International Journal of Academic Research and Development
ISSN: 2455-4197
Impact Factor: RJIF 5.22
www.academicsjournal.com
Volume 3; Issue 1; January 2018; Page No. 441-446

Formulation and evaluation of Chlorhexidine Gluconate topical gel

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Abstract
Topical therapies present a valuable therapeutic option for Osteoarthritis, Rheumatoid arthritis & ankylosing spondylitis pain management including several other inflammatory diseases. In context with topical application of a drug, critical barrier of epidermal membranes offer several diffusional resistance in the course of skin permeation. Thus, the developments of topical products which allow penetration of compounds into the skin require utilization of several approaches. Chlorhexidine is an antiseptic effective against a wide variety of gram-negative and gram-positive organisms, facultative anaerobes, aerobes and yeast. It is used as a topical anti-infective for skin, mucous membranes and as preservative for eye drops. Chlorhexidine is also used as an antiseptic ingredient used in mouth wash to prevent oral plaque, oral bacteria and in treating gingivitis. For the preparation of the gel formulation succinyl chitosan was used as gelling agent. Chlorhexidine gluconate is biguanide derivative having wide spectrum of antibacterial activity. It is highly effective against *S.* aureus and P. aeruginosa which are responsible for the development of infection in bedsore. Globally bedsore is a major problem in bedridden, old and debilitated patients. Incidence rates in hospitalized patients vary from 0–65.6% in USA and Canada and 2.2–60% in UK. The prevalence of pressure ulcers is 3–11% in hospitalized patients 2.5–24% in nursing-home patients and 17% in adults in community. Chlorhexidine gluconate is highly effective at pH 5-7.5 which is generally a pH of skin.

Keywords: Chlorhexidine gluconate, biguanide, bedsore etc.

Introduction
Chlorhexidine gluconate is biguanide derivative having wide spectrum of antibacterial activity. It is highly effective against *S.* aureus and P. aeruginosa which are responsible for the development of infection in bedsore. Globally bedsore is a major problem in bedridden, old and debilitated patients. Incidence rates in hospitalized patients vary from 0–65.6% in USA and Canada and 2.2–60% in UK. The prevalence of pressure ulcers is 3–11% in hospitalized patients 2.5–24% in nursing-home patients and 17% in adults in community. Chlorhexidine gluconate is highly effective at pH 5-7.5 which is generally a pH of skin. Succinyl chitosan is a natural biocompatible polymer having antimicrobial, wound healing, haemostatic, film forming and moisture retaining properties. The objective of present study is to develop and evaluate varies topical gel formulations of Chlorhexidine gluconate prepared using succinyl chetosan, Lutrol F-127 & Propylene glycol in different concentrations. The optimized formulation will be expected to be highly effective in treatment bedsore and would show synergistic antimicrobial activity against S. aureus and P. aeruginosa. Effectivity of the therapy in wound healing will be due to moisture retaining properties of gel.

Materials and methods
Chlorhexidine gluconate was obtained from Dr. Reddy's Pharmaceutical, Hyderabad, India as a gift sample. Chitin was obtained from Gadre marine Ratanagiri as a gift sample. Sodium hydroxide, acetone, sodium hypochlorite, isopropyl alcohol, succininic anhydride, ethanol, acetic acid, polyethylene glycol 400, sodium acetate, sodium chloride, citric acid and methanol were purchased from Haryana Scientific Emporium, Rohtak. Lutrol F-127 was obtained from Dr. Reddy's Pharmaceutical, Hydrabad, India.

Formulation development
Gel formulations were prepared using Lutrol F-127 (5, 10, 15, 20 and 25%) by using cold process. For the preparation of gels weighed quantity of Lutrol F-127 was placed in the beaker. To that weighed quantity of propylene glycol and Suc-chi was added. Then mixture was kept in an ice bath having temperature ranging from 2-4^{o} C on magnetic stirrer. Then accurate quantity of precooled distilled water was added and stirred for 30 mins. Then this dispersion was kept in a freezer overnight to remove the air bubble.

