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Microbial contamination of cosmetics and the pharmaceutical products, and their preservation strategies: A comprehensive review

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Abstract

A brief discussion of the numerous types of microbial contamination of the pharmaceutical and cosmetic items and their corresponding effects is attempted in this study. The pharmaceutical and cosmetic products are particularly vulnerable to microbial contamination, because they contain chemical compounds that encourage the microbial development. Contamination can potentially happen during production; storage, and/ or usage. These contaminants can cause a variety of unfavorable effects, including alteration of the consistency and appearance; phase separation, alteration or loss of activity, and even the emergence of toxicity in the contaminated items. Organizations such as the United States Food and Drug Administration (FDA) actively regulate the consumer safety by frequently recalling the potentially dangerous or contaminated products from the market. Therefore, to prevent microbial contamination and increase the shelf life of a product, a variety of preservatives are added to the final formulation. However, some of these preservatives may be toxic to the consumer as well. In this context, we have also reviewed the mechanisms of action of some of the most commonly used antimicrobial preservatives, including the organic acids; parabens, phenol, organomercurials, ethanol, chlorobutanol, benzalkonium chloride, chlorocresol, sodium benzoate, isothiazolinones, sodium sulfite, and sodium metabisulfite, in addition to the potential toxicity caused by them to the consumers.

Keywords: Cosmetics, Microbial contamination, Pharmaceutical products, Shelf-life, Antimicrobial preservatives

1. Introduction

In most of the cosmetics, abundant organic and inorganic compounds; growth factors, moisture, and essential minerals are present, thus providing optimum conditions for the microbial growth (Behravan *et al*., 2005). Meanwhile, several types of microbial contaminations can often take place in these cosmetics (Behravan *et al*., 2005). Cosmetics act as an important pathogen transmission medium in the people's daily life (Babalola and Eze, 2015). In such products, the different sources from which these unwanted microorganisms may arise involve the contaminated raw materials; the specific production processes for these personalized cosmetics, the unsanitary environment and equipment conditions, and the poor personal hygiene of the handlers (Kim *et al*., 2020). A previous study reported by Lundov *et al*., (2009) found that usually the cosmetic products with high moisture contents are more susceptible to microbial contamination. The microorganisms need readily accessible water in perceptible amounts for their proliferation. The water activity (a_w) of the aqueous preparations can be decreased by drying; addition of polyethylene glycol (PEG), and/ or high concentrations of sugars (Baird, 2011).

As a result of microbial growth, the pharmaceutical preparations can get inactivated through the breakdown of its active ingredients; thus resulting in the reduction of the drug efficacy (Campana *et al*., 2006). Hence, we should firmly avoid microbial contamination of the drugs (Baird, 2011). Drug registration control; production control (*i.e*., raw materials; the manufacturing process, and the finished product), and the distribution control, are the three major phases of the drug control system, which supervise and guarantee maintenance of the standards of the pharmaceuticals. These three steps work together to achieve the end result of avoiding the faulty products from reaching the consumer (Ratajczak *et al*., 2015).

A wide range of preservatives is used; either singly or in combination, to restrict growth of the microorganisms in the cosmetics and pharmaceutical products, and to improve the longevity of these products (Shaikh *et al*., 2016; Dao *et al*., 2018). An ideal preservative ought to possess a broad spectrum of antimicrobial activity; be effective in small quantities, be non-toxic, be non-irritating, be stable across a wide pH and temperature ranges, and be compatible with the other compounds used in the preparation (Shaikh *et al*., 2016). While formulating a multiphase product, the preservatives should have the capability of remaining in the aqueous phase (Elder and Crowley, 2012). However, because of their inherent toxicity; some preservatives may still have negative health impacts on the consumers (Shaikh *et al*., 2016; Dao *et al*., 2018).

2. Microbial contamination of cosmetics

Any formulation that is applied on the mouth; teeth, mucous membranes, or on the exterior parts of a person's body, is considered as a cosmetic product (Blanchin *et al*., 2007). Water; thickeners, pigments, emulsifiers, preservatives, glitters, and fragrances are essential components of the cosmetics (Tazeen *et al*., 2023). One of the most vital and dynamic criteria for determining the quality of cosmetics is their microbiological safety (Dimri, 2022). Although not all cosmetics need to be sterile, the consumer's safety of the products must be well-assured (Budecka and Kunicka-Styczyńska, 2014). Prior to their commercial release, the cosmetic products are examined for their microbiological durability and stability, in order to prevent bacterial contamination and proliferation (Kim *et al*., 2020). The contamination levels must be checked for the raw materials; especially those of natural origins, which are used in the production of cosmetics (Elmorsy and Hafez, 2016). Good manufacturing practice (GMP) and good quality control (GQC) is required to ensure this (Siegert,

2012). Hence, specific areas; where the chance of contamination is high, must be identified and controlled (Elmorsy and Hafez, 2016).

However, customized cosmetics that are produced by unskilled salespersons in the stores lacking specialized production facilities are very susceptible to microbiological contamination from the handlers; surroundings, and/ or utensils. Furthermore, cross-contamination may occur during the manufacturing process at the time of cosmetics transfer to the fresh containers. In the case of the freshly-made customized cosmetics, it has been observed that the pH values of cosmetics greater than 10 do not always ensure reduced microbiological counts (Kim *et al*., 2020).

Several pathogenic microorganisms; mainly *Escherichia coli*; *Burkholderia cepacia*, *Klebsiella oxytoca*, *Staphylococcus aureus*, *Enterobacter gergoviae*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Serratia marcescens*, are most commonly observed in the contaminated cosmetics (Neza and Centini, 2016). Heat treatment is very much crucial to control these microbial pathogens (Kim *et al*., 2020).

