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Design and Formulation of Inorganic Dispersed Organogel for Hemostasis

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ABSTRACT

Hemostasis occurs by dehydrating the injury site, concentrating clotting factors, delivering clotting agents such as thrombin and fibrinogen or forming a physical barrier against bleeding. Introducing an economical alternative to thrombin and fibrinogen based injectable solutions, which has a risk of strong coagulation activation into the circulatory system is the main objective. To form a physical barrier against bleeding an organogel base (hydrocarbon type) was chosen. Colloidal silicone dioxide was dispersed in mineral oil and vitamin K1 (phytonadione) under a mixer to form a powder gel base. Sifted Kaolin (Al2Si2O5(OH)4), a crystalline mineral, was dispersed in ultrapure water using a propeller mixer to form an inorganic gel. Once in the wound the material should solidify to prevent its loss to unaffected areas. Hence, sifted carbopol 974P NF was spread in ultrapure water using a propeller mixer. These two aqueous phases were added to the organogel base using a sorbitol-based nonionic surfactant (1%), sorbitan sesquioleate under a homogenizer to transport the aqueous phases into a main continuous phase. Comparing the viscosity results of F6 and F7, F7 showed a higher viscosity. Viscosity reduction due to increase in shear rate was slighter for F7 in comparison to F6. Introducing an organogel base to form a physical barrier, Kaolin, which functions as an absorbent and coagulation activator (Factor XII), vitamin K1, which has an effect on ɣ-carboxylation of coagulation factors and healing wounds, carbopol 974P NF, which is an excellent mucoadhesive showed a promising economical approach for hemostasis.

Key words: Organogel, Kaolin, Carbopol 974P NF, Vitamin K1, Hemostatic gel

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INTRODUCTION

Internal hemorrhaging is an approximately 50% foremost cause of death after traumatic injury on the battlefield. Hemostasis occurs by dehydrating the injury site, concentrating clotting factors, delivering clotting agents such as thrombin and fibrinogen or forming a physical barrier against bleeding (1-3). To decrease the time required to establish hemostasis commercial products such as Quickclot®, which include kaolin, a crystalline mineral that functions as an absorbent and coagulation activator, Floseal®, which uses gelatin and thrombin to promote clotting in an injectable form were developed (4,5). Organogels are incorporated with the hydrocarbons, animal/vegetable fats, soap base greases and the hydrophilic organogels. Included in the hydrocarbon type is a combination of mineral oils and heavy hydrocarbon waxes with a molecular weight of about 1300 (6). Vitamin K (coagulation vitamin) is a principal cofactor in the synthesis of active blood-clotting factors II, VII, IX, X, protein C and protein S as well as non-coagulation proteins such as osteocalcin and matrix Gla protein (7-9). Incorporating clays such as Bentonite and Kaolin in

water, offers a thixotropic colloidal gel. Kaolin's interaction with factor XII triggers blood coagulation pathway. Incorporating vitamin K, Thrombin, Tranexamic acid, Rutin and Ascorbic acid in colloidal gel strengthens kaolin properties (10-12). Introducing an economical alternative to thrombin and fibrinogen based injectable solutions, which has a risk of strong coagulation activation into the circulatory system is the main objective

MATERIALS AND METHODS

Materials:

Vitamin K1 (phytonadione)(USP/Ph.Eur.) was obtained from DSM Nutritional Products Ltd (Switzerland). Light Kaolin BP was purchased from Bescoat (India). White soft paraffin BP was from Cotton Tree (UK). Mineral oil USP was from CVS Health (USA). Carbopol 974P NF was obtained from Lubrizol Advanced Material, Inc. (USA). Aerosil 200® (Evonik Degussa) was purchased from Kanchan Rasayan Supplier (Delhi, India). Sorbitan sesquioleate was obtained from Merck (Germany). Methyl Paraben and Propyl Paraben were from AASHICHEM (India). All other chemicals and solvents were of analytical reagent grade.

