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Riboflavin (Vitamin B2) Injected Vehicle Production And Filtration Process

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ABSTRACT

The objective of this study is to develop and produce a non-GMP batch of generic Vitamin B2 injection vehicle to verify that the vehicle met specifications and passed through the recommended filters using the proposed filtration set-up. The study limit is to defining the manufacturing conditions and testing plan for a non-GMP batch of Generic Vitamin B2 injection vehicles. By using The Sartobran filters were chosen among other kinds of filters due to the higher filterability of the 2%CMC solution. Microbiological analysis of water, pharmaceuticals, and other liquids consider as a filtration unit. Pre-sterilized single-use filtration units help to mitigate the risks of secondary contamination and streamline your workflows.

More ever, the yield or the collected amount of filtrate from the Sartorius filters directly depends on the applied pressure on the liquid reservoir and the internal total surface area of the filter used.

Keywords: B2, injection vehicle, Riboflavin vitamin vehicle.

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INTRODUCTION

Vitamin B2 also called riboflavin, is one of eight B complex vitamins that are hydrosoluble, that's means B vitamins are water-soluble, meaning the body does not store them [1]. All B vitamins enhance the body to convert food (carbohydrates) into fuel (glucose), which is consumed to produce energy [1,2]. These B complex vitamins also accelerate the body's fats and protein metabolism. B complex vitamins are necessary for a healthy liver, skin, hair, and eyes. They are also used to help the central nervous system function properly [2,3]. As well as producing energy for the body, riboflavin works as an antioxidant agent, fighting damaging particles in the body known as free radicals' scavenger. Free radicals can damage cells and DNA and may contribute to the aging process, as well as the development of many health conditions, such as heart disease and cancer. Antioxidants, such as riboflavin, can fight free radicals and may reduce or help prevent some of the damage they cause [4,5]. Behavioral neuroscience is the controlled substance that is injected into the animal generally called a vehicle [6]. In the recent study of drug-induced behavior of an animal, a substance is injected into the animal. These behavioral types' tests are common in neuroscience behavioral, and they are often done in rodents such as mice or rats [7]. Usually, the injectable solid dosage forms soluble medications before injection in a suitable vehicle. Some polar chemicals are easily dissolved in a saline solution, which can be injected through the body for easy absorption into the bloodstream vessels. However, some substances are not dissolved very easily in a polar solvent like water or physiological saline (0.9%). Instead, they must be suspended in suitable viscous vehicles such as carboxymethyl cellulose. The nature of the substance determines which vehicle must be used [8].

MATERIAL AND METHODS

Vitamin B2 injection vehicle per the package insert of B2 Injection Vehicle is carboxymethyl cellulose (CMC)-based injection vehicle that contains sodium chloride, polysorbate 20 (Tween 20), citric acid anhydrous, sodium phosphate dibasic, and sodium chloride dissolved in water for injection (WIF) [9,10]. Different concentrations of CMC with low molecular weight made and analyzed for viscosity: 0.1, 0.25, 0.35, 0.45, 0.50, 0.55, 0.65, 0.75, 1.0, and 2.0%. All solutions were diluted with PBS and pH adjusted to approximately 6.9 [11-13]. The viscosity profile is shown in Figure 1. Besides, the required amount of sodium chloride is needed to adjust the osmolality of the final product chosen based on a development and trial study, Table 1. The raw materials used for injection vehicle production are detailed in Table 2. These raw materials are produced in a qualified GMP facility and meet worldwide GMP regulations.

