Chapter 5

Minimatrices of Amoxicillin

5.1 INTRODUCTION

Amoxicillin is a semisynthetic antibiotic with a broad spectrum of bactericidal activity against many gram-positive and gram-negative microorganisms. H. pylori is very sensitive to amoxicillin in vitro and in vivo. It acts by inhibiting the synthesis of bacterial cell wall (Walsh and Peterson, 1995). In particular, H. pylori lives deep within the gastric mucus layer and prolonged local application of drug is needed for its effective activity against the bacteria (Emami et al., 2006). Conventional immediate release formulations like tablet can not maintain bactericidal concentration of amoxicillin for longer duration. Cooreman et al found that after 60 min of oral administration of amoxicillin in the form of tablet, amoxicillin concentration in mucus was found below minimum bactericidal concentration. This led to incomplete elimination and high recolonization rate of H. pylori (Cooreman et al., 1993). This difficulty can be overcome by developing stomach specific drug delivery system of amoxicillin. Gellan gum based intra-gastric floating in-situ gelling system (Rajinikanth et al., 2007). gliadin nanoparticles (Umamaheshwari et al., 2004b), pH-sensitive chitosan / polyvinyl pyrrolidone based controlled drug release system (Risbud et al., 2000) and mucoadhesive microspheres (Liu et al., 2005) have been reported in literature. Aim of the present investigation was to develop multiparticulate formulation in the form of minimatrices for sustained delivery of amoxicillin in stomach.

5.2 MATERIALS

Amoxicillin trihydrate was received as a gift sample from Aristo Pharmaceuticals Ltd. (Mumbai, India). Colorcon Asia Pvt Ltd (Goa, India) availed Polyethylene oxide (polyox) WSR coagulant. Hydroxypropylmethylcellulose (HPMC) K100M CR and HPMC K4M as gift samples. Microcrystalline cellulose (Avicel PH102) and Carbopol 974P were obtained from Signet Chemical Corporation (Mumbai, India) and BF Goodrich Co. (Clevelend, OH) respectively. Xanthan gum, talc, magnesium stearate and polyvinylpyrrolidone (PVP) K30 were purchased from S.D.Fine Chemicals (Mumbai, India). Sodium bicarbonate, citric acid, isopropyl alcohol (IPA), hydrochloric acid were purchased from Qualigens Fine Chemicals (Mumbai, India).

5.3 METHODS

5.3.1 Preliminary Experiments

Preliminary experiments were carried out for selection of suitable excipients and suitable manufacturing method. Initially direct compression trial was taken as it involves minimum processing steps. As it was not feasible, non aqueous granulation technique was implemented using PVP K-30 solution in IPA as granulating agent. After finalizing granulation as a preparation technique, trials A-1 to A-8 (Table 5.1 and 5.2) were taken to ascertain role of individual excipient in the formulation. Further, combination of excipients were tried in formulation A-9 to A-14 (Table 5.3 and 5.4). During this optimisation exercise, formulations were mainly evaluated for buoyancy lag time, matrix integrity and drug release pattern. Key formulation components responsible for achieving minimum lag time and capable of sustaining drug release for longer duration were identified.

Table 5.1 Composition of preliminary trial batches

Ingredients (per minimatrix)	A-1		A-2		A-3		A-4	
	mg	%	mg	%	mg	%	mg	%
Amoxicillin trihydrate*	14.34	40.97	14.34	40.97	14.34	40.97	14.34	40.97
HPMC K4M	17.16	49.03	-	-	-	-	-	-
HPMC K100M	-	-	17.16	49.03	-	-	-	-
HPMC K100M CR	-	-	-	-	17.16	49.03	-	-
Polyox WSR coagulant	-	-	-	-	-	-	17.16	49.03
Sodium bicarbonate	1.05	3.00	1.05	3	1.05	3.00	1.05	3.00
Citric acid	0.35	1.00	0.35	1	0.35	1.00	0.35	1.00
PVP K30**	1.75	5.00	1.75	5	1.75	5.00	1.75	5.00
Talc	0.175	0.50	0.175	0.5	0.175	0.50	0.175	0.50
Magnesium stearate	0.175	0.50	0.175	0.5	0.175	0.50	0.175	0.50
Total weight	**35**	**100**	**35**	**100**	**35**	**100**	**35**	**100**

*Equivalent to 12.5 mg of amoxicillin, **Used as 5% w/v solution in IPA

Table 5.2 Composition of preliminary trial batches

Ingredients (per minimatrix)	A-5		A-6		A-7		A-8	
	mg	%	mg	%	mg	%	mg	%
Amoxicillin trihydrate*	14.34	40.97	14.34	40.97	14.34	40.97	14.34	40.97
HPMC K4M premium	-	-	-	-	-	-	-	-
HPMC K100M	-	-	-	-	-	-	-	-
HPMC K100M CR	-	-	-	-	-	-	-	-
Polyox WSR coagulant	-	-	-	-	-	-	-	-
Xanthan gum	17.16	49.03	-	-	-	-	-	-
Guar gum	-	-	17.16	49.03	-	-	-	-
Carbopol 974P	-	-	-	-	17.16	49.03	-	-
Crosscarmellose sodium	-	-	-	-	-	-	17.16	49.03
Sodium bicarbonate	1.05	3	1.05	3	1.05	3.00	1.05	3.00
Citric acid	0.35	1	0.35	1	0.35	1.00	.35	1.00
PVP K30	1.75	5	1.75	5	1.75	5.00	1.75	5.00
Talc	0.175	0.5	0.175	0.5	0.175	0.50	0.175	0.50
Magnesium stearate	0.175	0.5	0.175	0.5	0.175	0.50	0.175	0.50
Total weight	**35**	**100**	**35**	**100**	**35**	**100.00**	**35**	**100**

*Equivalent to 12.5 mg of amoxicillin, **Used as 5% w/v solution in IPA

Table 5.3 Composition of preliminary trial batches

Ingredients (per minimatrix)	A-9		A-10		A-11		A-12	
	mg	%	mg	%	mg	%	mg	%
Amoxicillin trihydrate*	14.34	40.97	14.34	40.97	14.34	40.97	14.34	40.97
HPMC K100M CR	12.87	36.77	8.58	24.51	6.83	19.51	5.43	15.51
Polyox WSR coagulant	-	-	8.58	24.51	6.83	19.51	5.43	15.51
Xanthan gum	-	-	-	-	3.5	10.00	3.5	10.00
Carbopol 974P	-	-	-	-	-	-	2.8	8.00
Compritol 888AT	4.29	12.26	-	-	-	-	-	-
Sodium bicarbonate	1.05	3.00	1.05	3.00	1.05	3.00	1.05	3.00
Citric acid	0.35	1.00	0.35	1.00	0.35	1.00	0.35	1.00
PVP K30	1.75	5.00	1.75	5.00	1.75	5.00	1.75	5.00
Talc	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Magnesium stearate	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Total weight	**35**	**100**	**35**	**100**	**35**	**100**	**35**	**100**

*Equivalent to 12.5 mg of amoxicillin, **Used as 5% w/v solution in IPA

Table 5.4 Composition of preliminary trial batches

Ingredients (per minimatrix)	A-13		A-14	
	mg	%	mg	%
Amoxicillin trihydrate*	14.34	40.97	14.34	40.97
HPMC K100M CR	5.43	15.51	5.43	15.51
Polyox WSR coagulant	5.43	15.51	5.43	15.51
Xanthan gum	3.5	10.00	3.5	10.00
Carbopol 974P	2.8	8.00	2.8	8.00
Sodium bicarbonate	1.05	3.00	-	-
Citric acid	0.35	1.00	-	-
Avicel PH 102	-	-	1.40	4.00
PVP K30**	1.75	5.00	1.75	5.00
Talc	0.175	0.50	0.175	0.50
Magnesium stearate	0.175	0.50	0.175	0.50
Total weight	**35**	**100**	**35**	**100**

*Equivalent to 12.5 mg of amoxicillin, **Used as 5% w/v solution in IPA

5.3.2 Drug excipient interaction study

After finalizing the excipients from preliminary experiments, their compatibility with amoxicillin was tested by Differential Scanning Calorimetry (DSC). Thermal behavior of drug and excipients, individually and in mixture, was recorded using an automatic thermal analyzer system (DSC-60, Shimadzu, Japan). Temperature calibration was performed using indium as the standard. Samples were sealed in an aluminum pan and heated from 30° to 300°C at a heating rate of 10°C/min under constant purging of dry nitrogen at 30 ml/min. Sealed empty pan was used as a reference.

5.3.3 Experimental design

Design of experiment has been widely used in pharmaceutical field to study the effect of formulation variables and their interaction on dependent (response) variables (Lewis et al., 1999; Li et al., 2003; Narendra et al., 2006). In the present study, central composite design (orthogonal) was used for formulation designing and optimisation.