The Scientific Committee on Consumer Safety Products (SCCS) has separated the cosmetics into two different classes. Those intended for use in children under the age of three years; mucous membranes, or regions surrounding the eyes belong to Category 1, whereas the rest of the cosmetic products belong to Category 2 (Scientific Committee on Consumer Safety, 2015). The skin and mucous membranes protect the body against the microbial attacks through various immunological defence mechanisms, thereby acting as naturally occurring mechanical barriers. However, the chances of microbial infections can increase in the case of any kind of mechanical damages to these membranes or in the slight trauma caused by the action of some cosmetics. Moreover, older people; children less than 3 years of age, and persons with compromised immune systems is more vulnerable to get affected by the microbially contaminated cosmetics. Therefore, items in Categories 1 and 2 should not include more than 100 colony-forming units (cfus) of the aerobic mesophilic bacterial/ ml/ or gram, and not more than 1,000 cfus of these bacteria/ ml/ or gram, respectively. Additionally, neither category of products should contain *Staphylococcus aureus*, *E. coli*, *P. aeruginosa*, or *C. albicans* in even one ml or gram of the cosmetic product (Scientific Committee on Consumer Safety, 2015).

Many people use cosmetics without realizing the risks that they pose to their health (Dimri, 2022). The most frequent method for cosmetics application is topical, and this category of products includes lotions; foams, sprays, aerosols, pastes, creams, gels, ointments, and suspensions (Almukainzi *et al*., 2022). However, the semi-solid products, such as cream and rinse-off items, are highly vulnerable to microbial contamination (Kim *et al*., 2020). Fungal growth is favored in some cosmetic creams that are often water-in-oil emulsions; with high concentrations of solutes and lowered aw values (Elmorsy and Hafez, 2016). High yeast and mold fungal counts are detected in oil samples; followed by gel, solution, and cream (Elmorsy and Hafez, 2016). These contaminations can cause significant infections; particularly in the immune-compromised users (Baird, 2011). Higher fungal contamination is also observed in dry powder-based cosmetics such as talc, compared with the other cosmetics like eyeliner; cream, and mascara. This high fungal contamination of the powder-based cosmetics may occur due to frequent contact with air; common use of skin powder pads, and low a_w value (Dadashi and Dehghanzadeh, 2016). Furthermore, high microbial contamination is found in the contour brushes and blushes because they are frequently in contact with air (Noor *et al*., 2020). The two yeast fungal isolates that are most frequently isolated from the mascaras and eyeliners are *Rhodotorula* spp. and *Candida* spp. Meanwhile, *Penicillium* is the most prevalent mold fungal genus, followed by *Rhodotorula* spp. and *Candida* spp., which are frequently isolated from the in-use skin powders (Dadashi and Dehghanzadeh, 2016). The risk of infection arises

when numerous people share a single cosmetic product, since each person has a different skin microflora (Yadav *et al*., 2023).

Compared with fungal contamination, the bacterial contamination is more prevalent in the cosmetic products with high moisture content (Dadashi and Dehghanzadeh, 2016). Hence, bacterial counts are found to be higher in the shampoo; followed by gel, solution, cream, and oil (Elmorsy and Hafez, 2016). Beauty blenders; lipstick, and lipgloss can harbor opportunistic bacterial pathogens; mainly *P. monteilii*; *P. aeruginosa*, *P. putida*, and *P. fulva*. *Citrobacter freundii* that belongs to the *Enterobacteriaceae* family is also spotted in the lipstick and lipgloss (Bashir and Lambert, 2020). *Staphylococcus haemolyticus*; *Staphylococcus saprophyticus*, *Staphylococcus cohnii*, and *Staphylococcus capitis* are occasionally encountered in the eyeliner; mascara, and lipgloss. *Streptococcus* spp. are the most predominant bacteria, followed by *Pseudomonas* spp. and *Acinetobacter* spp.; among those bacteria typically isolated from the in-use cosmetics of the eye and skin (Dadashi and Dehghanzadeh, 2016). For several eye infections such as conjunctivitis; keratitis, and ophthalmitis, *P. aeruginosa* is the principal pathogen (Dimri, 2022). In addition, *Staphylococcus aureus* contamination of the eye products has the potential to infect both the exterior and interior tissues of the eye, which include the cornea; tear duct, conjunctiva, and posterior chambers (Almukainzi *et al*., 2022). *Acinetobacter junii*; a Gram-negative bacterium, has been identified in several cosmetic products. *A. junii* contaminated eyeliner and mascara has the potential to cause corneal ulcers by damaging the eye epithelium (Tazeen *et al*., 2023). To control the local bacterial populations, the skin produces many bioactive compounds, such as peptides and even certain lipids with antimicrobial activities (Yadav *et al*., 2023).

The consumer safety is governed by SCCS in the majority of the European nations, which is in turn overseen by the European Commission's Directorate-General for Health and Consumer Protection (Michalek *et al*., 2019). Since 2005, non-conforming items have been recorded in the Rapex database (the European Union's Rapid Alert System for Dangerous Non-Food Products). From 2005-2018, about 104 cosmetic products were reported to bear microbiological risks in the Rapex database. Among those, more than half of the cosmetics have been produced in Germany (18.27 %); France (7.69 %), Czech Republic (6.73 %), Greece (6.73 %), and India (6.73 %). The recalled cosmetics majorly included skin-cleaning and skin-care products. Among all the cosmetic goods reported in the Rapex database, *P. aeruginosa*; a Gram-negative bacterium, is the most frequently discovered species, followed by another Gram-negative bacterium termed *Enterobacter gergoviae* (Michalek *et al*., 2019).

Some of the recently recalled cosmetics by the Food and Drug Administration (FDA) on account of microbial contamination are listed in Table 1 (Food and Drug Administration. 2022a).

3. Microbial contamination of the pharmaceutical products

The two categories of pharmaceutical items are sterile and non-sterile (Ratajczak *et al*., 2015). Sterile medications are typically used in otic; ocular, or parenteral applications, and are items that can come in contact with the mucosal epithelial tissue; internal organs, or injured skin (Sandle, 2016). On the other hand, strict monitoring of microbial contamination of the non-sterile drugs is required, so that these drugs remain therapeutically active and safe for the consumer use. The most commonly detected microbial contaminations in the non-sterile drugs are mainly several bacterial spp., including *Enterococcus* spp., *Bacillus* spp., *Micrococcus* spp., *Staphylococcus aureus*, *P. aeruginosa*, *Salmonella* spp., and mold fungi such as *Mucor* spp.; *Aspergillus* spp., *Rhizopus* spp., and *Alternaria* spp. (Ratajczak *et al*., 2015).