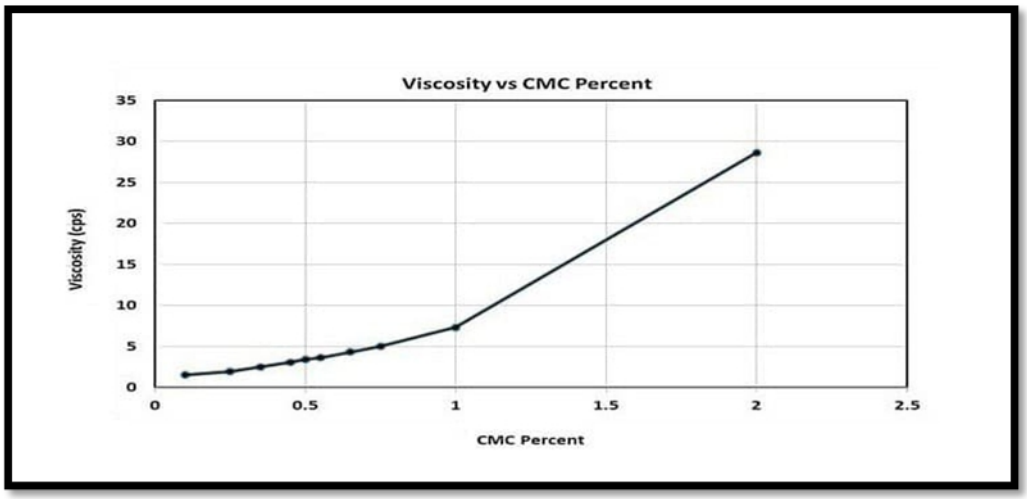

Figure 1: Viscosity of different concentrations of CMC solution

Therefore, based on the characterization results of injection vehicle (pH, viscosity, and osmolality) a composition of injection vehicle as mentions the core material Sodium Carboxymethyl Cellulose, USP, Low Viscosity with minimum amount 100 g, Water for Injection as 4850 g, Tween® 20 for min amount 0.5 g, Citric acid anhydrous, USP[1] as 1.5 g, Sodium phosphate dibasic, USP as 7.25 g and Sodium chloride for 35 g all these quantities consider as min level for five batch producers.

Table 1 Raw materials

Raw Material	Part No.	RM specification
CMC (Carboxymethylcellulose)	419895	RM00265
Sodium Chloride, low endotoxins	1.16224.5000	RM00234
Sodium Phosphate Dibasic Dihydrate	1.06576.5000	RM00266
Polysorbate 20	8.17072.1000	RM00267
Citric Acid	1.00241.5000	RM00264
Water for injection	NA	RM5160
CMC (Carboxymethylcellulose)	419895	RM00265

Table 2 Osmolality development study

NaCl	Osmolality (mOsm)
0.70%	277
0.75%	295
0.80%	309
0.85%	312

he vehicle concocted in a round bottom flask in a heating mantle with an overhead stirrer. First water was poured into the reactor and then CMC slowly mixed into the water. While mixing in the CMC, the solution heated to 72 ± 2°C by setting the heating mantle to 50%. When the solution reached 69-70°C the flask was removed from the heating mantle and placed on a plastic stand to cool and continue mixing. Once the solution reached at least 30°C, the sodium chloride, sodium phosphate, and Polysorbate 20 were added and allowed to mix for no less than 5 minutes or until all material was visibly dissolved. A pH meter was used to measure the initial pH of the solution [14]. A small amount of citric acid was added then the pH measured again. This process was repeated until the pH measured 6.8 – 7.2. The gross weight of the solution taken and the amount of water needed to be added to make the required volume of vehicle solution. The solution then allowed mixing for another three-five minutes.

RESULTS AND DISCUSSION

Autoclaving and several filters from Pall Corporation were tested with different parameters used for this purpose. The results for the development and characterization process are shown in Table 3 and Table 4. The autoclaving process could be a good candidate for the sterilization process of the 2% CMC solution without any significant changes in the properties of the solution (vehicle). On the other hand, all kinds of filters from Pall Corporation used were not feasible for the filtration and sterilization process due to early clogging and filtration difficulty. A new filter study was conducted with the small size of Sartobran 150 filter (P/N: 5231307H4—SS—B) with a specification and image listed in Table 5 for

filtration of three different 2% CMC lots. Also, the results of the filtration process shown in Table 6

Table 3 Results of formulation development batches using medium viscosity CMC

Lot Number	Concentration	Preparation Method	Yield	Average Viscosity, cPs
001-1	0.1%	RT	N/A	1.25
001-2 (unfiltered)	0.25%	RT	N/A	19.41
001-3 (filtered)	0.25%	RT	58.6	11.42
001-4	0.5%	RT	N/A	146.11
001-5	0.75%	RT	N/A	47.53
001-6	1.0%	RT	N/A	119.7
001-7	0.3%	RT	N/A	18.32

Table 4 Characterization of formulation development batches using low molecular weight CMC