Level of xanthan gum (X_1), rate controlling polymers [HPMC K100M CR : polyox WSR coagulant (1:1)] (X_2), carbopol 974P (X_3) and gas generating couple

[sodium bicarbonate: citric acid (3:1)] (X_4) were selected as formulation (independent) variables. The experimental design consists of total 26 experiments (Table 5.5), which include 16 factorial, 8 axial and 2 center points. The formulation variables and their levels were chosen from the knowledge obtained from the preliminary studies. In detail composition of all the formulations is shown in Table 5.6 to 5.12

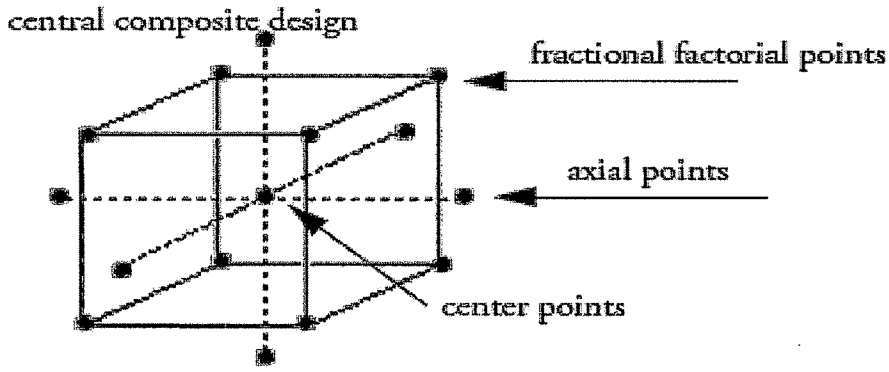


Fig 5.1 Diagram showing various points in central composite design

Buoyancy lag time (Y_1), drug release at 1 hour (Y_2), time required for 95% drug release (Y_3), swelling index (Y_4) and bioadhesive strength (Y_5) were studied as response (dependent) variables.

All the response variables were fitted to quadratic model and regression analysis was carried out to get a quantitative relationship between dependent and the analyzed independent variables. The equation can be given as

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 \quad (5.1)$$

where b_0 is arithmetic mean of 26 runs; b_i is an estimated coefficient for factors X_1, X_2, X_3 and X_4. All experimental results were computed by JMP software v5.1 (SAS Institute Inc., Cary, NC, USA)

Table 5.5 Formulation designing by central composite design

Formulation No.	Factor Levels			
	X_1	X_2	X_3	X_4
AGT 01	0	0	-1.48	0
AGT 02	1	1	-1	1
AGT 03	1	-1	-1	1
AGT 04	0	0	0	-1.48
AGT 05	-1	-1	1	-1
AGT 06	0	0	0	0
AGT 07	1	-1	1	1
AGT 08	0	0	0	1.48
AGT 09	1	1	1	1
AGT 10	0	-1.48	0	0
AGT 11	-1	1	1	-1
AGT 12	1	1	1	-1
AGT 13	-1	1	1	1
AGT 14	-1	-1	-1	-1
AGT 15	0	0	0	0
AGT 16	-1	1	-1	1
AGT 17	1	-1	1	-1
AGT 18	0	1.48	0	0
AGT 19	1	1	-1	-1
AGT 20	1.48	0	0	0
AGT 21	1	-1	-1	-1
AGT 22	-1	-1	1	1
AGT 23	-1.48	0	0	0
AGT 24	-1	1	-1	-1
AGT 25	-1	-1	-1	1
AGT 26	0	0	1.48	0
Coded values	**Actual values***			
	X_1	X_2	X_3	X_4
-1.48	6.83	4.55	4.55	2.28
-1	9	6	6	3
0	12	9	9	4.5
1	18	12	12	6
1.48	20.17	13.45	13.45	6.72

*Actual value indicates quantity per minimatrix (%w/w)

Table 5.6 Composition of by central composite design batches

Ingredients (per minimatrix)	AGT 01		AGT 02		AGT 03		AGT 04	
	mg	%	mg	%	mg	%	mg	%
Amoxicillin trihydrate*	14.34	40.97	14.34	40.97	14.34	40.97	14.34	40.97
Xanthan gum	4.20	12.00	6.30	18.00	6.30	18.00	4.20	12.00
Polyox WSR coagulant	1.58	4.50	2.10	6.00	1.05	3.00	1.58	4.50
HPMC K100M CR	1.58	4.50	2.10	6.00	1.05	3.00	1.58	4.50
Carbopol 974P	1.59	4.55	2.10	6.00	2.10	6.00	3.15	9.00
Sodium bicarbonate	1.18	3.37	1.58	4.50	1.58	4.50	0.60	1.71
Citric acid	0.39	1.13	0.53	1.50	0.53	1.50	0.20	0.57
PVP K-30**	1.75	5.00	1.75	5.00	1.75	5.00	1.75	5.00
Avicel PH102	8.04	22.98	3.86	11.03	5.96	17.03	7.26	20.75
Mg stearate	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Talc	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Total Wt	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*

*Equivalent to 12.5 mg of amoxicillin, **Used as 5% w/v solution in IPA

Table 5.7 Composition of by central composite design batches

Ingredients (per minimatrix)	AGT 05		AGT 06		AGT 07		AGT 08	
	mg	%	mg	%	mg	%	mg	%
Amoxicillin trihydrate*	14.34	40.97	14.34	40.97	14.34	40.97	14.34	40.97
Xanthan gum	3.15	9.00	4.20	12.00	6.30	18.00	4.20	12.00
Polyox WSR coagulant	1.05	3.00	1.58	4.50	1.05	3.00	1.58	4.50
HPMC K100M CR	1.05	3.00	1.58	4.50	1.05	3.00	1.58	4.50
Carbopol 974P	4.20	12.00	3.15	9.00	4.20	12.00	3.15	9.00
Sodium bicarbonate	0.79	2.25	1.18	3.37	1.58	4.50	1.76	5.04
Citric acid	0.26	0.75	0.39	1.13	0.53	1.50	0.59	1.68
PVP K-30**	1.75	5.00	1.75	5.00	1.75	5.00	1.75	5.00
Avicel PH102	8.06	23.02	6.49	18.53	3.86	11.03	5.71	16.31
Mg stearate	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Talc	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Total Wt	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*

*Equivalent to 12.5 mg of amoxicillin, **Used as 5% w/v solution in IPA

Table 5.8 Composition of by central composite design batches

Ingredients (per minimatrix)	AGT 09		AGT 10		AGT 11		AGT 12	
	mg	%	mg	%	mg	%	mg	%
Amoxicillin trihydrate*	14.34	40.97	14.34	40.97	14.34	40.97	14.34	40.97
Xanthan gum	6.30	18.00	4.20	12.00	3.15	9.00	6.30	18.00
Polyox WSR coagulant	2.10	6.00	0.80	2.27	2.10	6.00	2.10	6.00
HPMC K100M CR	2.10	6.00	0.80	2.27	2.10	6.00	2.10	6.00
Carbopol 974P	4.20	12.00	3.15	9.00	4.20	12.00	4.20	12.00
Sodium bicarbonate	1.58	4.50	1.18	3.37	0.79	2.25	0.79	2.25
Citric acid	0.53	1.50	0.39	1.13	0.26	0.75	0.26	0.75
PVP K-30**	1.75	5.00	1.75	5.00	1.75	5.00	1.75	5.00
Avicel PH102	1.76	5.03	8.05	22.99	5.96	17.02	2.81	8.02
Mg stearate	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Talc	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Total Wt	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*

*Equivalent to 12.5 mg of amoxicillin, **Used as 5% w/v solution in IPA

Table 5.9 Composition of by central composite design batches

Ingredients (per minimatrix)	AGT 13		AGT 14		AGT 15		AGT 16	
	mg	%	mg	%	mg	%	mg	%
Amoxicillin trihydrate*	14.34	40.97	14.34	40.97	14.34	40.97	14.34	40.97
Xanthan gum	3.15	9.00	3.15	9.00	4.20	12.00	3.15	9.00
Polyox WSR coagulant	2.10	6.00	1.05	3.00	1.58	4.50	2.10	6.00
HPMC K100M CR	2.10	6.00	1.05	3.00	1.58	4.50	2.10	6.00
Carbopol 974P	4.20	12.00	2.10	6.00	3.15	9.00	2.10	6.00
Sodium bicarbonate	1.58	4.50	0.79	2.25	1.18	3.37	1.58	4.50
Citric acid	0.53	1.50	0.26	0.75	0.39	1.13	0.53	1.50
PVP K-30**	1.75	5.00	1.75	5.00	1.75	5.00	1.75	5.00
Avicel PH102	4.91	14.03	10.16	29.02	6.49	18.53	7.01	20.03
Mg stearate	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Talc	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Total Wt	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*

*Equivalent to 12.5 mg of amoxicillin, **Used as 5% w/v solution in IPA

Table 5.10 Composition of by central composite design batches

Ingredients (per minimatrix)	AGT 17		AGT 18		AGT 19		AGT 20	
	mg	%	mg	%	mg	%	mg	%
Amoxicillin trihydrate*	14.34	40.97	14.34	40.97	14.34	40.97	14.34	40.97
Xanthan gum	6.30	18.00	4.20	12.00	6.30	18.00	7.05	20.14
Polyox WSR coagulant	1.05	3.00	2.35	6.71	2.10	6.00	1.58	4.50
HPMC K100M CR	1.05	3.00	2.35	6.71	2.10	6.00	1.58	4.50
Carbopol 974P	4.20	12.00	3.15	9.00	2.10	6.00	3.15	9.00
Sodium bicarbonate	0.79	2.25	1.18	3.37	0.79	2.25	1.18	3.37
Citric acid	0.26	0.75	0.39	1.13	0.26	0.75	0.39	1.13
PVP K-30**	1.75	5.00	1.75	5.00	1.75	5.00	1.75	5.00
Avicel PH102	4.91	14.02	4.94	14.10	4.91	14.02	3.64	10.39
Mg stearate	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Talc	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Total Wt	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*

