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In-Vitro Study on Dissolution Profiling of Choline Chloride Encapsulated by Different Microencapsulation Technologies and Active Forms

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ABSTRACT

An *in-vitro* dissolution study was conducted to determine the effect of various microencapsulation technologies commercially employed to protect choline chloride from rumen mimic dissolution and absorption in intestinal mimic solution using two different type of actives (Liquid choline chloride 75% and choline chloride crystal 98%). This study shows that choline chloride crystals granulated by using carbohydrate matrix and coated by hydrogenated vegetable oils through top-spray fluid bed process technology have minimal dissolution of 11.14% in rumen mimic solution, liquid choline chloride granulated and further coated by hydrogenated vegetable oils through top-spray fluid bed process shows 34.71% dissolution, Liquid choline chloride absorb on silicon dioxide and coated by hydrogenated vegetable oils by pan coating shows dissolution of 82.69%, Liquid choline chloride emulsified and spray congealed shows dissolution of 84.6% while liquid choline chloride dispersed with hydrogenated vegetable oils solubilised 100% in rumen mimic solution. Results from study concluded that choline chloride active taken in crystal form granulated with cellulose based polymer and coated through fluid bed top-spray process technologies have minimal degradation in mimic rumen solution and thus maximum rumen bypass efficacy.

Key words: Choline Chloride, Rumen, Microencapsulation, Fluid bed process, Dissolution

INTRODUCTION

Choline chloride, (2-hydroxy)- trimethyl ammonium chloride $C_5H_{14}ClNO$is a highly hygroscopic substance used in feed for choline supplementation. In cattle choline apart from other functions (membrane integrity, neurotransmission, fat mobilization from liver and methyl group donation) has been observed to increase milk production. Unprotected choline in rumen is not effective as measured by digestibility studies due to complete or partial degradation by rumen microbes before it even reaches the intestine (Erdman and Sharma, 1991). Hence, supplementation of unprotected choline (conveniently as it is salt and choline chloride) is ineffective way to increase the choline supply. Therefore, Rumen-protected form of choline has been developed to deliver choline with less degradation to the small intestine for absorption (Pawar *et al*., 2015). Rumen protected form of choline increases the supply of choline to the small intestine with increasing milk yield and milk components or alleviating development of fatty liver syndrome (Hartwell *et al*., 2000). Type of technology used in manufacturing rumen protected choline has a direct effect on its rumen protection. Therefore, this study was undertaken to study the efficacy of different microencapsulation techniques on rumen protection of choline chloride.

MATERIALS AND METHODS

Analytical grade of potassium dihydrogen phosphate, sodium hydroxide, sodium bicarbonate, disodium hydrogen phosphate decahydrate, sodium chloride, potassium chloride, magnesium chloride, magnesium chloride, conc HCl were procured from Merck. Oxalic acid was procured form Rankem, Diethylamine and acetone were procured from Fisher scientific. Pepsin(1:3000), pancreatin(8X usp) were procured from sigma Aldrich.

Mimic rumen solution: Mc Dougall Buffer solution: pH 6.5, Each litre of Mc Dougall buffer solution contained 7.43 g of sodium bicarbonate, 7.0 g of disodium hydrogen phosphate, 0.34 g of sodium chloride, 0.43 g of potassium chloride, 0.10 g of Magnesium chloride, 0.05 g of calcium chloride.

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Mimic Abomasum solution: Clarks Lub Buffer pH 2.0, Each litre of Clarks Lub Buffer solution contained 3.73 g of potassium chloride, 2.1 g of Conc HCl, 1 g of Pepsin (1:3000)

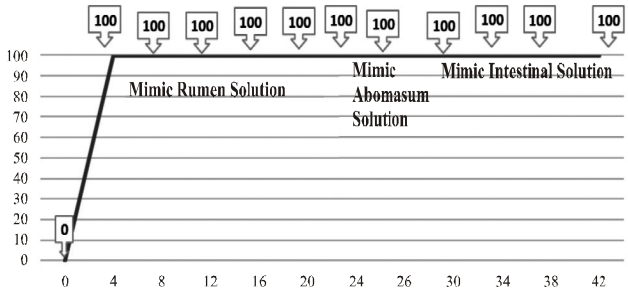
Mimic Intestinal Solution: pH7.8: Each litre of Mimic Intestinal solution contained potassium dihydrogen phosphate 68 g and 3.0 g of pancreatin (8X) USP.

