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EVALUATION OF CRITICAL MATERIAL ATRRIBUTES (CMA) AND CRITICAL PROCESS PARAMETERS (CPP) FOR FORMULATION OF PLGA BASED MICROSPHERE BY COACERVATION METHOD

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Abstract

Parenteral depot formulations have garnered significant attention due to their ability to provide controlled release of drugs. Despite advancements in depot formulation technology, the market availability of parenteral microsphere products remains limited. This scarcity can be attributed to challenges such as low yield, inconsistent batch-to-batch reproducibility, high costs of polymers, and the complexity of manufacturing processes. This study aims to systematically identify and optimize the critical material attributes and process parameters associated with the coacervation method used in PLGA based microsphere manufacturing. The results of this investigation provide conclusive insights into several key factors, including the selection of polymers based on desired release profiles (influenced by inherent viscosity), the impact of drug concentration on formulation, the effects of different types and rates of coacervating agent addition during microsphere production, and the optimization of organic solvent ratios. By assessing the influence of various parameters on critical quality attributes, this study contributes to enhancing the understanding and optimization of microsphere manufacturing via the coacervation method in parenteral depot formulations.

Keywords: Microsphere, Coacervation method, PLGA, CMA, CPP

1. Introduction

Pharmaceutical preparations meant to be administered via routes other than the gastrointestinal tract are referred to as parenteral formulations. These formulations are made especially for injection techniques like subcutaneous, intramuscular, and intravenous. The goal is to make it easier for the drug to enter the bloodstream directly, resulting in effective and quick absorption. This form of administration is specifically utilized for medications that have low gastrointestinal absorption or stability or that require a steady and timely commencement of therapeutic effects [1]. The drawbacks of parenteral formulations include the possibility of injection site infections, the need for expertise in administration methods, and the relatively limited self-administration potential as compared to oral medication [2].

Numerous obstacles can be addressed by strengthening aseptic conditions, choosing suitable excipients, and investigating innovative formulations that increase formulation stability. Depot formulations are crucial for reducing the frequency of doses. The pharmacokinetics and release properties of depot formulations are different from those of ordinary parenteral formulations. Depot formulations are made to release drugs steadily and long-term, giving a consistent and long-lasting therapeutic impact. Typical parenteral formulations, on the other hand, frequently show faster drug release and may need to be administered more frequently [3].

Depot formulations use a variety of techniques, including as encapsulation in polymer systems or biodegradable matrices, to ensure continuous release. A longer duration of action may result from this prolonged release, which could decrease the frequency of administration and thus increase patient compliance. To summarize, the main difference is in the release kinetics: normal parenteral formulations try to produce a rapid release of the medication, requiring more frequent dosing, whereas depot formulations strive for sustained, longer release.

Depot formulations that are designed to provide long-term drug release can take many different forms, such as injectable depots, polymeric matrices, implants, liposomes, and microspheres. Liposomes are spherical vesicles that contain medications, whereas microspheres and nanospheres are tiny particle structures. Implants are hard implants placed under the skin that control drug delivery by releasing the medicine gradually. In situ gel formulations allow for prolonged drug release by going through a phase change from liquid to gel. Encapsulating media consist of polymeric matrix, while injectable depots provide reservoirs at injection sites [4]. This range of formulations provides controlled and extended treatment effects while meeting complex medication delivery needs [5].

Microspheres, which are complex particulate entities with sizes ranging from 1 to 1000 microns, are the most advantageous of the investigated innovative dosage forms when it comes to stabilizing and prolonging the release of the medication. These tiny, spherical particles, which are made of various materials including polymers or proteins, are skilled drug carriers in cutting-edge drug delivery systems. Precisely engineered, microspheres contain medicinal substances and offer a novel approach to regulated and prolonged medication release. Microspheres are crucial for optimizing therapeutic interventions in a variety of medical applications because they can be

precisely manipulated in terms of size, composition, and surface features to create unique drug release profiles. The methods for preparing microspheres include: Emulsion-Solvent Evaporation, Spray Drying [6], Solvent Extraction/Evaporation, Electrospraying, Coacervation, Phase Separation/Quasi-Emulsion Solvent Diffusion, Supercritical Fluid Technology. Each method provides unique advantages and is chosen based on the specific requirements of the drug and the desired characteristics of the microspheres[7-8]. PLGA plays most prominent role in controlling the drug release from microsphere. Drug release profile can be tailored with the help of type of PLGA used. Many drug and process parameters influence the critical quality attributes of the microsphere which can be controlled by PLGA polymer [9-10] .

