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Release study of immunity Blend Beadlet- marker compound as vitamin C

Release study of beadlets and accumulated vitamin C release

The dissolution of immunity beadlets and release of vitamin C at physiological pH (6.8) was studied by preparing simulated intestinal fluid (SIF) without enzymes. The system consisted of 13.872 g Potassium dihydrogen phosphate and 35.084 g of disodium hydrogen phosphate and water was added to a final volume of 1000 ml. The final pH was adjusted to 6.8. About 900 ml of dissolution solution taken in the vessel. Then degassing the dissolution medium through sonication or other means is important since the presence of dissolved gases may affect results so the beadlet is placed within the medium in the vessels after it has reached sufficient temperature and then the dissolution apparatus (Electronic india ,model: 1912) is operated at 50 rpm. Then the samples were taken at 1, 4,8 and 12hours. About 10 ml of sample solutions collected from the apparatus in each time interval. Then the dissolution solutions are analysed by HPLC method.

HPLC Analysis

The HPLC used consisted of an LC-10AT VP Solvent Delivery System (Shimadzu), a model SCL-10 A VP System Controller (Shimadzu), and a Model SPD-10AVP UV Detector(Shimadzu) .Chromatographic separation was performed on C18 column at 25°C. The mobile phase is composed of ammonium dihydrogen phosphate (0.05 M, pH 3.0 adjusted by 85 wt% phosphoric acid) and acetonitrile at a ratio of 95:5 (v/v), with a flow rate of 1 mL/min. Sample and analytical standard were injected at a volume of 1 mL each. The detection wavelength for vitamin C was 245 nm, and the retention time was 5 minutes. To ensure the accuracy of analysis, vitamin C standard solutions in the range of 10-100 µ g/mL were freshly made every time before sample analysis. The concentration of vitamin C in samples was determined using the linear regression equation obtained from the respective standard calibration curves of vitamin C ($R^2 > 0.999$).

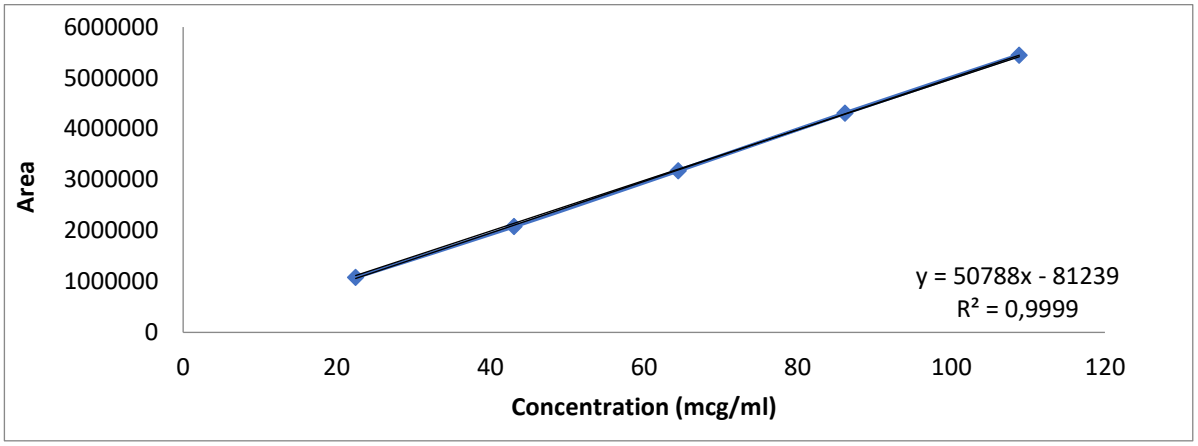

Fig 1 Calibration Curve of Vitamin C

$$\% \text{ Vitamin C loading} = \frac{\text{Amount of vitamin C} \times 100}{\text{Mass of beadlets}}$$

From the HPLC analysis found that average vitamin c content present in immunity blend beadlets are 250 mg with certain overages.