*Equivalent to 12.5 mg of amoxicillin, **Used as 5% w/v solution in IPA

Table 5.11 Composition of by central composite design batches

Ingredients (per minimatrix)	AGT 21		AGT 22		AGT 23		AGT 24	
	mg	%	mg	%	mg	%	mg	%
Amoxicillin trihydrate*	14.34	40.97	14.34	40.97	14.34	40.97	14.34	40.97
Xanthan gum	6.30	18.00	3.15	9.00	2.39	6.83	3.15	9.00
Polyox WSR coagulant	1.05	3.00	1.05	3.00	1.58	4.50	2.10	6.00
HPMC K100M CR	1.05	3.00	1.05	3.00	1.58	4.50	2.10	6.00
Carbopol 974P	2.10	6.00	4.20	12.00	3.15	9.00	2.10	6.00
Sodium bicarbonate	0.79	2.25	1.58	4.50	1.18	3.37	0.79	2.25
Citric acid	0.26	0.75	0.53	1.50	0.39	1.13	0.26	0.75
PVP K-30**	1.75	5.00	1.75	5.00	1.75	5.00	1.75	5.00
Avicel PH102	7.01	20.02	7.01	20.03	8.30	23.70	8.06	23.02
Mg stearate	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Talc	0.175	0.50	0.175	0.50	0.175	0.50	0.175	0.50
Total Wt	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*	*35.0*	*100*

*Equivalent to 12.5 mg of amoxicillin, **Used as 5% w/v solution in IPA

Table 5.12 Composition of by central composite design batches

Ingredients (per minimatrix)	AGT 25		AGT 26	
	mg	%	mg	%
Amoxicillin trihydrate*	14.34	40.97	14.34	40.97
Xanthan gum	6.30	18.00	7.05	20.14
Polyox WSR coagulant	2.10	6.00	1.58	4.50
HPMC K100M CR	2.10	6.00	1.58	4.50
Carbopol 974P	2.10	6.00	3.15	9.00
Sodium bicarbonate	0.79	2.25	1.18	3.37
Citric acid	0.26	0.75	0.39	1.13
PVP K-30**	1.75	5.00	1.75	5.00
Avicel PH102	4.91	14.02	3.64	10.39
Mg stearate	0.175	0.50	0.175	0.50
Talc	0.175	0.50	0.175	0.50
Total Wt	*35.0*	*100*	*35.0*	*100*

*Equivalent to 12.5 mg of amoxicillin, **Used as 5% w/v solution in IPA

5.3.4 Preparation of minimatrices

Required quantities of amoxicillin trihydrate, xanthan gum, polyox WSR coagulant, HPMC K100M CR, sodium bicarbonate, citric acid and Avicel PH102 were passed through sieve no.30 (Jayant Scientific Sieves, Mumbai, India) and properly mixed. PVP K30 solution (5% w/v) was prepared by dissolving it in isopropyl alcohol. This solution was slowly added to dry powder blend with proper kneading to obtain a granular mass of sufficient strength. Granulated mass was air dried at room temperature for 15-20 min and passed through sieve no.30. Sifted granules were dried at 40°-40°C for 20 min in tray dryer (Shree Kailash Industries, Baroda, India). Carbopol 974P was added in dried granules and mixed well. Magnesium stearate and talc were passed through sieve 40 and added to the granule blend. The blend was lubricated by proper mixing. Bulk density, tapped density and angle of repose of the lubricated granules was measured.

Lubricated granules were compressed into minimatrices on eight station rotary tablet compression machine (General Machinery Co., Mumbai, India) using 4 mm circular multi-tip punches. Compressed minimatrices were evaluated for weight

variation, thickness, hardness and friability as in process quality control parameters.

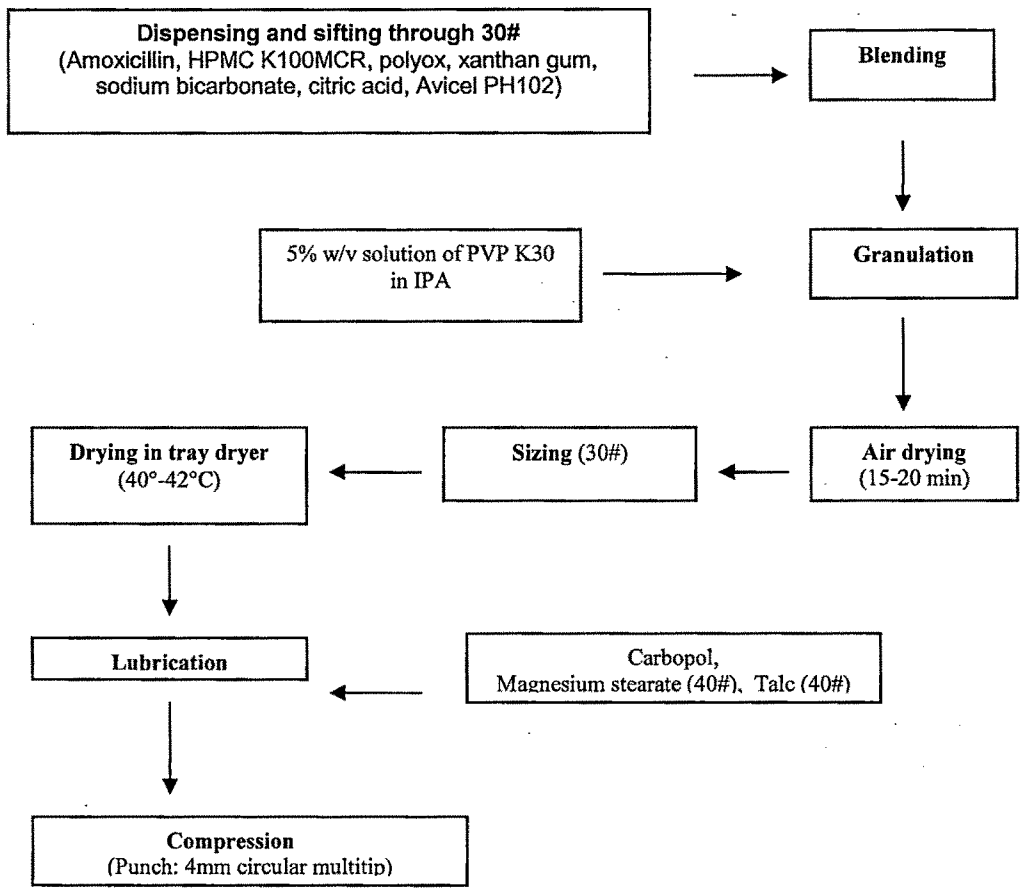


Fig 5.2 Flow diagram for preparation of minimatrices

5.3.5 Evaluation of granule properties

5.3.5.1 Bulk Density and Tapped Density

Suitably weighed quantity of the lubricated granules was transferred to 25 ml measuring cylinder. Volume occupied by the blend was measured and bulk density was calculated by the following equation

Bulk Density (B.D.)= Mass / Bulk Volume (5.2)

Granules in the measuring cylinder were subjected to 100 tappings by using density test apparatus (Electrolab, Mumbai, India) and the final volume occupied by the blend was noted. Tapped density was calculated by following equation

Tapped Density (T.D.) = Mass / Tapped Volume (5.3)

5.3.5.2 Compressibility Index and Hausner Ratio

Bulk density and tapped density can be used to calculate compressibility index and Hausner ratio which are measures of propensity of powder to flow and to be compressed (Amidon et al., 2009)

$$\text{Compressibility index} = (\text{T.D.} - \text{B.D.} / \text{T. D.}) \times 100 \qquad (5.4)$$

$$\text{Hausner ratio} = \text{T.D.} / \text{B. D.} \qquad (5.5)$$

Compressibility index below 10 indicate excellent flow properties of a blend while the value above 30 indicate poor flow. Hausner ratio between 1.0 and 1.11 indicates excellent flow while the value above 1.35 indicates poor flow.

5.3.5.3 Angle of Repose

Angle of repose determination is also one the method for estimating flow properties of the blend. It was determined by funnel method. Funnel was adjusted to certain height using tripod stand. Specific quantity of the lubricated blend was taken and it was passed through funnel until the heap formed touched tip of funnel. Angle of repose was determined by equation

$$\text{Angle of repose } (\theta) = \tan^{-1} h/r \qquad (5.6)$$

where h = height of the heap and r = radius of heap

5.3.6 Evaluation of minimatrices

5.3.6.1 Weight variation

Twenty minimatrices were individually weighed and percent weight variation was calculated.

5.3.6.2 Minimatrix dimensions

Diameter and thickness of the minimatrices was determined using vernier caliper.

5.3.6.3 Hardness

Hardness of the minimatrices was measured by using Monsanto type hardness tester.

5.3.6.4 Friability

Twenty minimatrices were weighed and transferred to the drum of friability test apparatus (Electrolab, Mumbai, India). Pan was rotated for 100 revolutions at the

speed of 25 revolutions per minute (rpm). Weight of the minimatrices after 100 revolutions was noted. Friability was calculated as:

Friability (%) = [(Initial weight – Final weight) / Initial weight] x 100 (5.7)

5.3.6.5 Drug content

Amoxicillin content was estimated by HPLC method. Twenty minimatrices were finely powdered. Powder equivalent to one minimatrix (35 mg) was taken in 100 ml volumetric flask. About 70-80 ml of mobile phase was added to it and sonication (Modern Industrial Corporation, Mumbai, India) was done for 20 min. Volume was made upto 100 ml by adding mobile phase. The solution was filtered using Whatman filter paper type I. One ml of the filtrate was diluted to 10 ml with mobile phase and 20 µl of the finally diluted solution was injected. Drug content was calculated from the peak area.

5.3.6.6 Buoyancy lag time and total buoyancy time

Lag time is the time interval between introduction of the minimatrices in the dissolution vessel to the time when these start floating towards the surface of dissolution medium. It was determined simultaneously during drug release study. Total time for which the formulation remains floated was also noted.