Design and formulation of topical inorganic dispersed organogel:

To form a physical barrier against bleeding an organogel base (hydrocarbon type) was chosen. Colloidal silicone dioxide (Aerosil® 200) was dispersed in mineral oil and vitamin K1 (phytonadione) under a mixer for 5 minutes to form a powder gel base. Sifted (Mesh No.20) Kaolin ($Al2Si2O5(OH)4$), a crystalline mineral, which functions as an absorbent and coagulation activator was dispersed in 8% ultrapure (Milli-Q) water using a propeller mixer with variable speeds (800-1200 rpm) to form an inorganic gel. Once in the wound the material should solidify to prevent its loss to unaffected areas. Hence, 2.5% sifted (Mesh No. 20) carbopol 974P NF, which is an excellent mucoadhesive, was spread in 10% ultrapure (Milli-Q) water using a propeller mixer with variable speeds (800-1200 rpm). These two aqueous phases were added to the organogel base using a sorbitol-based nonionic surfactant (1%), sorbitan sesquioleate with HLB value of 3.7 under a high shear mixer (homogenizer) (IKA Dispersers-T25 digital ULTRA-TURRAX®) to transport the aqueous phases into a main continuous phase. Finally, Methyl and Propyl parabens (Anti-bacterial and anti-fungal preservatives) were added to the main continuous phase.

Quality control tests for topically applied drug products:

According to ICH guidance Q6A, test procedures and acceptance criteria for new drug substances, universal test, which is applicable for topical inorganic dispersed organogel is description.

The acceptance criteria for a qualitative description is the final acceptable appearance of the finished dosage form and packaging. A visual examination should identify changes in color, adhesive migration (i.e. cold flow) for TDS, separations, crystallization, etc that are specific to the drug product .

Specific tests should be considered for topical inorganic dispersed organogel are antimicrobial preservative content, which should be based on levels of antimicrobial preservative necessary to maintain the product's microbiological quality at all stages throughout its proposed usage and shelf life and sterility, which is depended on the use of the dosage form, e.g., ophthalmic preparations, products that will be applied to open wounds or burned areas .

Antimicrobial effectiveness testing:

For category 2 products (topically used products made with aqueous bases or vehicles; nonsterile nasal products and emulsions, including those applied to mucous membranes), criteria for bacteria should be NLT (not less than) 2.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days. The criteria for yeast and molds is no increase from the initial calculated count at 14 and 28 days (13)

Sterility tests:

The minimum quantity to be used for each medium in insoluble preparations, creams and ointments to be suspended or emulsified is the contents of each container to provide not less than 200 mg. The test may be carried out using the technique of Membrane Filtration or by Direct Inoculation of the Culture Medium with the product to be examined. Appropriate negative controls are included. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted to 1% in isopropyl myristate, by heating, if necessary to not more than 40°. In exceptional cases it may be necessary to heat to not more than 44°. Filter as rapidly as possible (14).

RESULTS

Experimental formulation compositions are listed in Table 1. F_3 comparing to F_2 showed a lower viscosity due to less carbopol® 974p (mucoadhesive polymer) content. Comparing the viscosity results of F_6 and F_7, the above mentioned formulation compositions with up to 100g mineral oil for F_6 and up to 50g mineral oil, up to 100g soft paraffin for F_7, showed a higher viscosity for F_7. Viscosity reduction due to increase in shear rate from more than 10^2 was slighter for F_7 in comparison to F_6 due to presence of soft paraffin, which has a semi-solid consistency (Figure 1&2). Viscosity could also be modified with changes in amount of kaolin and carbopol® 974p. Sterility test results after 14 days for F_6 and F_7, which had 0.1% antimicrobial preservatives, showed no microbial growth in FTM and SCDM media. However, F_3 (with no antimicrobial preservative), which had the same formulation composition with F_6, showed microbial growth in FTM and SCDM media (Table 2). Antimicrobial preservative effectiveness testing results for F_6 and F_7 showed one log reduction in initial amount of E. coli, S. aureus, C. albicans, A. niger and two log reduction for P. aeruginosa after one-week incubation, which is acceptable. Log reduction of initial amount was acceptable for the above mentioned microorganisms after 14 and 28 incubation periods, too (Table 3). Clinical experiments are going to be held, determining the potential medical application of the sterile inorganic dispersed organogels.

