density

An accurately weighed quantity of the microspheres (W), was carefully poured into the graduated cylinder and the volume (Vo) was measured. Then the graduated cylinder was set into the density determination apparatus LAB INDIA. Bulk density and tapped density of microspheres were determined along with compressibility index and hausner's ratio and the results were reported in the Table 12. Bulk Density = Tapped density =

4. 8. 1 Compressibility index (Carr's indices)

Compressibility index was calculated by using the formulaWhere vo- initial volumevf- final volumeCi < 15 % shows good flow propertyCi > 25 % shows poor flow propertyCi > 50 % shows great potential problems. Ci 20 %- 40 % shows reasonable flow property.

4. 8. 2 Hausner's Ratio

Hausner's ratio was measured by the ratio of tapped density to bulk density. Hausner's ratio =

4. 9 Procedure for in vitro dissolution studies: 46

Guar gum microspheres equivalent to 100mg of drug were placed in US Pharmacopoeia basket type- I dissolution apparatus at 37± 0. 5 ºC with constant stirring rate of 50 rpm to prevent floating. The in vitro dissolution studies were performed at three different pH values: (i) 1. 2 pH (simulated gastric fluid) for two hours (ii) 6. 8 pH and (simulated intestinal fluid) for three hours. (iii) 7. 4 pH (simulated colonic fluid) for subsequent hours. 5ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced to maintain sink condition. The withdrawn samples were passed through whatmann filter paper and the amount of mebeverine released was determined by UV absorption spectroscopy at 263nm. Dissolution profiles of the formulations were analyzed by plotting drug release versus time plot shown in figure no 13. Data obtained was also subjected to kinetic treatment to understand the release mechanism and to understand the best fit model of the dissolution rate testing. The data generated for various formulations were depicted in Table No18.

4. 9. 2 In vitro drug release in rat caecal contents: 44

The ability of the most promising formulation of guar gum microspheres to release Mebeverine in the physiological environment of the colon was assessed by carrying out release studies in the rat caecal content release medium. Rats weighing 150-200g were kept on a normal diet and administered 1ml of 1% wt/vol solution of guar gum in water. This treatment was continued for seven days (to induce specific enzyme responsible for degradation of guar gum in vivo). Thirty minutes before the drug release

studies began, the rat was sacrificed, the abdomen was opened, stapled before and after the caecum, and the caecum was removed. The caecum bag was opened and caecum bag was opened and its contents were weighed and homogenized, then suspended in pH 7. 4 buffer solution to give the desired concentrations of caecal contents. The suspension was filtered and sonicated for 20 minutes to disrupt the bacterial cells. After sonication, the mixture was centrifuged at 2000rpm for 20minutes. Percentage drug release in In vitro dissolution medium containing rat caecal contents was shown in figure no 17.

4. 9. 1 Kinetics Modeling

Data obtained from dissolution studies was fitted to various kinetic equations. The kinetic models were used zero order equation (Q = Qo -kot) first order equation (ln Q = ln Qo - k1t) Higuchi's equation (Q = kht1/2) and Korsmeyer– Peppas equation log Qt vs. log t, where Qt is the cumulative amount of drug release at time t and Qo is the initial amount of drug present in microspheres. ko is the zero order release rate constant, k1 is the first order release rate constant, and kh is the diffusion rate constant. The coefficient of regression and release rate constant values for zero, first and Higuchi and Korsmeyer–Peppas models were computed in table no 14, 15, 16, 17 and the graphs were shown in figure no 13, 14, 15, 16.

4. 9. 3 In vitro release profile:

Dissolution studies were carried out for Mebeverine HCl loaded Guar gum Microspheres and Mebeverine HCl loaded Guar gum MicrospheresMedium: 900ml of the medium was useda) pH 1. 2 buffer for two hours (since the

average gastric emptying time is 2hours.)b) pH 6. 8 buffer for two hours (Average small intestinal transit time is 3 hours.)c) pH 7. 4 buffer for subsequent hours. Apparatus: USP Apparatus Type II (Basket type)Rotational Speed: 50 rpmTemperature: 37ºc ± 0. 5 ºcSampling Time: Every hourProcedure: 5ml of sample was withdrawn at predetermined time intervals and fresh dissolution medium was replaced. The withdrawn samples were analyzed and the amount of mebeverine HCl dissolved was determined by UV absorption.