Lot #	Description	pH	Viscosity	Observations	Filtration Yield%	Filter Type
111-01	Raw CMC (before autoclaving and filtration process)	6.9	34.5	NA	NA	NA
111-02	Coarse Filtered CMC (4.5um)	6.9	33.6	NA	NA	NA
112	Autoclave	6.9	25.8	NA	NA	NA
113	10mL Syringe Filter at RT	6.9	33.3	Clogged after few minutes	77	Sterivex SVGV01015
114	50mL Syringe Filter at RT	6.9	33.4	Clogged after few minutes	~16	Sterivex SVGV01015
115-01	50mL Syringe Filter at 60°C	6.9	33.9	Clogged after few minutes	NA	Sterivex SVGV01015
115-02	50mL Syringe Filter at 60°C	6.9	35.6	Clogged after few minutes	NA	Sterivex SVGV01015
116	Pressured at RT	6.9	25.9	Clogged after few minutes	NA	Fluorodyne Mini Kleenpak
117	Pressured at 40°C	6.9	29.3	Clogged after few minutes	NA	Fluorodyne Mini Kleenpak
118	Pressured at 37°C	6.9	31.5	Clogged after few minutes	NA	Fluorodyne Mini Kleenpak

Table 5 Specifications of Sartobran 150

Filtration Area	0.16 ft^2 | 0.015m^2
Prefilter/Endfilter Membrane	Cellulose acetate
Pore Size	0.45µm + 0.2µm
Max. Allowable Differential Pressure	58 psi at 20°C 29 psi at 80°C

Table 6 analysis of three CMC injection vehicle batches using Sartobran 150 filter

Lot #	0001	0002	0003

Max Pressure	50 psig	55 psig	49 psig
Elapsed Time	4 min	17 min	30 min
Prefilter Mass	440.5 g	991.4 g	2982.6 g
Yield	NA	97.60%	clogged
CMC Collected	380.9 g	967.3 g	1278.1 g
g/cm^2	n/a	n/a	8.5

It shows that this filter effectively filtered the injection vehicle. the experimental study of the lot (0003) was conducted to determine the filter efficiency by finding the maximum mass it could filter. The efficiency was more than 10 times higher than that for previous Pall Corporation filters used (Table 4). After confirming the Sartobran 150 as a viable filtering option, the Sartobran P size H8; P/N: 5235307H8--SS-A was chosen to scale up for a 5 L batch. The specification of these filters is shown in Table 7

Table 7 Specifications of Sartobran P, H8 filters

Filtration Area	1.1 ft^2 | 0.1 m^2
Prefilter/Endfilter Membrane	Cellulose acetate
Pore Size	0.45µm + 0.2µm
Max. Allowable Differential Pressure	72.5 psi at 20°C 29 psi at 80°C

In order to study the feasibility of using two Sartobran P, H8 filters in series for the sterilization of 2% CMC solution, the vehicle was prepared with double H8 filters of five lots that pressure gauge to find the desire result.

The study shows that double filtering will produce enough material so three batches were prepared with the new formulation and the double filter configuration to prove reproducibility, Table 8 showed the characterization and properties and result of (0004) patch

Table 8 double filter study set up

Experiment #	0004	
Prefilter Mass	6213.8 g	
CMC Collected	6015.5 g	
Yield	96.8%	
Filter	1	2
Starting Pressure	11 psig	11 psig
Max Pressure	54 psig	15.8 psig
Elapsed Time	13 minutes	14 minutes
pH	7.0	
Viscosity	32.6 cPs	
Osmolality	254 mOsm	

Primary packaging

Vitamin B2 injection vehicle is supplied in a standard vial, stopper, and seal assembly to fill the GLP clean room produced injection vehicle. The primary packaging is detailed in Table 9.

Table 9 Primary Packaging for Injection Vehicle Produced.

Component	Supplier	Part No.	Description
Vial	West	6800-0318	Schott manufactured 5 ml flint glass with blowback
Stopper	West	S10-F451	20 mm serum stopper in 4432/50 gray with FluroTec coating on plug and B2-40 coating on top. Stopper diameter is 18.90 mm.
Seals	West	54202038	20 mm FlipOff TruEdge, 6-Bridge, clear lacquer, 3003 H14 metal, Red button,

			polypropylene homopolymer plastic

CONCLUSION

Development of Vitamin B2 injection vehicle resulted in the production of a non-GMP batch and passed through the recommended filters (Sartobran P, H8 and Satobran P, H0 from Sartorius Company) using the proposed filtration setup. The Sartobran filters were chosen among other kinds of filters due to the higher filterability of the 2%CMC solution. Also, the integral structure of the Sartobran filters consists of mixed 0.45 and 0.20 µm pores within the same filter. More ever, the yield or the collected amount of filtrate from the Sartorius filters directly depends on the applied pressure on the liquid reservoir and the internal total surface area of the filter used. Besides, the autoclaving sterilization process is considered a feasible option for the sterilization of injection vehicle due to the ease of use and cost.

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