DISCUSSION

Introducing an economical alternative to thrombin and fibrinogen based injectable solutions, which has a risk of strong coagulation activation into the circulatory system is the main objective (15). To form a physical barrier against bleeding an organogel base (hydrocarbon type) was chosen (16). Increase in viscosity using 50% soft paraffin, 50% mineral oil rather than 100% mineral oil gave a semi-solid consistency, which forms a better physical barrier against bleeding. Using mixture of kaolin as an absorbent and coagulation activator, vitamin K1, coagulation activator and wounds healing agent, carbopol 974P NF, mucoadhesive agent, augments hemostasis. Multiple approaches to enhance therapeutic properties of hemostats were developed. These include Quickclot®, which incorporates kaolin, solely. Rhee P et al. reported that the main mechanism is the absorption of water and the rapid concentration of platelets and clotting factors, catalyze a rapid clot formation (17). Pozza M et al. reported using Celox® in massive traumatic bleeding, which is a chitosan (a mucoadhesive component that maintains the silica in contact with the wound) granule that fosters clot formation through adsorption and dehydration, and the advancement of red blood cell bonding (18). In this inorganic dispersed organogel, using carbomer 974P NF as a mucoadhesive agent increases the efficiency of kaolin in water absorption due to adherence time increase. Furthermore, Arnaud F et al. reported that the temperature generated by QuickClot® at wound sites reaches to an average of 61˚C, with the potential to rise to as high as 76˚C, causing thermal injury (19). In contrast, organogel base (hydrocarbon type) in inorganic dispersed organogel, due to its occlusive properties can be used as a dressing for minor burns (20). There is no reported safety concern related to the chitosan-based products and patients with known shellfish allergies may encounter problems with both chitosan powder and bandages (21). Bilgili H et al. stated that Dry Fibrin Sealant Dressing (DFSD), which consists of biological materials has the risk of transmission of infectious agents (viral agents), a reason why DFSD has not achieved FDA approval (22). Inorganic dispersed organogel has antimicrobial preservatives (methyl and propyl parabens), which prevents it from microbial growth. An emerging approach, hydrogels network focuses on incorporating synthetic silicate nanoplatelets, which are found to be cytocompatible with human stem cells and animal cells. However, it is not an economical approach (3). Although using kaolin and carbopol 974P NF gives a hydrogel network, mineral oil and soft paraffin forms a physical barrier against bleeding, which is a cost-effective approach.

CONCLUSION

Introducing an organogel base to form a physical barrier, Kaolin, which functions as an absorbent and coagulation activator (Factor XII), vitamin K1, which has an effect on ɣ-carboxylation of coagulation factors and healing wounds, carbopol 974P NF which is an excellent mucoadhesive showed a promising economical approach for hemostasis.

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Table 1. Experimental formulation compositions

Formulation Composition	Aerosil®	Vitamin K_1	Mineral oil	Carbopol® 974p	Kaolin BP	Methyl and propyl paraben	Sorbitan sesquioleate
F_1	3.0 gr	-	Up to 100	5.0 gr	15.0 gr	-	1.0 gr
F_2	3.0 gr	10.0 gr	Up to 100	5.0 gr	15.0 gr	-	1.0 gr
F_3	3.0 gr	10.0 gr	Up to 100	2.5 gr	15.0 gr	-	1.0 gr
F_4	3.0 gr	-	Up to 100	2.5 gr	15.0 gr	-	1.0 gr
F_5	3.0 gr	-	Up to 100	-	15.0 gr	-	1.0 gr
F_6	3.0 gr	10.0 gr	Up to 100	2.5 gr	15.0 gr	0.1+0.1 gr	1.0 gr
F_7	3.0 gr	10.0 gr	Up to 50 + Up to 100 (Soft paraffin)	2.5 gr	15.0 gr	0.1+0.1 gr	1.0 gr

Table 2. Sterility test (membrane filtration method)

Microorganism Type	Incubation time	Initial amount	Log reduction	Amount after log reduction	Acceptance Criteria
E.coli	7	0.5×10^6	1	0.5×10^5	NLT 1 log
E.coli	14	0.5×10^6	4	0.5×10^2	NLT 3 log
E.coli	28	0.5×10^6	0	0.5×10^6	Without change from day 14
P.aeruginosa	7	0.2×10^6	2	0.2×10^4	NLT 1 log
P.aeruginosa	14	0.2×10^6	4	0.2×10^2	NLT 3 log
P.aeruginosa	28	0.2×10^6	0	0.2×10^6	Without change from day 14
S.aureus	7	0.7×10^6	1	0.7×10^5	NLT 1 log
S.aureus	14	0.7×10^6	4	0.7×10^2	NLT 3 log
S.aureus	28	0.7×10^6	1	0.7×10^5	Without change from day 14
C.albicans	7	0.2×10^6	1	0.2×10^5	Without change from day 1
C.albicans	14	0.2×10^6	1	0.2×10^5	Without change from day 1
C.albicans	28	0.2×10^6	0	0.2×10^6	Without change from day 1
A.niger	7	0.15×10^6	1	0.15×10^5	Without change from day 1
A.niger	14	0.15×10^6	0	0.15×10^6	Without change from day 1
A.niger	28	0.15×10^6	0	0.15×10^6	Without change from day 1

