Chapter - 3

E X P E R I M E N T A L

EXPERIMENTAL

3.1 MATERIALS

* 5-Fluorouracil - gift sample from Hoffmann - La Roche, Switzerland.
* Terephthaloyl chloride, Ninhydrin - Fluka Chemie, Switzerland.
* L-Asparagine monohydrate, L-Arginine, L-Cystine, Tween 20, N,N'-methylene bisacrylamide, Tetraethyl methylene diamine - Loba Chemie Indo Austranal Co. Ltd., India.
* L-Glutamine, L-Lysine monohydrochloride, L-Citrulline, Acrylamide - Sisco Research Lab. Ltd., India.
* L-Ornithine monohydrochloride - S.D.'s Lab. Chem. Industries Ltd., India.
* Span 85 - Koch Light Laboratories Ltd., England.
* Sodium carbonate, Disodium hydrogen orthophosphate, Sodium chloride, Sodium hydroxide, glacial Acetic acid, Sodium acetate, Glucose (all A.R. grade) - Sarabhai M. Chemicals, India.
* Chloroform, Cyclohexane, Toluene, Ethyl acetate, Propanol, Diethyl ether, Glycerol (all A.R. grade) - Qualigens Fine Chemicals Ltd., India.
* Ammonium peroxo disulphate, Potassium dihydrogen phosphate, Sodium dihydrogen orthophosphate, (all G.R. grade) - E. Merck (India) Ltd., India.

* Pluronic F68 - BASF Wyendotte Corporation Ltd., U.S.A.

* isoButyl cyano acrylate - Sigma Chemical Corporation, U.S.A.

* Haemoglobin Powder - gift sample from Cadila Laboratories Ltd., India.

3.1.1 Buffer Solutions/Reagents

1) Phosphate buffer (pH 7.4)[198]

50 ml of 0.2M potassium dihydrogen phosphate solution was mixed with 39.1 ml of 0.2N sodium hydroxide solution and the volume was made upto 200 ml with distilled water.

2) Phosphate buffered saline (pH 7.0)[199]

2.5 gm of sodium dihydrogen orthophosphate, 2.523 gm of disodium hydrogen orthophosphate and 8.2 gm of sodium chloride were dissolved in sufficient distilled water to produce 1000 ml.

3) Acetate buffer (pH 5.0)[198]

13.6 gm of sodium acetate and 6 ml of glacial acetic acid were dissolved in sufficient distilled water to produce 1000 ml.

4) 0.45M Sodium carbonate solution

4.86 gm of sodium carbonate was dissolved in sufficient distilled water to produce 100 ml.

5) Ninhydrin Reagent[200]

0.2 gm of Ninhydrin was dissolved in 50 ml of distilled water and the volume made upto 100 ml with acetate buffer (pH 5.0). The reagent solution was always freshly prepared before use.

3.2 METHODS

3.2.1 Analytical Methods

[A] SPECTROPHOTOMETRIC DETERMINATION OF 5-FLUOROURACIL

The official methods for the assay of 5-fluorouracil from dosage forms involves the measurement of absorbance of the drug solution in acetate buffer (pH 4.7) at 266 nm (I.P., U.S.P.). As most of our studies involved phosphate buffered saline (pH 7.0), calibration curve for the spectrophotometric determination of 5-FU was taken in phosphate buffered saline after ascertaining that there was no significant difference in the calibration plots of 5-FU in the two systems.

(a) Calibration Curve for the Estimation of 5-Fluorouracil

50 mg of 5-fluorouracil was accurately weighed and dissolved in 100 ml of phosphate buffered saline. From this stock solution, aliquots of 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8

and 1.0 ml were withdrawn and transferred to 10 ml volumetric flasks. All the samples were made upto 10 ml with phosphate buffered saline to yield solutions of 0.5, 2.5, 5, 10, 15, 20, 30, 40 and 50 µg/ml 5-FU, respectively. Their absorbance was measured at 266 nm on Carl-Zeiss spectrophotometer using phosphate buffered saline as the blank.