Contamination of the pharmaceuticals by microorganisms may occur during manufacturing; storage, and/ or use by the consumer (Zeitoun *et al*., 2015). Gram-positive bacterial contamination of the

Table 1. Some recent FDA recall for cosmetics due to their microbial contamination (Food and Drug Administration. 2022a)

Date	Product brand and description	Recall reason	Risks
04/20/2022	Babyganics, Chamomile verbena bubble bath (Cosmetics)	Microbial contamination with *Pluralibacter gergoviae*	*P. gergoviae* does not typically make healthy people ill; also adults are less vulnerable than infants. But immunocompromised people or people with irritated or cracked skin, such as those having diaper rash, are prone to this infection.
03/18/2022	Jergens, Ultra Healing Moisturizer (Skin Care Products, Cosmetics)	Occurence of the bacterium *P. gergoviae*	*P. gergoviae* usually presents a higher risk of infections for the individuals having compromised immune systems than the healthy individuals.
05/07/2020	Becca Cosmetics, Light Shifter Brightening Concealer (Makeup, Cosmetics)	Potential Mold contamination	Although mold contamination is not likely to result in a serious health hazard; however, possibility of a brief eye and/or skin irritation or allergy exists.
03/24/2020	Saje Natural Wellness, Splish Splash Gentle Baby Wash (Skin Care, Cosmetics)	Presence of the bacterium *Pseudomonas aeruginosa*	Being an opportunistic bacterial pathogen, *P. aeruginosa* can cause infections and leads to the presence of bacteria in the blood; especially in people with compromised immune response.
05/24/2019	La Bella Extreme Sport, Styling Gel (Hair Products, Cosmetics)	Bacterial contamination (*Burkholderia cepacia* and collection of related strains)	While they seldom infect healthy persons; those with compromised immune systems, chronic lung disorders, and cystic fibrosis are particularly in danger.

pharmaceutical preparations is the most commonly reported, and can take place due to human interventions. Meanwhile, the Gram-negative bacterial contamination of such products is relatively lesser; however, the lack of control in the pharmaceutical plants; especially those involving water systems and raw materials, is the main cause of its occurrence (Jimenez, 2004). Due to storage in moist surroundings, the condensed water films may build up on the surface of the dry items such as the tablets or bulk oils; thus fostering the growth of fungi (Baird, 2011). On the contrary to their aim of use to protect the formulation

against the microorganisms, the preservatives can serve as other sources of microbial contamination. They can be employed as an immediate supply for microbial nourishment; especially if their levels become reduced, and if they are of an aromatic nature (Parker, 2002). Based on the release of acidic or basic metabolites by the microorganisms; the product pH varies accordingly and it may shift significantly, thus enabling secondary attack by the microorganisms that were previously prevented by the original product pH. The fruit juice-flavored syrups and similar products that have a low pH value of around 3-4 are more susceptible to mold or yeast attacks (Baird, 2011). Yeasts are capable of metabolizing the organic acids; thereby resulting in a pH hike, which further facilitates the secondary bacterial growth. On the contrary, the soap-based emulsions have a pH above 8, thus are seldom spoiled by the microorganisms (Baird, 2011).

An active or inactive ingredient that is used to create a pharmaceutical dosage form is referred to as a pharmaceutical raw material (Food and Drug Administration. 2022b). These substances are made using various techniques, including fermentation; chemical synthesis, Recombinant DNA or other biotechnology techniques, extraction from natural sources, and/ or a mixture of all these methods (Food and Drug Administration. 2022c). Most of the pharmaceutical raw materials; depending on their nutritional qualities and moisture concentrations, support some types of microbial development (Parker, 2002). Some drugs like the herbal pharmaceutical products contain natural raw materials; for which the antimicrobial pre-treatment is not practicable, hence making them much more prone to Gram-negative bacterial contamination. A lot of these herbal dosage forms are commercially available in the form of tablets; powders, and capsules, which are often found to be contaminated with *E. coli* and other Gram-negative bacteria of the *Enterobacteriaceae* family (Enayatifard *et al*., 2010; Ratajczak *et al*., 2015). Inappropriate preparation techniques; contaminated supplies, and machinery, in addition to careless or incorrect resource handling by the infected workers during manufacturing, may all contribute to such Gram-negative bacterial contamination (Zeitoun *et al*., 2015). Additionally, crops used to make herbal products can be unintentionally contaminated by organic fertilizers that have not fully decomposed, or by ruminant feces that potentially carry *E. coli* and other *Enterobacteriaceae* family bacteria (Ratajczak *et al*., 2015). The plant products, including starch; tragacanth, gum acacia, powdered rhubarb, and agar may be contaminated by *Streptococcus* spp.; *Erwinia* spp., *Lactobacillus* spp., *Pseudomonas* spp., and/ or *Bacillus* spp., which may cause human diseases (Jones *et al*., 2011). *Aspergillus flavus*; a fungal producer of potentially harmful aflatoxin B (AFB), is detected in a number of raw materials used in herbal formulations (Singh *et al*., 2008). Thus, the crude herbal substances may often be accompanied with high aflatoxin contamination (Tassaneeyakul *et al*., 2004).

Hefny *et al*., (2022) conducted a study using 360 samples collected from 30 branded non-sterile pharmaceutical drops, including analgesic; anti-spasmodic, mucolytic, and multivitamin drops. According to their findings, 285 (79.17 %) of the samples were devoid of viable bacterial populations, while 75 (20.83 %) of the remaining examined samples had lower bacterial levels with a count of 10-10^2 cfu/ ml. Moreover, 46 (12.78 %) of the samples contained 10-10^2 cfu/ ml of fungi, whereas 314 (87.22 %) of them were fungus-free. Compared with the earlier reports conducted by other authors, this study's total number of isolated microorganisms was lower. This may be attributed to the pharmaceutical companies, which have started adhering to the Good Manufacturing Practices (GMPs) over the past few years (Hefny *et al*., 2022).

Gurung and Rai, (2021) conducted a study including 30 non-sterile pharmaceutical samples; including 15 cough syrups and 15 multivitamin syrups from various manufacturers; collected from several sellers of Pokhara valley, Nepal. The results showed that 14 cough syrups (93.3 %) and 13 multivitamin syrups (86.67 %) were contaminated with bacteria, fungi, and/ or both. Compared with the samples of

cough syrup, the multivitamin syrup was less contaminated. The minerals and antioxidants found in the multivitamin syrups may be responsible for this variation.