Polymer selection
For the preparation of the gel formulation first of all alone succinyl chitosan was used as a gelling agent but the concentration of succinyl chitosan required is so more (up to 10 %) which is not cost-effective. For that a combination of gelling agent were used. In combination with a succinyl chitosan copolymers carbopol-940, Sodium CMC and Lutrol F-127 in different concentration were used. It was found that out of these three copolymers Suc-chi showed incompatibility with carbopol-940, Sodium CMC due to their precipitation in the gel formulation. Suc-chi was found compatible with Lutrol F-127; hence it was used as copolymer in gel formulation.

Process optimization

Chlorhexidine gluconate gel can be prepared by two methods i.e. hot and cold method. In hot method Lutrol F 127 was dissolved in water at approx. 70°C. Then Chlorhexidine gluconate was added into propylene glycol and that mixture was then added into the warm aqueous phase to form a homogeneous mass. The gel was form when the solution cools to room temperature.

In cold process for the preparation of gels weighed quantity of Lutrol F-127 was placed in the beaker. To that weighed quantity of propylene glycol, a Suc-chi was added. Then mixture was kept in an ice bath having temperature ranging from 2-4º C on magnetic stirrer. Then accurate quantity of precooled distilled water was added and stirred for 30 mins. Then this dispersion was kept in a freezer overnight to remove the air bubble.

Both methods of preparation will generally yield gels with comparable properties. But it was found that Chlorhexidine is unstable above 70ºC and also adding Lutrol F 127 too rapidly to the hot aqueous phase may result in the formation of lumps that will only dissolve after standing for several hours. Hence it was recommended that preferably "cold process" should be used for the preparation of gel.

Table 1: Concentration of various ingredients in gel formulation

Sample ID	Chlorhexidine gluco. Solution (%)	Suc-chi (%)	LutrolF-127	Propylene glycol (%)	Distilled water (%)
C9	1	1	15	5	78
C10	1	1	15	10	73
C11	1	1	15	15	68
C12	1	1	15	20	63
C13	1	1	20	5	73
C14	1	1	20	10	68
C15	1	1	20	15	63
C16	1	1	20	20	58
C17	1	1	25	5	68
C18	1	1	25	10	63
C19	1	1	25	15	58
C20	1	1	25	20	53

Evaluation of gels

Gels were evaluated for appearance, consistency, pH, viscosity, spreadability, skin irritation, drug content, content uniformity and invitro drug release.

Appearance

It was found that gel with 5 and 10 percent concentration of propylene glycol become a clear and transparent while gel with 15 and 20 percent concentration of propylene glycol becomes a whitish in colour. All the gels were smooth in texture and homogeneous in nature.

PH

pH of all gel formulation were found to be between 6 to 7 thus indicating suitability for topical application.

Spreadability

Easy spreadability is one of the important characteristic of any topical preparation as far as patient compliance is concerned. Moreover if gel spreads easily, its application to the concerned area would be more comfortable and also relatively small amount of gel would be required to cover the wounded area. Gel was considered good when it took minimum time to spread on a body surface.

It was found that spreadability of formulation C9-C15 were comparatively more than that of the other formulations, which indicate that the concentration of polaxomer affect the spreadability of the gel formulation i.e. as the concentration of polaxomer increases the spreadability gel decreased.

Drug content and content uniformity

Drug content and content uniformity of the gel formulations were determined by UV spectrophotometric method and it was found to be within limit except formulation C9, C18, C19 and C20 which deviate from the limit. It was found that such deviations were due to the higher concentration of the polaxomer in the gel, because of that gel became thick which lead to the uneven distribution of drug in the gel, hence show deviation from the limit.

Table 2: Data of evaluation parameter of gel formulation

Formulati--on code	Physical appearance	Texture	pH	Spread--ability (cm)	Viscosity (in Pascal Second)
C9	Transparent	S	6.91	2.5	0.185663
C10	Transparent	S	6.64	2.9	0.2428
C11	Whitish	S	6.92	3.0	0.2376
C12	Whitish	S	6.74	2.6	0.2812725
C13	Transparent	S	6.69	2.4	0.32467625
C14	Transparent	S	6.91	2.1	0.35479875
C15	Whitish	S	6.38	2.2	0.3470875
C16	Whitish	S	6.81	1.8	0.021816667
C17	Transparent	S	6.85	1.8	0.0983025