[B] PARTITION COEFFICIENT OF AMINO ACIDS

The diamine should diffuse from the aqueous phase to the interface during the interfacial polycondensation reaction for proper formation of the polyamide wall around the dispersed core material droplets. Hence, the partition coefficient of the amino acids between the aqueous and the organic phase was studied in the same amount and under the same conditions as those used in the preparation of the microcapsules. At the completion of the 'dummy' interfacial polymerisation process, the two phases were separated by centrifugation. The aqueous phase was collected, an aliquot of 0.1 ml was withdrawn from it and made upto 10 ml with phosphate buffered saline. Aliquots were withdrawn from this stock solution and transferred to 10 ml volumetric flasks. 2 ml of Ninhydrin reagent was added to each flask, the pH was adjusted to ≅6 using dilute hydrochloric acid and the volume was made upto 5 ml with distilled water. The samples were heated on a

boiling water bath for 20 minutes, cooled and the volume was made upto 10 ml with water. The absorbance was measured at 570 nm on Spectronic-20 (Bausch & Lomb) against a similarly treated blank in which the amino acid was excluded.

[C] CALIBRATION CURVE FOR THE ESTIMATION OF AMINO ACIDS

The amino acids were analysed quantitatively by the Ninhydrin method.[200] 25 mg of the amino acid was dissolved in 25 ml of 0.45M sodium carbonate solution. From this stock solution, aliquots of 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml were withdrawn and transferred to 10 ml volumetric flasks. 2 ml of freshly prepared Ninhydrin reagent was added to each flask, the pH adjusted to ≅6 with dilute hydrochloric acid and the volume was adjusted to 5 ml with distilled water. The flasks were heated on a boiling water bath for 20 minutes, cooled and the volume made upto 10 ml with water. This produced solutions having amino acid concentration of 1, 2, 4, 6, 8 and 10 µg/ml respectively. Their absorbance was measured at 570 nm on Spectronic-20 using an appropriate reagent blank.

3.2.2 Preparation of Microcapsules

[A] POLYAMIDE MICROCAPSULES

(a) Polyterephthalamide Microcapsules

(i) Preparation of Microcapsules

Various types of polyamide microcapsules were prepared by interfacial polycondensation as per a reported procedure.[104] Terephthaloyl chloride was used as the diacid chloride and diamino acids like L-Asparagine monohydrate, L-Arginine, L-Citrulline, L-Cystine, L-Glutamine, L-Ornithine monohydrochloride and L-Lysine monohydrochloride were used as the diamines.

0.01 moles of the amino acid was dissolved in 5 ml of 0.45M sodium carbonate solution and the beaker was immersed in an ultrasonic ice bath (4°-8°C). 5% Span 85 was added to an organic solvent mixture containing chloroform and cyclohexane (1:3) and the two phases were emulsified for 5 minutes in an ultrasonic vibrator (Vibronics) at 120 W to give a water-in-oil (w/o) emulsion. A solution of terephthaloyl chloride was prepared by dissolving 0.012 moles of terephthaloyl chloride in 25 ml of the organic solvent mixture and added at once to the w/o emulsion. The

system was allowed to sonicate for 3 minutes and then the reaction was quenched by adding 30 ml of the organic mixture. The organic phase was then separated by centrifugation (Remi centrifuge) and decantation and the microcapsules sedimented at the bottom were dispersed in 10 ml of a mixture of glycerin and Tween 20 (3:1) using a magnetic stirrer (Remi). After 5 minutes, 50 ml of phosphate buffered saline was added, the stirring was continued for 3 more minutes and then the dispersion was centrifuged. Excess Tween was removed from the microcapsules by repeated washings with 50 ml of phosphate buffered saline and subsequent centrifugation. Finally, the surfactant-free microcapsules were suspended in phosphate buffered saline, filtered and stored in a refrigerator till further use.

The flow chart for the preparation of polyterephthalamide microcapsules is given in Fig. 4.

