RESEARCH PAPER

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Formulation and characterization of polyterephthalamide microcapsules as carriers for the anticancer agent, 5-Fluorouracil

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Polyterephthalamide microcapsules were prepared by interfacial polycondensation reaction between diamino acids and a diacid chloride. the formulation conditions were optimized by varying parameters like mode of emulsification, time of emulsification, concentration of emulsifying agent, time of polymerisation and phase volume ratio till microcapsules of desired particle size range were obtained. These microcapsules are suggested as carriers for the anticancer drug, 5-Fluorouracil, for the purpose of drug targeting.

SEMIPERMEABLE microcapsules were first prepared by Chang[1] (1964) by interfacial polycondensation reaction between a diamine (hexamethylene diamine) and a diacid chloride (sebacoyl chloride) as carriers for hemoglobin. Because of their advantages like nontoxicity, nonimmunogenicity, nonspecificity, good payload capacity and ease of formulation, many workers investigated the formulation and optimisation of polyamide or Nylon microcapsules as carriers for various agents. Koishi at al.[2] (1969) and Shigeri at al.[3,4] (1969, 1970) investigated the formulation and characterisation of polyphthalamide microcapsules, whereas Madan[5] (1978) described the mechanism and technique of interfacial reactions. change[6] (1971) and Mori et al.[7,8] (1972,1973) described the preparation and characterisation of microcapsules containing L-Asparaginase, a antitumor agent, while Miyawaki et al.[9]. (1974) and Arakawa and Konodo[10] (1981) encapsulated Urease and Catalase respectively in Nylon microcapsules.

The present paper describes the formulation of polyterephthalamide microcapsules (prepared by reaction between various diamino acids (Asparagine, Arginine, Ornithine and Cystine) and the diacid chloride, terephthaloyl chloride as carriers for the anticancer agent, 5-Fluorouracil. These polyamides have an advantage over the conventional polyamides in that they are not only biodegradable but their polymeric wall material is stronger due to some degree of crosslinking occurring between the -COOH group of the amino acid and the -COCl group of the acyl chloride (Santo and Abend,[11] 1976) making the interfacial film more resistant to stresses like centrifugation and enzymatic attack. The paper also discusses the optimisation of formulation conditions of the mocrocapsules by a systematic and stepwise study of variable parameters mainly affecting the particle size and size distribution of the microcapsules.

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MATERIALS AND METHODS

Materials

Terephthaloyl chloride (Fluka Chemie, Switzerland), L-Asparagine monohydrate, L-Cystine and L-Arginine (Loba Chemie, India), L- Ornithine monohydrochloride (C.D's Lab. Chem. Industries, India), Span-85 (Koch Light Laboratories, England), Tween-20 (wilson Laboratories, India), Chloroform,

Cyclohexane (A.R. Grade, Qualigens Fine Chemicals, India), 5-Fluorouracil (gift from La Roche, Switzerland).

Method

The polyterephthalamide microcapsules were prepared by the interfacial polycondensation reaction between a diamino acid and a diacid chloride as described by Chang[1] (1964). To a definite volume of 0.45 M Na_2CO_3, equal volume of distilled water was added and 0.001 Moles of the diamino acid was dissolved in it. The solution was emulsified with 20 ml of an organic phase consisting of chloroform and cyclohexane (1:3) containing Span-85 as the emulsifier and then a solution of 0.0012 Moles of Terephthaloyl chloride in the same organic phase was added to the emulsion and the reaction was finally euenched with excess organic phase. The mixture was centrifuged, the supernatant organic phase was decanted off, the sedimented microcapsule pellet dispersed in 10 ml of a 5% Tween-20 solution and then diluted with 50 ml phosphate buffered saline. The microcapsules were then collected by centrifugation and repeated washings with buffered saline to remove excess Tween and then finally suspended in normal saline and stored in a refrigerator till further use.