Some of the recently recalled pharmaceutical products by the Food and Drug Administration (FDA) on account of microbial contamination are listed in Table 2 (Food and Drug Administration. 2022a).

Table 2. Some recent FDA recall for pharmaceuticals due to their microbial contamination (Food and Drug Administration. 2022a)

Date	Product brand and description	Recall reason	Risks
06/22/2022	CVS Health, Magnesium Citrate Saline Laxative Oral Solution, Lemon Flavor. (Drugs)	Microbial contamination with *Gluconacetobacter liquefaciens*	Consuming this contaminated product can elevate the risk of invasive infections in the immunocompromised persons; brought about by *G. liquefaciens*, which may have serious and potentially fatal health effects.
06/09/2022	Snore Stop, Nasal spray. (Drugs)	Microbial contamination with *Providencia rettgeri.*	It may lead to serious or fatal outcomes in the immunocompromised individuals, including pneumonia; disseminated fungal infection, invasive fungal rhinosinusitis, and/ or bacteremia/ sepsis. *P. rettgeri.* may cause infectious complications, which are anticipated to be less severe in the non-immunocompromised people.
06/07/2022	Allergy Bee Gone for Kids, Nasal Swab Remedy. (Drugs)	Presence of increased levels of *Bacillus cereus* in the product.	*B. cereus* may lead to serious or fatal outcomes in the immunocompromised patients, including pneumonia; disseminated fungal infection, invasive fungal rhinosinusitis, and/ or bacteremia/sepsis. Moreover, it may cause infectious complications, which are anticipated to be less severe in the non-immunocompromised patients.
05/02/2022	Fagron, SyrSpendSF 500mL, and 4L. (Drugs)	May contain *Burkholderia gladioli.*	*B. gladioli* is an opportunistic pathogen that often affects the people who have respiratory illnesses. Patients at higher risk include those with impaired immune systems, such as those with cystic fibrosis. Complications following transplants may also result from it.

12/30/2021	Taro, Clobetasol Propionate. (Drugs)	Occurence of *Ralstonia pickettii*.	If *R. pickettii* contamination is present in the product, systemic infections can occur in people with compromised immune systems or with those whose skin has been damaged (such as in cases of sunburn; psoriasis, or abrasions). This is because the product contains a corticosteroid component that improves the ointment's absorption. Presence of this bacterium in the bloodstream of a person, can lead to invasive, potentially fatal infections, including pneumonia; sepsis, bone marrow or bone inflammation, joint fluid and tissue infections, and meningitis.

4. Effects of microbial contamination

Due to their minimal nutritional requirements and metabolic flexibility, many spoilage bacteria can utilize different preparation ingredients as their substrates. Numerous substances, including thalidomide (*i.e*., used in the treatment of cancer); analgesics (*i.e*., paracetamol and aspirin), alkaloids (*i.e*., atropine and morphine), barbiturates, mandelic acid, and steroid esters can be digested by bacteria, and hence act as substrates for their proliferation (Baird, 2011). The presence of crude animal and vegetable products in the formulation may act as additional nutrient sources for the microbial growth and their metabolic processes (Murtaza *et al*., 2021). The majority of *Pseudomonas* spp. can flourish even in the demineralized water made *via* the ion-exchange approach, because it typically has enough nutrients. However, the acute pathogens need certain growth factors that are frequently missing in the pharmaceutical preparations; as a result, they are unable to multiply; however, they endure for a significant amount of time while still being viable and contagious (Baird, 2011). It has been observed that high concentrations of the opportunistic pathogens, or low concentrations of the harmful bacterial toxins that survive for a long period of time after death of the pathogens, are the main causes of damaged cosmetics or herbal products. As a consequence of microbiological contamination, the chemical; physical, and organoleptic characteristics of the drug can get altered, and they may also get converted into toxic products (Ratajczak *et al*., 2015). As a result, there can be changes in the appearance; changes or losses in activity, changes in consistency, and separation of phases in the pharmaceutical products (Dao *et al*., 2018).

4.1. Change in appearance

The original appearance of a pharmaceutical product can change due to contamination and visible microbial growth can be observed (Dao *et al*., 2018). Various shades of microbial pigments are produced by the contaminating microorganisms, resulting in discoloration or a change in the original color of the products (Baird, 2011). Meanwhile, lactose is commonly used as an excipient in some tablets (Murtaza *et al*., 2021). Following incubation for one week, the lactose tablets change their color from white to brown, and three weeks later, they become soften. The microbe-induced chemical alteration of the tablet components, such as starch, can also result in a color change (Obuekwe and Eichie, 2006). Moreover, as a consequence of microbial contamination, the development of cracks at the sides and on the rough surfaces of the tablets can be observed. Starch; as a binding agent in the tablets, once being broken down by the contaminating microorganisms and then further consumed as a nutrient for their growth, leads to a

lessened binding capacity and consequent development of cracks (Obuekwe and Eichie, 2006).

4.2. Change in activity

The primary advantage of the tablets and the capsules is that they deliver an accurate and complete amount of the active ingredient, which is necessary for its therapeutic action (Murtaza *et al*., 2021). Due to microbial contamination, the activity of these various active ingredients such as acetylsalicylic acid; caffeine, hydrocortisone, and atropine can become altered. Contamination and subsequent degradation of caffeine by the fungi can result in the formation of theophylline (1,3-dimethylxanthine), which has higher pharmacological effects; however, it is more toxic than caffeine. Soluble intercellular esterase of *A. lwoffii* is responsible for degradation of the acetylsalicylic acid or aspirin, resulting in the loss of biological activity of this product (Dao *et al*., 2018).

4.3. Change in consistency

As a result of fungal contamination by *Fusarium* spp.; *Aspergillus* spp., and *Penicillium* spp., the viscous cosmetic formulations containing cellulose as the chief ingredient may convert into a mass of slippery consistency. This is attributed to the action of the fungal hydrolytic enzymes, including β-glucosidase; exo-β-1,4-glucanase, and endo-β-1,4-glucanase on the crystalline cellulose; rendering it inappropriate for use (Dao *et al*., 2018). The thickening and suspending agents such as acacia; tragacanth, and carboxymethyl (CM)-cellulose, can be depolymerized by the microbial action; resulting in loss of the viscosity and sedimentation of the suspended ingredients. On the other hand, polymerization of the surfactants and sugar molecules in the shampoos; creams, and syrups by the microorganisms can lead to slimy and sticky masses. Furthermore, the fungal growth may lead to gritty textures of the creams (Baird, 2011).