(ii) Optimisation of Formulation Conditions

Although the general procedure for the preparation of polyamide microcapsules has been essentially the same in almost all the reports screened, variations in the formulation conditions have been observed.

Figure - 4

Flow chart for the preparation of polyterephthalamide microcapsules

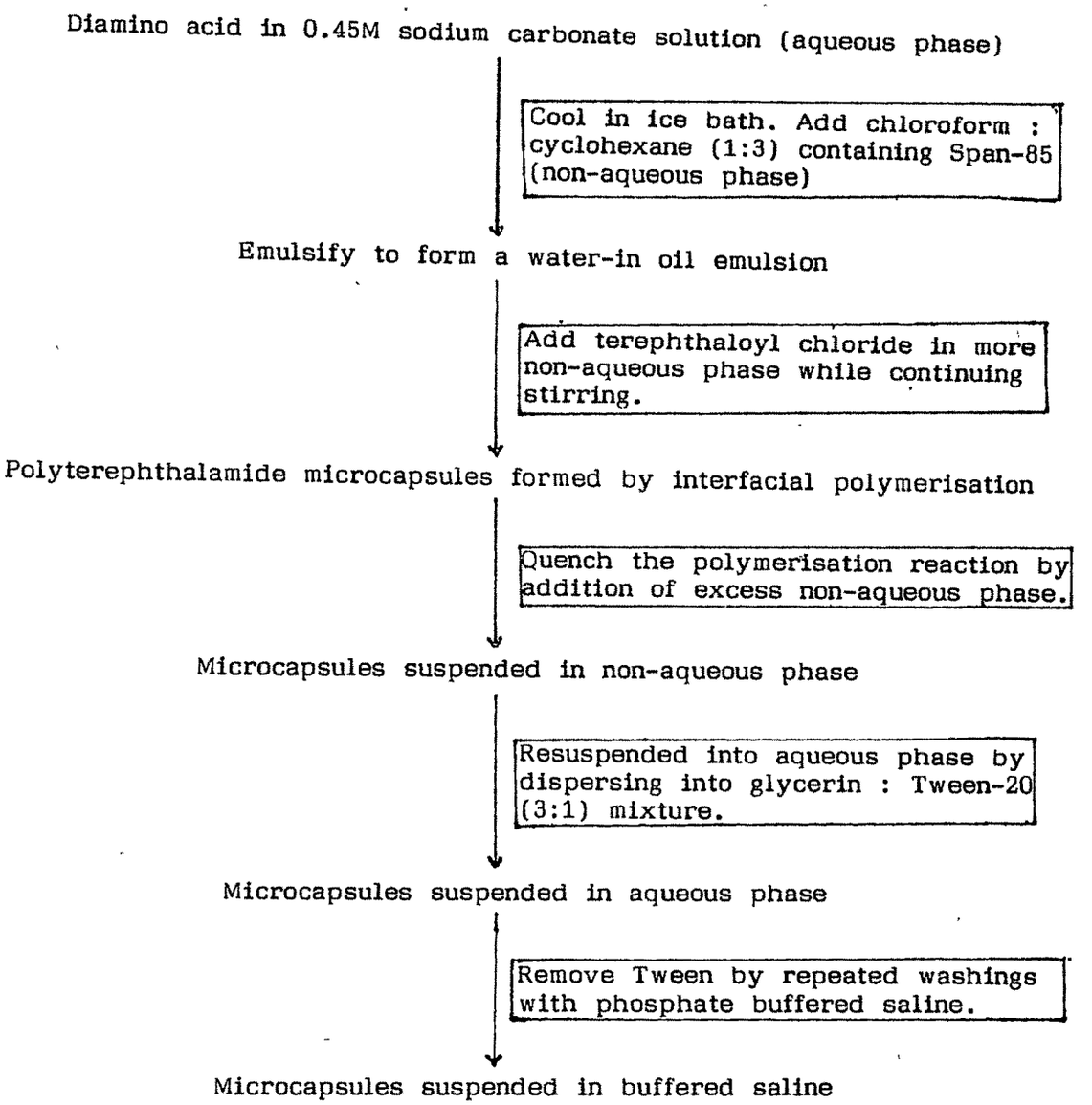

Table - 1

Reported Formulation Conditions for Preparation of Polyamide Microcapsule

No.	Aqueous Phase	Organic Phase	Span Concentration	Emulsification time	Polymerisation time	Reaction Temperature	Medium for Seperation	Size	Ref. No.
1.	0.45M Na_2CO_3	$CHCl_3$: Cyclohexane (1:4)	15%	n.s.	3 min.	0°C	50% Tween-20	35-75 um	107
2.	-do-	Benzene & $CHCl_3$: Cyclohexane (1:4)	-do-	1-3 min.	-do-	R.T.	-do-	n.s.	102
3.	-do-	$CHCl_3$: Cyclohexane (1:3, 1:4)	5% or more	5 min.	-do-	Icebath	-do-	n.s.	103
4.	$NaHCO_3$ - Na_2CO_3 buffer (pH 9.8)	$CHCl_3$: Cyclohexane (1:3)	10%	-do-	-do-	-do-	-do-	3-5 um	108
5.	-do-	$CHCl_3$: Cyclohexane (1:4)	1.5%	2 min.	45 sec.	-do-	25% Tween-20	70-110 um	110
6.	-do-	-do-	1%	1 min.	3 min.	4°C	50% Tween-20	80 um	111

(Table-1) Contd.

No.	Aqueous Phase	Organic Phase	Span Concentration	Emulsification time	Polymerisation time	Reaction Temperature	Medium for Seperation	Size	Ref. No.