This study is predicated upon the discernment of pivotal process parameters and material attributes crucial for the advancement and refinement of the coacervation methodology employed in the preparation of depot microspheres. Contemplating the heightened prevalence of anti-diabetic agents, Saxagliptin has been specifically chosen as the prototypical drug for formulation optimization endeavors [11]. The investigative focus revolves around elucidating the intricacies of the coacervation process, emphasizing the interplay between critical factors, and endeavoring to enhance the overall efficiency of microsphere development for sustained drug delivery.

2. Materials

Saxagliptin Hydrochloride was willingly provided as a gift sample by Torrent Pharmaceuticals, India. Purac polymer was provided by Corbion, Netherland. RESOMER® RG 503H, and, RESOMER® RG 504H was provided by Evonik, Germany. The organic solvents such as n-Heptane Dichloromethane and Methanol, were purchased from Finar Limited, India. Solvents used were of Injectable grade. Gift samples for Span 80 and Silicone oil 350 were provided by Croda, India and Dupont, USA respectively. All the chemicals used for the Quantification were of analytical grade.

3. Methods

3.1 Quantification of Saxagliptin Hydrochloride:

The quantification of API was done with the help of HPLC system (Shimadzu, Japan), by photodiode array detector and an enduring C18 column. The mobile phase consist of dibasic sodium phosphate buffer (pH 3.5) and acetonitrile, at a flow rate of 2 ml/min. Detection transpired at 213 nm, UV detector. The column and HPLC system were maintained at 45°C. This method was employed for the determination of the formulation drug count and its dissolution profile [12].

3.2 Preparation of Formulation

In the Coacervation technique, Saxagliptin, representing the drug phase, underwent dissolution in methanol, while the PLGA polymer, constituting the polymer phase, dissolved in dichloromethylene. The drug phase was subsequently introduced into the polymer phase, forming the dispersed phase. Employing silicone oil as the coacervation agent, this amalgamation underwent continuous stirring at a controlled rate. The resultant coacervation phase was then translocated to the quench phase, comprised of n-Heptane and Span 80, to induce microsphere solidification. Subsequent to this process, microspheres were systematically collected through a sieve bucket. Washing phases, including n-Heptane and Span 80, Ethanol and Span 80, as well as

n-Heptane, were sequentially employed to eliminate residual solvents. The resultant microspheres underwent drying at varying temperatures for the final drying phase [13].

3.3 Evaluation of Microsphere

3.3.1 Drug entrapment: A quantity of 10 milligrams of Saxagliptin microspheres was thoroughly weighed and placed into a volumetric flask. A proportionate amount of Acetonitrile was introduced to the volumetric flask, undergoing vortexing until the microspheres achieved dissolution. The resultant solution underwent further dilution, reaching approximately 80% of the total volume with acetonitrile, followed by a 15-minute sonication period. Ultimately, the volume was adjusted to the mark with acetonitrile. When necessary, the sample underwent additional dilution with acetonitrile to attain a concentration falling within the standard curve range. Subsequently, the sample was filtered through a 0.45-mm PVDF (polyvinylidene fluoride) syringe filter before subjecting it to HPLC analysis. The determination of the percentage Drug Loading (DL) was executed in triplicate sets, and the outcomes were reported as the average ± standard deviation (SD).

3.3.2 Dissolution study: In vitro drug release experimentation was conducted utilizing a Bottle Rotation Apparatus (Make: Electro lab) equipped with a 100 mL bottle assembly. Approximately one hundred milligrams, equivalent t 10.00 mg of Saxagliptin, of meticulously weighed microspheres were placed into the 100 mL bottle. Subsequently, 90 mL phosphate buffer solution was added to the bottle. The bottle was affixed within the bottle assembly, and the apparatus commenced operation at controlled temperature of 37°C (± 0.5°C) at 12 RPM. Sampling procedures were executed at predetermined intervals, and the samples were subjected to analysis through an HPLC method [14].