5.3.6.7 Drug Release Study

Drug release study was carried out in 900 ml of 0.1N HCl at 37±0.5°C using USP type II dissolution test apparatus (VDA 6-DR, Veego Instruments Corporation, Mumbai, India) at 50 rpm. Eight minimatrices (containing total 100 mg of amoxicillin) were introduced in dissolution vessel. Sample (5 ml) was withdrawn at 1,2,3,4,6,8,10 and 12 h and was replenished with equal volume of dissolution medium. After suitable dilution, amount of drug released was estimated by UV spectrophotometry (UV-1700, Pharmaspec, Shimadzu, Japan) at 229 nm.

5.3.6.8 Scanning Electron Microscopy (SEM) studies

SEM studies were carried out to observe surface features of the minimatrices of optimum formulation. Minimatrices were introduced in dissolution medium. Test parameters were same as mentioned in Drug release study. Minimatrices were withdrawn after 6 h and excess of dissolution medium was wiped off. Surface

features were observed at different magnifications using Scanning Electron Microscope JSM-5610LV (Jeol Ltd., Japan). Surface features of minimatrix before exposing to dissolution medium i.e dry minimatrix were also observed.

5.3.6.9 Kinetic modelling of drug release

Kinetic modelling was done by fitting the in vitro drug release data to zero-order release kinetics (equation 5.8), first-order release kinetics (equation 5.9), Higuchi's square root of time equation (equation 5.10) (Costa and Lobo, 2001) and Ritger and Peppas equation (equation 5.11) (Ritger and Peppas, 1987)

$$Q_t = Q_0 + K_0 t \qquad (5.8)$$

where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and K_0 is the zero order release constant

$$Q_t = Q_0 e^{-K_1 t} \qquad (5.9)$$

where Q_t is the amount of drug dissolved in time t, Q_o is the initial amount of drug in the solution, K_1 is the first order release constant

$$Q_t = K_H t^{1/2} \qquad (5.10)$$

where Q_t is the amount of drug dissolved in time t, K_H is the Higuchi release constant.

$$Q_t / Q_\infty = K_R t^n \qquad (5.11)$$

where Q_t is the amount of drug dissolved in time t, K_R is the release constant.

5.3.6.10 Fluid uptake study

This study was carried out by a new and convenient method using the baskets of dissolution test apparatus. Five minimatrices were placed in a basket which was immersed in a petridish having 100 ml of 0.1N HCl. Baskets were removed every hour, excess of the 0.1N HCl was soaked by tissue paper and final weight was measured. The study was carried upto 12 h. Fluid uptake capacity was expressed as swelling index and it was calculated by equation

$$\text{Swelling Index} = [(W_2 - W_1) / W_1] \times 100 \qquad (5.12)$$

Where W_1 = Initial weight of minimatrices and W_2= Weight of wet minimatrices at 12 h

5.3.6.11 Ex-vivo bioadhesion study

This study was carried out by using Instron tensiometer (Instron 1121, UK). On the upper jaw of tensiometer, single minimatrix was stuck using double sided adhesive tape and on the lower jaw goat stomach tissue (which was freshly

collected from local slaughter house) was fixed. Upper jaw, having 10 gm load cell, was lowered until it came in proper contact with the tissue and was kept as such for 20 seconds. Afterwards upper jaw was moved in upward direction at speed of 5 mm/min until the minimatrix was completely detached from the tissue. During the test, goat stomach tissue was wetted by adding 20 µl of 0.1N HCl. Force in "dynes/cm^{2}" required for this detachment was measured.

5.3.7 Optimisation of responses using desirability function

This method is used to achieve an optimum formula based on desirability functions. In desirability approach, each response is associated with its own partial desirability which varies from 0 to 1 according to closeness of the response to its target value. The desirability function is a guide to optimize the process or a formulation using multiple response data from a statistically planned experiment. During optimization of formulations, the responses have to be combined in order to produce a product of desired characteristics. The application of the desirability function combines all the responses in one measurement and gives the possibility of predicting optimum levels for the independent variables. The combination of the responses in one desirability function requires the calculation of the individual desirability (Mashru et al., 2005).

In the context of present investigation, suitable formulation should have low buoyancy lag time, low % drug release at 1 h, high time required for 95% drug release, high swelling index and high bioadhesion strength. The individual desirability for each response was calculated using the following methods.

Buoyancy lag time and % drug release at 1h were minimized in the optimization procedure, as suitable formulation should have low lag time, and should release less amount of drug at initial hour. Desirability of these responses were calculated using following equation:

$$d_1 \text{ or } d_2 = Y_{max} - Y_i / Y_{max} - Y_{target} \quad \text{for } Y_i > Y_{target} \qquad (5.13)$$

$$d_1 \text{ or } d_2 = 1 \quad \text{for } Y_i < Y_{target} \qquad (5.14)$$

where d_1 and d_2 is individual desirability for buoyancy lag time and % drug release at 1h respectively. The Y_{max} and Y_{target} indicate maximum and target (minimum) value of experimental result for respective response variables (Table

5.13). Y_i is the experimental result of the response variable for individual factorial design batch.

Time required for 95% drug release, swelling index and bioadhesion strength values were maximized in the optimization procedure, as suitable formulation should have high values for these response variables. Desirability function of these responses were calculated using the following equation:

$$d_3 \text{ or } d_4 \text{ or } d_5 = Y_i - Y_{min} / Y_{target} - Y_{min} \quad \text{for } Y_i < Y_{target} \qquad (5.15)$$

$$d_3 \text{ or } d_4 \text{ or } d_5 = 1 \quad \text{for } Y_i > Y_{target} \qquad (5.16)$$

where d_3 is desirability for time required for 95% drug release, d_4 for swelling index and d_5 for bioadhesive strength. The Y_{min} and Y_{target} indicate minimum and target (maximum) value of experimental result for respective response variables. Y_i is the experimental result of the response variable for individual factorial design batch.

The overall desirability values were calculated from the individual values by using the following equation:

$$D = (d_1 d_2 d_3 d_4 d_5)^{1/5} \qquad (5.17)$$

5.3.8 In vivo study by gamma scintigraphy

Gamma scintigraphy is a technique whereby the transit of a dosage form through its intended site of delivery can be non-invasively imaged in vivo via the judicious introduction of an appropriate short lived gamma emitting radioisotope. Standard radiolabeling techniques incorporate the radioactive marker in a finished product shortly before dose administration. Technetium 99m is the most commonly used metal atom in radiopharmaceuticals, probably 75 % of all radiopharmaceuticals include technetium 99m as the radionuclide. Technetium 99m has desirable physical properties for imaging purposes. It has 6 h half life and 140 KeV gamma photon that is emitted with high abundance (Hladik and Norenberg, 2000). In the present study minimatrices were radiolabelled by using Technetium Tc 99m pentetate as a radiolabelling agent. Small holes were made on one side of the minimatrices. Radioactive material having 6 milicurie of radioactivity was introduced using a fine needle (Ofori-Kwakye, et al., 2004). The holes were sealed using non-aqueous solution of ethyl cellulose to avoid leakage of the

radioactive material. Four radiolabelled minimatrices were introduced into hard gelatin capsule. In vivo gastric residence time of the optimum formulation was evaluated in three healthy human volunteers having age group of 25 to 35 years. There was no history of previous illness in near past. Volunteers were fasted overnight and radiolabelled minimatrices introduced in hard gelatin capsules were administered after a standard breakfast along with 250 ml of water. In vivo transit behaviour of the formulation was monitored by capturing the images by Gamma camera (e-cam signature series, Siemens).

5.3.9 Stability studies

Stability study is an integral part of the formulation development process. Optimised formulation was subjected to stability studies as per ICH guidelines. Samples were packed in aluminium pouches using 0.04 mm aluminium foil and charged for stability at 40°C/75%RH and 30°C/65%RH in stability chamber (Newtronic, Mumbai). Samples were withdrawn at 1, 2, 3 and 6 month interval and analysed for physical appearance, buoyancy lag time, drug content, bioadhesive strength and in vitro dissolution profile.

5.4 RESULTS AND DISCUSSION

5.4.1 Preliminary Experiments

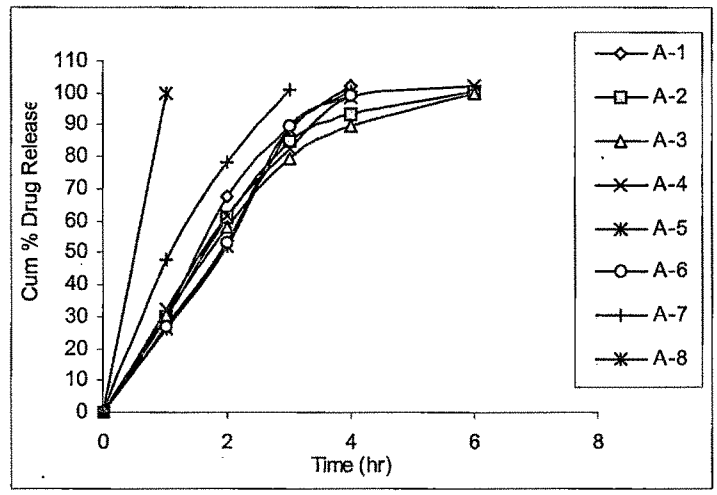
Preliminary experiments were conducted to ascertain feasibility for direct compression of minimatrices. But it was not successful due to poor flow of drug excipient blend. It was due to presence of major amount of amoxicillin trihydrate, a fluffy component, in the formula. Hence granulation technique was implemented. Non-aqueous solution of PVP K30 (5% w/v solution in IPA) was used as granulating agent. Granules obtained with this technique had sufficient granular strength and dried granules were having good flow properties. Preliminary experiments revealed that the agents responsible for gas generation must be added in the formulation in order to impart floating feature. Sodium bicarbonate is one such excipient which reacts in presence of acidic dissolution medium to generate carbon dioxide bubbles. These bubbles get entrapped in the formulation matrix and reduces its density. Thus it imparts floating feature to the formulation. For proper gas formation, acidic atmosphere is essential. After oral administration of a dosage form, this atmosphere can be provided by acidic gastric contents. Some times gastric pH may be elevated and in such cases gas