Comparative *in-vitro* dissolution studies were done mimicking the *in-vivo* condition in dairy cattle. All Sample were developed in-house by different technologies. Samples were coded as A,B,C,D & E based on technology used Sample A: Liquid choline chloride adsorb on silica and mixed with hydrogenated vegetable oil by solid dispersion technique Sample B: Liquid choline chloride emulsified and spray congeal by hydrogenated vegetable oils by spray freezing technology Sample C: Liquid choline chloride adsorb on silica, granulated and coated by hydrogenated vegetable oil in pan coater Sample D: Liquid choline chloride adsorb on silica, granulated and coated by hydrogenated vegetable oil via fluid bed processor technology Sample E: Choline chloride crystal granulated with cellulose based polymers and further coated by hydrogenated vegetable oil mix by fluid bed process technology. All products developed were having assay of 33.5% minimum determined with HPLC and non-aqueous titrimetric both.

USP -1 type apparatus with six paddle was used for determination of dissolution profile of all samples. Other conditions maintained included temp 39ºc and rpm of 100. Mimic rumen solution study was done for 24 h and sampling was done at interval of four hours. This solution was replaced with mimic abomasum solution for 2 h and sampling was done at the end of 26th h which again was replaced with mimic intestinal solution for 12 h and sampling was done at the interval of 4 h upto 42 h. Each time in sampling interval, 5 ml of solution was taken out and replaced with fresh equivalent amount of media solution. The pipetted out samples were filtered and analyzed in HPLC in isocratic condition and detector in positive polarity.

RESULTS AND DISCUSSION

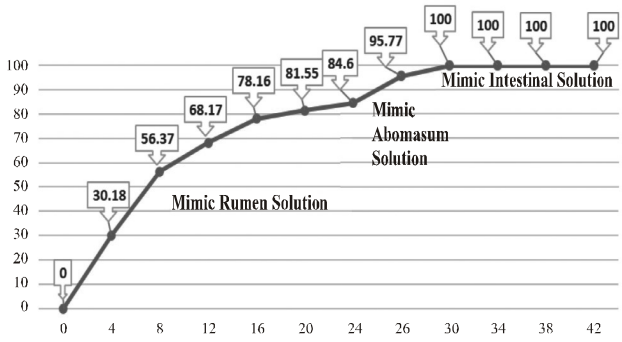
Graph 1: Dissolution profile of sample A



Sample A: Liquid choline chloride dispersed in hydrogenated vegetable oil matrix

Cumulative release profile of sample A shows 100% release within four hours in mimic rumen solution Eventually, cumulative release profile in Mimic abomasum solution and Mimic intestinal solution was also obtained 100%. It indicates that technique of solid dispersion with hydrogenated vegetable oils do not coat enough to resist its dissolution in given conditions.

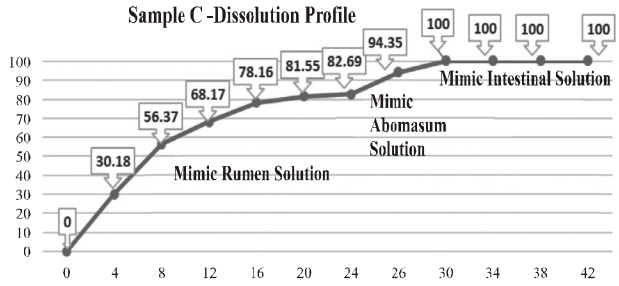
Graph 2: Dissolution profile of sample B



Sample B: Liquid choline chloride emulsified and spray congeal by Spray freezing technology

Cumulative release profile of Sample B shows 84.6% of claimed choline chloride released in 24 hours under rumen mimic solution, 95.77% released in abomasum while 100% released in intestine. Sample B was manufactured by mixing liquid choline chloride in melted hydrogenated vegetable oil and emulsified. The emulsified mix is further sprayed in cooling tower to congeal the fat matrix containing choline chloride resulting in microspheres of choline chloride.

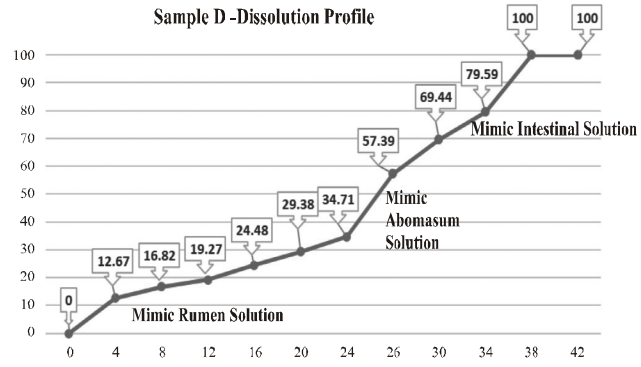
Graph 3: Dissolution profile of sample A



Sample C: Liquid choline chloride adsorb on silica, granulated and coated by hydrogenated vegetable oils by pan coating

Choline chloride in sample C was released 82.69% of claim in rumen mimic solution, 94.35% in abomasum and 100% in intestinal solution. It was manufactured by adsorbing liquid choline chloride on fine silicon dioxide granulated by adding binding agents and further coated with melted hydrogenated vegetable oil in pan coater resulting in microcapsules. This is an extension of core shell technology.