3.3.3 Particle size distribution: The representative microsphere sample was meticulously weighed and placed in a vial, to which water was subsequently added. The microspheres underwent external sonication for a duration of 5 minutes to ensure proper dispersion. Following the sonication, the sample was subjected to analysis utilizing a light scattering particle sizer, specifically the Malvern Mastersizer 3000. The resulting particle size distribution was observed and characterized through key parameters such as D10, D50, D90, and span values. These metrics provide valuable insights into the distribution of particle sizes within the analyzed sample [15].

3.3.4 % Yield: The microsphere obtained after the final step of the process can be quantified under the % yield formula which can be calculated from weight of product formed in relation to amount of polymer and API used in the process with multiplication to 100 [16].

3.4 Optimization trail for critical process parameters (CPP) and critical material attributes (CMA) [17][13][18]

3.4.1 Selection of the polymer: The primary selection of a distinctive polymer holds paramount importance in the formulation of parenteral sustained-release systems as polymer will decide the duration of the drug release from the formulation. The drug phase

involved the dissolution of equal amount of Saxagliptin in 1 gm of methanol. Diverse polymers, namely Resomer RG 504 H, Resomer RG 503 H, and Purac, constituted the polymer phase, with 950 mg of PLGA polymer dissolved in 8 gm of dichloromethylene. The dispersed phase, created by adding coacervation agent (silicone oil) under continuous stirring at a controlled rate, underwent subsequent transfer to the quench phase (n-Heptane + Span 80) to induce the solidification of microspheres. This systematic approach ensures a nuanced exploration of polymer characteristics and their impact on the sustained release of Saxagliptin. All the process parameters such as temperature, addition rate, and stirring efficiency were kept constant in all the trials.

3.4.2 Trial to optimize drug loading capacity: With reference to the above preliminary polymer trials, subsequent investigations were conducted, entailing diverse formulations with varying drug concentrations (10% and 15%). These formulations were systematically plan with polymers to assess and quantify the drug loading capacity as a percentage. This iterative approach seeks to discern optimal drug-polymer ratios, contributing to a comprehensive understanding of the intricate dynamics governing drug loading in the formulation.

3.4.3 Optimization of silicone oil addition rate: The coacervation phase, involving silicone oil, plays a pivotal role in the microsphere formation through the phase coacervation technique. Preliminary trials carried out to assess this role by systematically varying the addition time of the coacervation phase. In the ensuing investigation, distinct addition times, namely 2, 5, and 10 minutes, were scrutinized to learn their impact on the microsphere formation process. This trail is aim to identify the impact of the rate of addition of silicone oil on critical quality attributes of the formulation keeping all the process parameters constant.

3.4.4 Trail for selection of Silicone oil grade: The choice of silicone oil grade holds significance in shaping the distinctive characteristics of microspheres. Silicone oils with increase in viscosity grades exhibit enhanced stability indices during the coacervation process. The impacts of varying grades of silicone oil, specifically 350 CST and 1000 CST, were systematically assessed through a series of controlled experimental trials. This empirical investigation aims to verify the differential effects of silicone oil viscosity grades on the resultant microsphere attributes.

3.4.5 Trial to identify the impact of Silicone oil to DCM ratio: Ratio of silicone oil and DCM shows a significant role on particle size distribution. The effect of the ratio of Silicone oil (350 CST) and DCM were evaluated by performing the different trials with varying ratio of DCM to silicone oil I.e., 1:1.2, 1:1.4 and 1:1.6.

3.4.6 Optimization of quench phase quantity: Quench phase harden the microspheres formed during the coacervation process. Based on the literature it was noted that due to increase in quantity of quench phase, microspheres may become more stringent causing the impact on drug release profile. Trails were taken with two different quantity of quench phase to optimize the quantity of quench phase.

4. Results and discussion

4.1 Selection of the polymer: Microsphere formed with different polymers were of White to off white Colour, having satisfactory particle size Distribution and Drug entrapment. But Dissolution profile of FP1 was not as per the desired profile having significant amount of lag time. So, Resomer RG 503 H and Purac (55:45) were selected for further trails seen in Fig.1. It was found that as the viscosity of the polymer increases, drug release decreases significantly which may be due to the rigid matrix formation in microsphere describe in Table 1 causing slow breakdown of the polymer.