7.	0.45M Na_2CO_3 -HCl buffer (pH 8.4)	$CHCl_3$: Cyclohexane (1:5)	5%	-do-	-do-	-do-	Ethanol	10 um	112, 113
8.	0.45 M Na_2CO_3 -HCl buffer (pH 9.8)	$CHCl_3$: Cyclohexane (1:4)	1%	n.s.	3 min.	Icebath	Glycerin Tween-20	50-65 um	116
9.	-do-	-do-	5%	1 min.	-do-	4°C	30% Tween-20 & alcohol	20-30 um	117
10.	-do-	-do-	-do-	3 min.	10 min.	-do-	5% ethanolic Tween-20	n.s.	124
11.	n.s.	Mineral oil &/or $CHCl_3$, Cyclohexane	n.s.	20 sec.	10 min.	n.s.	$CHCl_3$ wash	n.s.	106

R.T. - Room Temperature, n.s. - not specified

Table - 2

Summary of the Various Parameters Studied for Optimisation of the Formulation Conditions for Polyamide Microcapsules

Batch No.	Parameter No.	Parameter	Level	Remarks
1a	1.	Mode of emulsification	Stirrer (Simple)	Solvent evaporation
1b			Magnetic stirrer	Large size
1c			Ultrasonic vibrator*	Optimum
2a	2.	Aqueous phase	0.1N NaOH	Negligible yield
2b			$NaHCO_3$ buffer (pH 9.8)	Less yield
2c			0.45M Na_2CO_3*	Optimum
3a	3.	Organic phase	CCl_4	Negligible yield
3b			$CHCl_3$	-do-
3c			Ether	-do-
3d			Mineral oil	Large size, difficult separation
3e			$CHCl_3$: Cyclohexane (1:3)*	Optimum
3f			$CHCl_3$: Cyclohexane (1:4)	Satisfactory

(Table-2) Contd.