Releasing Study

The concentration of vitamin C in samples was determined using the respective calibration curves of vitamin C in SIF (Y = 68.512X + 1538.2, R2= 0.999). To stabilize vitamin C, samples collected at pH 6.8 were also diluted with phosphoric acid (pH 3) before HPLC analysis. The cumulative release percentage of vitamin C from beadlets at time t (CR%) during *in vitro* dissolution was determined from the following equation

$$M_S = (c \times df)/ Vc \quad (5)$$

$$CR = (c \times df)/ V + \sum_o^{t-1} M_s \quad (6)$$

$$CR\% = (CR/M) \times 100\% \quad (7)$$

c is the amount of vitamin C in the withdrawn sample at time t (mg); D is the concentration of vitamin C in the diluted sample analyzed by HPLC at time t (mg/mL); df is the dilution factor of sample diluted for HPLC analysis, df = 1; Gc is the sample volume withdrawn at time t, Gc =10 mL; H is the cumulative release amount of vitamin C at time t (mg); G is the volume of dissolution solution, G=900 mL; $\sum_o^{t-1} M_s$ is the amount of vitamin C in the withdrawn samples prior to time t (mg); % is the cumulative release percentage of vitamin C from 500 mg beadlet at time t; *M* is the total amount of vitamin C in beadlets (mg).

Sr No.	Time (hr)	Area A	Conc. µg/ml	Amt. mg	CDR %
1	1hr	659345	14.58	72.91	29.16
2	4 hr	1508690	31.31	156.53	62.61
3	8 hr	2256356	46.03	230.13	92.05
4	12 hr	2352895	47.93	239.64	95.85

Table 1: Cumulative release of vitamin c in immunity Blend

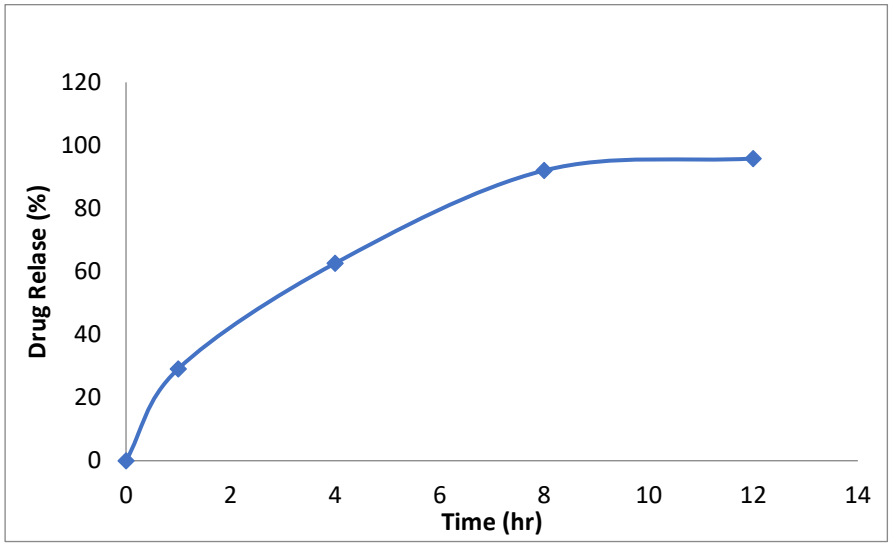

Fig 2: ***Vitamin C release from immunity blend Beadlets***

To obtain the rate of vitamin C release in simulated intestinal fluid (SIF) from beadlets, beadlets with an initial weight of ~ 500 mg were incubated in 900 mL of SIF (pH 6.8) solutions with a consistent rotation for a period of 8 h. Beadlets dissolved in SIF within 1hr. It can be observed that a burst release (25 %) of vitamin C after 1 hours and could release up to 85% up to 8 h. The release of vitamin C in SIF showed an initial release of 25 % in first 1 hr and 60% after in 4 hr, and an increase in release up to 90% after 8 h followed by a slight decrease.