Table 3. Sterility test (membrane filtration method)

Membrane Filtration method			
FTM microbial culture medium		SCDM microbial culture medium	
Culture medium volume (ml)	100	Culture medium volume (ml)	100
No. of medium container	1	No. of medium container	1
Sample volume (ml)	10 (No.6)	Sample volume (ml)	10 (No.6)
Incubation temp and period	14days/32.5±2.5˚C	Incubation temp and period	14days/32.5±2.5˚C
F_6 sterility result	Negative	F_6 sterility result	Negative
F_7 sterility result	Negative	F_7 sterility result	Negative
F_3 sterility result	Positive	F_3 sterility result	Positive

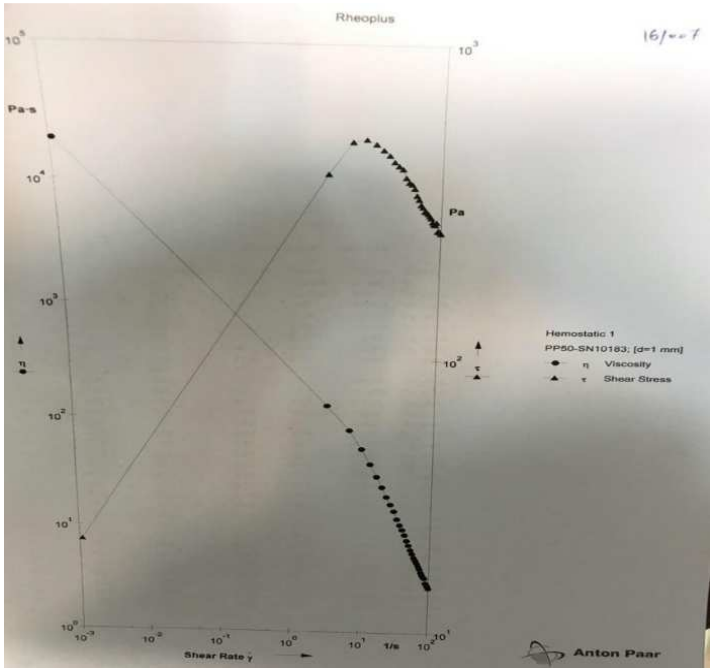

Figure 1. Viscosity and shear stress (F_7)

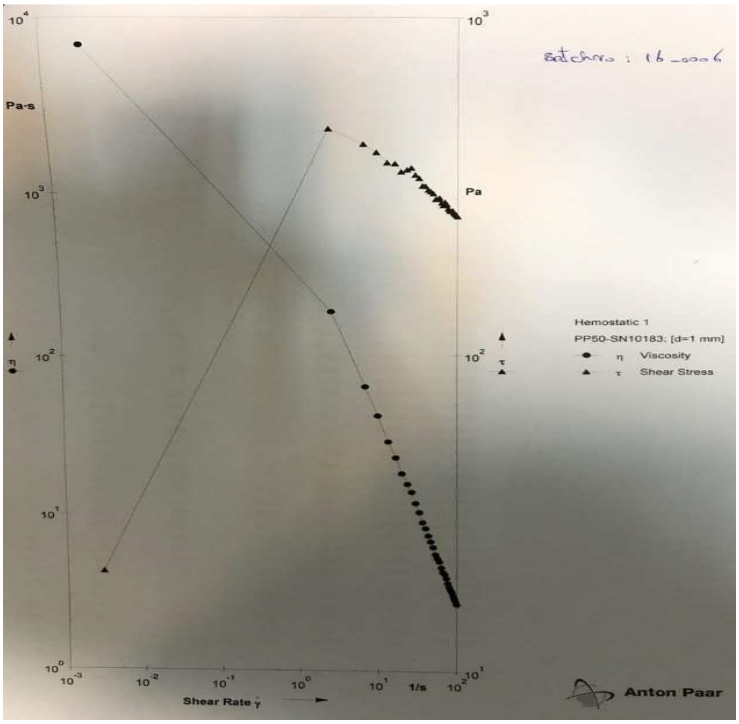

Figure 2. Viscosity and shear stress (F_6)

Declaration of Interest
The authors report no declarations of interest.