Table 1 Results of Polymer selection studies

Batch		**SC-1**	**SC-2**	**SC-3**
Parameter		**Resomer RG 504 H (5% DL)**	**Resomer RG 503 H (5% DL)**	**Purac (55:45) (5% DL)**
% Yield		87.95	85.45	88.64
% Drug Entrapment		76.7	74.2	80.5
PSD by malvern zitasizer 3000 (micron)	**D10**	25.523	32.823	33.625
	D50	40.827	47.652	46.755
	D90	65.397	62.837	71.358
	Span	0.977	0.630	0.807
% Drug Release at day 15		78.39	92.47	63.52

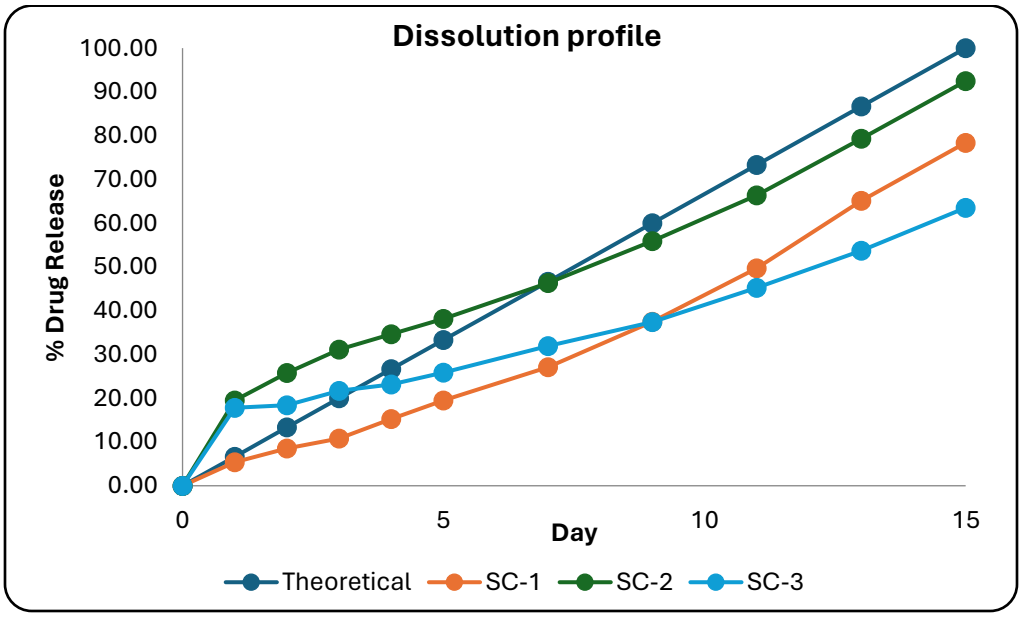

Figure 1 Drug release profile of polymer selection studies

4.2 Trial to optimize drug loading capacity: From the below results it can be concluded that, formulation with Purac polymer have high efficiency in entrapping the drug in comparison of Resomer RG503H polymer. Particle size distribution were found to be identical for all. But In dissolution study in Fig.2, it was found that formulation with Purac polymer have delayed release of API deviating from the targeted release profile. So for further optimization, Resomer RG503H as polymer with 5% drug loading is selected for further trial. The observation of below trail stats the importance of lactide to glycolide ratio in the polymer in Table2. With increase in lactide amount (55% in Purac) there is decrease in the drug release due to lipophilic nature of it.

Table 2 Results for optimization of drug concentration

Batch		**SC-4**	**SC-5**	**SC-6**	**SC-7**
Parameter		**Resomer RG 503 H (5% DL)**	**Resomer RG 503 H (10% DL)**	**Purac (55:45) (5% DL)**	**Purac (55:45) (10% DL)**
% Yield		86.75	84.92	86.15	83.86
% Drug Entrapment		77.5	80.2	82.5	85.7
PSD by malvern zitasizer 3000 (micron)	**D10**	33.154	32.468	33.795	33.629
	D50	45.835	47.285	48.373	47.519
	D90	65.393	63.733	69.429	72.879
	Span	0.703	0.661	0.737	0.826
% Drug release at Day 15		89.66	96.48	58.40	73.59