Batch No.	Parameter No.	Parameter	Level	Remarks
4a	4.	Emulsification time	1 min.	Improper emulsification
4b			3 min.	Broad size distribution
4c			5 min.*	Optimum
5a	5.	Span-85 concentration	1%	Unstable emulsion
5b			5%*	Optimum
5c			10%	Separation difficult
6a	6.	Temperature of reaction	Icebath (4°C)*	Optimum
6b			R.T. (25°C)	Coarse film, large size
7a	7.	Time of polymerisation	1 min.	Fragile film
7b			3 min.*	Optimum
7c			5 min.	Coarse film
8a	8.	Phase volume ratio	1:5*	Optimum
8b			1:4	Unstable emulsion
8c			1:3	Unstable emulsion

(Table-2) Contd.

Batch No.	Parameter No.	Parameter	Level	Remarks
9a	9.	Medium for collection	Alcohol	Particle aggregation
9b			50% Tween-20	prolonged washing time
9c			Glycerin-Tween (3:1)*	Optimum
10a	10.	Stabilizers	Egg albumin	Very large particles, none found suitable
10b			Bovine serum albumin	
10c			Casein	
10d			Gelatin	
10e			Polyethylene glycol-400	

previously. The drug-loaded, washed microcapsules were then suspended in sufficient volume of phosphate buffered saline to yield a suspension containing 5 mg/ml of 5-fluorouracil.

(b) Cross-linked Haemoglobin Microcapsules

(i) Preparation of Microcapsules

Haemoglobin was cross-linked with terephthaloyl chloride by an emulsification-reticulation procedure as described by Rambourg et al[117-118]. 105 mg of haemoglobin powder and 35 mg of glucose were dissolved in 3 ml of 0.45M sodium carbonate solution and emulsified with 10 ml of the organic mixture containing 15% Span 85. A further 15 ml of the organic phase containing 200 mg of dissolved terephthaloyl chloride was added to it and after 3 minutes, the reaction was quenched with 25 ml of the organic phase. The product was separated and collected by the procedure already described; Except in this case, 10 ml of 50% Tween 20 solution was used instead of glycerol-Tween mixture.

(ii) Optimisation of Formulation Conditions

The basic reaction conditions for the preparation of cross-linked haemoglobin microcapsules remained almost

Table - 3

Optimisation of Formulation Conditions for
Cross-linked Haemoglobin Microcapsules

Batch No	Parameter No.	Parameter	Level	Remarks
A1	A.	Concentration	5%	Very large size
A2		of Span 85	10%	Large size
A3			15%*	Optimum
B1	B.	Temperature of	Ice bath (4°C)	Low yield
B2		reaction	R.T. (25°C)*	Optimum
C1	C.	Aqueous : Organic	1:5	Denaturation of Hb
C2		phase ratio	1:4	-do-
C3			1:3*	Optimum

the same as those for polyamide microcapsules, except for concentration of Span 85, temperature of reaction and aqueous to organic phase ratio. These parameters were systematically optimized as shown in Table 3.

(iii) 5-Fluorouracil-loaded Cross-linked Haemoglobin Microcapsules

5-FU-loaded cross-linked haemoglobin microcapsules were prepared in exactly the same manner as 5-FU loaded polyterephthalamide microcapsules, after the formulation conditions were optimized.

[B] POLYACRYLAMIDE MICROCAPSULES

(a) Preparation of Microcapsules

Two methods were employed for the preparation of cross-linked polyacrylamide microparticles viz. i) Gel polymerisation method and ii) Emulsion polymerisation method.

i) Gel Polymerisation Method

600 mg of acrylamide and 200 mg of N,N'-methylene bis acrylamide (Bis) were dissolved in 10 ml of phosphate buffer (pH 7.4). 10 mg/ml of 5-FU was also dissolved in the buffer. 100 µl of a freshly prepared solution of 0.5 g/ml ammonium peroxodisulfate (ammonium

persulfate) was added as the catalyst and 100 µl of N,N,N',N' tetraethyl methylene diamine (temed) was added as the initiator. Within 10 minutes, the monomers polymerised to give a soft, translucent gel. This gel was repeatedly homogenized in a homogenizer with 40 ml portions of phosphate buffered saline in order to wash off the unreacted monomers and traces of the catalyst and initiator. After each wash, the gel was centrifuged and the sediment was rehomogenized. This process was continuted till particles of size less than 10 to 20 µm were obtained. The suspension was then filtered through a muslin cloth to remove larger particles, if any, and the filtrate was centrifuged. The sedimented microparticles were suspended in sufficient volume of phosphate buffered saline so as to get 5 mg of 5-FU per ml of suspension.