generation may be failed. Hence citric acid was included in the formulation (Liu and Fassihi, 2008). Sodium bicarbonate and citric acid act as gas generating couple. Talc and magnesium stearate were added as lubricants. Granulating agent, gas generating couple and lubricants were integral part of each formulation. In trial A-1 to A-8 (Table 5.1 and 5.2), different polymers and gum were tried individually. Formulation A-1, containing HPMC K4M, was having floating lag time of 8-10 min and it was capable of sustaining drug release only upto 4 hours. Formulation A-5 and A-6 containing xanthan gum and guar gum could sustain drug release upto 4 hours but it totally lack floating phenomenon. Formulation A-2, A-3 and A-4 containing HPMC K100, HPMC K100M CR and polyox, respectively, were capable to sustain drug release upto 6 h and had floating lag time of 8-10 min. Formulation A-7 containing carbopol 974P released whole amount of drug in 3 h while A-8 containing crosscarmellose sodium released whole drug in 1 h. Very less sustained release capability of A-7 may be due to highly hydrophilic nature of carbopol and its inability maintain integrity. Crosscaremellose has swelling and disintegrant property that is responsible for rapid matrix disintegration and rapid drug release.

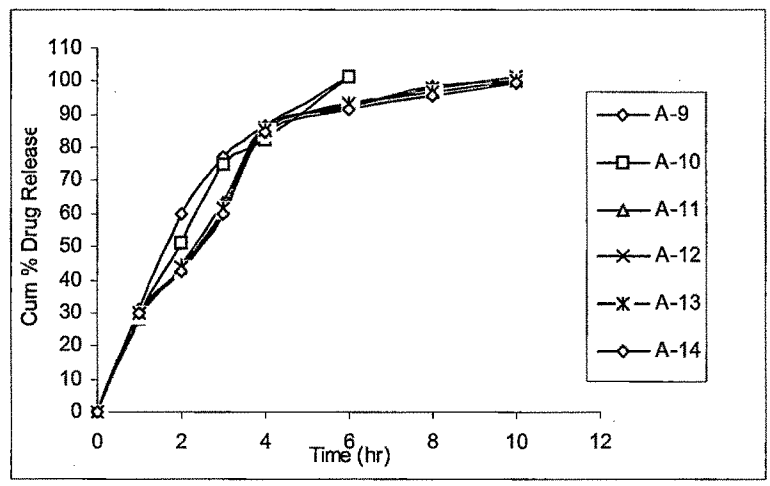
HPMC K100M CR based formulation A-3 showed somewhat promising sustained drug release pattern. But it could sustain drug release only upto 6 h. To further prolong this feature, need was felt to include some lipid based excipient which could sustain the release phenomenon due to its hydrophobic nature. Formulation A-9 was prepared by hot melt granulation of amoxicillin by compritol 888AT. But no promising results were obtained as the formulation could sustain drug release only upto 6 h.

In trial A-10, polyox WSR coagulant was introduced along with HPMC K100M CR in 1:1 proportion. This formulation has the results which were not promising than A-9. Xanthan gum has the property to form a viscous network after getting hydrated. In experiment A-11, xanthan gum was included in the formulation composition. Viscolysing property of gum might have helped to create obstacles in drug release channels formed by polymeric network. Thus A-11 could sustain drug release upto 10 h. Carbopol 974P is a bioadhesive polymer having strong hydrophilic property which swells in presence of acidic dissolution media (Li et al., 2002). Swelling phenomenon can further enhance sustained drug release feature

as it increases diffusional path length of drug release channel (Miranda et al., 2006). This component was added in granulation stage in formulation A-12 which can sustain drug release upto 10h. Whether incorporation of this component in lubrication stage can affect the drug release was ascertained in trial A-13 which indicated no significant difference. Role of gas generating couple in achieving buoyancy feature was ascertained by trial A-14 as it was conducted without incorporating sodium bicarbonate and citric acid. The formulation absolutely failed to float which clearly indicated that presence of this component is must in the formulation. The preliminary experiments offered tentative guidelines regarding selection of the formulation components and method of its preparation. Further systematic optimisation studies were carried out by an experimental design approach to find out appropriate level of an individual component.



5.3 Dissolution profile of preliminary trial batches (A-1 to A-8)



5.4 Dissolution profile of preliminary trial batches (A-9 to A-14)

5.4.2 Drug excipient interaction study

DSC thermogram for amoxicillin showed that it has endothermic peak at 134.9°C (Fig 5.5). The mixture containing amoxicillin and other formulation excipients also showed an endothermic peak at the same temperature as shown by an individual amoxicillin. It clearly indicated that there was no interaction between amoxicillin and the excipients.

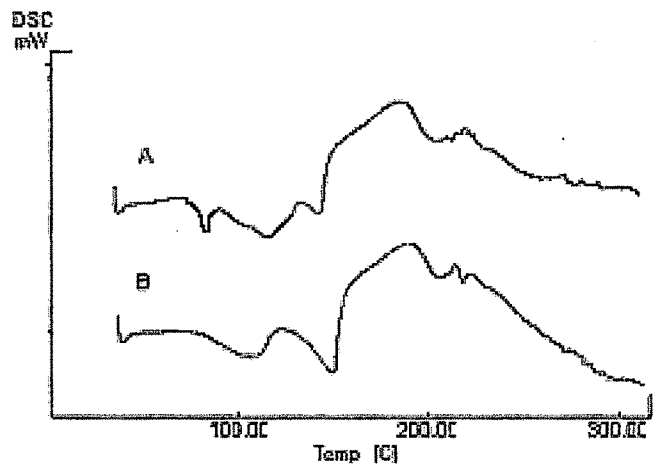


Fig. 5.5 DSC thermogram (A) Amoxicillin along with excipients and (B) Pure amoxicillin

5.4.3 Experimental design

Preliminary experiments in the laboratory revealed that HPMC K100M CR and polyox coagulant along with xanthan gum played significant role in sustaining drug release while carbopol 974P was important for maintaining matrix integrity (Deshpande et al., 1997) and sustaining drug release. Sodium bicarbonate and citric acid had prominent role in imparting buoyancy phenomenon. Total buoyancy time depends on the overall entrapment of the gas in the matrix network formed by polymers, gum and carbopol. Hence these four components were selected as formulation variables for systematic optimisation studies. Results of the experiments carried out as per central composite design are shown in Table 5.13. Regression coefficients obtained by correlating formulation and response variables, using the mathematical relationship (equation 5.1) are shown in Table 5.14. Such polynomial equations can be used to draw

conclusions by considering sign i.e. positive or negative and magnitude of the coefficient (Li et al., 2001). High values of coefficient of determination (R^2) indicate good fit. The prediction profiler correlating independent and response variables is shown in Fig 5.7

5.4.4 Evaluation of granule properties

For all the designed formulations, bulk density was found between 0.42 and 0.58 gm/cm^3. Angle of repose was between 30° and 40° which indicates good flow properties (Marshall, 1987). Good flow properties are important for avoiding weight variation problems during compression of the minimatrices.

5.4.5 Evaluation of minimatrices

5.4.5.1 Weight variation

Weight variation was 6.1% calculated with respect to theoretical weight of the minimatrix. It was found satisfactory as it is less than 10% which is allowable limit for the uncoated tablets having unit weight less than 80mg (IP, 2007).

5.4.5.2 Minimatrix dimensions

Diameter of the minimatrices was 4.0 mm and thickness was between 2.8 and 3.1 mm

5.3.5.3 Hardness and Friability

Hardness of the minimatrices was 2-3 kg/cm^2 and friability was about 0.12% which indicates sufficient mechanical strength.

5.4.5.4 Drug content

All the formulations were found to contain 99.2 to 100.9% of added amount of amoxicillin per minimatrix. Drug content was estimated as per the procedure described in method section. Sonication was necessary in the procedure as the minimatrices contained polymers which have tendency to hold drug in the matrix network. Preliminary experiments confirmed 20 min of sonication time to be sufficient for complete drug extraction from the matrix network.

5.4.5.5 Buoyancy lag time and total buoyancy time

Aim of the present research work was to develop a formulation having gastroretentive capabilities which can be achieved by imparting floating and

gastric mucoadhesive properties. Hence buoyancy lag time is very important parameter for the developed formulations. Short lag time may ensure immediate floating of the minimatrices and may further avoid settling of the formulation in lower part of stomach and ultimately avoid escape of the formulation from pyloric sphincter (Streubel et al., 2006). Buoyancy lag time varied from 7 to 32 min for the developed formulations (Table 5.13). Least lag time of 7 min and maximum lag time of 32 min was observed for formulation AGT 09 and AGT 04 respectively. Least lag time might be observed in formulation AGT 09 due to presence of higher amount of gas generating couple (X_4) as compared to other formulations.