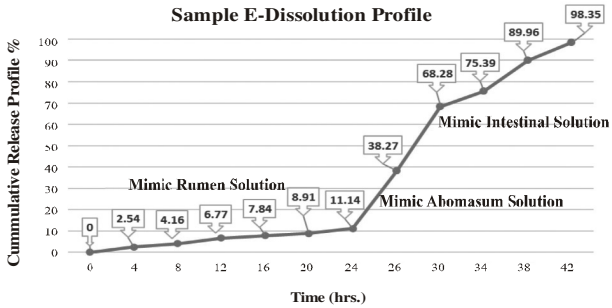
Graph 4: Dissolution profile of sample D



Sample D: Liquid choline chloride absorbed on silica, granulated and coated by hydrogenated vegetable oil by fluid bed processor technology

Cumulative release profile of Sample D shows choline chloride release of 34.71% in rumen solution, 57.39% in abomasum solution and 100% in intestinal solution. It was manufactured by absorbing liquid choline chloride on fine silicon dioxide granulated by adding binding agents and further coated with melted hydrogenated vegetable oil in fluid bed process te chnology. This results in microcapsules via core shell technology.

Graph 5: Dissolution profile of sample E

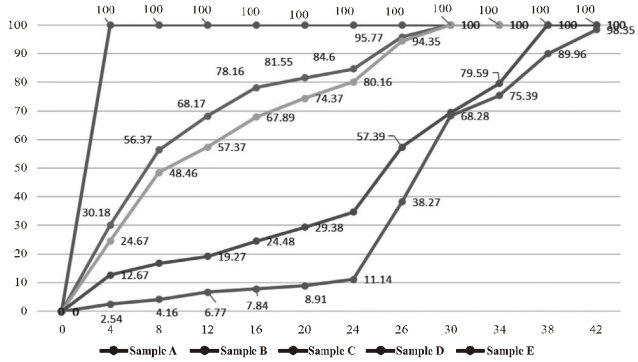


Sample E: Choline chloride crystal granulated with carbohydrate matrix and further coated by Hydrogenated Vegetable Oils by fluid bed process technology

Cumulative release profile of Sample E shows choline chloride release of only 11.14% in rumen solution followed by 38.27% in abomasum solution and sequential release of upto 98.35% in intestinal solution. It was manufactured by using choline chloride 98% crystal as active which were initially granulated and further coated with mix plant based vegetable oil. Coating was based on core shell technology where individual active is binded and coated to form microencapsulates.

There was significant difference in solubility of choline chloride microencapsulated product using different actives and technologies employed. Product made with choline chloride crystal with fluid bed process technology had shown minimum solubility in rumen mimic solution, thus maximum rumen protection while active (liquid choline chloride absorbed on silica and dispersed with hydrogen vegetable oils showed maximum solubility in rumen mimic solution and thus no rumen protection.

Graph F: Comparative dissolution profile



S. No.	Technology	Dissolution in Rumenmimic solution pH 6.5 (0-24 h)	Dissolution in Abomasum mimic solution pH 2.0 (24-26 h)	Dissolution in Intestinal mimic solution pH 7.8 (26-38 h)
1	Solid Dispersion	100%	100%	100%
2	Spray Congealing	84.6%	95.77%	100%
3	Pan coating	82.69%	94.35%	100%
4	Fluid Bed Process with liquid choline chloride as active	57.39%	69.44%	100%
5	Fluid Bed process with choline chloride crystals as active	11.14%	38.27%	98.35%

CONCLUSIONS

Choline chloride is well known nutrient used for non- alcoholic fatty syndrome (NAFS). However, it is degraded by microbes in rumen if not protected. True protection is necessary for actual use by animal at intestine. Choice of active and technology of coating effects the choline availability at intestine. Product (Sample C,D,E) which are coated by core-shell technology (FBP & Pan coating) are more protected from rumen degradation as compared to product (Sample B) manufactured by spray congealing technology (matrix coating) and product manufactured by solid dispersion technology (Sample A). Choice of active also has a direct impact on release profile. Choline chloride in crystal form is more preferred form for microencapsulation as compared to liquid choline chloride.

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