ii) Emulsion Polymerisation Method

Acrylamide and N,N' methylene bisacrylamide (Bis) were dissolved in 5 ml of phosphate buffer (pH 7.4). the solution was cooled to 4°C by immersing the beaker in an ice bath and 100 µl of a freshly prepared solution of ammonium persulphate (0.5 g/ml) was added to it. The aqueous phase was then emulsified in an organic phase consisting of toluene and chloroform (3:1), using Pluronic F68 as the emulsifying agent.

After 5-10 minutes, 100 µl of N,N,N',N' tetra ethyl methylene diamine (temed) was added to initiate the polymerisation reaction. The stirring was continued for half-hour and thereafter the microparticles were separated by centrifugation and dispersed in alcohol. The polyacrylamide microparticles were washed and recovered in the same manner as described earlier for polyamide microcapsules.

(b) Optimisation of Formulation Conditions by Emulsion Polymerisation Procedure

A glance at the reported methods (Table 4) indicates a wide variation in the choice of the levels of various parameters like : monomer to cross-linking agent ratio, concentration of Pluronic F68, time of emulsification, time of polymerisation, aqueous to organic phase ratio, temperature of reaction etc. Hence, all these variable parameters were first optimized before preparing drug-loaded microparticles. (Table 5)

(c) Drug-loaded Polyacrylamide Microcapsules

After the formulation conditions were optimized, drug-loaded polyacrylamide microparticles were prepared by dissolving 10 mg/ml of 5-fluorouracil in the phosphate buffer (pH 7.4) alongwith the monomer and cross-linking agent. Thereafter,

Table - 4

Summary of Reported Formulation Conditions for Polyacrylamide Microcapsules

No.	Acrylamide :Bis ratio*	Aqueous : Organic phase ratio*	Concentration of emulsifier*	Polymerisation time	Particle size	Ref.
1.	19:1	1:67	1.25% v/v	30 min.	50 - 250 um	134
2.	3:1	1:12	0.08% w/v	10 min.	10 um	135,137,138
3.	3:1	1:5	0.32% w/v	30 min.	n.s.	136
4.	3:1	n.s.	n.s.	5 min.	1-5 um	139
5.	19:1	1:60	0.03% w/v	n.s.	n.s.	140
6.	11:1,5.5:1	1:40	0.25% w/v	20 min.	0.2-0.5 um	150
7.	3:1	1:40	0.25% w/v	20 min.	0.3-0.5 um	148
8.	3:1	1:6.25	0.32% w/v	30 min.	182-585 um	151

* - Calculated from given amounts

n.s. - not specified

Table - 5

Optimisation of Formulation Conditions for Polyacrylamide Microcapsules

Batch No.	Parameter No.	Parameter	Level	Remarks
a1	1.	Pluronic concentration	0.05%	No polymerisation
b1			0.1%	-do-
c1			0.5%	Broad size distribution
d1			0.75%	Satisfactory
e1			1.0%*	Optimum
f1			2.0%	Difficulty in isolation
a2	2.	Aqueous to organic	1:10	No polymerisation
b2		phase volume ratio	1:5*	Optimum
c2			1:4	Large size
d2			1:3	-do-
e2			1:2	Difficulty in isolation
a3	3.	Mode of emulsification	Ultrasonic vibrator	Gel formation
b3			Magnetic stirrer*	Optimum
c3			Simple stirrer	Solvent evaporation

(Table-5) Contd.