C18	Transparent	S	6.74	1.8	0.03315
C19	Whitish	S	6.69	1.9	0.043045333
C20	Whitish	S	6.81	1.9	0.03404225
Plain chlor gel	Transparent	Smooth	6.71	2.3	0.1983
Marketed	Transparent	Smooth	6.65	2.5	0.2143

Table 3: Data of drug content and content uniformity of gel formulation

Parameters / Formulation code	Drug content (µgm/ml)	Content uniformity (%)
C9	16.3589	80.82067
C10	19.9750	99.36583
C11	19.4505	97.057
C12	20.7584	102.1602
C13	19.3189	96.5945
C14	19.5898	97.89733
C15	20.4914	102.6873
C16	20.1741	101.8637
C17	20.6113	102.8438
C18	18.7424	93.654
C19	22.2287	110.8355
C20	25.1153	125.5635
Plain chlor gel	19.3452	96.55717
Marketed	20.4328	101.8498

***In-vitro* drug release**

In-vitro release of Chlorhexidinegluconate from gel formulation was studied on Keshary Chien diffusion cell using cellophane membrane. From the result it was found that as the concentration of the polaxomer increase from 10 percentupto 25 percent the drug release from the gel get decrease significantly. Gel formulation C10 show better release than that of the plain Chlorhexidinegluconate gel as well as the marketed gel formulation (fig.1).