4.4. Separation of phases

Emulsions are the most commonly used cosmetics, and they can be categorized as creams or lotions based on their consistency. Compared to lotions, the creams have a thicker or heavier consistency (Salvioni *et al*., 2021). These semi-solid biphasic emulsions contain aqueous and oil phases in a pre-determined ratio (Dao *et al*., 2018). Although the oil-in-water type of emulsion is more popular to the consumers; however, it poses a high risk of contamination, because of the substantial amount of water used in its preparation, which represents an ideal environment for the bacterial growth (Shkreli *et al*., 2022). Separation of phases of this emulsion can occur as a result of lipolysis, which releases fatty acids from oils; thereby lowering the pH and promoting coalescence of the oil globules (Baird, 2011). *Pseudomonas* spp.; mainly *P. aeruginosa*, is potentially capable of adversely affecting the emulsions; as they can liquefy the gelatin and break down the hydrocarbons and fats (Dao *et al*., 2018). Phase separation of the oil-water and olive oil emulsions can also be performed by *Trichoderma viride*; *A. flavus*, and *A. niger* (Bloomfield, 2006).

5. Preservation strategies for the cosmetics and pharmaceutical products

Various preservation techniques are used by the cosmetics and pharmaceutical products manufacturers to prevent microbial contamination; without altering the characteristics of these products. Such preservation can be achieved by the use of both natural and synthetic chemical preservatives (Halla *et al*., 2018).

The term "preservative" refers to any synthetic or natural substance used to extend the shelf life of several products, including medications and cosmetics, by preventing microbial growth or by the subsequently-caused deleterious chemical alterations (Shaikh *et al*., 2016). Along with the natural chemicals such as a salt or alcohol, the preservatives can also be manufactured using synthetic substances. The need for this stems from the fact that the compounds that are organic or natural are not always healthy, as compared with those that are synthetically produced. In fact, the artificial preservatives have been used in food and

drug preparations for a very long time, since they function well in their minimal concentrations (Bhalerao and Bhosale, 2023). Although the chemical preservatives cannot completely prevent the products spoilage; however, a variety of them is used to combat the microbiological instability in the pharmaceutical and cosmetic products, by slowing down the microbial spoilage. However, the preservative activity against a specific group of microorganisms can hinder it from acting as a broad-spectrum antimicrobial agent. To solve this particular problem in the preservatives application, a specific combination of them is often preferred (Dao *et al*., 2018).

Several microbial preservation approaches are employed from the early production stages to the ultimate consumption stages (Halla *et al*., 2018). Preservatives must be added especially to such pharmaceutical products that have high aw to prevent microorganisms from altering and degrading them during storage (Shaikh *et al*., 2016). Careful handling of the preservatives in the manufacturing plant protects the consumers from the adverse effects of these preservatives in the final ready to use formulation (Dao *et al*., 2018). However, the quality of natural cosmetics must never be compromised by using harsh preservatives (Shaikh *et al*., 2016).

Despite the fact that many synthetic cosmetics and food additives are regarded as harmless; however, a few of them have been shown to be carcinogenic and toxic, thus limiting their use. It is crucial to realize that the toxicity; whether high or low, is an essential characteristic feature of these preservatives. Hence, nowadays; the preservatives are studied and handled with greater precision than ever before, and the use of the proper preservative in the right concentration is essential for the product to be safe and effective (Shaikh *et al*., 2016; Bhalerao and Bhosale, 2023). The Food and Drug Administration (FDA) has the primary legal responsibility for regulating the appropriate use of the preservatives. The manufacturers and/ or additional sponsors must first obtain a FDA approval before employing a compound that they have already been given a license (Bhalerao and Bhosale, 2023). Also, before employing any preservative; its minimum microbicidal concentration (MMC) and minimum inhibitory concentration (MIC) should be determined and taken into consideration (Dao *et al*., 2018).

Hence, considering all these factors, an ideal preservative must possess compatibility with the other employed substances during the preparation of pharmaceutical and cosmetic products; be non-toxic, less irritable, effective in low concentrations, stable in activity over a wide range of pH and temperature, and remains in the aqueous phase when a multi-phase product is formulated (Shaikh *et al*., 2016; Dao *et al*., 2018).

5.1. Choosing the right preservative

Successful preservation and physicochemical stability of the preservatives depend on a number of parameters that affect their antimicrobial efficacy (Bhalerao and Bhosale, 2023).

5.1.1. Stability

The preservative formulation; volatility, temperature, pH during usage, solubility, and partition in the water/ oil (W/O) or the oil/ water (O/W) emulsions are some of the parameters that may have an impact on durability of the preservatives. In fact, the product is unpreserved when parabens and the other lipophilic preservatives disperse in the lipid phase of the O/W emulsions. Additionally, the pH also influences the degradation or modification of the preservative action. For instance, parabens cease to be effective in the alkaline compositions; because at this pH, they begin to dissociate (Bhalerao and Bhosale, 2023).

5.1.2. Compatibility

According to the recent study conducted by Bhalerao and Bhosale, (2023), a suitable preservative must be compatible with the chemical components of the cosmetic and pharmaceutical formulations, such as the surfactants; solvents, colors, perfumes, and the other advertising additives. In this sense, the

antagonistic effects of some of the pharmaceutical components may render a number of the preservatives inactive. Talc, for example; hinders the antibacterial efficacy of methylparaben by over 90 %. On the other hand, some of the preservatives and substances such as the active compounds in sunscreen may interact cooperatively to provide a positive impact. EDTA is notable for its ability to work with a wide range of chemical preservatives; it disrupts the cell wall of bacteria, thus renders it easier to expose the cell to the other antimicrobial compounds.

The physical compatibility of a cosmetic product is also essential. The incorporation of a preservative may alter the overall appearance of a cosmetic or medicinal product. Depending on what kind of containers employed for packaging them, the preservatives' composition and activities may change in the cosmetic and pharmaceutical products. The lipophilic preservatives frequently have a greater possibility of being absorbed by the containers. Certain preservatives are incompatible with specific containers, such as polyethylene with specific phenolic compounds and mercury or nylon with parabens (Bhalerao and Bhosale, 2023).