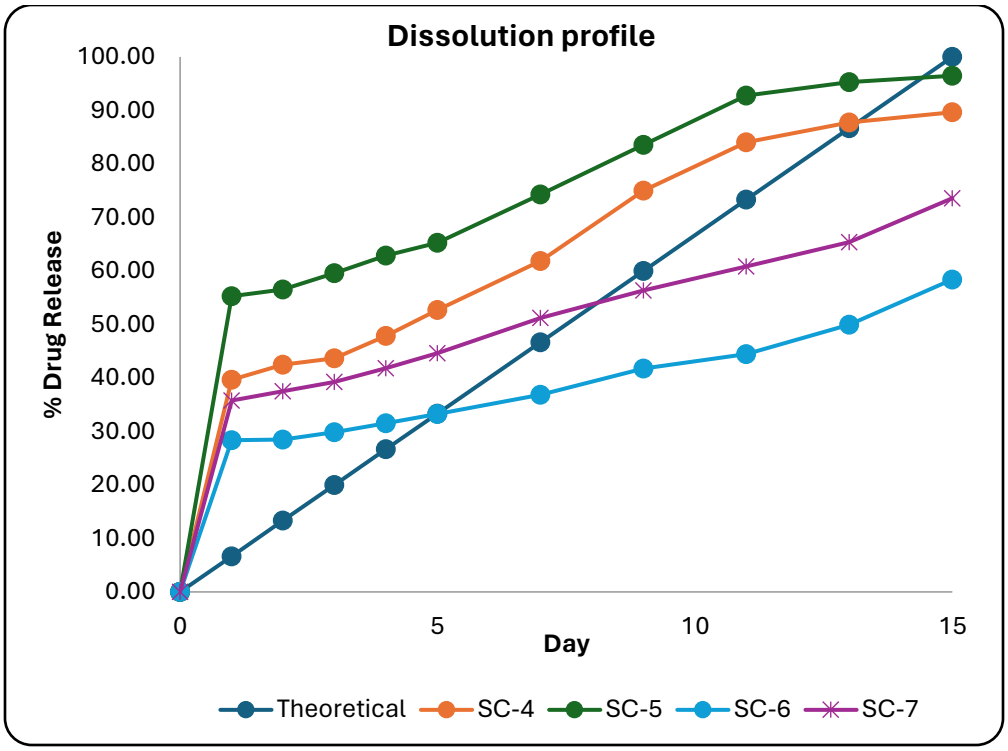

Figure 2 Drug release profile for optimization of drug concentration

4.3 Optimization of silicone oil addition rate: It was found that as the rate of addition of silicone oil decreases there is increase in the particle size distribution of the microsphere and more delayed profile of the drug release in Fig.3. There was no major impact of rate on %yield and entrapment seen in Table3. So based on the desired release profile and smaller particle size distribution, 2 mins silicone oil addition time were finalized for further trials.

Table 3 Results of change in Coacervation phase addition time studies

Batch		**SC-8**	**SC-9**	**SC-10**
Parameter		**Resomer RG 503 H (5% DL) (2 min)**	**Resomer RG 503 H (5% DL) (5 min)**	**Resomer RG 503 H (5% DL) (10 min)**
% Yield		87.23	82.40	84.62
% Drug Entrapment		72.7	70.8	73.2
PSD by malvern zitasizer 3000 (micron)	**D10**	35.639	34.419	35.624
	D50	48.294	57.328	62.942
	D90	65.732	75.845	88.389
	Span	0.623	0.723	0.838
% Drug release at Day 15		87.83	70.57	51.04