As can be seen from the results of regression analysis in Table 5.14 and prediction profiler in Fig 5.7, gas generating couple [sodium bicarbonate : citric acid (3:1)] (X_4) significantly decreased buoyancy lag time. Sodium bicarbonate and citric acid react in presence of acidic dissolution medium and generates carbon dioxide which gets entrapped in polymer matrix and decreases density of the minimatrix (Li et al., 2001). Sodium bicarbonate alone can react with gastric fluid to produce carbon dioxide. But citric acid was also included in the formulation to assure that an acidic microenvironment within the swelling matrix is maintained. This may contribute to continuous generation of carbon dioxide in the matrix independent of external changes in the pH environment (Liu and Fassihi, 2008). Xanthan gum has a tendency to form a viscous gel. Formation of viscous gel entraps the gas bubbles inside the matrix and minimizes chances of bubbles getting escaped from the polymer network channels. This in turn led to floating behavior of the minimatrices for longer duration. After swelling of the polymers in the matrix network for sometime, the system becomes hydrodynamically balanced due to fluid penetration. This phenomenon also significantly contributes in overall buoyancy time of the formulation (Bardonnet et al 2006). All the developed formulations were capable to float until their matrix integrity was maintained. It lasted upto 12 h for few formulations. Physical integrity of the formulations was maintained due to presence of carbopol which becomes viscous in presence of water and tends to bind the mixed polymeric system together and reduces matrix erosion (Li et al., 2002).

5.4.5.6 Drug release study

Sustaining drug release is very important aspect for maintaining drug concentration for longer time in the stomach, which is residence site of *H. pylori.* Maintaining effective drug concentration for longer time may completely eradicate *H. pylori* infection (Cooreman et al, 1993). Hence core goal of the present research work was to prepare a formulation having gastroretentive capability with sustained drug release feature.

Drug release can be sustained for longer time by retarding initial hour release to maximum possible extent. Xanthan gum (X_1), rate controlling polymers (HPMC K100M CR and polyox) (X_2) and carbopol 974P (X_3) were found to play important role in decreasing drug release at initial hour. Drug release at 1 h (Y_2) was 32.5% for formulation AGT 09 which contains high level of gum, polymers and carbopol while it was 53.3% for AGT 14 containing lowest level of these formulation variables. Results of regression analysis (Table 5.14), from its negative sign and magnitude and Prob>F value indicate that xanthan gum has significant role in retarding drug release at first hour as compared to HPMC, polyox and carbopol.

HPMC is a neutral hydrophilic polymer. The polymer molecular chains of HPMC hydrate in contact with water, entangle and form a gel matrix (Tritt-Goc and Pislewski, 2002). When exposed to water, carbopol becomes viscous and thus tends to bind the mixed polymeric system together (Li et al., 2002). During hydration process, channels are formed in the matrix network which are responsible for drug diffusion. After coming in contact with water, xanthan gum forms very viscous network. This network is particularly built up in the drug diffusion channels formed by polymeric network and ultimately may be responsible for retarding drug release in initial hour. The channel blockage might have enhanced with increasing gum level which might be responsible for decrease in initial hour drug release. Xanthan gum (X_1) and rate controlling polymers (X_2) have significant effect on the % drug release at 1h (Y_2). Higher level of X_1 and X_2 is necessary that for decreasing drug release at initial hour.

Time required for 95% drug release (Y_3) was also increased due to xanthan gum, rate controlling polymers (HPMC K100M CR and polyox) and carbopol 974P. Formulation AGT 09 and AGT14 required 9.39 and 3.31 h respectively for 95%

drug release. Less time may be required for Formulation no. AGT 14 as the matrix may not be capable to sustain drug release for longer time due to low level of X_1, X_2 and X_3 and the observation was vice versa for AGT 09 containing high levels of these formulation variables. Results of regression analysis in Table 5.14 indicate that X_1, X_2 and X_3 have significant effect on Y_3 while magnitude of regression coefficient shows maximum influence of X_2. Similar observation is presented as a prediction profiler in Fig.5.7.

Xanthan gum, HPMC, polyox and carbopol together played crucial role in sustaining drug release. Xanthan gum decreased drug release at initial hour due to its rapid viscolysing property. HPMC and polyox were particularly responsible for sustaining drug release at later period. So the drug release was found to be high initially and then gradually decreased. The diffusional spaces inside the gelling system are controlled by the molecular weight of the polymer (Siepe et al., 2008). Diffusion is the predominant drug release mechanism from high molecular weight HPMC and polyox matrices which swell to a higher extent. Swelling phenomenon increases matrix size and therefore diffusional path length is increased (Miranda et al., 2006). Drug entity present in the matrix core may ultimately be requiring more time to travel towards the matrix surface. This phenomenon may be responsible for increased time required for 95% drug release. HPMC and polyox take some time to get hydrated and swell. As this process is time dependent, drug release might be sustained in later hours due to these polymers. Since the swelling capacity depends on amount of polymer present in the formulation, as concentration of X_2 increased from -1 level to +1 level, time required for 95% drug release (Y_3) was rapidly increased as shown in Fig 5.7.

Carbopol is a water-insoluble but water swellable crosslinked polymer with molecular weight approximately $2x10^6$ Da. Swelling occurs due to the uncharged -COOH group that hydrates by forming hydrogen bonds with the imbibing water, thus extending the polymer chains. Swelling of this polymer contribute partially to the floating behavior of the GRDDS. When exposed to water, carbopol becomes viscous and thus tends to bind the mixed polymeric system together and reduces erosion of GRDDS (Li et al., 2002). This viscous network ultimately results in sustained drug delivery phenomenon.

Table 5.13 Experimental Results

Formulation No.	Buoyancy lag time (min)	Drug Release at 1h (%)	Time for 95%drug Release (h)	Swelling Index	Bioadhesion ($x10^3$dynes/cm^2)
	Y_1	Y_2	Y_3	Y_4	Y_5
AGT 01	17	43.4	4.39	345.8	6.8
AGT 02	16	41.1	5.93	501.2	7.9
AGT 03	12	45.1	4.51	317.2	7.2
AGT 04	32	46.3	4.42	342.2	9.3
AGT 05	21	48.6	4.44	204.9	12.9
AGT 06	15	45.6	4.44	296.6	10.1
AGT 07	10	38.1	6.25	453.9	12.2
AGT 08	8	46.9	4.45	329.5	9.7
AGT 09	7	32.5	9.39	341.0	17.9
AGT 10	19	47.1	3.34	258.6	9.2
AGT 11	23	42.2	7.58	200.2	15.3
AGT 12	22	35.2	9.20	412.6	17.1
AGT 13	14	39.5	7.88	179.1	16.9
AGT 14	25	53.3	3.31	314.0	7.0
AGT 15	15	45.6	4.44	296.6	8.9
AGT 16	18	48.1	5.86	215.5	8.7
AGT 17	21	40.9	6.21	443.0	17.5
AGT 18	16	42.3	5.85	393.2	11.8
AGT 19	21	38.5	7.47	382.5	11.2
AGT 20	22	31.9	5.94	466.8	8.2
AGT 21	24	45.2	4.51	423.7	7.1
AGT 22	15	49.2	4.36	225.5	10.2
AGT 23	18	50.6	4.33	159.0	8.8
AGT 24	22	47.3	5.97	289.3	8.3
AGT 25	14	52.6	3.32	250.3	7.0.
AGT 26	16	45.4	6.90	310.0	18.2

5.4.5.7 *Scanning Electron Microscopy (SEM) studies*

SEM studies shown that the minimatrix surface is intact in dry state (Fig. 5.6a). After exposure to dissolution medium, polymers present in the matrix absorbs surrounding dissolution medium and starts swelling. Diffusion channels created in the matrix network have openings on the surface. These surface openings which act as drug release channels are clearly observed in SEM images the form of pores on minimatrix surface (Fig. 5.6b,c,d). SEM Images for the exposed minimatrices were captured at different magnification of 25x, 50x and 100x.

Table 5.14 Regression coefficients for response variables

Term	Y_1		Y_2		Y_3		Y_4		Y_5	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
b_0	16.80	-	45.36	-	4.12	-	310.40	-	9.17	-
X_1	-0.64	0.4007	-4.51	< 0.0001	0.64	< 0.0001	90.84	< 0.0001	0.54	0.1197
X_2	-0.17	0.8220	-2.73	0.0007	1.28	< 0.0001	4.33	0.7316	1.28	0.0020
X_3	-1.00	0.1981	-2.06	0.0048	0.89	< 0.0001	-14.05	0.2781	3.55	< 0.0001
X_4	-5.32	< 0.0001	-0.20	0.7371	-0.06	0.5929	-10.07	0.4310	-0.38	0.2527
X_1^2	1.01	0.3657	-1.81	0.0565	0.54	0.0037	-2.34	0.8982	-0.22	0.6424
X_2^2	-0.13	0.9031	-0.24	0.7811	0.30	0.0693	3.57	0.8454	0.69	0.1623
X_3^2	-0.59	0.5921	-0.38	0.6647	0.78	0.0003	4.49	0.8068	1.60	0.0052
X_4^2	1.01	0.3657	0.62	0.4797	0.23	0.1564	8.10	0.6595	0.24	0.6200
X_1X_2	-0.19	0.8250	0.29	0.6720	-0.08	0.4792	6.88	0.6304	-0.13	0.7336
X_1X_3	-0.44	0.6076	-0.09	0.8971	0.18	0.1529	17.83	0.2260	0.44	0.2473
X_1X_4	-0.81	0.3474	-0.06	0.9264	-0.09	0.4542	5.59	0.6951	-0.44	0.2473
X_2X_3	-0.19	0.8250	-0.39	0.5696	0.20	0.1094	-17.36	0.2378	0.41	0.2737
X_2X_4	0.44	0.6076	0.06	0.9264	-0.07	0.5523	5.68	0.6907	0.46	0.2230
X_3X_4	-0.56	0.5109	-0.64	0.3556	0.13	0.2809	4.01	0.7786	-0.18	0.6346
R^2	0.8442	-	0.9012	-	0.9676	-	0.8466	-	0.9383	-

EC indicates Estimated Coefficient;
The terms having Prob > F values very small (< 0.05) indicate that these have significant effect on the response variables.