Batch No.	Parameter No.	Parameter	Level	Remarks
a4	4.	Time of emulsification	5 min.	In efficient emulsification
b4			10 min.*	Optimum
c4			15 min.	No significant effect on size
d4			20 min.	-do-
a5	5.	Time of polymerisation	10 min.	No polymerisation
b5			20 min.	Very less yield
c5			30 min.	Less yield
d5			1 hour*	Optimum
e5			2 hours	Excess polymerisation
f5			3 hours	-do-
a6	6.	Temperature of	Icebath (4°C)	Slow polymerisation rate
b6		polymerisation	R.T. (25°C)*	Optimum

polymerisation and separation of the microparticles was conducted as per the method described earlier. The washed microparticles were suspended in sufficient phosphate buffered saline so as to get a suspension containing 5 mg/ml of 5-FU.

[C] POLY (ISOBUTYL CYANO ACRYLATE) MICROPARTICLES

Poly (isobutylcyanoacrylate) [PiBCA] microparticles were prepared by two different methods :

i) Anionic polymerisation method

ii) Emulsion polymerisation method.

i) Anionic Polymerisation[172]

15 mg of 5-fluorouracil was dissolved in 1.5 ml of 0.01M of hydrochloric acid containing 0.5% Tween 20. 20 µl of isobutylcyanoacrylate (iBCA) monomer was dispersed in it with stirring on a magnetic stirrer (Remi) at the highest speed. The stirring was continued for 2 hours and the resultant dispersion was neutralized to pH 7.0 with 1M sodium hydroxide solution. The PiBCA microparticles were then collected by centrifugation, suspended in phosphate buffered saline, and stored in a refrigerator till further use.

ii) Emulsion Polymerisation[160]

25 mg of 5-fluorouracil was dissolved in 2 ml of

distilled water and this aqueous phase was emulsified with 20 ml of the organic phase consisting of chloroform and cyclohexane (1:4) on a magnetic stirrer (Remi), using 5% Span 85 as the emulsifier. After 5 minutes, 20 µl of the monomer was added to the emulsion and stirring continued. After 30 minutes, continuous stirring, the reaction was quenched by addition of 40 ml of the organic phase. The microparticles were separated and collected as per the method already described for polyamide microcapsules.

NOTE : As the isobutylcyanoacrylate monomer is highly reactive, it tends to polymerise spontaneously even upon exposure to atmosphere during handling or transfer. Hence, a stock solution was prepared by diluting 0.1 ml monomer to 2 ml with cyclohexane in a sealed vial.

3.3 EVALUATION OF MICROCAPSULES

3.3.1 Particle Size Analysis

Particle size analysis of the microcapsules was carried out according to the standard procedure[201] using Ore microscope (МИН-9, Russia; least count 0.25 µm) at 600 X magnification. A minimum of 100 particles were counted for each batch and the average diameter along with the frequency distribution was calculated.

3.3.2 Photomicrographs of Microcapsules

Photomicrographs of the microcapsules were taken on Olympus-ECE-Bi-I microscope (Olympus Optical Co. Ltd., Japan) at 600 to 1000 X magnification.

3.3.3 Drug Entrapment in Microcapsules

The drug entrapment in the microcapsules was studied by an indirect method as it was not possible to lyse the microcapsules completely and estimate the content of 5-fluorouracil entrapped within. The amount of unentrapped drug was estimated by analysing the aqueous supernatant and washings collected during isolation and purification of the microcapsules. The amount of the drug entrapped in the microcapsules was calculated by subtracting the amount unentrapped from the original amount of 5-FU added to the system prior to polymerisation.

(i) Method for Assay of 5-FU from Microcapsules

Tween 20 was found to interfere with the absorption spectrum of 5-fluorouracil at 266 nm. Hence, it was necessary to develop a modified procedure for estimating the drug from the aqueous washings containing Tween 20. 5-FU was extracted from the aqueous samples using the method reported by Lavreshin.[54] The procedure involved the use of ethyl acetate : propanol (7:3) as the extraction medium, an aqueous to

organic phase ratio of 1:1 and extraction time of 4 minutes. After extraction, the drug was estimated from the organic phase at 272 nm on Carl-Zeiss spectrophotometer using a similarly treated blank.