5.1.3. Safety

Furthermore, Bhalerao and Bhosale, (2023) added that when used in cosmetic or pharmaceutical preparations; the preservatives may cause severe intolerance reactions. Therefore, selecting effective and non-toxic products typically becomes a primary concern. It is also imperative to consider the risks and safety hazards associated with handling the antibacterial compounds during the preparations production. In a previous study conducted by Neza and Centini, (2016), about 24 cosmetic products from various countries had to be recalled, since they contained certain levels of benzalkonium chloride (1 %); triclosan (0.4 %), methyldibromo glutaronitrile (0.025-0.36 %), and methylisothiazolinone (0.025-0.36 %), which exceeded those permitted by the European Regulation 1223/2009.

5.1.4. Cost

A crucial marketing factor is the cost of the cosmetic and pharmaceutical compounds (Bhalerao and Bhosale, 2023). The price of the cosmetics is influenced by a number of key factors, including the cost of production; the cost of delivery, and the cost of product advertising. For instance, starch and many scleroproteins is used in several different cosmetic formulations, because they are widely available and reasonably priced (Bhalerao and Bhosale, 2023).

5.2. Different widely used preservatives

The most widely employed preservatives in the cosmetics and pharmaceutical final products include the following:

5.2.1. Organic acids

The organic acids are widely used as preservatives because of their broad antimicrobial spectrum (Dao *et al*., 2018; Halla *et al*., 2018). Bhalerao and Bhosale, (2023) attributed this activity to their high acidity, thus it is difficult for the bacteria and mold fungi to survive in their presence. One major factor that affects the specific activity of each organic acid is the variation in pH. Dao *et al*., (2018); Punia Bangar *et al*., (2022) reported that growth of the spoilage bacteria and fungi is inhibited by organic acids, such as acetic; benzoic, lactic, sorbic, propionic, acid and formic acid. This is because, the organic acids; in general, cause pH imbalance within the microbial cells, which, in turn, leads to deleterious effects in them, including membrane disruption; inhibition of metabolic reactions, and accumulation of toxic anions (Dao *et al*., 2018). In addition, the organic acids inhibit the growth of the contaminating yeasts by generating extreme stress responses in order to re-establish homeostasis, which exhaust the stored energy required for proper growth and the other metabolic functions in these yeast fungi (Dao *et al*., 2018). Some particular organic acids, like formic; acetic, propionic, butyric, and benzoic acids, cause acidification of the environment external to the microbial cell; rendering it unsuitable for its growth (Stratford and Eklund, 2003).

Even the plasma membrane's fluidity becomes influenced by the medium-or long-chain organic acids (Halla *et al*., 2018). In their un-dissociated forms, several organic acids, including propionic acid; benzoic acid, formic acid, and sorbic acid, can penetrate the microbial lipid membrane and upon internalization; due to the neutral pH of the cell cytoplasm, they dissociate into anions and protons. Consequently, this affects the internal pH of the cytoplasm; thereby altering the isoelectric pH of the component amino acids of several essential enzymes that are involved in many vital processes, including glycolysis; cell signaling and active transport, in addition to disrupting the proton-motive force (PMF) (Halla *et al*., 2018). The anionic acids being negatively-charged; chelate the positively-charged metal ions, or essential trace elements of the shell of the microorganisms, and thereby removing them (Stratford and Eklund, 2003). Besides, once inside the microbial cells; benzoic acid hampers the active transport of some amino acids and oxo-acids, while sorbic acid inhibits the essential metabolic enzymes, including fumarase; aspartase, and succinate dehydrogenase, and it also inhibits germination of the bacterial endospores (Ortega Morente *et al*., 2013). The individual activity of each acid varies; depending on a number of intrinsic and extrinsic factors, including changes in the pH (Ricke, 2003). At an acidic pH, the ionic equilibrium of the weak acids shifts to an un-dissociated state; thus having high permeability across the plasma membrane of the contaminating microbial cells. Hence, at an acidic pH, the organic acid preservatives being weak acids possess greater antimicrobial activities (Dao *et al*., 2018).

5.2.2. Parabens

According to a recent study reported by Alaba *et al*., (2022), manufacturers of the pharmaceutical and cosmetic products frequently employ parabens to maintain the microbiological purity of the chosen products, thus prolong their shelf life. Three of the most popular paraben compounds are methylparaben; propylparaben, and butylparaben (Watson, 2022). The antimicrobial activities of parabens are mostly directed against the Gram-positive bacteria, rather than the Gram-negative ones. Compared with the bacteria, these parabens however are more efficient against the moulds and yeast fungi (Sandler, 2017). These preservatives can be used either alone; or to overcome their stability issue, they can be used in combination with other parabens or with other antimicrobial agents, including 2-5 % propylene glycol or imidurea (Dao *et al*., 2018). The broad-spectrum antimicrobial activity of parabens is stable over a pH range of 4-8, and increases as the chain length of the alkyl moiety of a particular paraben increases. However, an increase in the chain length causes a decrease in the aqueous solubility of a paraben. In these cases, paraben salts; especially sodium salts, are often added in the formulations to overcome their low solubility issues. However, this treatment may result in an increase in the pH of the weakly-buffered formulations (Sandler, 2017). The parabens, in general, cause inactivation of the microbial cytoplasmic membranes by interfering with them (Soni *et al*., 2005). Parabens react with the free amino acids; particularly glutamic acid and aspartic acid, leading to an inhibition of the protein synthesis, including the production of essential enzymes such as ATPase and phosphotransferase (Garner *et al*., 2014). Parabens also inhibit the DNA and RNA syntheses; affect the transport of nutrients across the cell membrane, interact with the mechano-sensitive channels by allowing leakage of the cytoplasmic materials, and specifically inhibit the mitochondrial oxygen consumption in fungi (Ito *et al*., 2015; Halla *et al*., 2018). However, due to a long list of potential side effects; parabens, which are present in the majority of the creamy or liquid cosmetics, are representing a greater risk (Alaba *et al*., 2022). For instance, Monna, (2022) revealed that parabens may mislead the body by thinking that they are hormones, as they are known as endocrine disruptors. Meanwhile, if this leads to an imbalance in the level of hormones, the human body may experience several problems. Parabens can also lead to allergies and skin rashes (Alaba *et al*., 2022). Hence, the international organizations have set standards for the appropriate

levels of paraben that are used in the different cosmetic products (Al-Halaseh *et al*., 2022). The cosmetics contain parabens in concentrations that range from 0.01 to 0.3 %, as the Cosmetic Ingredient Review highlighted that these compounds are safe at concentrations up to 25 % (Becker, 2022). Methyl-β-D-maltoside and methyl-β-D-maltotrioside can be used as alternatives to parabens, as these compounds have the potency to inhibit the microbial enzymatic metabolism; with lower toxicity than that of parabens (Marçon *et al*., 2013).