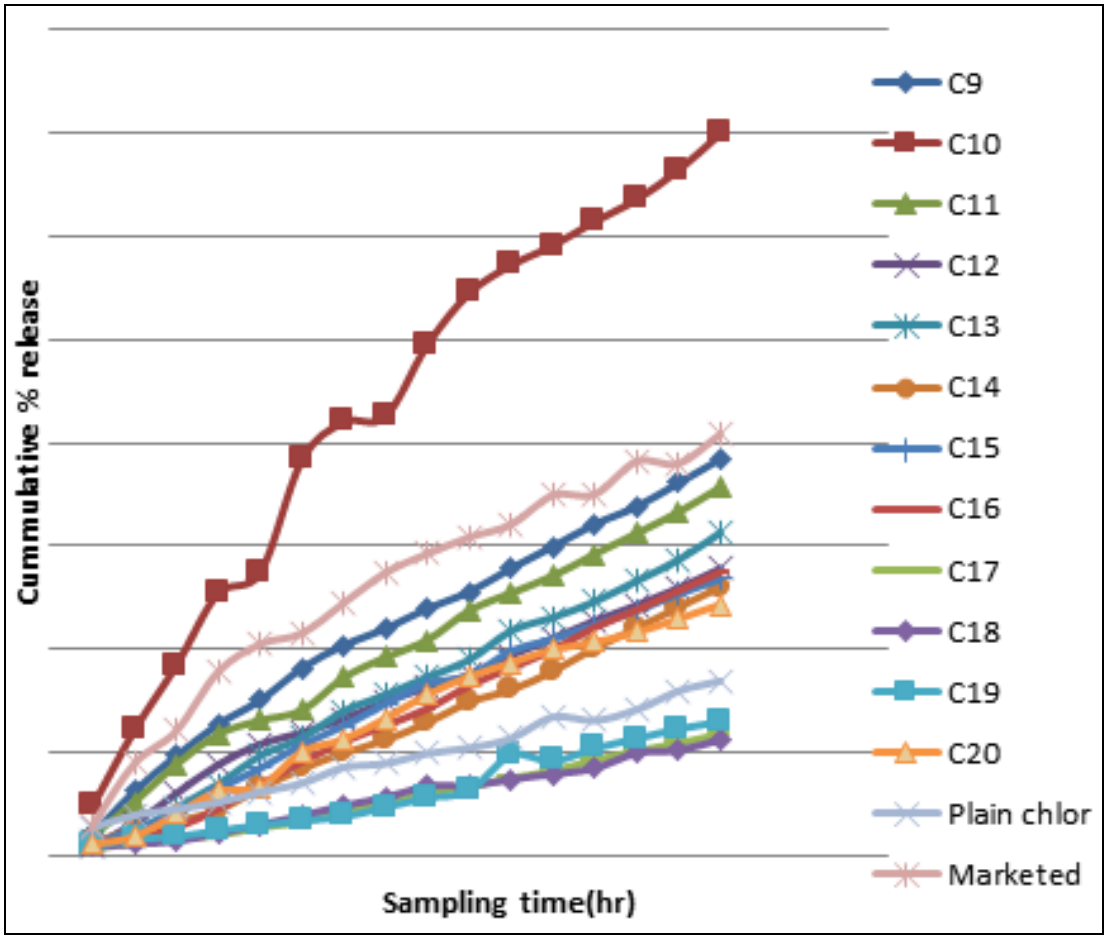

Fig 1: *In-vitro* release pattern of various gel formulation

Sampling time/cumulative % release	c9	c10	c11	c12	c13	c14	c15	c16	c17	c18	c19	c20	Plain	Marketed
0.5	2.09496	4.831848	2.012855	0.966196	1.391188	1.31379	1.127616	1.238593	0.74732	0.895404	0.923589	1.164524	2.7722	3.0496
1	6.28968	12.19564	5.248538	3.175562	2.384324	2.53412	2.559588	1.704957	1.24284	1.16919	1.671572	1.903847	3.9638	8.974
1.5	9.69783	18.43764	8.875863	6.062116	4.687733	3.37112	4.198794	2.779595	1.674802	1.444575	1.878883	4.102804	4.5437	12.1167
2	12.76476	25.42502	11.78062	8.846898	6.956999	4.45824	6.514532	4.581165	2.01523	2.213063	2.482051	6.259747	5.1693	17.8357
2.5	15.06503	27.3843	13.13135	10.82771	9.706565	6.56081	8.4913	6.870914	2.79065	3.052521	2.981109	6.690332	6.2052	20.5122
3	18.19264	38.31588	14.10759	11.93411	11.44054	8.45606	10.76495	9.130352	3.289108	3.908003	3.413239	10.08304	7.0335	21.556
3.5	20.40466	42.00138	17.26575	13.28404	14.04352	9.97507	12.59608	11.08979	4.466546	4.905323	3.808619	11.30378	8.5828	24.4863
4	21.99226	42.52644	19.22903	14.97759	15.58294	11.22598	14.74842	12.65631	5.047106	5.678875	4.739029	13.32881	8.944	27.4232
4.5	23.97446	49.46664	20.91378	16.38999	17.33426	12.90906	16.67831	14.27638	6.129684	6.753363	5.630518	15.6505	9.9336	29.2518
5	25.56253	54.45848	23.75488	17.51356	19.00453	14.90519	17.45659	16.48687	6.714257	6.813804	6.327701	17.37987	10.4615	30.8445
5.5	27.85776	57.17083	25.45875	19.4429	21.80175	16.08603	19.83447	18.23309	7.555277	7.443038	9.782955	18.70248	11.3831	32.09478
6	29.95781	59.06828	27.1112	21.06247	23.10187	17.87787	21.01354	19.97965	8.245429	7.938695	9.182345	19.99616	13.4662	34.9148
6.5	32.12552	61.40965	29.22446	22.77311	24.62906	20.12154	22.35319	22.13994	9.284967	8.508214	10.44174	20.68307	13.1218	35.0153
7	33.8226	63.53223	31.23696	24.27155	26.63151	22.05271	23.76171	23.83222	10.16827	9.973936	11.36412	21.65508	14.1723	38.2093
7.5	36.09976	66.36619	33.28171	25.92757	28.66564	24.08218	25.37515	25.65872	10.90716	10.24928	12.30895	22.97005	15.9644	37.9954
8	38.4095	69.88343	35.67512	27.82022	31.27609	26.02818	26.80055	27.48784	11.79696	11.26352	12.998	24.33806	16.9534	40.8151