5.4.5.8 Kinetic modelling of drug release

In vitro drug release data was fitted to zero order, first order, Higuchi and Ritger and Peppas equation. Curve fitting exercise provided release rate constant and coefficient of determination. The optimum formulation AGT 09 best fitted Higuchi's equation which correlates the drug release rate with square root of time.

5.4.5.9 Fluid uptake study

The degree of hydration of the polymer is one of the determining factors for velocity of drug release from the swellable matrices (Michailova et al., 2001). Mobility of the polymer chains and thus drug diffusion significantly depends on the water content of the matrix system. At high water content, polymer chain relaxation takes place with volume expansion giving high swelling of the system (Siepmann and Peppas, 2001). Xanthan gum, HPMC, polyox and carbopol have the property to absorb water and get hydrated. Thus % fluid uptake depends on

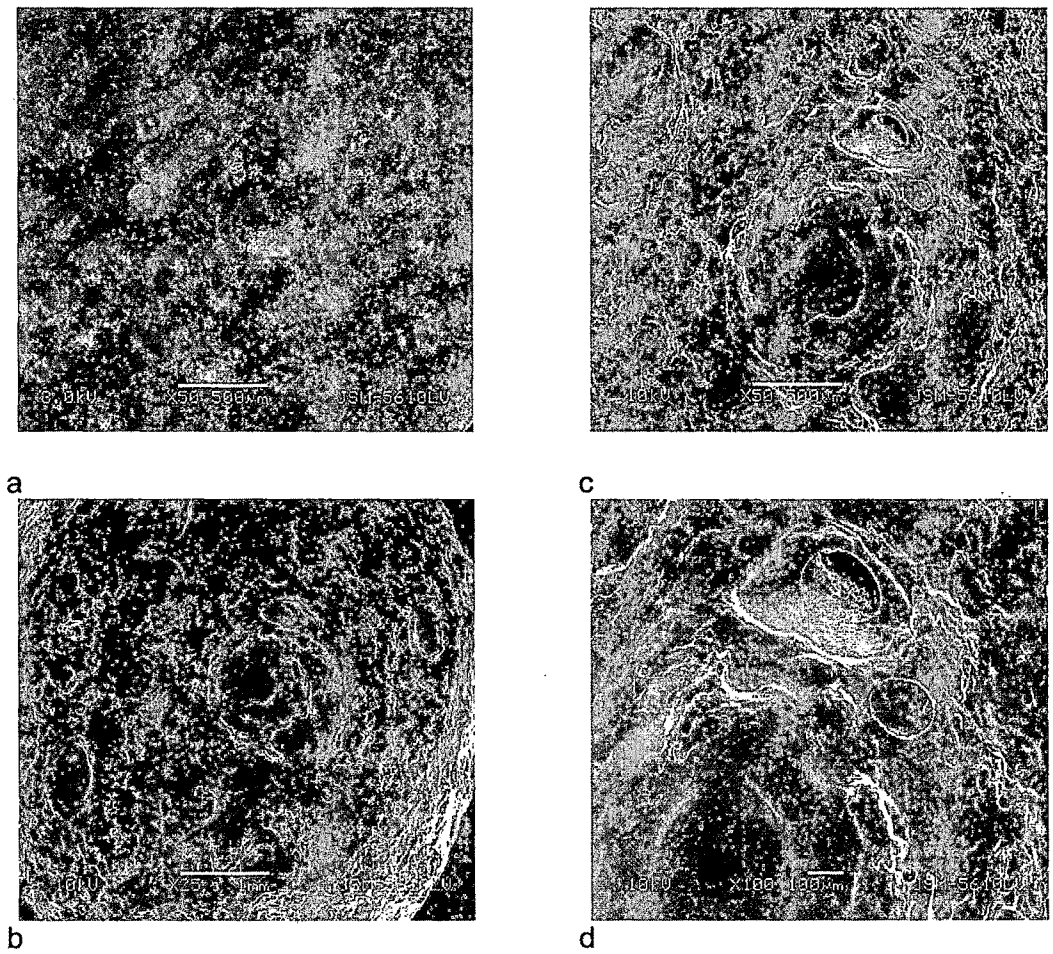


Fig 5.6 SEM images of formulation AGT 09 (a) Surface of dry minimatrix at 50x; minimatrix surface after 6 h exposure in dissolution medium (b) at 25x (c) at 50x (d) at 100x

the amount of these components present in the formulation. Results of the fluid uptake study indicate that amount of xanthan gum has prominent effect on this parameter. Formulation AGT 20 containing highest amount of xanthan gum was having swelling index 466.8 while formulation containing least amount (AGT 23) has value of 159. Significance of the effect of xanthan gum on swelling index can be interpreted from regression analysis values in Table 5.14 and can be observed from prediction profiler in Fig.5.7. This effect may be due to water holding and viscolysing property of xanthan gum. In case of the formulations containing lower amount of xanthan gum, swelling index values may be less because the matrix may not be capable to hold water for longer duration. X_1 and X_2 in combination increased swelling index to maximum extent.

Table 5.15 Results for kinetic modelling of drug release

Formulation	Zero order		First order		Higuchi		Ritger & Peppas		
No.	R^2	K_o	R^2	K_1	R^2	K_H	R^2	K_R	n
AGT 01	0.5433	6.20	0.4646	0.051	0.8044	28.43	0.7629	0.5437	0.78
AGT 02	0.6451	6.62	0.6008	0.061	0.8818	29.14	0.8711	0.4874	0.71
AGT 03	0.5636	6.21	0.5143	0.051	0.8232	28.26	0.8094	0.5468	0.66
AGT 04	0.5741	6.36	0.5338	0.051	0.8320	28.82	0.8245	0.5447	0.64
AGT 05	0.5428	6.02	0.5132	0.046	0.8092	27.71	0.8064	0.5799	0.64
AGT 06	0.5426	6.09	0.4857	0.049	0.8063	27.96	0.7842	0.5598	0.68
AGT 07	0.7314	7.19	0.6958	0.076	0.9274	30.49	0.9269	0.5774	0.53
AGT 08	0.5400	6.04	0.4919	0.047	0.8058	27.78	0.788	0.5709	0.58
AGT 09	0.7984	7.04	0.7520	0.085	**0.9643**	**29.13**	0.9531	0.3604	0.59
AGT 10	0.5149	5.98	0.4505	0.045	0.7838	27.81	0.5909	0.5807	0.75
AGT 11	0.7324	6.84	0.7511	0.067	0.9360	29.13	0.433	0.4485	0.96
AGT 12	0.7762	6.97	0.7444	0.080	0.9538	29.11	0.5514	0.378	0.95
AGT 13	0.7968	7.17	0.8104	0.078	0.9663	29.75	0.4192	0.3976	0.98
AGT 14	0.4953	5.72	0.4737	0.039	0.7713	26.91	0.5512	0.6253	0.77
AGT 15	0.5719	6.32	0.5246	0.051	0.8295	28.68	0.6867	0.5428	0.81
AGT 16	0.6313	6.42	0.6576	0.054	0.8766	28.50	0.463	0.531	0.91
AGT 17	0.7346	7.14	0.7238	0.073	0.9331	30.33	0.4819	0.4265	0.94
AGT 18	0.6079	6.38	0.5567	0.056	0.8581	34.13	0.7673	0.5128	0.83
AGT 19	0.7350	7.07	0.7045	0.075	0.9169	34.09	0.9309	0.416	0.55
AGT 20	0.6620	7.05	0.5510	0.076	0.8837	35.37	0.8369	0.4029	0.82
AGT 21	0.5844	6.39	0.5443	0.053	0.8628	35.22	0.8356	0.5359	0.61
AGT 22	0.5282	6.00	0.4895	0.045	0.8299	34.66	0.7882	0.5851	0.56
AGT 23	0.5338	6.06	0.5077	0.044	0.8286	34.79	0.8017	0.5865	0.56
AGT 24	0.6495	6.47	0.6825	0.056	0.8860	33.99	0.9271	0.5195	0.47
AGT 25	0.5075	5.85	0.4851	0.041	0.8195	34.57	0.7831	0.6163	0.57
AGT 26	0.6016	6.34	0.5778	0.053	0.8614	34.35	0.8565	0.5324	0.63

5.4.5.10 Ex vivo bioadhesion study

Gastroretention can be achieved by floating property of the formulation. Imparting gastric mucoadhesive feature can further strengthen the gastroretention phenomenon. For introducing this feature, carbopol 974P was added in the formulation. Carbopol is polyanionic in nature and contains primarily a large number of carboxylic groups that have a good tendency to form hydrogen bond with sialic acid residues of mucin glycoproteins. Hence it is a better mucoadhesive agent (Dodou et al., 2005; Adhikary and Vavia, 2008). Positive

sign and magnitude of regression coefficients in Table 5.14 indicates significant influence of carbopol 974P (X_3) on bioadhesion parameter. Increase in carbopol concentration increased the bioadhesive strength which can be observed from the prediction profiler in Fig.5.7. HPMC, a long-chained and non-ionic polymer, has also limited bioadhesive property. It could be due to formation of physical or hydrogen bonding with the mucus components. Presence of this component also enhance overall bioadhesion of the formulation. Ultimately bioadhesive property of the optimized formulation could assist the tablet to stay in the upper part of gastrointestinal tract and enhance the gastroretention along with the floating feature (Chavanpatil et al., 2006).