Several batches of polyterephthalamide microcapsules were prepared under different conditions and their particle size analysis carried out in order to determine the optimum conditions of formulation of the microcapsules. The variable parameters studied were: mode of emulsification, time of emulsification, concentration of emulsifying agent, time of polymerisation, temperature of polymerisation and phase volume ratio.

Particle size analysis was carried out using Ore MμH-9 Microscope (Russia) at 600X magnification and photomicrographs were taken on Olympus ECE Bi-I microscope (Japan).

After the optimum formulation conditions were established, microcapsules containing 5-Fluorouracil were prepared according to the method already described using aqueous solution of the drug for emulsification.

RESULTS & DISCUSSION

Table 1 summarizes the arithmetic mean particle diameter and standard deviations of various batches of microcapsules prepared. The frequency distribution plots of the same are shown in **Figs. 1 and 2.** The optimum conditions were identified as those wherein the microcapsules showed smallest mean diameter and least standard deviation.

The microcapsules prepared using a magnetic stirrer (Remi) at highest speed (≅ 2000 rpm) gave larger particles with a relatively wide size distribution (Fig.1) while the microcapsules prepared using Ultrasonic Vibrator (Vibronics) were smaller with a narrower range of size distribution.

The time of emulsification is a crucial factor as it ultimately influences the size of the microcapsules formed because the polymer film is going to cost the emulsion droplet. Hence, lesser the emulsion droplet size, lesser will be the particle size. Emulsification time of 1 min, gave a broad size distribution (Fig.1) and partial separation of aqueous phase indicating incomplete emulsification, whereas an emulsification time of 5 min. gave a narrow distribution with minimum size and standard deviations.

Low concentration (1%) of Span-85, the emulsifying agent was insufficient for complete emulsification and gave big particles whereas above 5%, the Span concentration did not significantly influence the particle size (Fig.2). Moreover, the product prepared using 10% Span was difficult to separate. Hence, though the standard deviation of this batch was least, a Span concentration of 5% was considered optimum.

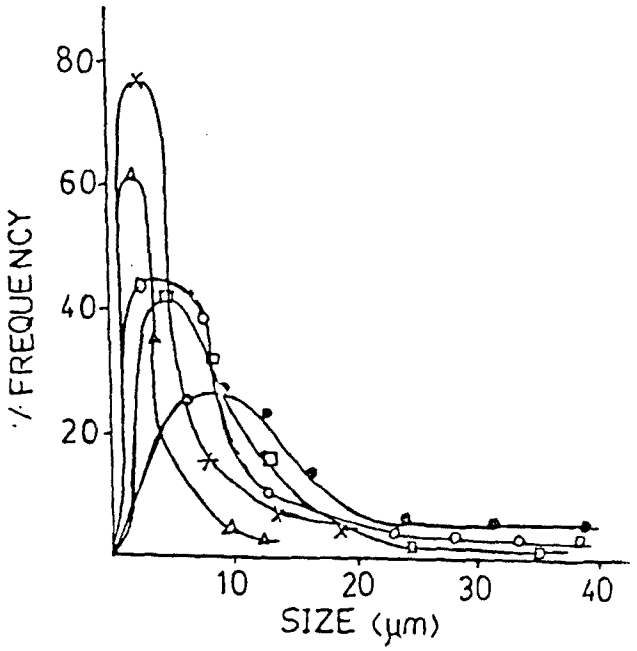

Fig.1: Effect of mode of emulsification and time of emulsification on the particle size of polyterephthalamide microcapsules
Key: o — Magnetic Stirrer; o — Ultrasonic Vibrator; □— 1 min.; x — 3 min.; — 5 min.

The time of polymerisation is another important parameter in the formulation of polyamide microcapsules because greater the reaction time, thicker will be the polymer film formed around the core droplets which may reduce the permeability to the core molecule. Hence, it is important that the reaction is quenched as soon as a polymer film of uniform thickness is obtained. Although a polymerisation time of 1 min. gave very small particles, (dm - 2.25 μm), the polymer film was very fragile and broke down upon centrifugation; whereas a polymerisation time of 5 min. gave big particle size with wide size distribution (**Fig.2**). Hence, a 3 min. polymerisation time was considered optimum as it gave small size, least standard deviation and was found to withstand the stress due to centrifugation.