(ii) Calibration Curve for Estimation of 5-Fluorouracil by Extraction

50 mg of 5-fluorouracil was dissolved and made upto 50 ml with phosphate buffered saline. Aliquots of 0.02, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4 and 0.5 ml were withdrawn and made upto 5 ml with the buffer, giving final drug concentration of 4, 10, 20, 30, 40, 50, 60 80 and 100 µg/ml respectively. The aqueous solutions were then extracted with 5 ml of the organic phase consisting of ethyl acetate : propanol (7:3) for 4 minutes and the absorbance of the organic layer was measured against an appropriate blank at 272 nm on Carl-Zeiss spectrophotometer.

Recovery studies were conducted using known quantities of 5-FU in the organic phase in order to validate the accuracy of the established analytical method.

3.3.4 Infrared Spectroscopy of the Microcapsules

The infrared spectrum of each type of polymeric microcapsule was recorded in potassium bromide disc on a Perkin-Elmer Infrared Spectrophotometer (Model 1420).

3.3.5 In vitro Drug Leaching Studies

As all the products were stored as suspensions in phosphate buffered saline, in vitro drug leaching studies were essentially conducted to estimate the extent of drug leaching from the microcapsules during storage. The microcapsules suspended in 5 ml of phosphate buffered saline and stored in a refrigerator were sampled at periodic time intervals upto 2 months and analysed for 5-FU content after suitable dilution.

3.3.6 In vitro Drug Release Studies

A quantity of microcapsules equivalent to 5 mg of 5-FU was suspended in 10 ml of phosphate buffered saline in a vial. The vials were maintained at 37 ± 1°C upto 6 hours and at regular time intervals, samples were withdrawn, diluted and analysed for 5-FU content spectrophotometrically.

3.3.7 In vivo Organ Distribution Studies

In vivo organ distribution studies of 5-fluorouracil from various batches of microcapsules were carried out on healthy albino rats (Wistar strain) of either sex, weighing between 250 to 350 gms. Groups of four rats were used for each set of study. The animals were fasted overnight prior to the study, anaesthetized with solvent ether and 1 ml of the microcapsule suspension, equivalent to 5 mg

of 5-FU, was injected via the femoral vein. The animals were sacrificed at time intervals ranging from half-hour to 48 hours and their vital organs like liver, lungs, spleen, kidneys and intestine were removed. After weighing, all the organs were cut into small pieces and homogenized in four times their weight of chilled phosphate buffered saline, using a tissue homogenizer. The homogenate was centrifuged (Remi centrifuge) at the highest speed for ≅30 minutes, the clear homogenate was collected and its volume noted. A measured aliquot of the homogenate was withdrawn into a 5 ml volumetric flask and the volume was made up with phosphate buffered saline. This aqueous phase was extracted with an equal volume of the organic mixture consisting of ethyl acetate and propanol as per the method described earlier and the organic layer was analysed for 5-FU content against a similarly prepared blank using respective organs of untreated rats.

(i) Calibration Curve for Estimation of 5-Fluorouracil in various Organs

As already reviewed, most of the reports in literature describe HPLC methods for estimating 5-FU in biological samples. As majority of the reported HPLC procedures first involve extraction of 5-FU from the biological sample using an organic solvent, we developed a method involving extraction of 5-FU from the tissue homogenate using a solvent system recommended for HPLC separation and estimating the drug from the organic phase spectrophotometrically.

25 mg of 5-FU was dissolved in 50 ml of phosphate buffered saline and aliquots of 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4 and 0.5 ml were added to 5 ml of clear tissue homogenates obtained from the respective organs of untreated rats. The samples were extracted with the reported organic solvent mixture (ethyl acetate : propanol) and the absorbance of the organic phase was measured at 266 nm on Hitachi-2000 Spectrophotometer against an appropriate blank. Triplicate runs of all calibration curves were conducted.