5.4.6 Optimisation of responses using desirability function

Overall desirability values were calculated for all the designed formulations (Table 5.16). The Y_{max} and Y_{target} values are 32 and 7 for floating lag time and 53.3 and 31.9 for % drug release at 1 h. Respective Y_{min} and Y_{target} values are 3.31 and 9.39 for time required for 95% drug release, 159 and 501.2 for swelling index and 6.8 and 18.2 for bioadhesive strength (Table 5.13). These values were introduced in equations 5.13 to 5.16. The formulation having highest desirability value was said to be optimum because the overall desirability is calculated from the individual desirability values for each response variable. Highest desirability value of 0.87 was found for formulation AGT 09. Hence it was said to be an optimum formulation.

Table 5.16 Overall desirability values

Formulation No.	Overall desirability	Formulation No.	Overall desirability
AGT 01	0.00	**AGT 14**	0.00
AGT 02	0.43	**AGT 15**	0.31
AGT 03	0.25	**AGT 16**	0.29
AGT 04	0.00	**AGT 17**	0.63
AGT 05	0.25	**AGT 18**	0.53
AGT 06	0.34	**AGT 19**	0.55
AGT 07	0.66	**AGT 20**	0.45
AGT 08	0.37	**AGT 21**	0.00
AGT 09	**0.87**	**AGT 22**	0.25
AGT 10	0.00	**AGT 23**	0.00
AGT 11	0.41	**AGT 24**	0.29
AGT 12	0.74	**AGT 25**	0.00
AGT 13	0.45	**AGT 26**	0.57

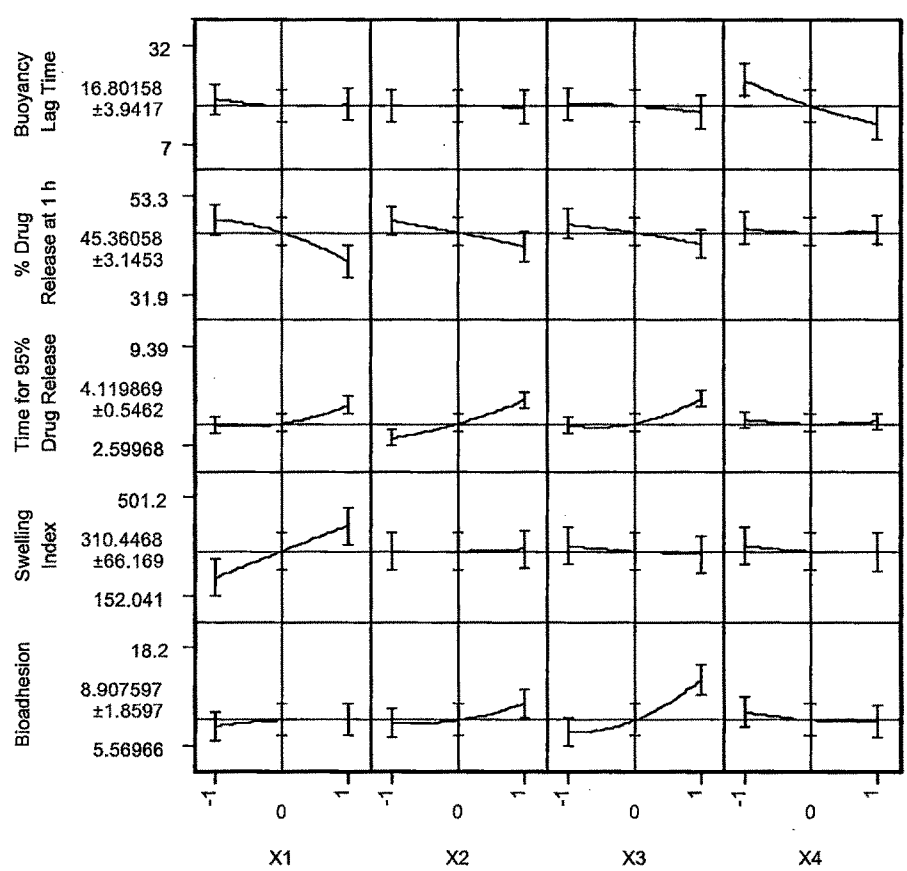


Fig 5.7 Prediction profiler correlating formulation and response variables

5.4.7 In vivo study by gamma scintigraphy

Four radiolabelled minimatrices were put in hard gelatin capsule and it was administered to the volunteers. Initial image was captured immediately after administration (0 h) which is shown in Fig 5.8. As the capsule was intact, it is observed as single entity. Afterwards the capsule was disintegrated and minimatrices were released which were clearly observed at 2 h. Images were captured every hour to ascertain location of the minimatrices. The images captured at 0, 2, 6, 8 and 9 h are shown here. Minimatrices were observed in stomach till 8 h. Image taken at 9 h did not show their presence in stomach which shows that the developed minimatrices are having minimum gastric residence time of 8 h. Till 6 h the minimatrices were intact and afterwards its erosion was started,

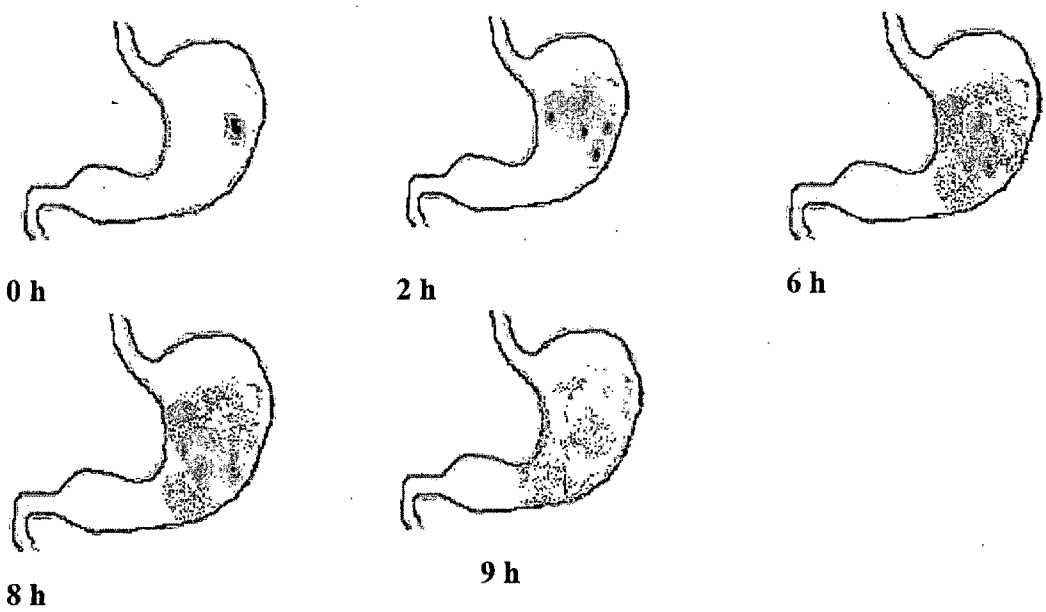


Fig 5.8 Gamma scintigraphy images showing gastric residence time of minimatrices

5.4.8 Stability studies

Stability samples of the optimum formulation (AGT 09) were periodically withdrawn at 1, 2, 3 and 6 months from stability chamber and analysed for buoyancy lag time, drug content and dissolution profile. The results were compared with initial samples. There was no significant change in amoxicllin content. Dissolution profile of stability samples were compared with initial sample

profile by using similarity factor and it ranged from 82-92 which indicated their similarity.

Table 5.17 Stability data

Parameter	Initial	Storage condition and duration			
		30°C/65%RH		40°C/75%RH	
		3 Months	6 Months	3 Months	6 Months
Buoyancy lag time (min)	7	8	10	10	11
Assay (%)	99.5	98.7	99.1	98.5	97.3

5.5 CONCLUSION

Multiparticulate formulation in the form of minimatrices having floating and mucoadhesive feature were developed. Formulations were designed by central composite design. Xanthan gum, rate controlling polymers (HPMC K100M CR and polyox WSR coagulant), carbopol 974P and gas generating couple were selected as formulation variables. Preliminary formulation trials were carried out for screening suitable excipients and preparation technique. Direct compression was not feasible. Hence non- aqueous granulation technique was used for preparation of the minimatrices. Prepared minimatrices were evaluated for thickness, hardness, friability, buoyancy lag time, drug release pattern, swelling properties, bioadhesion strength and drug content. Formulation AGT 09 was found as best formulation which was selected by desirability approach. It was having minimum buoyancy lag time, high bioadhesion strength and was capable to sustain drug release upto 12 h. Drug release profile of this formulation best fitted to Higuchi's equation. Gastric residence time of the formulation was determined in healthy human volunteers by gamma scintigraphy technique which shown that the minimatrices stayed in stomach till 8 h. stability studies of the optimum formulation was carried out as per ICH guidelines and the formulation was found stable at accelerated condition till 6 months as there was no significant change in drug content and drug release pattern. Minimatrices can be manufactured by using existing tablet manufacturing facility with cost effective approach. Hence the developed GRDDS may be explored as an effective tool in the management of *H. pylori* associated gastric complications as it has therapeutic as well as manufacturing advantages.