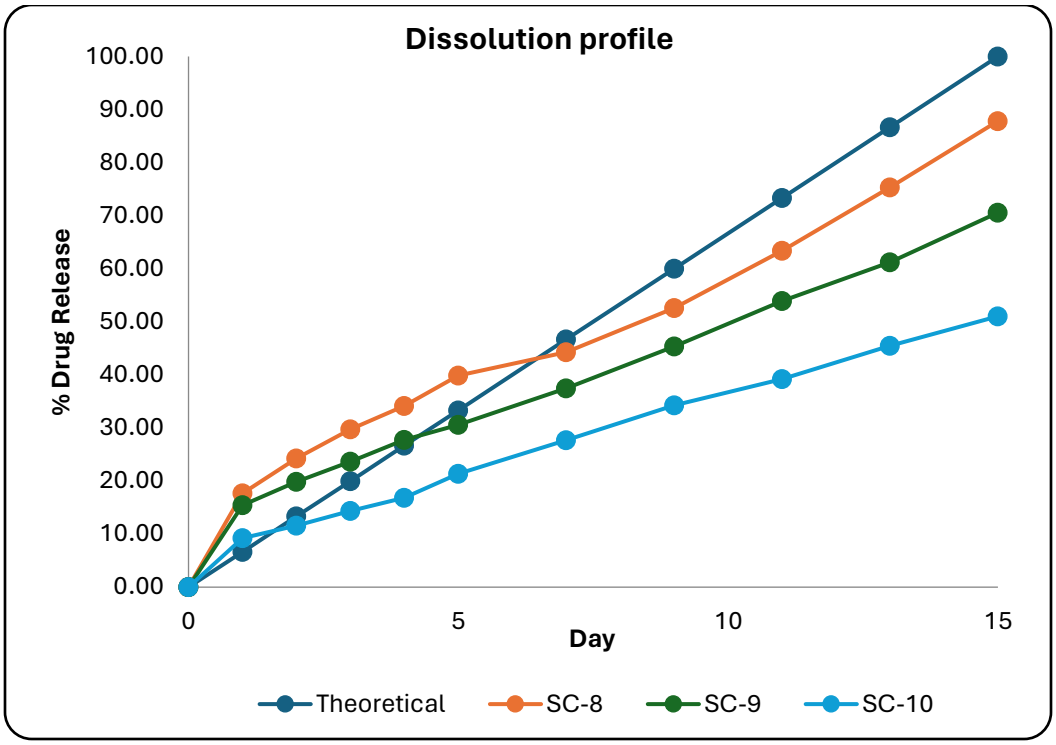

Figure 3 Drug release profile of Coacervation phase addition time studies

4.4 Trail for selection of Silicone oil grade: It was found that as the amount of silicone oil Increases there was uniform increase in the particle size distribution of the formulation due to increase in the viscosity of the dispersed phase seen in Table 4. Linear trend was not observed with regards to the dissolution profile in Fig.4. It was observed that to achieve linear release of drug, DCM to silicone oil ratio should be 1:1.4.

Table 4 Results of Silicone oil (350 CST):DCM ratio studies

Batch		**SC-11**	**SC-12**	**SC-13**
Parameter		**Resomer RG 503 H (5% DL) (1.2:1)**	**Resomer RG 503 H (5% DL) (1.4:1)**	**Resomer RG 503 H (5% DL) (1.6:1)**
% Yield		72.48	83.91	75.42
% Drug Entrapment		65.5	71.9	63.6
PSD by malvern zitasizer 3000 (micron)	**D10**	22.749	34.385	56.429
	D50	35.254	46.529	83.176
	D90	47.525	64.826	124.721
	Span	0.703	0.654	0.821