5.2.3. Phenol

Phenol is extensively employed in the cosmetic and parenteral formulations as an antibacterial preservative (Dao *et al*., 2018). The various kinds of plant phenols include simple phenols; flavonoids, phenolic acids, anthocyanins, tannins, stilbenes, lignins, and lignans (Rathee *et al*., 2023). They are generally used to extend the product shelf life by inhibiting the development of oxidative rancidity; contamination, discoloration, degradation, and any other undesired changes. Meanwhile, due to their natural origins, the phenolic compounds are frequently used in place of parabens in the cosmetics, since they are smooth; do not irritate the skin, and do not release formaldehyde. Furthermore, Rathee *et al*., (2023) added that phenols are more advantageous than the synthetic items in several ways, as they are widely available; reliable, inexpensive, and have a few side effects. Although phenol is a broad-spectrum antimicrobial agent, its activity against the molds is not very rapid (Dao *et al*., 2018). It shows its antimicrobial activity in a concentration-dependent manner. Phenol shows a bacteriostatic effect at low concentrations and a bactericidal effect at high concentrations. At high concentrations, the phenol is very toxic; thus before its addition to any formulation, the concentration of the phenol must be checked properly. It has been observed that; with an increase in temperature, the antimicrobial activity of phenols also increases (Dao *et al*., 2018).

5.2.4. Organomercurials

Thimerosal; also known as thiomersal, is an organomercurial compound, having appreciable bacteriostatic and fungistatic activities (Kadri, 2017). It is frequently utilized for preservation purposes of the cosmetic products, including mascaras; make-up removers, soap-free cleansers, and of the pharmaceutical preparations such as eye- ear- and nose drops and ointments; topical medications, antiseptic sprays, and tinctures of Merthiolate (Geier *et al*., 2015). However, thimerosal is not much effective against the molds and their spores. Its antimicrobial activity is dependent on the pH value, i.e. it exhibits bactericidal activity at acidic pH, and displays bacteriostatic and fungistatic activities at neutral or alkaline pH (Kadri, 2017). However, several researches revealed that thimerosal has potential adverse effects on the human cells (Geier *et al*., 2015). Recently, Azevedo *et al*., (2023) highlighted that although thimerosal is used in vaccinations and is effective; however, there is an ongoing controversy over its safety, due to the potential dose-response effects on the human central nervous system (CNS).

As antimicrobial preservatives, the phenylmercuric salts such as phenylmercuric borate and phenylmercuric nitrate are frequently utilized in the ophthalmic; cosmetic, parenteral, and topical preparations, due to their effectiveness against the bacteria and fungi (Dao *et al*., 2018). As the pH increases, these salts become more effective in their action (Maiti *et al*., 2016). However, these salts have toxic effects since mercury may deposit on the lens; cornea, eyelids, and conjunctiva; besides having slower antibacterial action against the heavy inocula of *P. aeruginosa*; in particular (Maiti *et al*., 2016). Nowadays, in the pharmaceuticals and cosmetics; the phenylmercuric acetate is used as an alternative to the toxic phenylmercuric borate, and the less soluble and toxic phenylmercuric nitrate. However, phenylmercuric borate causes less irritation compared with phenylmercuric acetate and phenylmercuric nitrate. On the other hand, phenylmercuric nitrate has the benefit of being not precipitating in the formulations; a property of virtue by which it can

replace the phenylmercuric borate and phenylmercuric acetate when needed (Dao *et al*., 2018). All the organomercurials increase the microbial cellular permeability by binding to the sulfhydryl (-SH) groups of the cell membrane proteins; in addition, they kill the microorganisms by hampering their internal cell respiration (Maiti *et al*., 2016).

5.2.5. Ethanol

In cosmetics and pharmaceutical formulations, ethanol and its aqueous solution is extensively used at varying concentrations as an antimicrobial preservative. The antimicrobial activity of ethanol increases when used in combination with edetate salts or edetic acid (Quinn, 2017). In general, denaturation of proteins or inhibition of their biosynthesis serves as the mechanism of action of ethanol (Bhalerao and Bhosale, 2023). In *E. coli*; as a common bacterial contaminant, ethanol increases the cell-envelope permeability and decreases the peptidoglycan cross-linking, leading to its death. Quite interestingly, it has been observed that *E. coli* alters the composition of its membrane lipids when exposed to an ethanol stress (Haft *et al*., 2014). Additionally, phenoxyethanol may cause bacterial membrane lysis at low doses (Bhalerao and Bhosale, 2023). When non-ionic surfactants are introduced; ethanol's activity is hindered, and it becomes ineffective against the bacterial spores (Quinn, 2017).

5.2.6. Chlorobutanol

Chlorobutanol is used as an antibacterial preservative in the parenteral and/ or ophthalmic dosage forms, and in the non-aqueous preparations (Hanson, 2017). In addition, it is the active ingredient in several oral sedatives and topical anaesthetics. Furthermore, chlorobutanol was once advertised in several nations as an antiseptic mouthwash and a preservative in the topical pastes; intravenous vitamin formulations, and several other medications (Jeong *et al*., 2022). However, as it shows slow antibacterial activity, it is combined with other preservatives in some commercially available eye drops (Maiti *et al*., 2016). Chlorobutanol causes disorganization of the lipid bilayer of the bacterial cell membrane and increases the cell permeability; resulting in cell lysis (Dao *et al*., 2018). However, this recorded antibacterial activity of chlorobutanol decreases above pH 5.5 (Hanson, 2017). The ophthalmic solutions preserved with chlorobutanol cause less damage to the human corneal epithelial cells than benzalkonium chloride, due to slower incurrence of the cytotoxic effects of the former (Maiti *et al*., 2016).