REFERENCES

1. Ostomel T.A, Shi Q, Stucky G.D. Oxide hemostatic activity. J. Am. Chem. Soc. (2006)128; 8384-8385.
2. Spotnitz W.D, Burks S. Hemostats, Sealants and Adhesives: Components of the surgical toolbox. Transfusion. (2008) 48: 1502-1516.
3. Gaharwar AK, Avery RK, Assmann A, Paul A, McKinley GH, Khademhosseini A, Olsen BD. Shear-thinning nanocomposite hydrogels for the treatment of hemorrhage. ACSNANO. (2014)8; 9833-9842.
4. Ostomel T.A, Shi Q, Stoimenov PK, Stucky GD. Metal oxide surface charge mediated hemostatsis. Langmuir. (2007)23; 11233-11238.
5. OZ MC, Rondinone JF, Shargill NS. Floseal matrix. J. Card. Surg. (2003)18; 486-493.
6. Loyd V, Allen JR. Compounding gels. Current and practical compounding information for the pharmacist. (2014)4; 1-6.
7. Shearer MJ. Vitamin K. Lancet. (1995)345; 229-234.
8. Shearer MJ. Role of vitamin K and Gla Proteins in the pathophysiology of osteoporosis and vascular calcification. Curr. Opin. Clin. Nutr. Metab. Care. (2000)3; 433-438.
9. Marinova M, Lutjohann D, Westhofen P, Watzka M, Breuer O, Oldenburg J. A validated HPLC method for the determination of vitamin K in human serum – First application in a pharmacological study. The open clinical chemistry journal. (2011)4; 17-27.
10. Baker SE, Sawvel AM, Zheng N, Stucky GD. Controlling bioprocesses with inorganic surfaces: layered clay hemostatic agents. Chem. Mater. (2007)19; 4390-4392.
11. Arnaud F, et al. Comparison of 10 hemostatic dressings in a groin transection model in swine. J. Trauma Acute care surg. (2009)67; 848-855.
12. Bowman PD, Wang X, Meledeo MA, Dubick MA, Kheirabadi BS. Toxicity of aluminum silicates used in hemostatic dressings toward human umbilical veins endothelial cells, Hela cells, and Raw267.4 mouse macrophages. J. Trauma acute care surg. (2011)71; 727-732.
13. USP general chapters, Antimicrobial effectiveness testing, USP39, 51, 111-114.14. USP general chapters, Sterility tests, USP39, 71, 136-143.
14. Xie X, et al. A novel hemostatic sealant composed of gelatin, transglutaminase and thrombin effectively controls liver trauma-induced bleeding in dogs. Acta Pharmacol. Sin. (2013)34; 983-988.
15. Loyd, V; Allen, JR; Compounding gels. Current and practical compounding information for the pharmacist. (2014)4; 1-6.
16. Rhee P, Brown C, Martin M, Salim A, Plurad D, Green D, et al. QuikClot use in trauma for hemorrhage control: case series of 103 documented uses. J Trauma. (2008)64; 1093-1099.
17. Pozza M, Millner RW. Celox (chitosan) for hemostasis in massive traumatic bleeding: experience in Afghanistan. Eur J Emerg Med. (2011)18;31–33.
18. Arnaud F, Tomori T, Carr W, McKeague A, Teranishi K, Prusaczyk K, et al. Exothermic reaction in zeolite hemostatic dressings: QuikClot ACS and ACS+. Ann Biomed Eng. (2008)36;1708–1713.
19. Phillips LG, Robson MC, Heggers JP. Treating minor burns, ice, grease, or what? Postgrad Med (1989) 85; 219-222.
20. Waibel KH, Haney B, Moore M, Whisman B, Gomez R. Safety of chitosan bandages in shellfish allergic patients. Mil Med. (2011)176;1153–1156.
21. Bilgili H, Kosar A, Kurt M, Onal IK, Goker H, Captug O, et al. Hemostatic efficacy of Ankaferd Blood Stopper in a swine bleeding model. Med Princ Pract. (2009) (18);165–169.