%Drug release at day 15		85.45	87.83	58.44

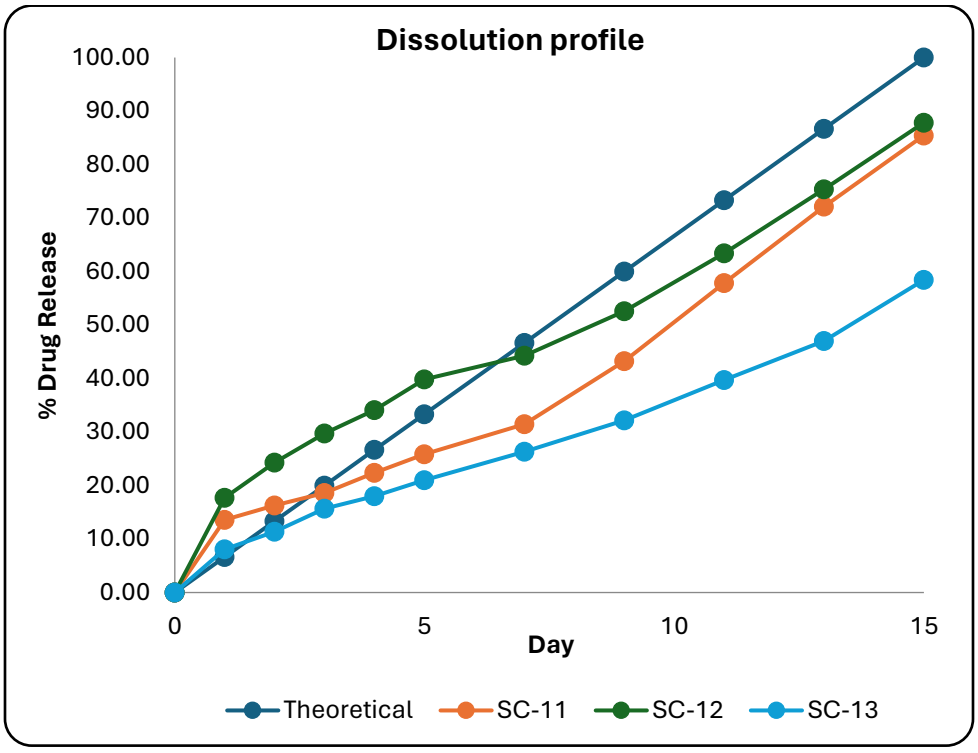

Figure 4 Drug release profile of Silicone oil (350 CST):DCM ratio studies

4.5 Trial to identify the impact of Silicone oil to DCM ratio: It was observed that formulation with silicone oil 1000 CST have broader particle size distribution as compared to formulation formed with 350 CST. It was also noted that release profile in Fig.5 was quite more sustained in formulation formed with 1000 cst due to the possible reason of formation of highly viscous phase causing stringent matrix formation. So based on the observation in Table 5, silicone oil with 350 cst were selected for further trails.

Table 5 Results of Silicone oil grade selection studies

Batch		**SC-14**	**SC-15**
Parameter		**Resomer RG 503 H (5% DL) Silicone oil 350**	**Resomer RG 503 H (5% DL) Silicone oil 1000**
% Yield		82.58	79.56
% Drug Entrapment		69.5	71.3
PSD by malvern zitasizer 3000 (micron)	**D10**	35.249	42.635
	D50	47.418	62.489
	D90	63.945	91.938

	Span	0.605	0.789
% Drug release at Day 15		87.83	63.69

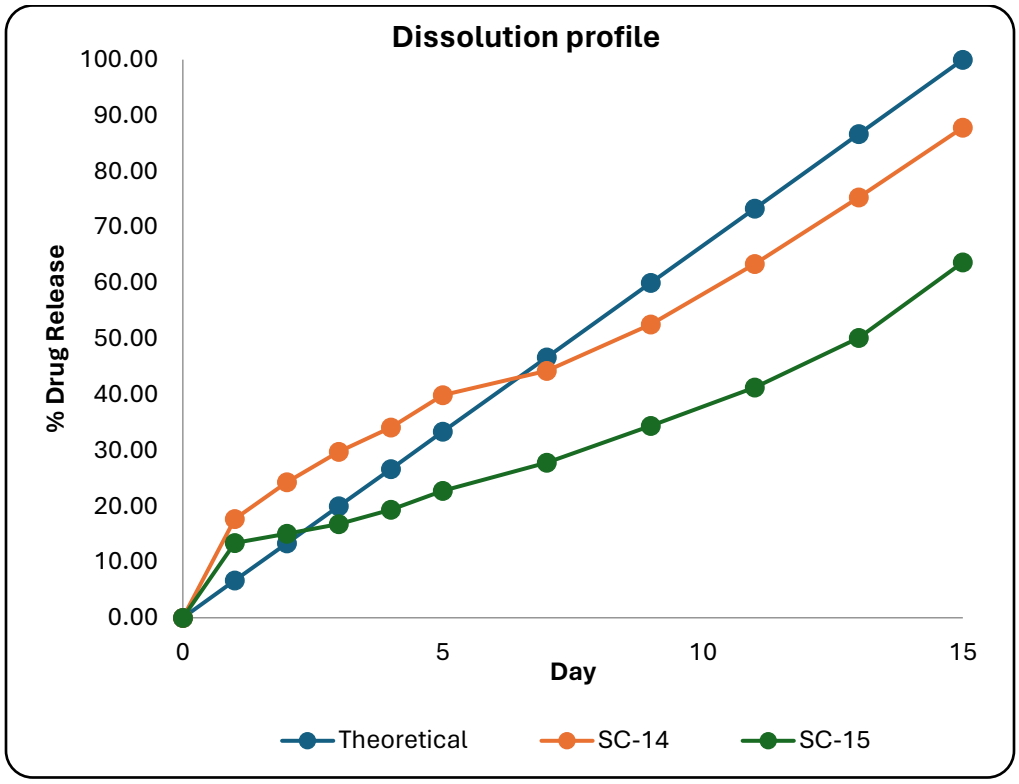

Figure 5 Drug release of Silicone oil grade selection studies

4.6 Optimization of quench phase quantity: Based on the analytical trials of particle size distribution, drug entrapment, % yield and dissolution in Fig.6 it was observed that there were no significant impact of quench phase quantity on the formulation. Thus we can concluded that there is not much impact of quench phase on critical quality attributes of microsphere describe in Table6.