Ice bath temperature was required to minimize evaporation of organic phase, reduce the particle

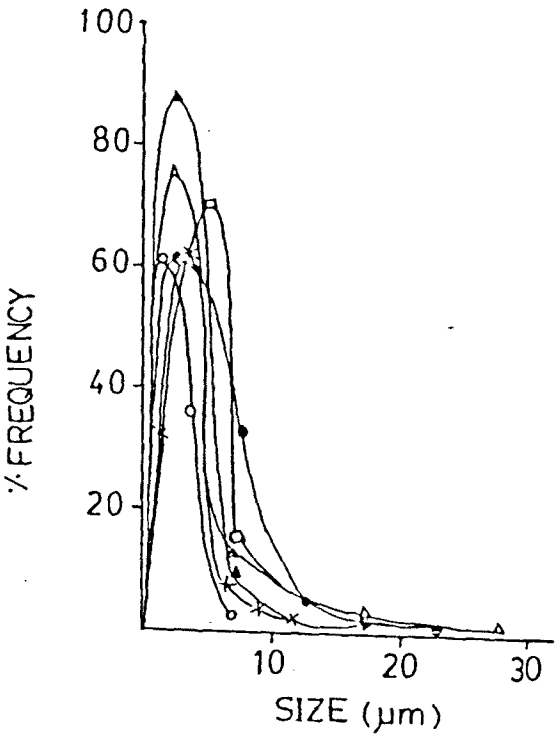

Fig 2 : Effect of concentration of Span and time of Polymerisation on the Particle Size of Polyterephthalamide Microcapsules
Key : □- 1% ; Δ - 5% ; ▲ - 10%; o- 1min. ; x - 3 min. ; ● - 5 min.

size, dissipate the heat of reaction and to render the rate of reaction slower. Reaction at room temperature will not only hasten the rate of reaction, leading to the formation of coarse and uneven films, but it will also increase the rate of evaporation of the organic phase, thuse increasing the phase volume ratio. It was observed that a minimum phase volume ratio of 1:5 (aqueous to organic phase) was required. At higher phase volume ratios, the emulsion was found unsta .e and some amount of aqueous phase was found to separate out due to inefficient emulsification.

Thus, the optimised formulation conditions for the preparation of polyterephthalamide microcap-

Table 1 : Particle size distribution of polyterephthalamide microcapsules prepared under various conditions

Order of evaluation	Parameter	Level	Mean diameter (μm ± S.D.)
1.	Mode of emulsification	Magnetic Stirrer	11.55 ± 4.84
		Ultrasonic bath*	7.9± 2.71
2.	Emulsification time	1 minute	6.9 ± 4.71
		3 minutes	4.75 ± 2.39
		5 minutes*	3.43 ± 0.93
3.	Span-85 concentration	1%	5.21 ± 3.05
		5%*	3.65 ± 2.08
		10%	3.34 ± 1.01
4.	Polymerisation time	1 minute	2.25 ± 0.61
		3 minutes*	3.38 ± 0.57
		5 minutes	4.72 ± 2.09
5.	Reaction temperature	Room (28°C)	4.36 ± 2.72
		Ice bath (5-8°C)*	3.38 ± 0.97

* indicates optimum level.

sules are: Aqueous: Organic phase volume of 1:5, stirring and emulsification in an ultrasonic vibrator maintained at icebath temp., Span-85 concentration of 5%, emulsification time of 5 min and polymerisation time of 3 min. The resulting microcapsules were spherical, with a thin coating of polymer and having an average size of 3-4 μm. The drug payload capacity of these microcapsules was found to be 70-75%.

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