Fig 2

Summary
Topical therapies present a valuable therapeutic option for Osteoarthritis, Rheumatoid arthritis & ankylosing spondylitis pain management including several other inflammatory diseases. In context with topical application of a drug, critical barrier of epidermal membranes offer several diffusional resistance in the course of skin permeation. Thus, the development of topical products which allow penetration of compounds into the skin require utilization of several approaches. Chlorhexidine is an antiseptic effective against a wide variety of gram-negative and gram-positive organisms, facultative anaerobes, aerobes and yeast. It is used as a topical anti-infective for skin, mucous membranes and as preservative for eye drops. Chlorhexidine is also used as an antiseptic ingredient used in mouthwasth to prevent oral plaque, oral bacteria and in treating gingivitis. For the preparation of the gel formulation succinyl chitosan was used as gelling agent. In the combination with a succinyl chitosan co-polymers such as carbopol-940, sodium CMC and Lutrol F 127 were used. It was found that out of these three co-polymer succinyl chitosan showed incompatibility with carbopol-940, sodium CMC due to their precipitation in the gel formulation. Succinyl Chitosan was found compatible with Lutrol F 127; hence it was used as co-polymer in gel formulation. Polymers were characterized using parameters such as solubility, pH, moisture content, ash content, bulk density, tapped density and angle of repose. Gel formulations were prepared with Lutrol F 127(5, 10, 15, 20, and 25%) by using cold process. Hot process was not found to be suitable for preparation of the gel as it was found that chlorhexidine is unstable above 70 C and also adding Lutrol F 127 too rapidly to the hot aqueous phase may result in the formation of lumps that will only dissolve after standing for several hours. Various batches of formulations were prepared and characterized using parameters like appearance, consistency, pH, viscosity, spreadability, drug content, content uniformity and in-vitro drug release. The gels were visually inspected for colour, texture and clearity. It was found that gel prepared with 5 and 10 % concentration of propylene glycol was clear and transparent in appearance while gel with 15 and 20 % concentration of propylene glycols was a whitish in colour. 1g of the prepared gel was mixed with 100 ml of suitable solvent. Aliquots of different concentrations were prepared by suitable dilutions after filtering the stock solution and absorbance was measured. Drug content and content uniformity was calculated using the equation, which was obtained by linear regression analysis of calibration curve. C9, C18, C19, and C20 which deviate from the limit. It was found that such deviations were due to the higher concentration of the polaxomer in the gel, because of the gel become thick which lead to the uneven distribution of drug in the gel; hence show deviation from the limit. Spreadability was expressed in terms of time in seconds taken by two slides to slip off from gel and placed in between the slides under the direction of certain load lesser the time taken for separation of two slides, better the spreadability. It was found that spreadability of formulation C9-C15 were comparatively more than that of the other formulations, which indicate that the concentration of polaxomer affect the spreadability of gel formulation i.e as the concentration of polaxomer increases, the spreadability of gel decreased.

Conclusion
The absorbance maxima of chlorhexidine gluconate was found as 231.5 nm which was selected for UV analysis. It was found to be almost colourless or pale yellowish, clear or slightly opalescent liquid, almost odourless. The melting point of chlorhexidine was found to be 132- 136° C. The molecular weight of the drug was found to be 505.46 gm. It was found to be miscible with water, soluble in ethanol (95%) and in acetone. Chlorhexidine gluconate was prepared by using two methods i.e. hot and cold method. In hot method chlorhexidine is unstable above 70° C and also adding Lutrol F 127 too rapidly to the hot aqueous phase may result in the formulation of lumps that will only dissolve after standing for several hours. Hence, it was recommended that preferably "cold process" should be used for the preparation of gel. Characterization of topical gel was performed on the basis of appearance, consistency, pH, viscosity, spreadability, drug content, content uniformity and in-vitro drug release. Gel formulation with 5 and 10% concentration of propylene glycol became clear and transparent while gel with 15 and 20% concentration of propylene glycol became a whitish in colour. Spreadability of formulation C9-C15 were comparatively more than that of the other formulations, which indicated that the concentration of polaxomer affected the spreadability of the gel formulation i.e. as the concentration of polaxomer increases, the spreadability of gel decreased. It was found that drug content and content uniformity of formulation C9, C18, C20 deviated from the limit which may be due to the higher concentration of the polaxomer in the gel, imparting thickness to the gel which lead to the uneven distribution of drug in the gel, hence showed deviation from the limit. In-vitro release study was done with the help of Keshary Chien Diffusion Cell. Gel formulation C10 showed better release than that of plain chlorhexidine gluconate gel as well as marketed gel formulation.

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