Table 6 Results of Increase in Hardening/Quench phase volume studies

Batch		**SC-16**	**SC-17**
Parameter		**Resomer RG 503 H (5% DL) hardening 6.725**	**Resomer RG 503 H (5% DL) hardening 13.45**
% Yield		81.76	78.47
% Drug Entrapment		73.5	70.8
	D10	35.235	33.846

PSD by malvern zitasizer 3000 (micron)	**D50**	48.644	47.254
	D90	65.374	63.289
	Span	0.620	0.623
% Drug release at day 15		87.83	76.38

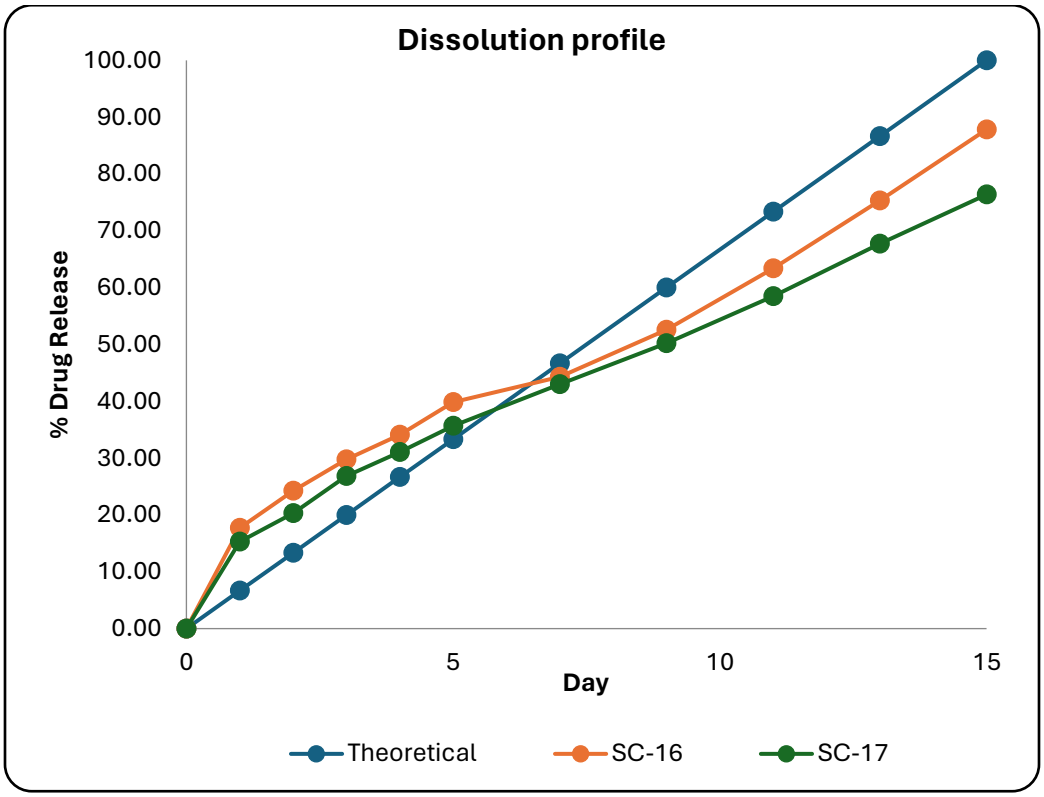

Figure 6 Drug release profile of Increase in Hardening/Quench phase volume studies

5. Conclusion

From the above trails it can be said that microsphere tailoring with desired quality attributes is possible by changing the process and material attributes concurrently. Quality attributes of microsphere depends on multiple factors which need to be identify. Proper QbD based design space can be formed for optimal formulation development. Citing the complex process, reproducibility can be gained by controlling the critical steps during the manufacturing process. Hence, it can be concluded that by controlling the critical steps of process and by selecting the ideal materials, coacervation method can help to formulate the microsphere with desired characteristics.

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