5.2.7. Benzalkonium chloride

A quaternary ammonium salt such as benzalkonium chloride is widely used as an antimicrobial preservative in the cosmetic and pharmaceutical formulations (Kibbe and Quinn, 2017). It is one of the most frequently used eye drop preservatives; although it has been reported to cause ocular damage to some extent (Datta *et al*., 2017). The recent study conducted by Bhalerao and Bhosale, (2023) reported that benzalkonium chloride is effective against some strains of *Mycobacterium*; *Trichophyton*, and *P. aeruginosa*. The anti-Pseudomonal action of benzoalkonium chloride increases when combined with EDTA; benzyl alcohol, 2-phenylethanol, and/ or 3-phenylpropanol (Bhalerao and Bhosale, 2023). This compound denatures the protein components of the microbial cytoplasmic membrane; leading to its degradation (Dao *et al*., 2018). Furthermore, benzalkonium chloride has the potency to directly target the mitochondrial Complex I in the eukaryotic microbial contaminants; as a result, it inhibits the mitochondrial ATP synthesis (IC_{50}, 5.3 µM) and O_2 consumption (IC_{50}, 10.9 µM) (Datta *et al*., 2017).

5.2.8. Chlorocresol

In both of the cosmetic and pharmaceutical formulations; chlorocresol is commonly employed as an antibacterial preservative. Various forms of skin infections are treated with a fixed dose formulation of neomycin sulphate; clobetasol propionate, and chlorocresol (Patel *et al*., 2022). Chlorocresol is effective against the bacteria, the fungi including the

molds and yeasts, and their spores (Nema, 2017). In the bacteria; higher chlorocresol concentrations cause the cytoplasmic components to coagulate, which results in an irreversible cellular damage. However, in fungi; chlorocresol stimulates leakage of the cell components, due to the damage it causes to their plasma membrane. When combined with other antimicrobial preservatives such as 2-phenylethanol; chlorocresol shows synergistic effects in its elevated antimicrobial activity (Dao *et al*., 2018). In an acidic medium, chlorocresol is an efficient antimicrobial preservative; however, its activity decreases with the increase in pH, thus as the pH increases beyond 9, it completely loses its activity. Moreover; in the presence of non-ionic surfactants, the efficacy of chlorocresol decreases (Nema, 2017).

5.2.9. Sodium benzoate

Sodium benzoate is largely utilized in cosmetics and pharmaceutical products as an effective antibacterial preservative due to its high water solubility. At 2-5 % w/w, it may be used as a tablet lubricating agent. It is found in oral medicines at concentrations of 0.02-0.5 %; parenteral drugs at 0.5 %, and cosmetics at 0.1-0.5 %. However, sodium benzoate utility as a preservative is restricted, because it is effective only across a limited pH range (He, 2017). Moreover, sodium benzoate has been found to be genotoxic; clastogenic and neurotoxic, and occasionally have a disagreeable flavor. In addition, sodium benzoate is responsible for cell cycle changes and intercalation into the DNA double-helical structure (Bruna *et al*., 2018). Later, Walczak-Nowicka and Herbet, (2022) added that sodium benzoate; when used as a preservative, can react with vitamin C and produces the carcinogenic benzene. Furthermore, sodium benzoate has a potential promise for several therapeutic applications used in the management of many disorders, including schizophrenia; depressive disorders, autistic spectrum disorders (ASDs), and neurodegenerative disorders.

5.2.10. Isothiazolinones

Isothiazolinones are heterocyclic organic compounds. Among them, methylchloroisothiazolinone or methylisothiazolinone is most often used as a preservative in the shampoos; toiletries, skin creams, and lotions (Yim *et al*., 2014). It is possible to manipulate the methylchloroisothiazolinone and methylisothiazolinone individually, or in the form of a 3:1 mixture (Corrêa *et al*., 2022). They display antimicrobial activity by oxidizing the proteins; especially the thiol (-SH) groups of the cysteine residues. This oxidation inhibits the proper functioning of the structural proteins present in the cell wall and cell membrane of the microbial contaminants, and may also inhibit the enzyme metabolism (Lambert, 2012). Methylchloroisothiazolinone is more effective than methylisothiazolinone alone. To be effective; methylisothiazolinone needs to be used at higher concentrations. However, as it may cause irritation and chemical burns; it is usually not used alone, and hence often combined with other preservatives (Yim *et al*., 2014).

5.2.11. Sodium sulfite and sodium metabisulfite

Both sodium sulfite and sodium metabisulfite act as potential antimicrobial preservatives (Cook and Foan, 2017; Sienkiewicz and Driver, 2017). Sodium sulfite behaves as a preservative in the cosmetics and pharmaceutical preparations, including the parenteral formulations; oral preparations, inhalations, and topical formulations. At 1 % w/v and at low pH, sodium sulfite is often used against the fungi (Sienkiewicz and Driver, 2017). Sodium metabisulfite is also most effective at low pH and can be used as an antimicrobial preservative in the oral formulations such as syrups (Cook and Foan, 2017). However, sodium metabisulfite may cause allergic contact dermatitis; a Type IV hypersensitivity reaction (Oliphant *et al*., 2012). These reactions are usually clinically significant and are associated with the use of drugs and personal care items containing sodium sulfite and sodium metabisulfite (Warshaw *et al*., 2021).

Conclusion

A lot of cosmetic and pharmaceutical products are often contaminated with the microorganisms. The acceptable level of contamination varies across the different products; based on their usage and functions. Microbial contamination may not only render the product ineffective but it may also adversely affect the user; especially if the contaminating microorganisms are pathogenic in nature. Thus, it is of great concern for the cosmetic and pharmaceutical manufacturers to control the microbial contamination of their final products; so as to ensure the consumers safety and satisfaction. A wide range of antimicrobial preservatives is used in these products to reduce the microbial contamination; some of which may show synergistic effects. Thereby, a combination of preservatives is often used to increase their antimicrobial efficacy. However, some preservatives may have adverse effects on the human health. So, to ensure safety of the consumers; it is important to use the right preservative in an optimum concentration during the manufacture of these cosmetic and pharmaceutical products, which are of magnanimous societal needs.

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Conflict of interest

The authors declare that there is no conflict of interests.

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